MANIPULATING PIG PRODUCTION VI

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1. Swine - Australasia - Congresses. 2. Swine - Australia - Congresses. 3. Swine - Growth - Congresses. 4. Swine - Nutrition - Congresses. 5. Swine - Reproduction - Congresses. 6. Swine - Lactation - Congresses. 7. Swine - Genetics - Congresses. 8. Swine - Health - Congresses. 9. Meat - Quality - Congresses. 10. Agriculture - Environment - Congresses. I. Cranwell, P.D. Peter Donald, 1937 - , II. Title.

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CONTENTS

A.C. DUNKIN MEMORIAL LECTURE

REVIEW:

REPRODUCTION, LACTATION AND PRE-WEANING GROWTH

REVIEW:

ONE-PAGE PAPERS:

MEAT QUALITY AND MEAT HYGIENE

SYMPOSIUM: Strategies to improve consistency of pork quality - Towards 2010

ONE-PAGE PAPERS:

GENETICS AND ANIMAL BREEDING

ANIMAL HEALTH

ONE-PAGE PAPERS:

APPLIED NUTRITION AND NUTRIENT DIGESTIBILITY

SYMPOSIUM: Sustaining supply and improving the utilisation of feed grains by the pig industry

ONE-PAGE PAPERS:

PIG PRODUCTION AND THE ENVIRONMENT

SYMPOSIUM: The impact of pig production on the environment, and opportunities for future control

ONE-PAGE PAPERS:

GROWTH, DEVELOPMENT, HEALTH AND ENVIRONMENT

ONE-PAGE PAPERS:

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The Proceedings, 'Manipulating Pig Production VI', contains 117 one-page papers, three Reviews and three Symposia, a total of 313 pages. As is the policy of the Association, all one-page papers, Reviews and Symposia were reviewed by external referees (at least two per paper). The committee of APSA and the editor gratefully acknowledges the assistance generously given during 1997 by the following referees and by those who wish to remain anonymous or who were inadvertently omitted from the list.

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PREFACE

These are the Proceedings of the Sixth Biennial Conference of the Australasian Pig Science Association (APSA). The first APSA meeting was held in 1987 and since then has been recognised as the major forum for scientific exchange within the Australian pig industry. The major strength of this meeting is the broad range of disciplines covered and a major focus for the committee is to ensure that this comprehensive base is maintained. Topics for invited reviews and symposia have been selected to meet both the current interests of the industry as well as ensuring diversity.

The Proceedings include a record number of one-page papers (117) which is an indication of both the amount and quality of research being conducted in Australasia as well as confirmation of the respect which the APSA meeting and proceedings have gained. Much of this research is supported by the Australian Pig Industry through the Pig Research and Development Corporation who are to be congratulated on their continued support of pig researchers and APSA. The growing trend towards international contributions also continued confirming the international recognition of APSA and it's activities.

Within the pig industry there is a growing interest in delivering a consistent high quality product in a sustainable manner. Symposia and submitted papers within these proceedings address these issues. A comprehensive review of recent advances in pig genetics is presented here while the reproductive consequences of early weaning are also discussed. The often made comparison between pigs and humans is further explored in a discussion on the pig as a biomedical model.

Our association has always maintained a rigorous editorial and review process for the Proceedings. The current editor Mr P.D. Cranwell has continued to ensure that material presented at the meeting and published in the Proceedings meet these standards. In addition, the committee have taken the position that one-page contributions appearing in the Proceedings are not abstracts but rather refereed (by at least two external referees) one-page papers.

I would like to thank the Organising Committee for their efforts during the past two years. The Committee consisted of Dr N.J. Gannon (Secretary), Mr R.J. Smits (Treasurer), Dr R. J. van Barneveld (Vice President), Dr. B.P. Mullan (Past President), Assoc Prof R.J. Love, Dr A.J. Peacock, Dr R.D. Warner and Dr I.H. Williams The editor Mr P.D. Cranwell also served as a non-voting member of the organising committee. Dr A.J. Peacock provided local support for the committee and this was complemented by the staff of ACTS Pty Ltd.

Early in 1994 APSA lost one of its most active members with the untimely death of Dr. Ted Batterham. To recognise the major contribution that Ted made to APSA and pig science in general a Batterham Memorial award was established in 1995 and has again been awarded this year. The award was made possible through the generous financial support of the following national and international companies: BASF, Farmstock, Heartland Lysine (US), Purina Mills (US), Rhône-Poulenc Animal Nutrition and Ridley Agriproducts.

F.R. DUNSHEA
President
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A REVIEW - GROWTH, DEVELOPMENT AND NUTRIENT METABOLISM IN PIGLETS AND INFANTS

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Abstract

This paper is concerned with comparisons of pig and human growth and metabolic regulation and focuses on a discussion of the usefulness of the piglet as a model for the human infant. In the first section the effects of colostrum, milk and insulin-like growth factor I (IGF-I) on tissue growth and protein synthesis in the neonate are considered. There is some evidence to suggest a functional role for milk-borne IGF-I, but it is clear that there are other, unknown factors in colostrum and milk that are equally important. The next section considers the use of piglets to quantify nutrient metabolism during total parenteral nutrition. The efficiencies with which low-birth-weight (LBW) infants and artificially-reared piglets utilise dietary energy and protein are very similar and it is argued that 'requirements' defined in piglets are of direct relevance to those of human infants. The next section is concerned with the hormonal regulation of synthesis of tissue protein. Recent investigations of tissue responses to feed intake in sucking piglets are considered and it is concluded that, in the neonate, insulin plays a key role in the regulation of muscle, but not hepatic, protein synthesis. The final sections consider isotopic investigations of digestibility and fuel metabolism. Recent work suggests that the intestinal mucosa may be a critical site of synthesis of conditionally essential amino acids and that enteral amino acids play a special role in maintaining these pathways of biosynthesis. Other studies of intestinal metabolism indicate clearly that nutritionally significant quantities of dietary amino acids are metabolised by the small intestinal tissues and suggest that amino acid oxidation may be the principal source of mucosal energy. Piglets are ideally suited for coordinated nutritional, metabolic and molecular investigations of growth regulation and have the advantage that the information generated can find use in both animal husbandry and paediatric investigation.

Introduction

Paediatric background

Because of the marked improvements in perinatal care that have occurred over the last 25 years, many neonates that are delivered prematurely or retarded in growth survive beyond the immediate postpartum period. The nutritional support of these infants presents substantial conceptual and practical challenges (Heird and Gomez, 1996). Apart from the support of early growth, the perinatal nutrition of these infants also has a substantial bearing on their long-term cognitive and behavioural development (Lucas, 1990,1992). Furthermore, there is now an awareness that the environment, especially the nutritional circumstances, of the 'normal' fetus and neonate can have long-term effects on the subsequent susceptibility of the individual to chronic diseases (Barker, 1995). These observations are well established at a descriptive and epidemiological level, but the underlying mechanisms remain obscure. While chronic degenerative diseases may not be a primary concern of those whose focus is on farm animals bred for meat production, adequate early development is crucial to the growth potential and efficiency of productive species. Thus, understanding normal postnatal development, the factors that affect it and the mechanisms whereby these factors act is of equal importance to the care of human infants as well as the care of meat-producing animals.

Why use piglets ?

For obvious ethical reasons, experiments that are based on the manipulation of the growth and development of human infants must be of very limited scope. Advances in the understanding of this subject will continue to depend heavily on the use of suitable animal models. At the outset, it is important to recognise explicitly that the main utility of animal models is in the establishment of general principles. While a given species might be useful for the study of one physiological function, it could be quite unsatisfactory as a model for another. Furthermore, the decision to use a particular species as a model of human function is often based on practical as well as theoretical considerations, an apposite example of which is the domination of the sheep in research on fetal fuel metabolism (Battaglia, 1989).

At the practical level the pig is an attractive candidate for studies of postnatal growth and development. The newborn piglet is sufficiently large to enable quite extensive surgical manipulation, but sufficiently small that other costs associated with the experiment are minimised. In addition, because the physiological and metabolic development of the newborn pig is well advanced, piglets can be easily raised artificially, even by total parenteral nutrition (TPN, see below) and the high voluntary appetite and equally high growth potential of the piglet allow studies over a wide range of nutrient intakes and growth rates. Finally, a significant advantage of the piglet as an experimental model for neonatal development is that the information so gained may not only help to understand aspects of human growth and development, but will also be of specific importance to animal production. In the authors' opinion, the same cannot be said with equal force about any other species.

Scope of the paper

The proceedings of a recent conference (Tumbleson and Schook, 1996) on the many uses of pigs as models of human physiology and disease amounted to two volumes containing 1000 pages. Clearly, a complete discussion of this subject is well beyond the scope of a single paper and therefore the present paper will consider three main areas of research:

1. Studies aimed at understanding the potential benefits of species-homologous milk to neonatal growth and development.
2. Studies of nutrient needs and the hormonal regulation of protein metabolism during the suckling period.
3. Recent isotopic investigations of the regulation of bioavailability of organic nutrients and their metabolism.

These three subjects have been selected, partly because each represents a focus of much current research, and partly because they illustrate the important role that the piglet could play in other investigations of subjects of direct paediatric relevance.

Pig and human growth and development

Comparisons of the developmental biology of pigs and human beings have been presented many times (Pond and Haupt, 1978; Widdowson *et al.*, 1979; Tumbleson and Schook, 1986, 1996; Miller and Ullrey, 1987; Shulman *et al.*, 1988; Moughan *et al.*, 1992). Therefore, the following discussion intends to highlight only the specific aspects that bear on the present paper's theme. In pigs and humans major portions of intestinal, visceral, skeletal muscle and neural development are well advanced at birth. For example, in both species, birth occurs in the midst of the developmental spurt of brain-mass accretion (Dobbing and Sands, 1979). Furthermore, because both species are omnivorous (as opposed to carnivorous or ruminant), it is reasonable to assume that the fundamental aspects of the regulation of their digestive function (Moughan *et al.*, 1992) and fuel metabolism (Duée *et al.*, 1996) will be similar and, as discussed below, many comparisons underscore this point.

There are, however, some important differences. In some respects, the newborn pig is less mature than the full-term human infant (Moughan *et al.*, 1992; Sangild *et al.*, 1996). The newborn piglet is much smaller in relation to its mature weight than the human and, indeed, many other mammals (Reeds, 1990). Thus, a much higher proportion of the pig's eventual weight gain occurs after birth. In some respects, this is an advantage for the design of experiments, because the substantial growth capacity of the piglet enables its rate of protein deposition to be manipulated over a wide range (e.g., Séve *et al.*, 1986). The full-term pig and full-term infant also differ with regard to their gross body composition. In the pig (Hausman *et al.*, 1991, 1992), the terminal stages of adipocyte maturation occur after birth and, presumably as a consequence, the body fat mass of the newborn pig is very low. Furthermore, the newborn piglet, like the low-birth-weight infant (LBW infant; Cornblath and Schwartz, 1993), is vulnerable to peripartum hypoglycaemia (Pégorier *et al.*, 1981), and because of its low fat stores, it is particularly reliant on milk fat intake for the maintenance of gluconeogenesis (LeDividich *et al.*, 1991; Lepine *et al.*, 1993).

There are also differences in gastrointestinal development between the full-term neonates of pigs and humans. Although the gastrointestinal tissues of the piglet make a much higher contribution to body weight than they do in the human neonate, some aspects of gastrointestinal function of the pig are less well developed at birth (Moughan *et al.*, 1992; Buddington and Malo, 1995; Sangild *et al.*, 1996). The newborn pig has a particularly marked ability to acquire macromolecules via mucosal endocytosis, while macromolecular absorption by the mucosa of the human is largely a fetal function and is manifest in human infants that are born prematurely (Goldblum *et al.*, 1989; Hutchens *et al.*, 1991). The amount of sucrase-isomaltase is also lower in the neonatal pig, although in this context it should be pointed out that the early developmental appearance of this enzyme in the human may be the exception of mammalian development, rather than the rule (Grand *et al.*, 1976). On the other hand, the postnatal changes in the expression of other brush border enzymes, and especially the relatively slow rate at which lactase activity decreases during the first few weeks of postnatal life (Kelly *et al.*, 1991), render the pig a better analogue of human development than the rodent. As a consequence of these differences, a number of authors (e.g., Shulman, 1993; Borum, 1993; Ball *et al.*, 1996) have suggested that the neonatal piglet is most appropriate as a model of the LBW infant.

The major area in which the pig is more advanced developmentally than the human is in relation to skeletal muscle function. The contribution of skeletal muscle to the body protein stores of the piglet is higher than that of the newborn human and, of course, the piglet is born mobile. This implies that the maturational program of muscle protein expression and neuromotor control of the pig is essentially complete at birth. However, the relatively poor coordination of muscle function characteristic of the newborn baby seems more a reflection of the development of motor control than of muscle development; from the perspective of maturation of muscle enzymes, the piglet and the human neonate are remarkably similar (Swatland and Cassens, 1973; Colling-Saltin, 1978; LeFaucheur *et al.*, 1995).

Mammary secretions and the regulation of neonatal growth and development.

Paediatric background

An obvious area of concern with regard to the early nutritional support of LBW infants is their long-term development. Aside from severe functional defects, such as cerebral palsy (Bhushan *et al.*, 1993), there is longstanding evidence for permanent deficits in intellectual function in small-for-gestational age (SGA) infants (Babson and Phillips, 1973; Henrichsen *et al.*, 1986). Although it has become almost universally believed that the consumption of colostrum and species-homologous milk confers developmental benefits, objective evidence to support this view is difficult to find.

An exception to this can be found in the results (Figure 1) of an extensive prospective study of the long-term influence of early feeding on the development of cognitive function in LBW infants (Lucas *et al.*, 1992, 1994). These studies generated three notable observations. First, the beneficial effects of appropriate feeding were more

apparent in SGA infants, than in those that were born with a body weight that was appropriate for their gestational age (AGA infants). Second, a higher intake of protein and trace nutrients provided by a nutrient-dense preterm formula not only had a marked effect on weight gain over the first month of life, but was also of long-term benefit to the infants' psychomotor development. Third, and most important, the benefits to subsequent development of a higher energy and protein intake in early infancy could be reproduced by the consumption of breast milk alone, even though the consumption of breast milk conferred no early advantage in terms of weight gain.

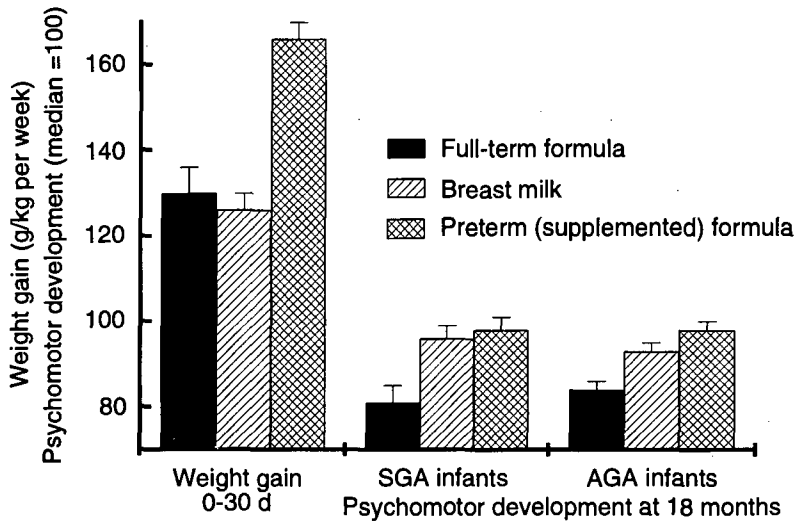

Figure 1. *Effect of diet on post-natal weight gain and psychomotor development in low-birth-weight infants (SGA, small for gestational age; AGA, appropriate for gestational age) (Lucas et al., 1994).*

Effects of colostrum and milk in piglets

Since the work of Widdowson (e.g., Widdowson *et al.*, 1976), that showed large effects in tissue growth following the ingestion of colostrum by piglets, it has also been held widely that colostrum has specific growth-stimulatory effects. There is also some limited evidence (Stack *et al.*, 1989) to suggest that the consumption of breast milk by LBW infants increases the rate of whole-body protein turnover at any given protein intake. However, until recently, the specificity of colostral effects, and in particular whether there were effects manifest only in specific tissues, has not been tested systematically. In order to obtain such information, Burrin *et al.* (1992b, 1995, 1997) carried out experiments in newborn piglets to identify whether the consumption of colostrum has effects on synthesis of tissue protein that cannot be reproduced by a similar macronutrient intake provided by a formula diet. Not surprisingly, the results (see also Mitton and Garlick, 1992; Davis *et al.*, 1997) showed that merely feeding a newborn animal produces a rapid increase in insulin, insulin-like growth factor I (IGF-I) and tissue-protein synthesis. Moreover, in comparison with mature porcine milk (which likely supplied bioactive molecules but lower quantities of nutrients than colostrum) or a supplemented formula (which supplied the same quantities of nutrients found in colostrum, but no known bioactive factors), feeding colostrum had additional stimulatory effects on protein synthesis in some, but not all, tissues.

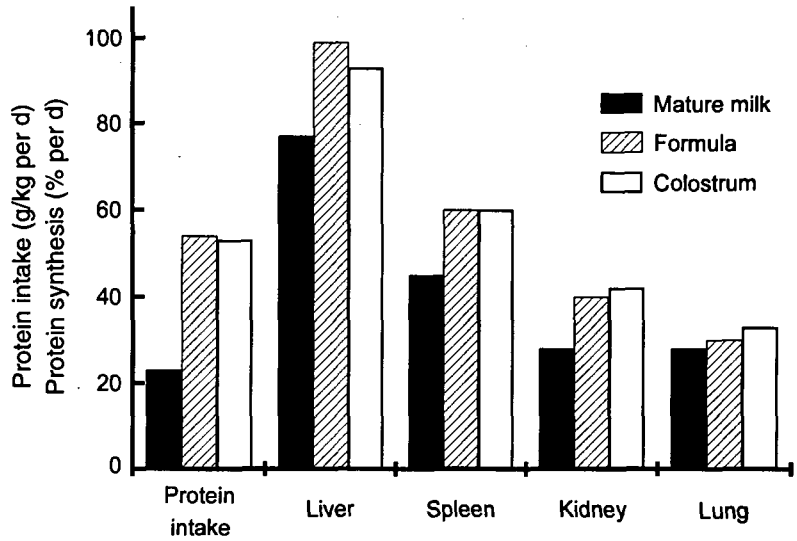

Figure 2. *Tissues of the newborn pig in which protein synthesis apparently responds primarily to nutrient intake (Burrin et al., 1992b, 1995, 1997).*

The results obtained by Burrin *et al.* (1992b, 1995, 1997) are shown in Figures 2 and 3. With the exception of the jejunum, the visceral tissues appeared to respond primarily to nutrient intake (Figure 2); it was the 'peripheral' tissues, specifically skeletal muscle, heart and brain (Figure 3), that exhibited an additional protein synthetic response to colostrum. Recent work by M.L. Fiorotto (unpublished) suggests that the stimulatory effect of colostrum on muscle-protein synthesis is confined to proteins associated with contractile function, and that the consumption of colostrum increases the level of expression of their mRNAs. The central question, of course, is: what is the mechanism underlying this effect?

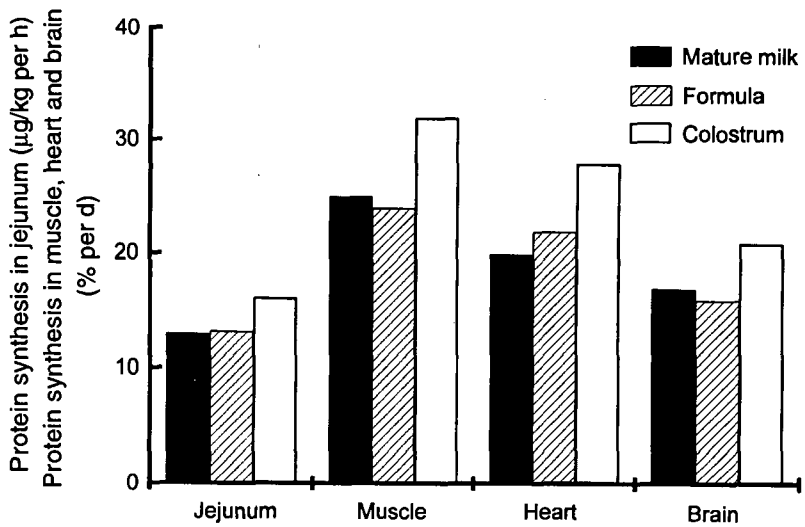

Figure 3. *Tissues of the newborn pig in which protein synthesis responds to colostrum (Burrin et al., 1992b, 1995, 1997).*

Possible role of milk-borne IGF-I

Given the fact that in Burrin's studies (Burrin *et al.*, 1992b, 1995, 1997), it was the peripheral tissues that apparently responded to colostrum, it would seem reasonable to propose that the response reflects the influence of the absorption of specific colostral components. Because both colostrum and mature milk contain a variety of well-recognised hormones and peptide growth factors (Klagsbrun, 1978; Shing and Klagsbrun, 1987; Francis *et al.*, 1988; Grosvener *et al.*, 1993; Odle *et al.*, 1996), and because the neonatal pig mucosa has a well-documented ability to absorb macromolecules, it is a natural presumption that the stimulatory effects of colostrum on synthesis of peripheral tissue protein reflect the systemic effects of a peptide growth factor that has been absorbed from the diet. Although there have been some investigations of the effects of the ingestion of other growth factors (Zijlstra *et al.*, 1994) and hormones (Shulman, 1990; Shulman *et al.*, 1992), particular effort has been put into the study of the role of IGF-I in the regulation of the growth of the sucking piglet (Xu *et al.*, 1994; Burrin *et al.*, 1996; Donovan *et al.*, 1996, 1997).

Although the consumption of colostrum is associated with a rise in concentration of circulating IGF-I, it is difficult to argue that the growth stimulation from the ingestion of colostrum is due to systemic effects of the IGF-I absorbed from colostrum. Studies with ^{125}I-labelled IGF-I (Donovan *et al.*, 1996) administered orally have shown that the peptide survives intact in the small intestine and, moreover, binds to the mucosal surface. However, when given in the quantities normally found in colostrum, little IGF-I is absorbed intact in newborn piglets (Donovan *et al.*, 1997). Even the ingestion of large quantities of IGF-I added to a nutrient-dense formula does not alter circulating IGF-I to a greater extent than that associated with feeding formula alone (Burrin *et al.*, 1996; Burrin, 1997). Furthermore, with the possible exception of small effects on the pancreas (Xu *et al.*, 1994) and liver (Burrin *et al.*, 1996), to the present authors' knowledge, no growth-stimulatory effects from the ingestion (as opposed to the systemic administration) of IGF-I have been demonstrated in the extra-intestinal tissues of piglets.

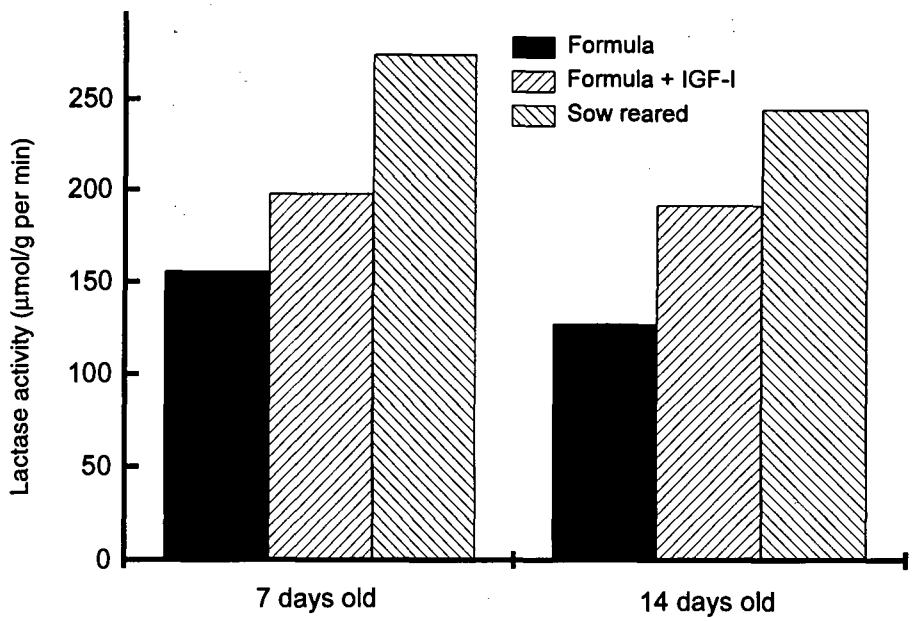

Figure 4. *Effect of IGF-I and natural milk on lactase expression in piglets (Houle et al., 1997b).*

The ingestion of IGF-I does, however, have some local stimulatory effects in the small intestine. The ingestion of pharmacological quantities of IGF-I over the first 4 days

of life (Burrin *et al.*, 1996), increases the mass of the small intestine and its mucosal protein and villous height. In addition, although lower, 'physiological', amounts of IGF-I apparently have no effects on mucosal mass, Houle *et al.* (1997b) have found that supplementation of a milk replacer with IGF-I increases the level of lactase in small intestinal, especially jejunal, mucosa (Figure 4).

The mechanism for the effects of IGF-I and sow's milk on lactase and sucrase expression is at this stage unknown. Dissecting this at the mechanistic level may prove difficult. Because the synthesis of lactase involves both complex glycosylation and proteolytic processing, there is a good likelihood that both pre- and post-translational mechanisms are important to the regulation of the level of lactase in the brush-border membrane. Dudley *et al.* (1993, 1994, 1996) have developed techniques that allow the measurement of lactase mRNA abundance as well as the *in vivo* rates of prolactase synthesis and post-translational processing. Burrin *et al.*, 1994 and Dudley *et al.*, 1996 (Figure 5) have shown that colostrum produces a marked increase in prolactase synthesis but, paradoxically, temporarily lowers the efficiency of processing prolactase to the brush border.

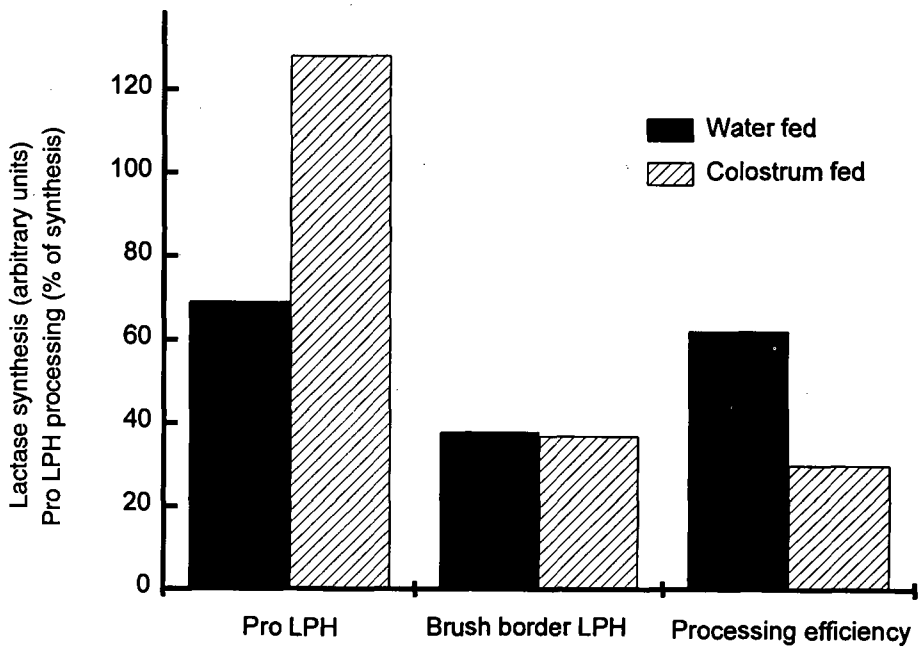

Figure 5. *Effect of colostrum on pro- and mature lactase (LPH) synthesis in newborn pigs (Dudley et al., 1996).*

In contrast, very recent results (S.M. Donovan and M.A. Dudley, personal communication; Houle *et al.*, 1997a) suggest that prolonged ingestion of an IGF-I supplemented formula simultaneously up-regulates gene transcription of lactase, prolactase translation and the efficiency with which the primary translation product is processed and translocated to the brush border. This mechanistic work, carried out in piglets (see also Dudley *et al*, 1997a) may well prove of importance to understanding the regulation of disaccharidase expression in humans, particularly in view of the changes in lactase activity (James, 1971) and gene transcription (Nichols *et al.*, 1997) that are associated with severe malnutrition in children, and of the prevalence of hypolactasia in many adults (Escher *et al.*, 1992).

Other components of milk

Apart from the results concerning the specific effects of IGF-I (which in themselves have some interesting therapeutic implications, see for example Tomas *et al.*, 1992; Lemmey *et al.*, 1994), Houle *et al.* (1997b) made an observation that is reminiscent of the dissociation of early growth and long-term development that Lucas *et al.* (1992; 1994) noted with regard to the development of human milk-fed LBW infants. In the study reported by Houle *et al.* (1997b), sow-reared piglets had slightly lower body weights and significantly lighter small intestines than artificially-reared piglets, but their levels of mucosal lactase were higher, even than those of the piglets that had received IGF-I-supplemented formula. This observation is interesting in at least three respects. First, it has proved notoriously difficult to quantify, in infants, a distinct benefit of human milk. The observation of Houle *et al.* (1997a, b) in the piglet provides quantitative, and hence objective, evidence of a benefit of natural feeding. Second, because the effect on lactase was prolonged at least until 14 days post-partum, the observation supports the implication of the results published by Kelly *et al.* (1991), that the subtle changes in milk composition that occur as lactation proceeds act in support of the development of piglets at any given stage of development. Third, the observation suggests that the beneficial effects of both colostrum and mature milk exceed those of a single growth factor, implying that there are other components of both colostrum and milk that support intestinal and perhaps other aspects of development.

Of course, mammary secretions and artificial milk replacers differ in many other respects (Grosvener *et al.*, 1993; Hamosh, 1997). Milk contains highly specific patterns of nucleotides (Gil and Uauy, 1995), polyamines (Buts *et al.*, 1995; Motyl *et al.*, 1995; Bardocz *et al.*, 1995) and oligosaccharides (Stahl *et al.*, 1994); and, at least in the pig, the free glutamine concentration of milk is very high (Wu and Knabe, 1994). Milk also contains relatively high amounts of ω3-polyunsaturated fatty acids. These fatty acids and their desaturation products are critical components of the plasma membranes of neurones and retinal cells (Purvis *et al.*, 1982), and their consumption undoubtedly affects the rate at which they are incorporated into the plasma membranes of cells in the central nervous system of the neonate (Arbuckle *et al.*, 1994). There is, moreover, some evidence to suggest that the consumption of ω3-fatty acids by sucking infants enhances the development of their visual function (Innis *et al.*, 1994). Finally, as milk contains considerable quantities of cholesterol, it is particularly interesting that Schoknecht *et al.* (1994) have shown growth and behavioural defects in neonatal pigs whose parents had been selected for low plasma cholesterol levels, and that these deficits could be corrected by the addition of cholesterol to the formula of the diet that these piglets consumed.

The piglet as a model for total parenteral nutrition (TPN) of the newborn

Paediatric background

Following the demonstration that the intravenous administration of elemental nutrient solutions was capable of providing adequate nutrition to animals, TPN was used successfully in an infant with severe short-gut syndrome (Wilmore and Dudrick, 1968). During the early years of this technique there were continual changes in the composition of TPN solutions as various problems with regard to amino acid and lipid metabolism became apparent. In the early stages, these major changes in the composition of TPN could be easily justified. Even so, as Heird and Gomez (1996) have pointed out, many quantitative aspects of the nutrient needs of very-low-birth-weight infants (infants born at <1500 g), and the consequent design of solutions to support these needs, remain at best poorly understood. Unfortunately, in part because the solutions that are now used 'do no harm' and in part because the functional end points (e.g., neural development) that are now sought are becoming more subtle, alterations in current TPN solutions are difficult to justify ethically and even more difficult to assess quantitatively. A strong case can be made therefore for the use of appropriate animal models to produce at least approximations of optimum nutrient mixtures.

Parenterally-nourished piglets

The piglet has proved a useful model, particularly in relation to the study of the nutrient requirements for weight gain and protein deposition. For example, in Figure 6 a comparison of the relationship between energy intake and energy deposition of LBW infants (Kashyap *et al.*, 1994) and artificially-reared piglets (Campbell and Dunkin, 1983a) are presented. The relationships are remarkably similar, given the differences in the species, the diets and the environment, and presumably reflect the rather obvious point that the fundamentals of energy metabolism must apply, irrespective of species. Furthermore, the partial efficiency of protein deposition is similar in the 'formula-fed' piglet (0.88 in piglets consuming a 13 protein energy % diet) and infant (0.72 in infants consuming an 18 protein energy % diet). Thus, quantitative aspects of the nutritional requirements of LBW infants and full-term piglets may well be quite similar, providing the differences in the growth potential of LBW infants (Thomson *et al.*, 1968) and full-term piglets are borne in mind.

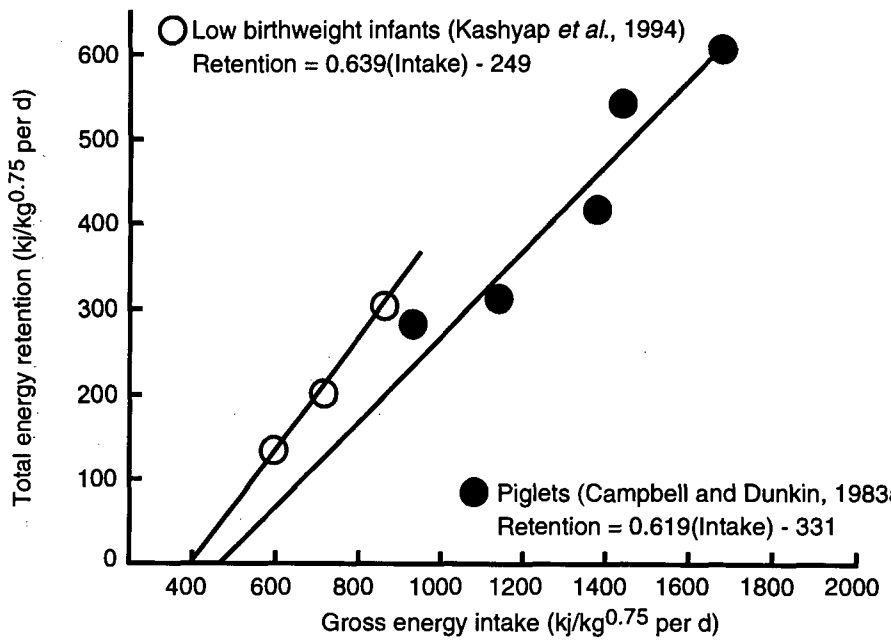

Figure 6. *The relationship between energy intake in low-birthweight infants and artificially-reared piglets.*

Over the last 15 years, the piglet has been used to study various aspects of the impact of TPN on growth and function. In the 1980s, Shulman and Fiorotto (Shulman and Fiorotto, 1984; Fiorotto, 1986) investigated the effects of different fuel mixes on the changes in the body composition of piglets maintained by TPN. The driving force for these investigations was the fact that the energy required to maintain the high rates of growth of LBW infants cannot be supplied easily by the use of glucose in the TPN solution. There have been similar studies of the ability of the newborn piglet to utilise long chain (Borum, 1993) and medium-chain fatty acids (Odle *et al.*, 1991a). Many aspects of the results of these studies underscore the metabolic similarities of the newborn piglet and the LBW infant (Shulman, 1993).

The most extensive recent studies of the piglet as a model for determining nutrient requirements of neonates maintained by TPN have been those of investigators at the University of Guelph (Wykes *et al.*, 1993, 1994; Ball *et al.*, 1996). From the perspective of the current discussion, these studies are particularly useful because they demonstrate the

utility of the pig as a 'test bed' for studying the nutrient needs of LBW infants. They also show the ease with which the powerful combination of nutritional manipulation and isotopic investigations of fuel metabolism can be used in piglets to determine macronutrient requirements. The traditional approach to the determination of amino acid requirements, and the approach that has generated all current recommendations for humans as well as laboratory and farm animals has been based on the measurement of nitrogen balance. However, in the early 1980s, V.R. Young and his group at Massachusetts Institute of Technology (MIT) pointed out that because the nutritional essentiality of amino acids resided in their carbon structures, measurements of the rate of oxidation of the carbon skeleton of an amino acid should, in principle, allow direct estimates of the requirements for the amino acid in question. The group then carried out a large series of measurements (summarised by Young 1987; Young *et al.*, 1989) in which they infused humans with ^{13}C-labelled essential amino acids and measured the point on the intake/oxidation curve at which the carbon catabolism of the test amino acid increased. The group have subsequently used the approach to issue a major challenge to currently recommended dietary amino acid allowances for adult humans.

At approximately the same time as Young was developing his approach, Kim *et al.* (1983) developed, in pigs, a carbon oxidation method that was the reverse of the MIT method. They argued that when the genetic potential for protein deposition is limited by inadequate supplies of a limiting amino acid, then, by definition, the other essential amino acids are present in excess and must be oxidised. It follows from this that if a *non-limiting* amino acid is labelled and used as the metabolic tracer of protein status, its oxidation rate will fall as the intake of the *limiting* amino acid in the diet approaches a quantity that allows the growth potential of the animal to be realised. Results obtained in piglets maintained by TPN, illustrated in Figure 7, show the ability of the approach to determine not only the median requirement but the range of requirement among the animals. Ball *et al.* (1996) are now not only utilising this technique to gain further insights into the requirements of other amino acids, such as threonine, in piglets but are using the data as a starting point for similar determinations in LBW infants.

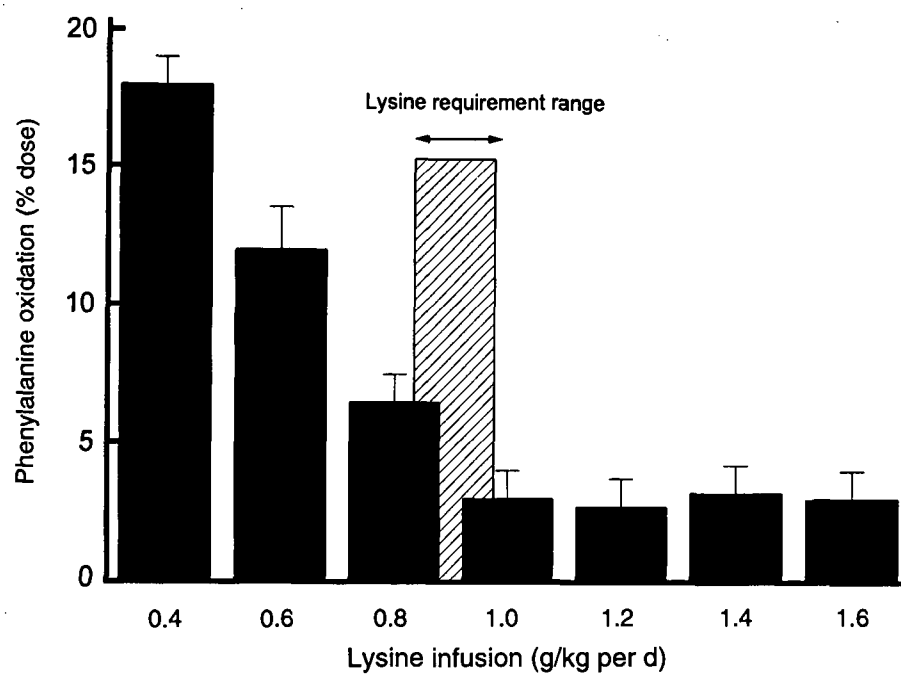

Figure 7. *Influence of lysine input on [^{14}C]-phenylalanine oxidation in piglets maintained by TPN (House, 1995; Ball et al., 1996).*

The studies discussed above were, of course, focused on general aspects of the organic macronutrient needs of infants. Other aspects of TPN have also been studied in the piglet. One of the ironies of the use of TPN is that it is often instituted because of a derangement in mucosal function of the small intestine. Yet, in piglets, as in other species, exclusive TPN slows the growth and compromises the function of the mucosa of the small intestine (Shulman, 1988; Adeola *et al.*, 1995). Dudley *et al.* (1997b) have shown recently that the synthesis of total mucosal protein and lactase is significantly inhibited by parenteral nutrition and interestingly, that the relationship between lactase synthesis and the abundance of the lactase mRNA is altered in a way that results in a more efficient processing of the primary translation product.

Unfortunately, other than the general impression that the mucosal cells have a 'need' for the provision of enteral nutrients, the mechanisms that underlie the deleterious effects of TPN on mucosal mass and function remain poorly identified. There has, for example, been a long standing interest in the role of glutamine as a trophic factor in the mucosa (reviewed by Burrin and Reeds, 1997). Despite some evidence in the rat (Haque *et al.*, 1996), Burrin *et al.* (1991,1992a) were unable to document effects of glutamine supplementation on the mucosal mass of piglets maintained by TPN. Nevertheless, this is an important area of clinical nutrition in which the piglet might provide a particularly appropriate model for more mechanistic studies of the changes in intestinal metabolism and local trophic factor expression that are associated with TPN.

The piglet as a model for the investigation of the hormonal regulation of growth

As has already been pointed out, the inherently high growth rate of the piglet makes it a particularly useful model for the study of growth regulation. There is, of course, a large literature on practical aspects of nutritional regulation of the early growth of the pig, and this area was a particular interest of Tony Dunkin (Campbell and Dunkin, 1983a, 1983b, 1983c). One characteristic of growth of particular interest is the fact that the weight-specific rate of protein deposition slows exponentially from birth to maturity. For much of this period, the slowing in growth reflects a parallel fall in the weight-specific intake of protein. However, over the suckling period, the rate constant of protein deposition declines more than the weight-specific intake. Apparently over the period from birth to approximately a month of age, there is a progressive fall in the nutritional efficiency of protein deposition (i.e., the mass of protein deposited per unit of protein intake above maintenance).

Food intake and synthesis of tissue protein

One key feature of natural feeding is its episodic nature. It has been argued (Garlick *et al.*, 1983; Garlick and Grant, 1988) that the key to understanding the regulation of postnatal protein deposition might be the understanding of the metabolic response to meals. The stimulation of net protein deposition that occurs after a meal could involve changes in either or both protein synthesis and degradation, and studies by Davis *et al.* (1993b, 1996) have shown that from birth to weaning, there is a marked developmental fall in the responsiveness of muscle protein synthesis to nutrient intake. This parallels a decline in the capacity of muscle to synthesise protein, as measured by the RNA:protein ratio. However, in visceral tissues, neither the capacity for protein synthesis nor the responsiveness of protein synthesis to refeeding changes to any significant extent. These data alone lend support to the general idea that protein deposition is regulated in a somewhat different manner in the peripheral and visceral tissues (Reeds *et al.*, 1993). They are reminiscent of the observations of Burrin *et al.* (1995, 1997) with regard to tissue protein synthetic responses to the ingestion of colostrum.

Potential role of insulin

It has been argued (Garlick and Grant, 1988; Mortimore *et al.*, 1987, Reeds *et al.*, 1992a, 1993) that insulin may be the critical acute regulator linking protein intake and

tissue protein turnover. T.A. Davis (personal communication) has extended this general view to propose that, at least regarding the regulation of muscle growth, the developmental changes in the protein synthetic response to feed intake primarily reflect alterations in tissue responsiveness and sensitivity to insulin. Thus, there is an exponential relationship between circulating insulin concentrations and muscle protein synthesis in piglets (Figure 8). Furthermore, it is clear that the asymptotic value for protein synthesis is higher in the younger pigs. Because of this, Davis has argued that a given concentration of insulin will stimulate protein anabolism more markedly in younger animals. One consequence of this is that the immediate increase in amino acid concentrations that follow a protein meal may be lower in younger as opposed to older animals. This, in turn, will lower the rate of postprandial amino acid catabolism and thereby maximise the proportion of the ingested protein that is deposited (see also Benevenga *et al.*, 1993; Reeds *et al.*, 1997a).

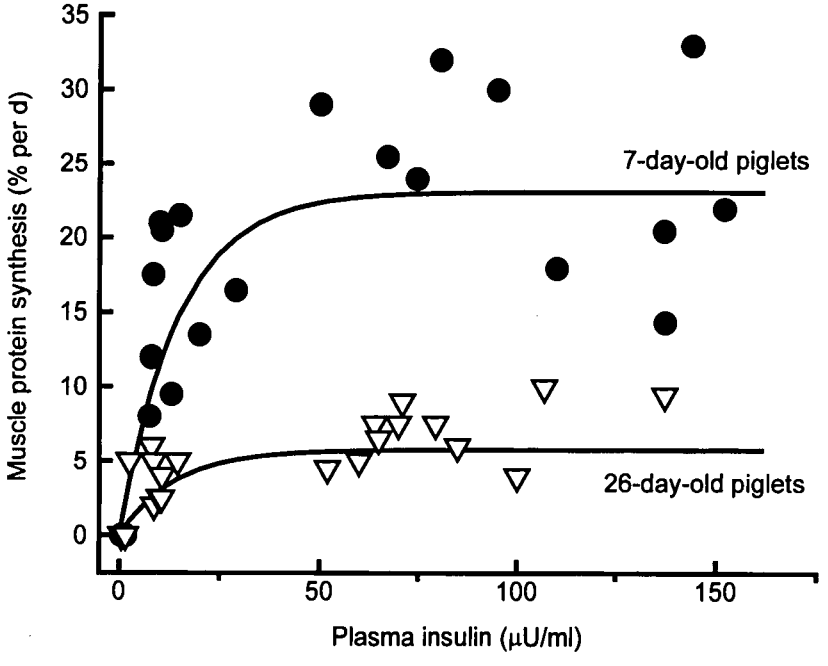

Figure 8. *Plasma insulin and muscle protein synthesis in fasted then re-fed piglets at two ages (Davis and Burrin, unpublished).*

The data shown in Figure 8 are only correlative and do not prove a specific effect of insulin. To investigate this further, Wray-Cahen *et al.* (1997a) have used a new, rapid, enzyme-based amino acid assay (Beckett *et al.*, 1996), to develop an 'amino acid clamp' technique that allows the formal quantification of the dose response relationships among insulin, amino acid utilisation and tissue protein synthesis. The relationship between insulin concentrations and whole-body amino acid utilisation, as measured by the rate of amino acid infusion necessary to maintain the essential amino acids constant under hyper-insulinaemic conditions is shown in Figure 9. The data show that, under conditions in which amino acid and glucose levels are maintained at fasting values, the stimulation of whole-body amino acid utilisation by insulin in 7-day-old pigs is approximately twice as great as that found in somewhat older but still sucking, piglets. Furthermore, the ED_{50} (effective dose for 50% of pigs) for the insulin effect on protein synthesis is lower in 7-day-old pigs (18 μU/ml) than in 26-day-old pigs (45 μU/ml).

Recent studies (Wray-Cahen *et al.*, 1997b) of the effect of insulin on tissue protein synthesis (rather than whole-body amino acid utilisation), have not only shown differences among tissues that parallel those associated with the feeding response, but have obtained results that suggest that the stimulation of tissue protein synthesis does not account for all of the response of whole-body amino acid accretion to insulin. In the study of Wray-Cahen *et al.* (1997b) protein synthesis in the skeletal muscle of sucking pigs proved also to be very sensitive to insulin (ED_{50} of < 20μU/ml). Although the maximum response of muscle protein synthesis to insulin was lower in the older animals, the ED_{50} showed no developmental change. In the liver on the other hand, protein synthesis appeared to be insensitive to insulin and responded primarily to the rise in amino acid concentrations. It seems likely that the regulation of proteolysis is the key insulin-regulated process in the liver *in vivo* as well as *in vitro* (Lardeaux and Mortimore, 1987; Mortimore *et al.*, 1987) and thus plays an important role in the regulation of protein accretion.

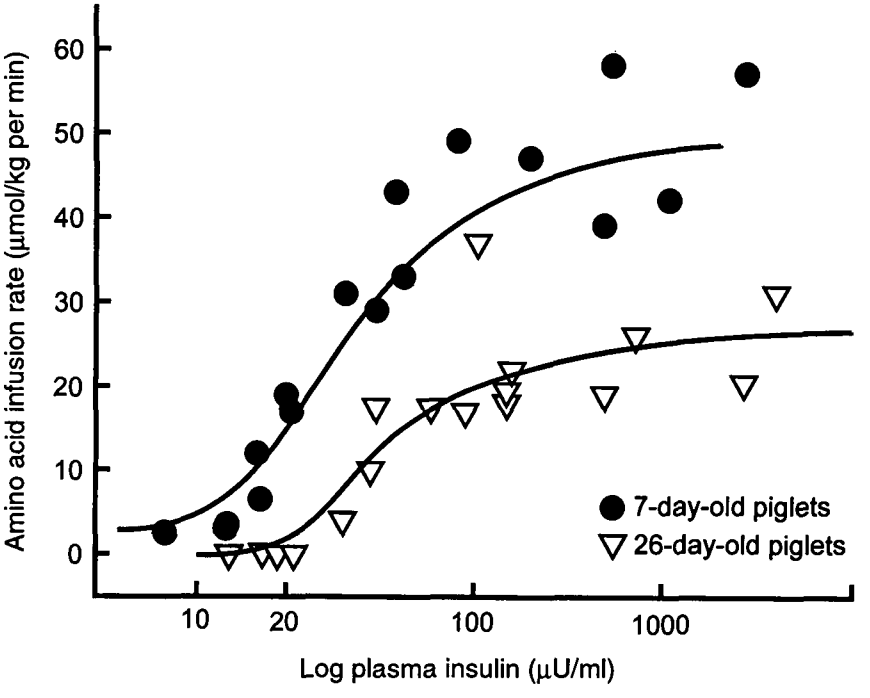

Figure 9. *Whole body amino acid utilization in piglets during a hyper-insulinaemic euamino-acidaemic clamp (Wray-Cahen et al., 1997a).*

These recent studies emphasise the general practicality of carrying out complex metabolic and endocrinological studies in piglets. The authors believe that the piglet may provide a particularly practicable model for much more detailed investigations of growth regulation at both the metabolic and molecular level. At the very least, the availability of a model organism that is not only growing rapidly, but that lends itself to imposition of highly specified metabolic and hormonal conditions, opens up a large number of possibilities for the study of other aspects of the endocrinological control of metabolism and growth.

Nutrient metabolism

Neonatal metabolism

Glucose and lipids

As was pointed out in the introduction, the newborn piglet is characterised by very low fat stores. During the first 2 days of life, like the LBW infant (Cowett *et al.*, 1988; Cornblath and Schwartz, 1993), the unfed, newborn piglet is quite susceptible to hypoglycaemia. This 'immaturity' is of importance to both species because the contribution of the brain to whole-body metabolism is at its highest in the newborn period and all evidence suggests that glucose is the preferred fuel for cerebral metabolism (see Flecknell and Wootton, 1989 for data obtained in piglets). There has, therefore, been continuing interest in glycaemic regulation in both piglets (Girard *et al.*, 1992) and infants, especially LBW infants (Cornblath and Schwartz, 1993). In Figure 10, data are compared for whole-body glucose turnover in pigs (Flecknell *et al.*, 1980; Wykes *et al.*, 1997) and infants and children (Bier *et al.*, 1977; Bougnères, *et al.*, 1989; Keshen *et al.*, 1997) following a relatively short fast. As recognised originally by Flecknell *et al* (1980), the relationship between body weight and glucose turnover in the two species is strikingly similar. These data serve to underscore the similarities in energetic efficiency that were highlighted earlier. Interestingly, the relationship diverges quite abruptly at or around 35 kg body weight. This is presumably related to the marked difference in maturity of the 35-kg pig and the 35-kg human and, as discussed by Bier *et al.* (1977), is probably related to the body weight at which brain growth ceases (Reeds *et al.*, 1993).

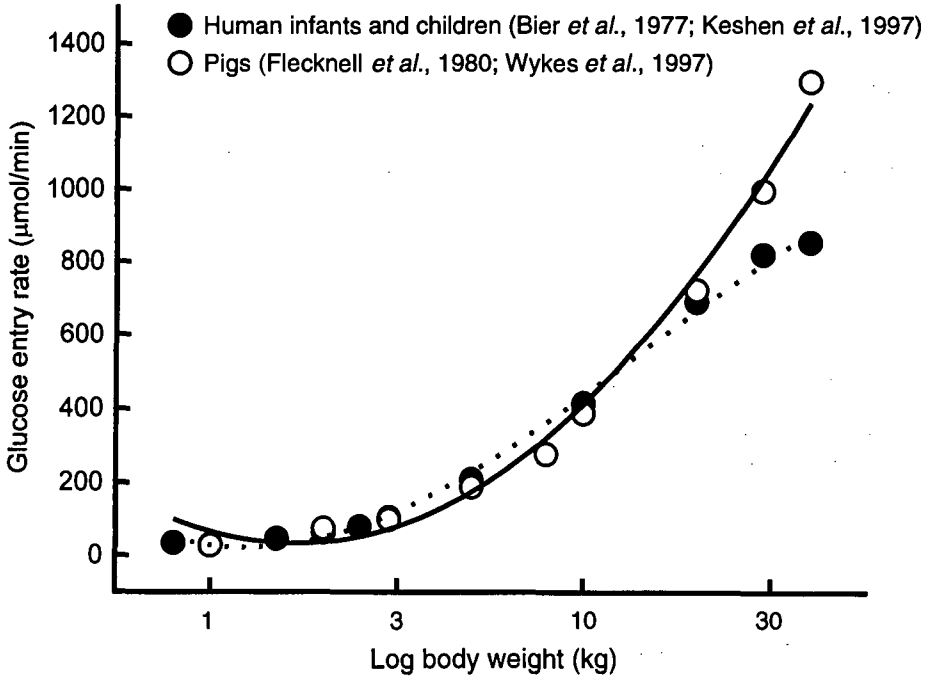

Figure 10. *Relationship between body weight and fasting glucose turnover in pigs and humans.*

The most extensive investigations of glucose and lipid metabolism in the newborn pig have been those summarised by Duée *et al.* (1996). This group has established the key importance of glucagon to the initial activation of gluconeogenesis (Duée *et al.*, 1985); the importance of hepatic fatty acid metabolism as a source of ATP and reducing equivalents for the support of hepatic gluconeogenesis (Pégorier *et al.*, 1985); and the important role of

substrate availability (Pégorier *et al.*, 1981). All three central metabolic observations appear to apply to infants, especially SGA infants (Haymond *et al.*, 1974; Sabel *et al.*, 1982; Bougnères *et al.*, 1989). Derangements in glucagon physiology (Bloom and Johnson, 1972) and glucose production (Kalhan *et al.*, 1977) have also been noted in the infants of diabetic mothers.

However, newborn piglets and full-term infants do differ metabolically in one important respect. Although the neonates of both species appear to be able to utilise ketone bodies (Tetrick *et al.*, 1995; Bougnères *et al.*, 1982; De Boissieu *et al.*, 1995; Odle *et al.*, 1991b), the ability of piglets to generate ketone bodies from fatty acids seems severely limited (Pégorier *et al.*, 1983; Odle *et al.*, 1995; Odle, 1997). In this respect, newborn piglets are similar to SGA infants (Sabel *et al.*, 1982). However, this similarity is probably not a reflection of immaturity but of the different organisation of lipid metabolism in the two species (Pond and Mersmann, 1996).

Amino acid biosynthesis

From the strict perspective of protein deposition, milk supplies a somewhat imbalanced mixture of amino acids. Studies in premature infants (Stack *et al.*, 1989; Kashyap *et al.*, 1994) show that the biological value of human milk (approximately 0.75) is surprisingly low. The main sources of the imbalance are the low contents of cysteine, arginine and glycine and the relatively high concentrations of leucine and glutamate in milk proteins (Davis *et al.*, 1993a, 1994). Arginine, cysteine and glycine are critical for the synthesis of molecules, such as glutathione, creatine, taurine and nitric oxide (discussed by Reeds and Hutchens, 1994), which are not only directly linked to protein synthesis, but play crucial roles in immune function and peroxidative defences as well as skeletal muscle and central neural function.

The calculation of the relationship, in sucking mammals, between intake of arginine, cysteine and glycine, and the rate at which they are deposited in body protein, indicates that biosynthesis may make a highly significant contribution to the supply of all three amino acids (Davis *et al.*, 1993a). However, there is remarkably little direct information on the pathways of biosynthesis of these amino acids in the neonate. There is now intriguing evidence from stable isotope studies that suggests that extremely-low-birth-weight infants (ELBW infants; birth weight < 1000 g) when maintained by TPN may have a very limited capacity for cysteine and proline synthesis (Miller *et al.*, 1995a,b). Furthermore, a recent study (Castillo *et al.*, 1995) of arginine biosynthesis that used intravenous tracers in LBW infants gave equivocal results in that some infants appeared to synthesise arginine while others did not.

However, it is still not certain whether ELBW infants are incapable of synthesising arginine, cysteine and glycine, or whether the results reflect the intravenous route of the infants' nutrition and the fact that the metabolic tracers were given intravenously. Recent work in the piglet has served to re-emphasise the role of the gut in amino acid biosynthesis, particularly in relation to arginine (Blachier *et al.*, 1993; Wu and Knabe, 1995; Flynn and Wu, 1996) and proline synthesis (Murphy *et al.*, 1996). Critically, the work of both Blachier *et al.* (1993) and Wu and Knabe (1995) in neonatal piglets, in addition to recent work based on the study of enteral [U-^{13}C]-glutamate metabolism in one-month-old piglets (Reeds *et al.*, 1997b), suggest that the intestinal mucosal cells of the pig are capable of complete arginine synthesis. In this respect, the pig appears to differ markedly from the rat (Featherstone *et al.*, 1973). Furthermore, studies of arginine biosynthesis in both humans (Berthold *et al*, 1995; Beaumier *et al.*, 1995) and piglets (Reeds *et al.*, 1997b), and of proline synthesis (Murphy *et al.*, 1996) in pigs, are suggesting that dietary (enteral) precursors appear to be the preferred, if not the obligatory, substrates for these biosynthetic pathways. These observations are reopening an old debate regarding the sources and precursors for the biosynthesis of the group of amino acids (arginine, proline, cysteine, tyrosine and glycine) now generally termed conditionally essential (Laidlaw and Kopple, 1987).

Isotopic studies of protein digestion

One of the most frustrating aspects of the interpretation of dietary recommendations, certainly for macronutrients, is the inadequate nature of data on bioavailability. In many cases, the information is based on apparent digestibility (i.e., intake - faecal losses), and formulating availability in this manner begs many important practical and metabolic questions. In the absence of more extensive information, the values are often given as constants (see for example Dewey *et al.*, 1996) yet there are many examples of complex interactions between the diet and the recipient animal that clearly influence the efficiency with which specific dietary constituents support the metabolic needs of the organism. Apposite examples of such phenomena are provided by the late Dr Batterham's work on the availability of lysine (Batterham *et al.*, 1990; Ball *et al.*, 1995) and by the literature on the influence of so-called anti-nutritional factors (Barth *et al.*, 1993; Savelkoul *et al.*, 1994).

From the perspective of protein and amino acid nutrition, understanding amino acid bioavailability requires essentially two sets of information: one related to protein digestibility, the other to the metabolic transformations that the amino acids undergo once they are removed from the lumen. Expressing protein and amino acid availability in terms of apparent faecal digestibility is at best inadequate and at worst completely misleading, both nutritionally and metabolically. Even the simple consideration of N digestibility is flawed by the influence of bacterial activity in the large, and perhaps the small intestine. For the same reason, apparent faecal amino acid digestibility is a meaningless term.

In one sense, the measurement of the ileal outflow of amino acids provides a useful measure because the difference between this value and the known rate of protein-bound, amino acid intake measures the maximum value for the net availability of amino acids for the support of all productive processes. However, the measurement of ileal outflows cannot be simply transformed into estimates of digestibility because the ileal digesta are a complex mixture of variable quantities of amino acids from the diet and from mucosal, pancreatic and biliary sources. The central problem is how to distinguish among the various contributors. There is now a considerable literature on the use of dietary proteins labelled with either ^{15}N (Mahé *et al.*, 1994; Roos *et al.*, 1995; Gausseres *et al.*, 1996) or ^{13}C (Boirie *et al.*, 1995) in feeding trials in both pigs and humans. On the basis of the relative intestinal flows of total- and ^{15}N-protein, it seems that many common dietary proteins are highly digestible, certainly more digestible than is suggested by measurements of apparent faecal nitrogen digestibility. This is a very useful approach to study some aspects of dietary protein metabolism, particularly in relation to studies on the regulation of digesta flow (Gaudichon *et al.*, 1994; Mahé *et al.*, 1996), but the method suffers from the kinetic problems that accrue from the first-pass incorporation of the labelled dietary protein into the protein of the mucosa (Leterme *et al.*, 1996). To that extent it seems likely that the method may well systematically underestimate actual protein digestibility.

The other isotopic approach that has been investigated in both pigs (Souffrant *et al.*, 1986; 1993; de Lange *et al.*, 1990; Lien *et al.*, 1997a, 1997b) and humans (Shulman *et al.*, 1995; Gaudichon *et al.*, 1996) is to label the subject. This method, apparently first applied systematically by Souffrant *et al.* (1986), involves the prolonged administration of a ^{15}N-labelled amino acid so that pancreatic and mucosal proteins are labelled to isotopic equilibrium (Figure 11). Once this condition is attained, the measurement of the isotopic enrichment of ^{15}N in the ileal digesta (de Lange *et al.*, 1990) or in the faeces (Shulman *et al.*, 1995) gives, in theory at least, a direct measure of the fractional contribution of endogenous amino acid nitrogen to the total nitrogen in the digesta. If total ileal (or faecal) nitrogen flow is then measured, the absolute amounts of endogenous (labelled) and exogenous (unlabelled dietary) nitrogen can be calculated. Data from the paper published by de Lange *et al.* (1990) are presented in Figure 12. In this study, endogenous protein contributed between 50% (canola diet) and 82% (wheat diet) of the total ileal protein. The apparent ileal digestibility of the various protein sources underestimated their actual digestibility by between 12% (canola meal) and 26% (barley). Data for the contribution of endogenous nitrogen to ileal nitrogen in formula-fed piglets (53%), and to faecal nitrogen in formula-fed infants (51%) are remarkably similar (Shulman *et al.*, 1995; N.J. Gannon and P.J. Reeds unpublished).

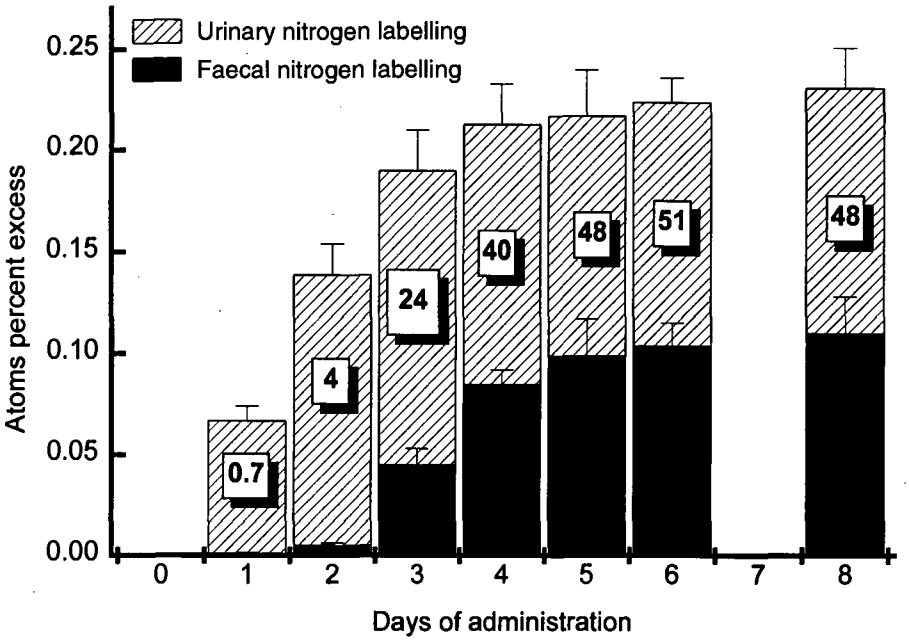

Figure 11. *Time course of urinary and faecal [^{15}N] labelling in infants receiving a constant oral input of [^{15}N]-glycine. Numbers within the columns are percent endogenous contribution to faecal nitrogen.*

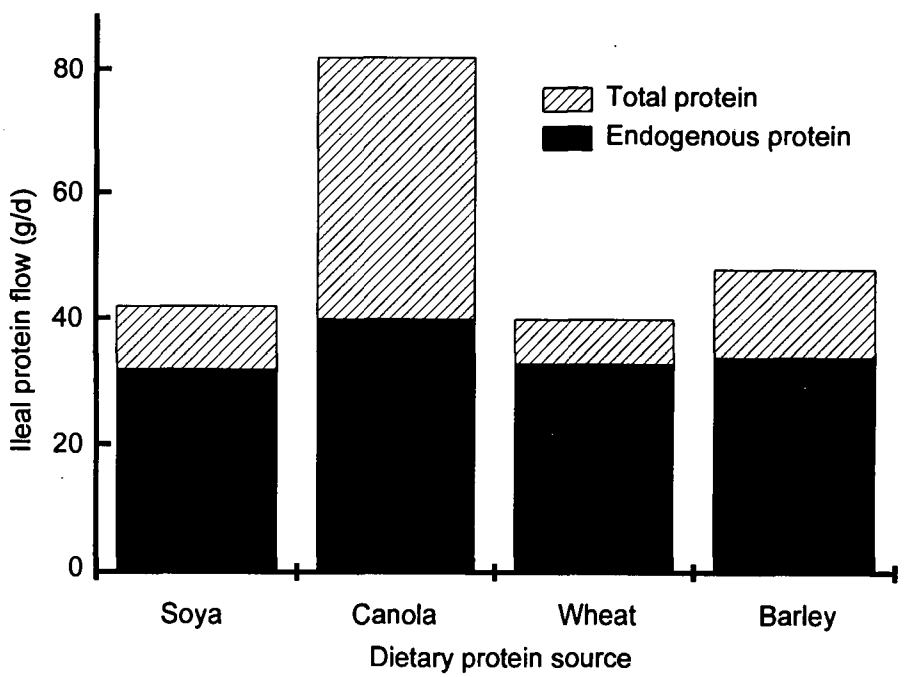

Figure 12. *Contribution of dietary and endogenous protein to the ileal digesta of pigs infused intravenously with [^{15}N]-leucine (de Lange et al., 1990).*

Although the ^{15}N-dilution technique has given invaluable information, there are some doubts with regards to its absolute accuracy. The main drawback is that to this date the major publications have been based on the use of single amino acids, in particular ^{15}N-leucine. Leucine was originally chosen as a tracer because of the presumption that it would freely label other amino acids via transamination. Unfortunately, this does not seem to be the case. The isotopic enrichment of other amino acids, even of isoleucine and valine, is less than that of the tracer leucine (de Lange *et al.*, 1992; Lien *et al.*, 1997a, 1997b) and the ^{15}N-label is not transferred to two key essential amino acids: lysine, frequently the first limiting amino acid, and threonine, an amino acid that contributes 25% of the core protein of the intestinal mucins (Roberton *et al.*, 1991). Thus, measurements of total ^{15}N and leucine ^{15}N-labelling give somewhat different estimates of endogenous protein secretion and hence of absolute protein digestion (de Lange *et al.*, 1992; Lien *et al.*, 1997a, 1997b). The problem is compounded further by the fact that measurements of the actual labelling of mucosal protein are rarely made and the calculations are based on plasma amino nitrogen labelling. For this reason alone, it is suspected that the data in the literature may also systematically underestimate the contribution of endogenous protein to the ileal protein flow.

One way of avoiding the first problem is to use mixtures of ^{15}N-amino acids. These can be prepared from algae (Gannon *et al.*, 1994) or in legumes labelled in hydroponic culture (Grusak and Pezeshgi, 1994). Furthermore, in unpublished studies, N.J Gannon has shown that even after a 10-day infusion of [U-^{15}N]-amino acids, the steady-state ^{15}N-enrichment of ileal protein is only 60% of the ^{15}N-enrichment of systemic amino acids. This suggests first, that dietary protein makes a significant contribution to ileal mucosal protein synthesis (see below) and second, that the use of plasma amino acid labelling will lead to an underestimate of the true rate of mucosal protein secretion.

Isotopic studies of intestinal amino acid metabolism

Even though isotopic and non-isotopic measurements, such as the homoarginine (Barth *et al.*, 1993) and ileal amino acid composition methods (Fan *et al.*, 1994), of protein digestion suggest that it is very efficient and approaches 100% with many common dietary proteins, results from related studies using a different approach (Rerat *et al.*, 1988, 1992; Ebner *et al.*, 1994) suggests that less than 100% of the amino acids that have exited the lumen appear in the portal vein. Furthermore, it is clear that the portal appearance varies markedly among amino acids. In Figure 13 the recent measurements by Stoll *et al.* (1997b) of the steady state portal appearance of different amino acids in rapidly growing, frequently fed piglets are presented. Four points are evident from these data. First, despite the fact that the animals were growing at a high rate (at least 30 g/kg per d), no more than 60% of the dietary essential amino acid intake appeared in the portal circulation. Second, the portal appearance of methionine and threonine (see also Rerat *et al.*, 1992), was less than that of other essential amino acids. Third, there was no net absorption of glutamate, aspartate (Windmueller and Spaeth, 1980) or, critically, cystine (see also Ebner *et al.*, 1994). Fourth, the appearance of alanine, arginine and tyrosine exceeded their intake, thereby providing clear non-isotopic evidence of the net synthesis of these amino acids within the tissues of the portal drained viscera. It is clear, therefore, that digestibility does not necessarily equal systemic availability, and that the chemical score of the diet is not necessarily the same as that of the mixture of amino acids available for extra-intestinal tissue protein metabolism.

There are three reasonable explanations for the difference between true protein digestibility, which approaches 100%, and portal amino acid appearance, which does not. First, it is possible that significant amounts of dietary protein are absorbed into the portal blood as small peptides. There is some evidence that peptide absorption might be substantial in calves (Koeln *et al.*, 1993). However, it appears to be a minor contributor to portal amino acid appearance in goats (Blackwell *et al.*, 1996), and B. Stoll and P.J. Reeds (unpublished) have been unable to obtain any evidence that there are nutritionally significant quantities of circulating peptides in milk-fed piglets. Second, it is possible that the true absorption of dietary amino acids into the portal blood is efficient, but because there is continuing utilisation of arterial amino acids by the portal drained viscera (see for

example Yu *et al.*, 1990), the net portal balance systematically underestimates the true portal absorption. The third possibility is that there is substantial utilisation of dietary amino acids by the mucosa, as suggested by a number of studies in adult humans (Hoerr *et al.*, 1993; Biolo *et al.*, 1992; Matthews *et al.*, 1993a). The most effective, if not the only way to investigate the latter two possibilities is to combine isotopic tracers with portal balance measurements. Stoll *et al.* (1997a, 1997b, 1997c) have recently completed a series of studies in piglets based on simultaneous intragastric and intravenous infusions of multiple amino acids labelled with stable isotopes and the results from these studies will be discussed below.

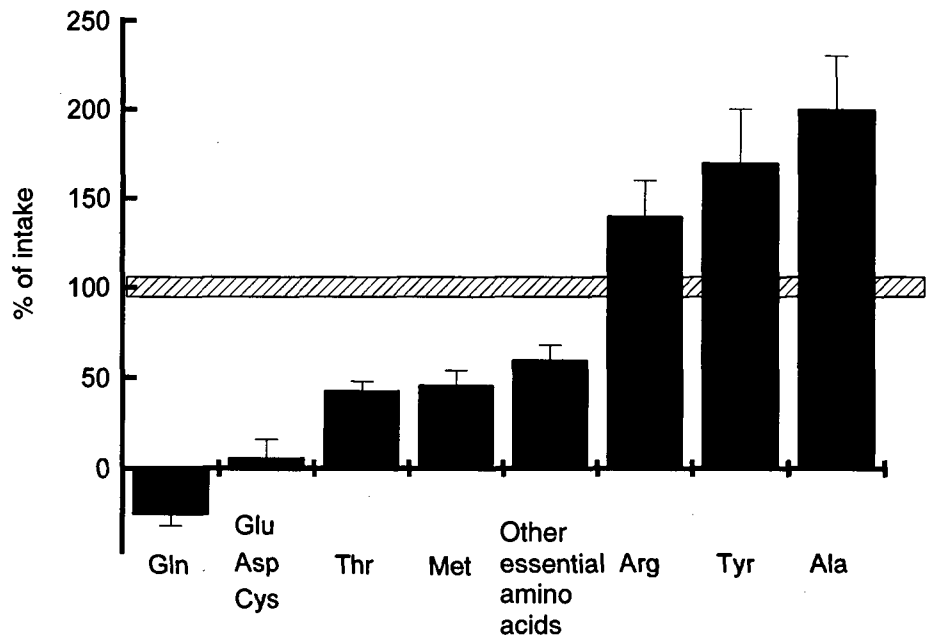

Figure 13. *Portal appearance of different amino acids in piglets fed once hourly with a milk-replacer (Stoll et al., 1997b).*

Using stable isotopes to quantify mucosal amino acid metabolism

Before discussing the results of the Stoll *et al.* studies (Stoll *et al.*, 1997a, 1997b, 1997c), it is appropriate to discuss the tracer approach that was used in the studies. A major difference between stable isotopic and radioisotopic analysis is that mass spectrometers measure labelled molecules, while radioactive counting techniques measure isotopic atoms. Gas chromatograph/mass spectrometers can therefore separate a given compound, say phenylalanine, into molecules (so called isotopomers) that contain ^{12}C, ^{14}N, ^{16}O and ^{1}H from molecules that contain 1,2...x atoms of ^{13}C, ^{15}N, ^{18}O and ^{2}H. It is therefore possible to infuse one labelled form of an amino acid, such as phenylalanine containing 9-^{13}C-atoms [U-^{13}C]-phenylalanine as in the Stoll *et al.* studies (Stoll *et al.*, 1997a, 1997b, 1997c) via the stomach and a second labelled form (phenylalanine containing 5-^{2}H-atoms) via a peripheral vein. In this way, it is possible to quantify both the appearance of the enteral tracer and the disappearance of the systemic tracer across the portal drained viscera, and to relate both to the portal amino acid mass balance. The appearance of the tracer amino acid then measures the true absorption of the dietary amino acid uncomplicated by simultaneous removal of the arterial amino acid.

In the studies of Stoll *et al.* (1997a,b,c) algal protein uniformly labelled with ^{13}C was used as the dietary tracer. This brings with it three technical advantages. First, the data

relate more directly to the portal availability of amino acids derived from a protein, as opposed to a free amino acid. Second, the mass spectrometric detection of molecules containing >3 ^{13}C-atoms is very reliable. Third, the technique allows the simultaneous investigation of multiple amino acids. In Figure 14 the mass (net) and the tracer (total) appearance of threonine, leucine, lysine and phenylalanine in portal blood, expressed in proportion to total and tracer amino acid intake are compared. The results show that with the notable exception of threonine, the tracer balance is greater than the mass balance. This difference indicates that there is continuing utilisation of arterial leucine, lysine and phenylalanine by the portal drained viscera. However, the data also indicate quite clearly that much less than 100% of the known disappearance of the labelled amino acids from the lumen appeared in the portal blood and suggest that the intestinal cells metabolised at least 30% of the labelled essential amino acids that they absorbed from the intestinal lumen.

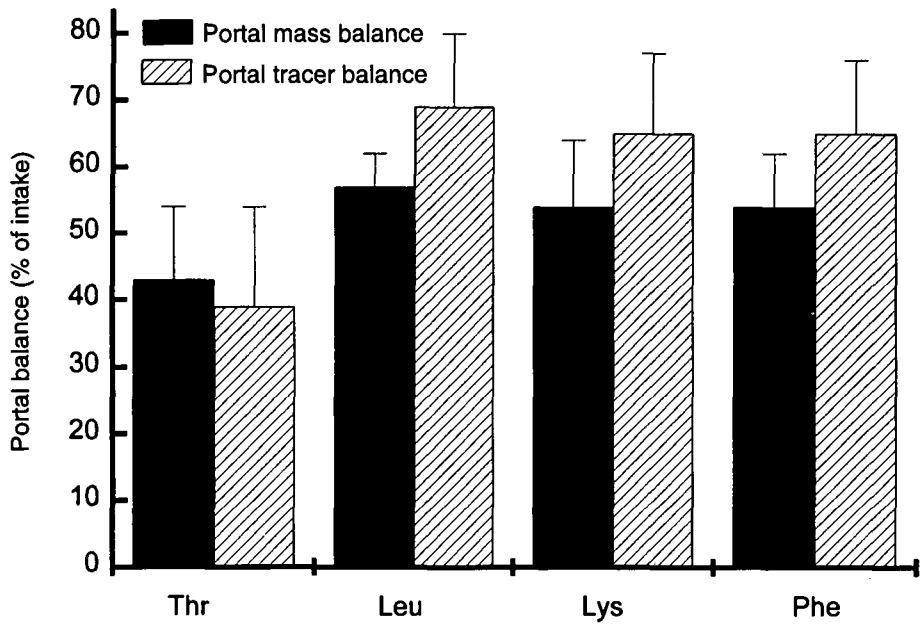

Figure 14. *Portal mass balance and [U-^{13}C]-tracer balance of essential amino acids in fed piglets (Stoll et al., 1997b).*

The next important question arising from these data is: what is the fate of these amino acids in the mucosal cells? Given the very high rate of protein synthesis in the mucosa of young pigs (Dudley *et al.*, 1994), it is reasonable to assume that protein synthesis represents the major pathway of utilisation of dietary amino acids. However, direct measurements of the incorporation of the enteral tracer into mucosal protein showed that this was not so. The relationship, for individual animals, between the total first-pass utilisation of threonine, leucine, lysine and phenylalanine (as determined from the portal tracer balance) and their incorporation into mucosal protein as described by Stoll *et al.* (1997b) are presented in Figure 15. The figure illustrates two very important points. First, there is substantial variation between animals. This, in fact, reflects differences in mucosal protein mass among the animals and leads to the conclusion that the mass of the mucosa has a nutritionally significant effect on the systemic availability of essential amino acids. Second, the slope of the line is 0.15. In other words, on average only 15% of the total intestinal metabolism of the dietary essential amino acids resulted in incorporation of the amino acids into mucosal protein. This leads to the equally important, but unexpected, conclusion that the major mucosal fate of dietary amino acids is not mucosal protein synthesis but catabolism and oxidation. This conclusion was also

confirmed by Stoll *et al.* (1997b) by direct measurements of ammonia balance across the portal drained viscera, from which the data suggest that about 25% of the dietary protein was catabolised by the mucosa in first pass. Indeed it appears that under the conditions of this experiment, the oxidation of dietary amino acids was the dominating source of energy for the mucosa.

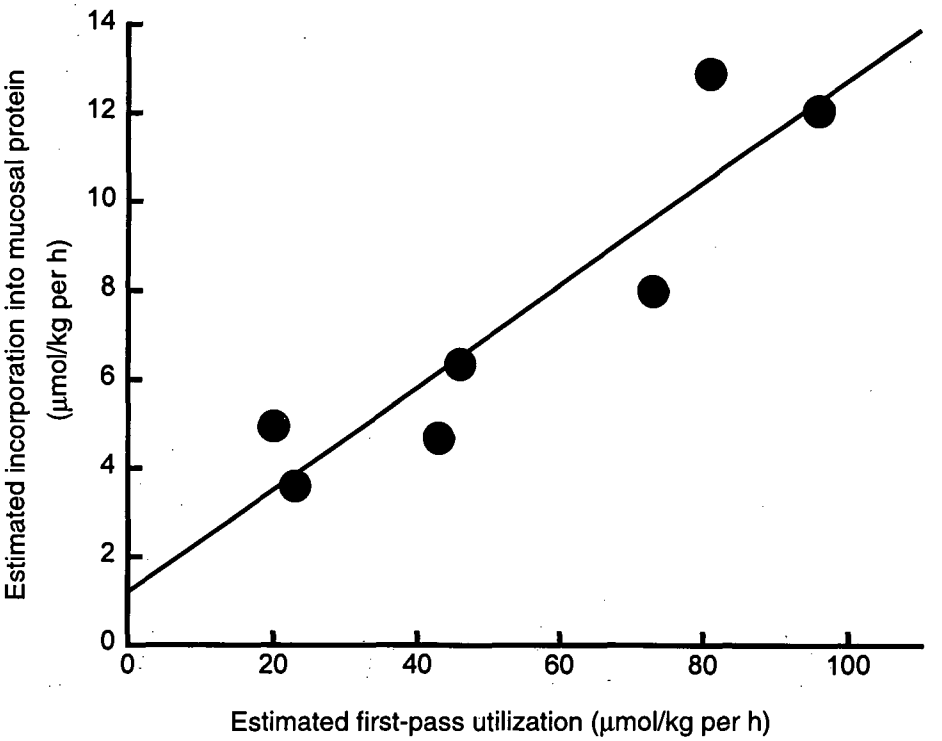

Figure 15. *Individual animal values for total intestinal metabolism and mucosal protein incorporation of intragastric [U-^{13}C]-essential amino acids in fed piglets (Stoll et al., 1997b).*

The results from the experiments discussed in this section raise the equally important question of the source of amino acids for mucosal protein synthesis. It has been known for many years that the mucosal cells can utilise both luminal and arterial amino acids for protein synthesis (Alpers, 1972). Two lines of evidence from work by Dudley *et al.* (1994) and Stoll *et al.* (1997a, 1997b) suggest that in the fed state, the uptake of arterial amino acids makes a minor contribution to the mucosal free amino acid pool, but a major contribution to mucosal protein synthesis. The first line of evidence is based on measurements of the labelling of the precursor form of lactase, prolactase (Dudley *et al.*, 1994). Because this protein is an intermediate for brush border lactase synthesis, its turnover rate is extremely high. During a constant infusion of a labelled amino acid, prolactase can be brought to isotopic steady state (Figure 16) and under this condition, by definition, it measures directly the isotopic enrichment of the pool of amino acids from which it derives. Thus the plateau labelling can be used to probe the relationship between the labelling of the free amino acid and the protein synthetic precursor pool, in much the same way as apo-lipoprotein B-100 labelling can be used to investigate hepatic amino acid kinetics (Parhofer *et al.*, 1990; Reeds *et al.*, 1992b; Jahoor *et al.*, 1994; Bhattiprolu *et al.*, 1994; Stoll *et al.*, 1996). Results from two recent experiments (Dudley *et al.*, 1997b and Wang *et al.*, unpublished; Table 1) show quite clearly that the plateau isotopic enrichment of prolactase is *higher* than that of the free amino acid pool, suggesting a preferential utilisation of arterial leucine for mucosal protein synthesis.

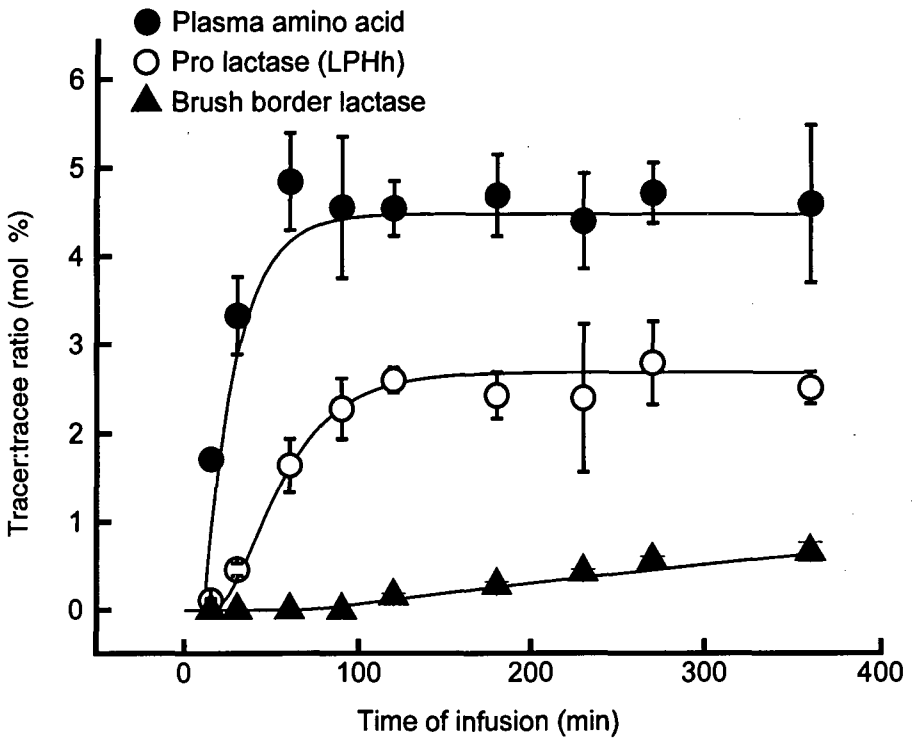

Figure 16. *Phenylalanine and leucine labelling kinetics of pro- and mature (BB) lactase in fasted piglets (Dudley et al., 1997c).*

Table 1. Relationship between the labelling (mol percent) of plasma, mucosal and prolactase leucine in piglets fed with a conventional (H. Wang and M.A. Dudley, personal communication) or an elemental diet (Dudley *et al.*, 1997b) and infused intravenously with ^{2}H-leucine (mean ± SD). (Because prolactase comes to isotopic steady state it measures directly the labelling of the amino acid from which it was derived).

	Plasma-free leucine	Mucosal-free leucine	Prolactase leucine
Conventional diet	4.0 ± 1.0	1.2 ± 0.2	1.8 ± 0.2
Elemental diet	4.9 ± 0.8	1.5 ± 0.9	2.2 ± 0.9

The second line of evidence is provided by experiments in which different tracers of phenylalanine were given intravenously and intragastrically (Stoll *et al.*, 1997a, 1997b). The results of these studies (Table 2) show quite clearly that the relative contributions of the intragastric and intravenous tracers to the mucosal-free and protein-bound pools of phenylalanine differ markedly. Furthermore, the results imply that while arterial phenylalanine contributed only 30% of the mucosal free phenylalanine it contributed 60% of the mucosal protein.

Table 2. The labelling (mol percent) of mucosal free and protein bound phenylalanine in piglets, fed a milk-replacer diet and infused intragastrically with [U-^{13}C]-phenylalanine and intravenously with [^{2}H]-phenylalanine (mean ± SD).

	Mucosal-free phenylalanine	Mucosal-protein phenylalanine	Protein:free
Intravenous tracer	3.30 ± 0.31	1.10 ± 0.16	0.32 ± 0.04
Intragastric tracer	0.48 ± 0.02	0.27 ± 0.02	0.56 ± 0.03
IG:IV	6.53 ± 0.55	3.86 ± 0.42	0.59 ± 0.10

Intestinal glutamate metabolism

The data on portal balance and protein synthesis discussed above suggest that amino acid metabolism is substantial in the mucosa, and this introduces the final aspect of intestinal metabolism in the piglet that will be discussed. Although in our studies it appeared that the mucosal cells were catabolising 25% of the dietary amino acids, dietary glutamate, dietary aspartate and arterial glutamine accounted for approximately 60% of this total. This result is similar to that obtained in both rats (Windmueller and Spaeth, 1975, 1980) and in humans (Matthews *et al.*, 1993b). However, there is much suggestive evidence that other pathways of mucosal metabolism involving glutamate and glutamine might be nutritionally significant. Studying this aspect of mucosal metabolism is, however, difficult because there are multiple potential inputs of glutamate, especially in the mucosa.

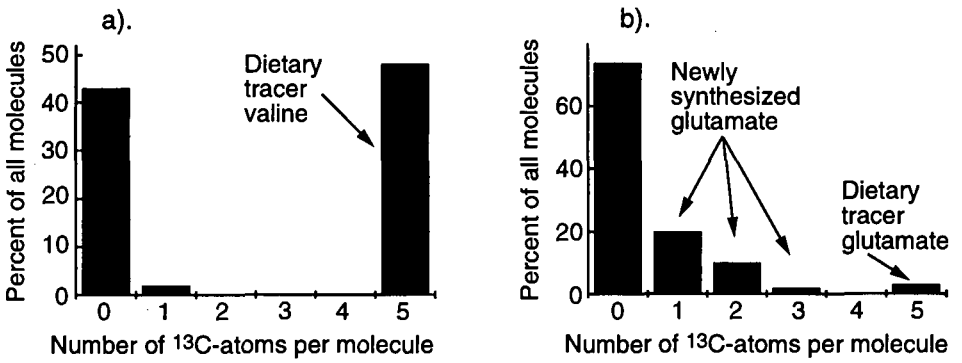

Figure 17. *a). Distribution of labelled valine molecules in egg protein. b). Distribution of labelled glutamate molecules in egg protein.*

However, the study of glutamate metabolism is simplified by another advantage of the use of multiple ^{13}C-labelled tracers. This arises from the fact that the compounds metabolised in the central pathways of intermediary metabolism are formed from the condensation of molecules containing 1 (CO_2), 2 (acetyl CoA) and 3 (pyruvate) carbons. Thus if a metabolic precursor, such as glucose, glutamate or glutamine, is introduced into a biological system in its [U-^{13}C]-form, any resynthesis of the tracer metabolite will return labelled molecules containing no more than 3-^{13}C atoms. This phenomenon is shown strikingly in the results presented in Figure 17. In this study (Berthold *et al.*, 1991) fed a laying hen for 27 d with a diet containing [U-^{13}C]-protein, and measured the ^{13}C-distribution in amino acids isolated from egg protein. The main result from this study was that labelled essential amino acids (valine in the figure) were present in the protein only as unlabelled or completely labelled forms, while amino acids that could be synthesised by the hen (glutamate in the figure) contained virtually no original tracer molecules. They were, however, highly enriched with isotopomers containing 1,2, and 3-^{13}C atoms that had become incorporated via the synthesis of the amino acid.

In recent experiments, Reeds *et al.* (1996) have taken advantage of this feature of the labelling pattern of recycled substrates to investigate the utilisation of enteral glutamate by the mucosa. In these studies, fed piglets received intragastric infusions of [U-^{13}C]-glutamate. These results (Reeds *et al.*, 1996) revealed that although more than 95% of the dietary glutamate was metabolised in first pass, there was substantial recycling of glutamic acid molecules within the mucosa. As a result, the mucosal glutamate pool was labelled in a complex fashion (Figure 18). However, the most intriguing result was that two key end products of glutamate metabolism, glutathione (Reeds *et al.*, 1997b) and arginine (Reeds *et al.*, 1997c), had an isotopomer pattern that was essentially identical to that of the dietary (tracer) glutamate, and markedly different from the mixed glutamate pool. In other words, there is not only a mechanism in the enterocytes that channels arterial essential amino acids to mucosal protein, but a separate mechanism that channels dietary non-essential amino acids to intermediary metabolism.

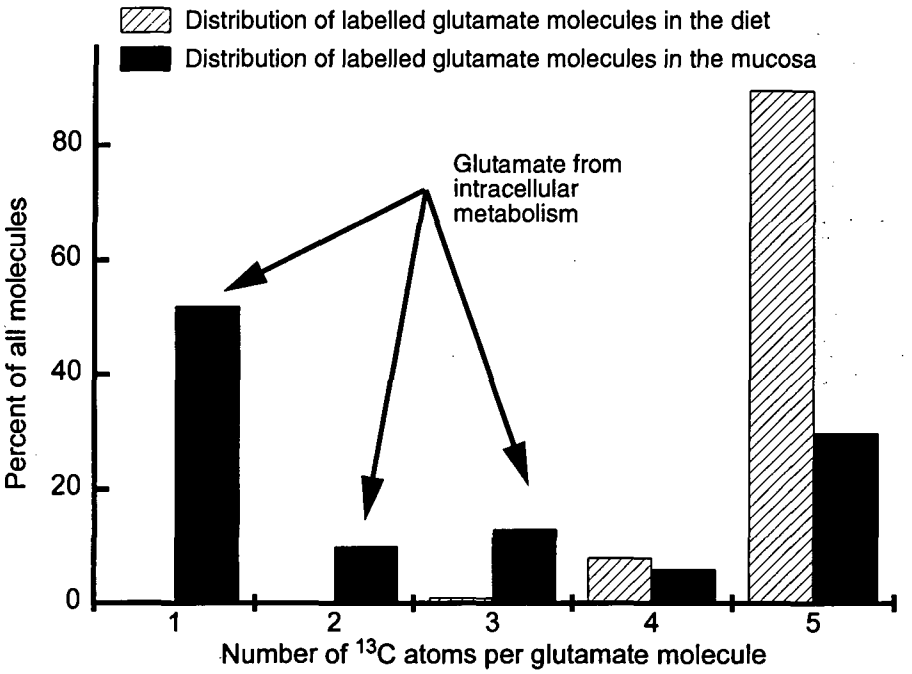

Figure 18. *The distribution of ^{13}C-isotopomers in mucosal glutamate during an intragastric infusion of [U-^{13}C]-glutamate (Reeds et al., 1997a).*

This dual balance/isotopic approach to studying intestinal metabolism is very powerful, and can be applied to many other metabolic problems (Reeds *et al.*, 1997d). However, the observations that have been discussed are not only of importance to understanding the nutrition of the piglet, and hence of direct relevance to animal husbandry, but pose important new questions with regard to the metabolism of infant mammals in general.

Conclusion

This paper has discussed a number of aspects of neonatal growth, development and metabolism but has by no means covered all aspects of this exciting and important field of interest. The size, growth rate and resilience of the piglet make it an extremely attractive model from a practical point of view. The bulk of the metabolic evidence supports strongly the utility of studies in piglets as a basis for understanding more of the physiology, metabolism and nutrition of human infants.

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A REVIEW - MANAGEMENT AND NUTRITION OF THE EARLY WEANED SOW

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Abstract

Productivity of the commercial sow herd is determined by a number of factors impinging on both fertility and management of dry sow and farrowing space. The current interest in segregated early weaning (SEW) as a management tool is driven in part by its potential advantages in maximizing occupancy and usage of relatively expensive farrowing facilities. Although understanding of those mechanisms underlying interactions between nutrition and reproduction in the sow is improving, the adoption of lactation lengths substantially shorter than three weeks have been associated with depressions in fertility. The severity and form of these depressions (ranging from extended weaning to oestrus intervals and increased non-productive sow days to decreased subsequent litter size) are dependent upon genotype and its interaction with environmental/management factors, e.g., *pre-* and *post-partum* nutrition. This review considers the growing literature concerning the physiological basis for suppressed fertility in sows undergoing shortened lactation lengths with emphasis on the primiparous sow. Potential control points within the hypothalamic-pituitary-ovarian complex are outlined, followed by practical considerations of nutritional management. The authors hypothesize a role for protein balance as a potential determinant of subsequent fertility. In light of described physiological mechanisms, alternative management approaches are suggested, including nutritional approaches, litter manipulations and pharmacological intervention. Ultimately, however, management recommendations will be determined by the herd in question by considering genotype and economic considerations of the operation concerned.

Introduction

Traditionally, the primary measure of breeding herd efficiency has been pigs born (or weaned) per sow per year. However, within North America and increasingly elsewhere, this is considered to be a relatively poor measure of breeding herd efficiency since it does not accurately reflect economic efficiency. An alternative measure is pigs born (or weaned) per farrowing crate per year. This measure takes account of the fact that the most expensive sow housing on the farm is the farrowing crate. In order to maximize farrowing crate efficiency, firstly, the crates must be occupied and secondly, they must annually house as many litters as possible. The first objective can be met by meeting breeding targets (taking farrowing rate into account) and the second objective met by reducing the duration of individual sow occupancy (i.e., lactation length).

If the objective of management is simply to maintain farrowing crate occupancy, then short lactation lengths are not necessary since, as discussed below, any economic advantage from potential litters per crate per year may be largely offset by reductions in sow performance. However, a move to earlier weaning does allow an expansion of the sow base within existing facilities with the capital costs incurred being limited to the less expensive dry sow accommodation. Earlier weaning may also form an integral part of the herd health program. Indeed, the current interest in segregated early weaning (SEW) was driven by the belief that specific pathogens could be eliminated from the progeny by implementing a pathogen-specific lactation length. This in turn allows for improved grower/finisher performance. Suggested examples have been a 10 d lactation to eliminate *Pasteurella multocida* (atrophic rhinitis) and *Mycoplasma hyopneumoniae* (enzootic

pneumonia) and a 21d lactation to eliminate *Actinobacillus (Haemophilus) pleuropneumoniae* and transmissable gastroenteritis (Alexander and Harris, 1992). However, expectations have been overly optimistic and it is now accepted that while shorter lactation lengths are invaluable in the control of these diseases, they do not necessarily eliminate them, especially when the immune status of the sow herd has not been adequately stabilized.

The objective of this paper is to review the effects of lactation length on sow reproductive performance and also how management can have an effect on the expression of sow fertility. The possible mechanisms whereby lactation length may affect fertility necessitates an understanding of the association between stage of lactation and the physiologic and metabolic status of the sow. It is not our intention to repeat earlier reviews but to draw relevant conclusions from them. The final part of this paper will address management strategies that could be employed to overcome the deleterious effects of short lactation lengths on sow fertility.

Effects of lactation length on reproductive performance

It has been known for many years that short lactation lengths are associated with extended and more variable weaning to oestrus intervals (Van der Heyde, 1972; Svajgr *et al.*, 1974; Cole *et al.*, 1975; Aumaitre *et al.*, 1976; Varley and Cole, 1976a; Kirkwood *et al.*, 1984a) making it more difficult to meet breeding targets. Recently, a negative association was confirmed between the length of the weaning to oestrous interval and the duration of the oestrous period in conventionally weaned sows (Nissen *et al.*, 1997). It was further established that ovulation occurred at approximately 70% of the way through the oestrus period, regardless of its duration, and that peak fertility occurred when breeding was at about 10 h before ovulation. If the same associations occur in early weaned sows where a longer weaning to oestrus interval is expected, then oestrus detection and mating management may need to be re-evaluated for SEW systems.

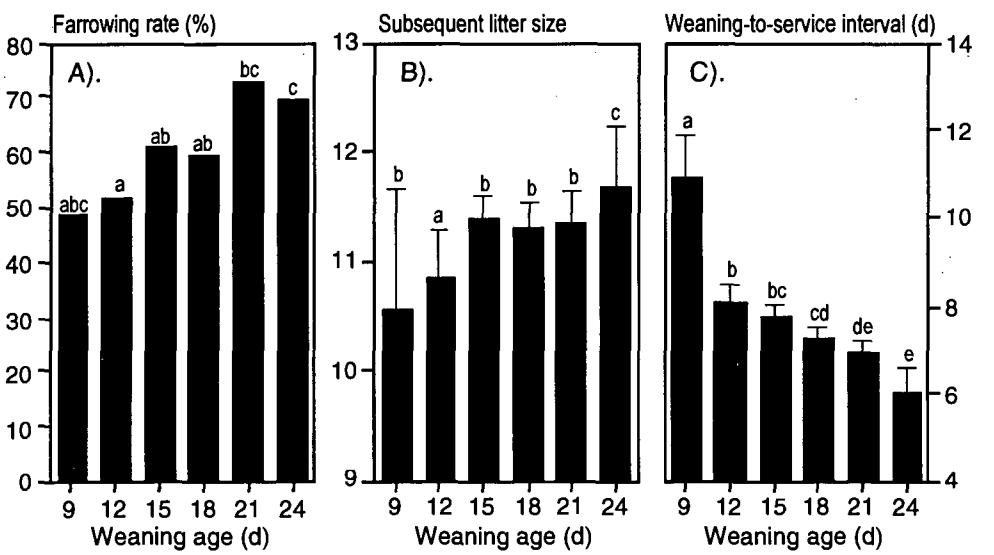

Figure 1. *The influence of weaning age on A). farrowing rate percentage, B). subsequent litter size and C). weaning-to-service interval, derived from commercial PigCHAMP databases. Weaning age groups comprise age ± 1 d, e.g., 9 ± 1, 24 ± 1 (Dial et al., 1996b).*

Short lactation lengths also reduced farrowing rates and subsequent litter sizes (Pay 1973; Cole *et al.*, 1975; Kirkwood *et al.*, 1984a; Clark and Leman 1987; Xue *et al.*,1993; Mabry *et al.*, 1996; Marsteller *et al.*, 1997). The effect on subsequent litter size is the result of increased embryo mortality, there being no effect of lactation length on ovulation rate (Varley and Cole 1976b; Marsteller *et al.*, 1997). The effects of lactation length on fertility

are illustrated in Figure 1, which is based on PigCHAMP data (Dial *et al.*,1996b). Each of the above effects will have a cost associated with them, although the relative economic impact of each effect may vary among farms. However, a recent analysis of Pig Champ data found that, on average, more than 90% of the weekly variance in weaner production was accounted for by numbers of females served weekly (Dial *et al.*, 1996a). It is increasingly accepted in the expanding North American industry that pig throughput is second only to pig price in influencing profitability. Consequently, the importance of meeting breeding targets and the potential adverse effect of unpredictable returns to oestrus are evident.

Endocrine changes from late gestation and farrowing to breeding

Despite the empirically well-established relationship between lactation length and fertility, there is a paucity of literature describing the physiological mechanisms underlying fertility reductions observed in sows weaned at substantially less than 21 d. Whether reproductive compromise occurs via 'premature disruption' of, and therefore an inadequate, sucking stimulus, nutritional/metabolic sequelae or uterine-ovarian insufficiencies is unclear. Data are accumulating, however, to support the involvement of the entire hypothalamic-pituitary-ovarian axis in determining the length of time for return to oestrus to occur, and both the quantity and quality of oocytes/embryos resulting from the post-weaning mating.

As in all mammals, the basic elements of the reproductive axis of the pig comprise a 'central' signalling unit, consisting of the hypothalamus [secreting luteinizing hormone-releasing hormone (LHRH)] and anterior pituitary [secreting the gonadotrophins - luteinizing hormone (LH) and follicle- stimulating hormone (FSH) under the influence of pulsatile LHRH]. Follicle stimulating hormone and LH, in turn, stimulate 'local' ovarian follicular and oocyte growth, maturation and, ultimately, ovulation.

Modulation of reproduction potentially occurs, therefore, via both central and local ovarian mechanisms. An understanding of the relative importance of each mechanism during lactation and the post-weaning period, and how their function may be modulated by environmental/management factors, may indicate explanations for, and potential solutions to, fertility depressions observed in SEW systems.

Modulation of the hypothalamic-pituitary axis

The return to fertile oestrus post weaning requires an appropriate gonadotrophin stimulus to a sensitive ovary for the establishment of the final stages of ovarian follicular maturation (Foxcroft and Hunter, 1985). Follicles emerge from the primordial population, becoming available for recruitment into the preovulatory pool during lactation, or even during the final stages of gestation in sows subjected to short lactation lengths. Morbeck *et al.* (1992), for instance, have estimated a period of 19 d for the advancement of antral follicles into the preovulatory cohort. Dynamic changes in the intra-ovarian and intra-follicular environments and in the development of sensitivity to gonadotrophin stimulation result in a so-called heterogenous population of follicles (Foxcroft and Hunter, 1985). Such heterogeneity may have important consequences for follicle and oocyte quality at ovulation and subsequent embryo viability, as discussed more fully in later sections.

Suckling-mediated effects on gonadotrophin secretion

Luteinizing hormone secretion during gestation is characterised by high amplitude, low frequency pulsatility, as the LHRH pulse generator operates under the inhibitory influence of circulating progesterone negative-feedback. This steroid inhibition may be mediated by endogenous opioidergic peptide (EOP) inhibition (Willis *et al.*, 1996). Luteinizing hormone pulsatility is not totally suppressed in very late gestation (Parvizi *et al.*, 1976; Ziecik *et al.*, 1983), suggesting that chronic suppression of hypothalamic LHRH secretion or decreased pituitary sensitivity to LHRH is not an inevitable consequence of gestation in sows. The persistence of episodic LH secretion in late gestation may have important consequences for ovarian responses to enhanced gonadotrophin secretion

following early weaning. Although a similar pattern of LH secretion predominates during established lactation, evidence from studies of sows in early lactation and sows weaned immediately after farrowing (zero-weaned sows) indicates a potentially active LHRH pulse generator and pituitary. De Rensis *et al.* (1989) reported active LH secretion from 24-36 h *post-partum* in both sows which suckle their piglets and zero-weaned sows. Later studies have reported longer periods of active secretion and a high degree of variation among sows in patterns of LH secretion in the immediate *post-partum* period (De Rensis *et al.*, 1993a). Luteinizing hormone secretion was also suppressed by the end of the second day *post-partum* in sows which suckle their piglets (De Rensis *et al.*, 1993b). A number of neuroendocrine mechanisms, including opioidergic pathways (De Rensis *et al.*, 1993b), appear to mediate the suckling inhibition of LHRH secretion at this stage. Certainly the pituitary is sensitive to exogenous LHRH stimulation from early lactation onward (Stevenson *et al.*, 1981). Pituitary sensitivity has been reported to increase (Bevers *et al.*, 1981) or remain static (Rojanasthien *et al.*, 1987) *post-partum*, suggesting that suckling-induced suppression of LH pulsatility is via suppression of LHRH secretion alone or in combination with changes in pituitary sensitivity.

Pituitary sensitivity to the negative and positive feedback effects of circulating oestrogen appear to alter during lactation (Elsaesser and Parvizi, 1980; Cox *et al.*, 1989b; Sesti and Britt, 1993) and have been related to sow reproductive performance (see the review by Varley and Foxcroft, 1990). Abnormally high follicular-fluid oestradiol concentrations have been reported in zero-weaned sows, potentially as a consequence of dysfunction of oestradiol positive feedback and a compromised LH surge (De Rensis *et al.*, 1993a). The gonadotrophin escape from inhibition is further demonstrated by increasing basal levels of LH with duration of lactation (Stevenson and Britt, 1980; Stevenson *et al.*, 1981; Kirkwood *et al.*, 1984a), possibly reflecting a lower sucking intensity during later lactation. Following weaning there is a rapid recovery in hypothalamic stores of LHRH (Cox and Britt, 1982). Whether the rate of recovery of LHRH stores is affected by previous lactation length has not been addressed, although Sesti and Britt (1993) reported an increase in pituitary LH as lactation proceeds.

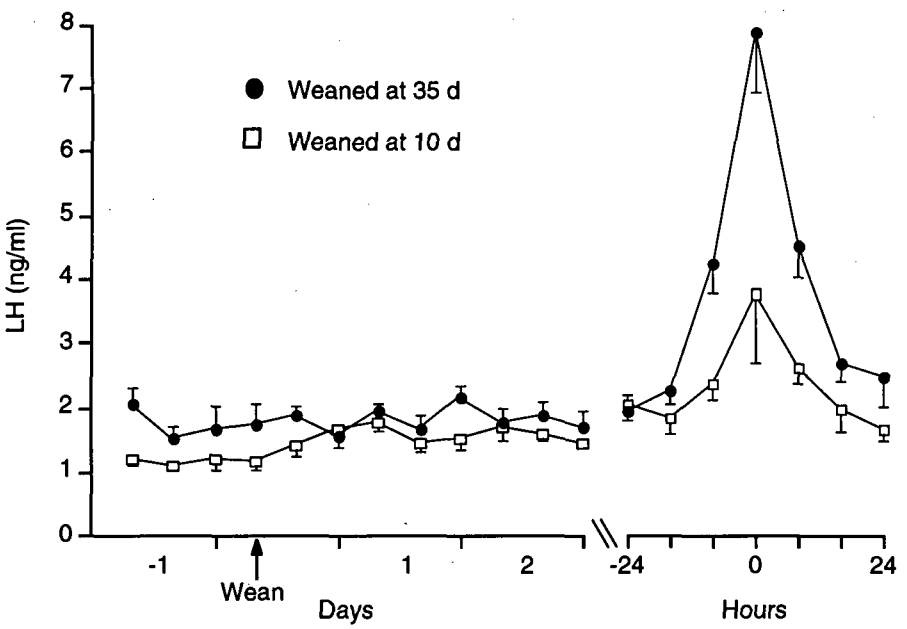

Figure 2. *Suppression of mean LH concentrations and attenuation of the preovulatory LH surge in sows weaned at 10 d versus 35 d (from Kirkwood et al., 1984a).*

Plasma levels of LH are also reported to show at least a transient increase during the first 24 h after weaning following lactation lengths of 21 d or more (Edwards and Foxcroft, 1983). Again, whether a similar increase occurs at weaning after short lactation lengths has yet to be investigated. It is known that lower basal LH levels are maintained during the weaning to oestrus interval in early weaned sows (Kirkwood *et al.*, 1984a) and, further, that the magnitude of the preovulatory LH surge is greatly attenuated in sows having had short lactation lengths (Edwards and Foxcroft, 1983), as demonstrated in Figure 2 (Kirkwood *et al.*,1984a). The biological significance of these observations has not been determined, but the administration of exogenous LHRH to sows at the time of breeding subsequent to a 12 d lactation was without effect on reproductive performance (Kirkwood, unpublished data).

The primary inhibition to preovulatory follicular maturation following farrowing in sows appears to be via a suckling-induced suppression of LHRH/LH secretion. Manipulations of this inhibition, e.g., via zero-weaning, may compromise both follicular development and the ultimate ovulatory signal, the LH surge, by influencing circulating steroid concentrations and pituitary sensitivity. It is feasible, therefore, that weaning at substantially less than 21 d may result in dysfunction of the hypothalamic/pituitary surge affecting both the timing, pattern and duration of ovulation itself, although such a hypothesis has not been adequately tested.

Metabolic modulation of gonadotrophin secretion

Empirical observations of the relationship between conformational characteristics (e.g., backfat thickness) and reproductive function have long formed the basis of successful breeding herd management. Such approaches in research continue to evolve and consider in more detail the potential underlying physiological and endocrinological mechanisms coordinating metabolic state and fertility. The considerable losses in backfat endured by modern sows during their first lactation (Whittemore *et al.*, 1980) and their correlations with decreased subsequent fertility (King *et al.*, 1982) have emphasized the challenges facing the sow, particularly the primiparous sow. More extreme management strategies, e.g., utilizing modern, extremely feed-efficient and lean female lines, potentially places further pressure on an already severely challenged animal. Management systems emphasizing optimal utilisation of relatively expensive farrowing space will almost certainly result in a parity distribution skewed towards primiparous animals, as sow inventory increases and culling decisions place emphasis on reproductive efficiency. Current indices of sow productivity include factors such as the ultimate size of the weaned litter and the non-productive days (i.e., 'empty' days prior to the sow being successfully bred). Since reproductive performance of sows with few parities is particularly sensitive to adequate nutrition, she remains the principal challenge to optimal sow productivity.

If fertility of the primiparous sow is a consequence of her overall metabolic state, future difficulties might be obviated by allowing her a period of recovery before rebreeding. Indeed, larger litter sizes have been reported from first/second parity sows bred on their second, as opposed to first, post-weaning oestrus (Clowes *et al.*, 1994). This effect, however, was not observed in sows of later parities. Interestingly, weaning-to-first-oestrus intervals were no different among treatment groups or parities. Zak *et al.* (1997a) also reported only small differences in weaning to oestrus intervals among sows fed *ad libitum* during a 28 d lactation and those undergoing feed-restriction during different stages of lactation. Remarkably, however, ovulation rates were reduced by lactation feed-restriction. These results contrast strongly with earlier findings including those of Koketsu *et al.* (1996a) who, using a 21 d lactation model, reported greatly delayed return to oestrus in feed-restricted sows. Prior analysis of production figures from large commercial units by the Minnesota group (Koketsu *et al.*, 1994) had also established a correlation between reduced, and altered patterns, of lactational feed intake and subsequent reproductive failure in sows of various parities. These findings strongly suggest genotype differences in the pattern of reproductive response to lactational insult. It appears that different genotypes may either undergo a self-imposed, partial "skip-a-heat" (i.e., delayed weaning to oestrus interval; Figure 3 from Dial *et al.*, 1996b) or commit

to resumption of cyclicity, despite subsequent reductions in fertility associated with suboptimal follicular/oocyte quality (i.e., lowered ovulation rates and embryo survival).

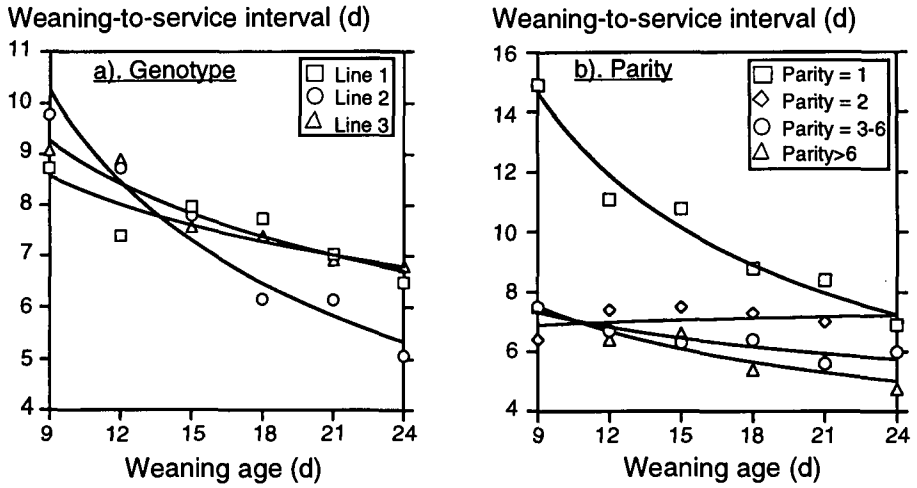

Figure 3. *The influence of a). genotype and b). parity on weaning to service interval and weaning age (Dial et al., 1996b).*

A metabolically-driven modulation of hypothalamic LHRH secretion and consequent alteration in pituitary LHRH-sensitivity and gonadotrophin release might be predicted to have major implications for reproductive function in the metabolically challenged lactating sow. In fact, short-term variations in lactational feed-intake in the sow have been correlated with alterations in the pattern of LH secretion (Tokach *et al.*, 1992), even though mid-lactation LH pulsatility in the sow is already extremely low, due to the direct inhibitory actions of suckling on the LHRH pulse generator described above. Tokach *et al.* (1992) correlated reduced LH concentrations from the second week of a 28 d lactation with extended weaning-to-oestrus intervals in primiparous sows. Similarly, Koketsu *et al.* (1996c) reported reduced LH pulsatility in sows undergoing feed restriction during 1 week of, or an entire, 21 d lactation, which also correlated with an extended weaning-to-oestrus interval. Complete suppression of lactational LH secretion in sows undergoing feed restriction either for the first 21 d or the last week of a 28 d lactation, in addition to a LH response to resumption of *ad libitum* feeding from day 21 to 28, were reported by Zak *et al.* (1997a).

Luteinizing hormone secretion is acutely sensitive to nutritional status in prepubertal gilts, as illustrated by the results of Booth *et al.* (1994, 1996) and Cosgrove *et al.* (1991, 1993). A short period of maintenance feeding, followed by realimentation, resulted in a suppression and then rapid resumption of LH pulsatility. Intravenous administration of glucose solutions, isocaloric with *ad libitum* meals, to feed-restricted gilts also resulted in rapid elevations of gonadotrophin secretion (Booth, 1990). Similar observations have been made in growth-restricted lambs (Ebling *et al.*, 1990). The inability of meal ingestion *per se* to invoke similar responses [e.g., no LH response to gastric distension via low energy sugar beet fibre in gilts (Formigoni *et al.*, 1996), or ingestion of water iso-volumetric with a full feed in fasted sheep (Foster *et al.*, 1995)], suggests a role for energy intake in the control of gonadotrophin, at least prepubertally. Tokach *et al.* (1992) found that LH secretion was not elevated following glucose injection in lactating sows, although Kemp *et al.* (1995) reported increased lactational LH pulsatility and post-weaning preovulatory LH surges in sows fed a carbohydrate- versus fat-rich lactation diet. Whilst these results do not rule out a potential role for glucose in regulation of the LHRH pulse generator in the sow, they suggest a complexity beyond a direct relationship to peripheral glucose concentrations.

Indeed, peripheral blood sugar concentrations may be of only limited relevance to the environment of those cells directly responsible for LHRH secretion. Elevated brain glucose levels have been suggested to mediate nutritionally-enhanced LH secretion via direct actions on the LHRH neuron. Bucholtz *et al.* (1996) suppressed LH pulse frequency in sheep without altering peripheral glucose concentrations by injecting the glycolytic inhibitor 2-deoxyglucose (2DG) into the lateral cerebral ventricle, suggesting a direct, 'central' site of action of glucose. Parenteral 2DG decreased LH pulsatility but also lowered peripheral glucose concentrations. In general, the brain is relatively well buffered by metabolic and endocrine systems against gross fluctuations in blood sugar availability. Whilst similar studies have yet to be conducted in the sow, it seems unlikely that simple alterations in peripheral glucose concentrations will be responsible for altering hypothalamic LHRH secretory activity unless they are associated with decreased energy availability to neurons themselves.

Peripheral and central glucose concentrations are regulated by the antagonistic actions of insulin and glucagon, and insulin responses to meal ingestion are rapid. Cosgrove *et al.* (1992) and Booth *et al.* (1996) reported greater and more sustained elevations of plasma insulin in realimented versus feed-restricted prepubertal gilts, associated with increased plasma LH. Injections of long- and slow-acting insulin to gilts on high- or low-energy diets resulted in elevated ovulation rates and appeared to increase pulsatile LH secretion, but not mean LH concentration, in a subset of animals (Cox *et al.*, 1987).

If insulin does indeed modulate LHRH secretion it is most likely at the level of the median eminence, since insulin receptors have been detected in the basal hypothalamus of the rat (Havrankova *et al.*, 1978). It has been suggested that cerebrospinal fluid concentrations of insulin, acting as integrated long-term reflections of humoral fluctuations, may constitute a central modulator of LHRH release (Woods *et al.*, 1979). However, long-term intravenous infusion of glucose to feed-restricted rams, while raising peripheral insulin and glucose concentrations, did not affect LH concentrations (Boukhliq *et al.*, 1996). Even blockade of the insulin response to realimentation in the rhesus monkey did not abolish associated increases in LH secretion (Williams *et al.*, 1996). In a rather limited study, intracerebroventricular (ICV) injection of insulin has been reported to increase LH pulsatility in gilts (Cox *et al.*, 1989a) and Zak *et al.* (1997a) reported depressions in insulin and LH concentrations in lactating primiparous sows subjected to feed restriction compared with controls fed *ad libitum*. Support for a direct effect of insulin on the hypothalamic pulse generator is equivocal from similar approaches in the sheep. Intracerebroventricular injection of insulin in growth-restricted ewe lambs did not increase LH (Hileman *et al.*, 1993), although a single peripheral injection of glucose in feed-restricted lambs resulted in elevated LH pulsatility (Branum *et al.*, 1997). The coordination of the prime stimulatory mechanism of reproduction, i.e., the hypothalamic-pituitary LHRH pulse generator, with nutritional status via such a transient and highly variable factor as blood insulin concentrations seems both unlikely and inappropriate.

The growth hormone/insulin-like growth factor (GH/IGF) complex of endocrine signalling does comprise a physiological system which appears to respond to alterations in metabolic state which are appropriate to the coordination of reproduction locally and may underly longer term, centrally mediated responses. Growth hormone (GH) secretion from the anterior pituitary exerts its anabolic effects predominantly via the stimulation of IGF-I synthesis and secretion from a wide range of tissues including hepatic, uterine and ovarian follicular cells (see Thissen *et al.*, 1994 for review). Plasma GH concentrations are inversely related to metabolic state in the pig. Booth *et al.* (1994) reported elevated GH in feed-restricted gilts, and several workers (Baidoo *et al.*, 1992b) reported higher lactational GH concentrations in metabolically challenged sows. Baidoo *et al.* (1992a) suggested a central role for reduced insulin and IGF-I and increased cortisol and GH in inhibiting the activity of the hypothalamic-pituitary-ovarian axis. Paradoxically, IGF-I concentrations are suppressed under the same circumstances, suggesting an uncoupling of the GH/IGF-I axis during metabolic challenge, probably via a reduction in hepatic GH receptors and IGF-I synthesis (Charlton *et al.*, 1993). Lowered insulin status has also been implicated in the reduction of tissue sensitivity to GH stimulation (Clemmons *et al.*, 1985). Protein turnover is driven towards accretion by IGF-I, and it is noteworthy that primiparous sows

bred on their first post-weaning oestrus exhibited higher circulating IGF-I concentrations at mating than those bred at their second (Clowes *et al.*, 1994). This result is potentially indicative of the physiological priority of the primiparous sow to return to her ideal protein accretion curve rather than maximize fecundity (see below). Zak *et al.* (1997a) also reported depressions in circulating IGF-I in lactating primiparous sows subjected to feed restriction compared with controls fed *ad libitum*.

However, the concentration of IGF-I does not respond with a similar rapidity as LH and insulin following realimentation of feed-restricted gilts (Cosgrove *et al.*, 1992; Charlton *et al.*, 1993; Booth *et al.*, 1996) and, therefore, appear unlikely to be directly mediating immediate changes in LH/LHRH activity, although a long-term role for IGF-I modulation of LHRH remains a possibility. It has been proposed that IGF-I modulates LH secretion in the gilt under *in vitro* conditions (Whitley *et al.*, 1995). Booth *et al.* (1996) noted a biphasic response of gonadotrophin secretion to realimentation, the longer-term increases in LH being temporally correlated with improved metabolic status as demonstrated by elevated IGF-I concentrations after 7 d of re-feeding. Only after 7 d of re-feeding were both baseline insulin and IGF-I concentrations higher in re-fed versus restricted gilts. Perhaps either or both hormones, acting synergistically, exert a direct effect on the LHRH pulse-generator. The family of epidermal-like growth factors (epidermal-like growth factor, transforming growth factors-α and -β) and nerve growth factor have been implicated in developmental plasticity of the LHRH pulse generator (Ojeda *et al.*, 1990). The roles of such factors in regulating alterations in the reproductive status of the adult are unknown.

If protein balance is a prime mediator of the reproductive status of the sow, a number of aforementioned mechanisms conceptually describe a role for circulating amino acids and gonadotrophin secretion. Dietary amino acids may modulate LHRH activity directly and reductions in their concentrations, e.g., during feed restriction or metabolic challenge, may disrupt gonadotrophin secretion. Central noradrenergic pathways have been indicated as stimulatory to LHRH release in several species including the pig (Parvizi and Ellendorf, 1978; Chang *et al.*, 1993) and have been shown to be sensitive to precursor (tyrosine) availability in the diet (Fernstrom, 1983). Cameron and Schreihofer (1995) reported that administration of the β-adrenergic antagonist, phenoxybenzamine, partially prevented the rise in LH associated with realimentation of fasted male monkeys. Tyrosine is also a precursor for dopamine secretion, a catecholaminergic neurotransmitter present in the hypothalamus and has a clearly established role in modulating LH secretion (Thiery *et al.*, 1995). Fasting of rats for 5 d suppressed LH concentrations and the brain turnover of dopamine and noradrenaline. Conversely, treatment with catecholaminergic antagonists did not restore LH pulsatility to control values (Pirke and Spyra, 1981). In a more recent study infusion of tyrosine, phenylalanine and tryptophan failed to alter gonadotrophin secretion and ovulation rates in luteal phase ewes (Downing *et al.*, 1997). However, tyrosine treatment of maintenance-fed ewes increased LH secretion (Hall *et al.*, 1992) and tyrosine concentrations have been positively correlated with LH pulsatility in the lactating dairy cow (Zurek *et al.*, 1995). Tyrosine and tryptophan, the amino acid precursor of serotonin, compete with other large neutral amino acids (LNAA) for transport mechanisms across the blood-brain barrier, and diets enriched with tryptophan have been reported as altering feed intake and plasma concentrations of other LNAA in lactating sows (Libal *et al.*, 1997). It remains feasible, therefore, that alterations in dietary composition or protein turnover rate may interfere with precursor availability and the activities of neuronal systems impinging on the LHRH neuron during metabolic challenge. Such hypotheses, however, remain untested in the pig but should, perhaps, receive rather more attention as the focus on reproduction-interactions in the sow focuses on questions of protein.

Modulation of the ovarian follicle and oocyte

A well-established literature documents empirical observations of nutritional influences during gestation and lactation on ovarian function (Baidoo, 1989; Koketsu *et al.*, 1996a; Zak *et al.*, 1997a). A distinction is seldom made, however, between hypothalamic-pituitary and direct ovarian effects. Recent studies have begun to focus on

discrete mechanisms which mediate follicular development within the porcine ovary and the relative sensitivity of the intraovarian environment to external and internal nutritional cues, affecting both follicular and oocyte maturation. The recruitment and maturation of the preovulatory cohort of ovarian follicles in the pig is acutely dependent upon appropriate gonadotrophin stimulation (Foxcroft and Hunter, 1985) and gives rise to a population of follicles heterogenous in maturity (Hunter and Wiesak, 1990). The time for maturation of antral follicles to the preovulatory stage has been estimated as 19 d in the prepubertal gilt (Morbeck *et al.*, 1992). Whether a direct comparison is appropriate between follicular development in the prepubertal gilt and the late-gestating/early-lactating sow is unclear. It remains feasible, however, that gestational nutrition and metabolic status has profound effects on the subsequent responses of the ovary to changing gonadotrophin stimulation in lactation. As lactation progresses, the gradual rise in both FSH and LH and increase in LH pulsatility results in an increase in the population of medium (5-8 mm), oestrogenic follicles in the ovary. Thus, alterations of feed-intake or metabolic status of lactating sows may have an impact on subsequent preovulatory follicular populations and ovulated oocyte quality, a concept described as "follicular imprinting" (Zak *et al.*, 1997b). Thus nutrition in each stage of the breeding cycle of the sow becomes interdependent. Specifically, even metabolic status in late gestation may become a critical factor in SEW systems employing short (10 d) lactation lengths. Under such circumstances fertilizable oocytes may be ovulating from follicles which were undergoing initial maturation *pre-partum*. Lactation length itself may, therefore, impose fundamental effects on ovarian function. Those mechanisms which mediate follicular/oocyte sensitivity to gonadotrophin stimulation have become of particular relevance to our understanding of how nutrition may regulate reproduction.

Influences on the ovarian follicle

Metabolic hormones do exert direct effects on follicular dynamics in the pig. Insulin treatment, irrespective of changes in gonadotrophin secretion, increased ovulation rates of cyclic gilts (Cox *et al.*, 1987), stimulated follicular steroid synthesis and/or decreased follicular atresia in cyclic (Matamoros *et al.*, 1990) and PMSG-treated (Matamoros *et al.*, 1991) gilts. Insulin treatment of gilts made artificially diabetic by streptozotocin treatment produced similar results (Meurer *et al.*, 1991). Further, cessation of insulin therapy in mid-luteal diabetic gilts increased follicular atresia without affecting gonadotrophin receptor binding, suggesting gonadotrophin-independent, insulin-mediated mechanisms (Cox *et al.*, 1994). Certainly, insulin exerts potent effects on granulosal cell differentiation and function *in vitro* (May and Schomberg, 1981; Amsterdam *et al.*, 1988). Realimentation-induced increases in follicular aromatase activity and/or development have also been associated with marked increases in insulin status, without consistent alterations in GH status (Cosgrove *et al.*, 1992; Charlton *et al.*, 1993; Booth *et al.*, 1994). Interestingly, control of granulosa cell glucose utilization may differ from peripheral mechanisms since changes in follicular fluid glucose concentrations do not parallel those in the periphery (Britt *et al.*, 1988).

Follicular function may also be determined by the effects of longer-term endocrine, paracrine and autocrine signalling (for review see Roche, 1996). While short-term feed-restriction of the prepubertal gilt appears to uncouple hepatic GH receptor-stimulated IGF-I expression (high GH concomitant with low IGF-I mRNA expression), ovarian IGF-I mRNA expression remained unaltered (Charlton *et al.*, 1993) and follicular IGF-I concentrations remain considerably higher than those in plasma. Follicular IGF-I concentrations also appear to be inversely related to follicular size in swine (Charlton *et al.*, 1993). Ovarian IGF-I synthesis may, therefore, mitigate the impact of acute nutritional challenges to ovarian function. Considerable data now exists describing the role of IGF-I and other growth factors in the porcine ovary (see reviews by Adashi *et al.*, 1990; Guidice, 1992; Hammond *et al.*, 1993; Ojeda and Dissen, 1994).

Ovarian IGF-I gene expression (Samaras *et al.*, 1993) and follicular fluid concentrations (Hammond *et al.*, 1985) increase during spontaneous and gonadotrophin-(Hammond *et al.*, 1988) and GH-induced (Bryan *et al.*, 1989) development during the follicular phase, falling after ovulation (Samaras *et al.*, 1992). Similar, though not identical, results have been reported during follicular development in the weaned sow

(Howard and Ford, 1992). Insulin-like growth factor-I does have mitogenic effects on ovarian cells, increasing DNA synthesis and cellular differentiation (Baranao and Hammond, 1984; Maruo *et al.*, 1988). The most potent actions of IGF-I, however, are apparent as interactions with those of gonadotrophins and other growth factors, e.g., enhancing gonadotrophin stimulated steroidogenesis (Veldhuis and Rodgers, 1987) and transforming growth factor-β (TGF-β) stimulated cellular proliferation and differentiation (May *et al.*, 1988). An added degree of complexity underlying these actions is the regulation of growth factor bioavailability by binding proteins (e.g., IGFBP) which is influenced by the gonadotrophin (Mondschein *et al.*, 1990) and steroid milieu (Samaras *et al.*, 1993). In general, porcine follicular growth is inversely related to concentrations of the specific binding proteins IGFBP-2, 4 and 5, although the latter two may actually increase in preovulatory follicles (Hammond *et al.*, 1992). Data emerging from *in situ* hybridization studies of follicles from cyclic gilts subjected to nutritional challenges have also raised the possibility of a role for IGF-II in mediating intra-follicular regulation (A. Dawson, personal communication). It seems conceivable, therefore, that gross alterations in the metabolic state of the lactating sow (particularly the generally catabolic primiparous animal), associated with major alterations in protein and fat mobilization mediated via dynamic changes in insulin/IGF status, result in altered follicular maturation and, potentially, oocyte quality.

Transforming growth factor-α (TGF- α) is expressed in both thecal and granulosal cells of a number of species (see Mulheron and Schomberg, 1993), including the pig (Singh and Armstrong, 1995). Both TGF- α and its receptor (Skinner and Coffey, 1988; Roberts and Skinner, 1991) are expressed in bovine thecal and granulosal cells suggesting both paracrine and autocrine methods of action (see Armstrong and Webb, 1997 for review). A so-called 'juxtacrine' mode of action for the EGF family has also been proposed due to their synthesis as a transmembrane precursor (Massague, 1990), allowing cell to cell communication of similar or different phenotypes, e.g., granulosa and theca. Transforming growth factor-α inhibits the steroidogenic actions of gonadotrophin-stimulation (May and Schomberg, 1989) while stimulating cell proliferation. The apparently opposing actions of TGF-α and IGF-I on ovarian cell function may actually constitute a coordination of follicular development, allowing concomitant somatic growth and steroidogenesis, prerequisites for the preovulatory follicle (Ojeda and Dissen, 1994). Conversely, TGF-α has been reported as stimulatory to oestrogen production of granulosal and thecal cells in the prepubertal gilt (Gangrade *et al.*, 1991). It is, therefore, feasible that the follicular cell response to TGF-α is dependent upon previous exposure to gonadotrophin hormones. Whether the extended suppression of high frequency LH pulsatility associated with suckling and catabolism in the lactating sow (Zak *et al.*, 1997a) results in an ovarian sensitivity which equates with that of the prepubertal period remains unknown.

Influences on the oocyte

An exquisitely close relationship between the granulosal cells of the cumulus oophorus and the oocyte regulates development and maturation of the germ cell within the follicle. Critical to this regulation is the existence of gap junctions between cumulus cells and the oocyte, allowing interactions between somatic cells (cumulus, granulosal and thecal) and germ cells (for review see Buccione *et al.*, 1990), including a potentially nutritive role (Brower and Schultz, 1982). Interactions between oocyte maturation and follicular derived factors have been implicated in the pig (Ding and Foxcroft, 1994a; Ding and Foxcroft, 1994b). Germinal vesicle breakdown and resumption of meiosis during final oocyte maturation appears to be dependent on disruption and/or modification of gap junctions between the oocyte and cumulus cells, (e.g., Moor and Heslop, 1981). Such disruption overcomes the influence of meiosis arresting factors or allows oocyte responses to meiosis stimulating factors. Epidermal growth factor treatment has been shown to influence these (Eppig and Downs, 1987; Downs *et al.*, 1988) and other (Ding and Foxcroft, 1994b) processes. Porcine oocytes incubated in IGF-I enriched media possessed increased blastocyst cell numbers compared with controls following fertilization (Xia *et al.*, 1994).

Zak *et al.* (1997b) reported shifts in follicular populations away from larger follicles towards medium/smaller follicles in primiparous sows subjected to feed-restriction during a 21d lactation compared with sows fed *ad libitum*. Oocytes from sows fed *ad libitum* exhibited improved *in vitro* maturational ability *per se*. Furthermore, follicular fluid from larger follicles harvested from the same sows was better able to support *in vitro* maturation of oocytes harvested from randomly selected prepubertal gilts, when compared with follicular fluid obtained from previously feed-restricted sows. It seems highly probable, therefore, that IGF-I, TGF-α and -β and a variety of other growth factors and binding proteins play important roles in selection of pre-ovulatory follicles and likely, therefore, in oocyte maturation. They may thus form a tangible link between metabolic status and ovarian function (Figure 4).

A plethora of mechanisms will potentially mediate nutritional influences on reproduction. The intimate interactions between metabolic status and nutrition during defined stages of the sow's breeding cycle predicates an integrated approach to nutritional management, dependent upon which production system is adopted. Certainly, nutritional considerations will be of importance in the adoption of a relatively extreme management structure such as SEW.

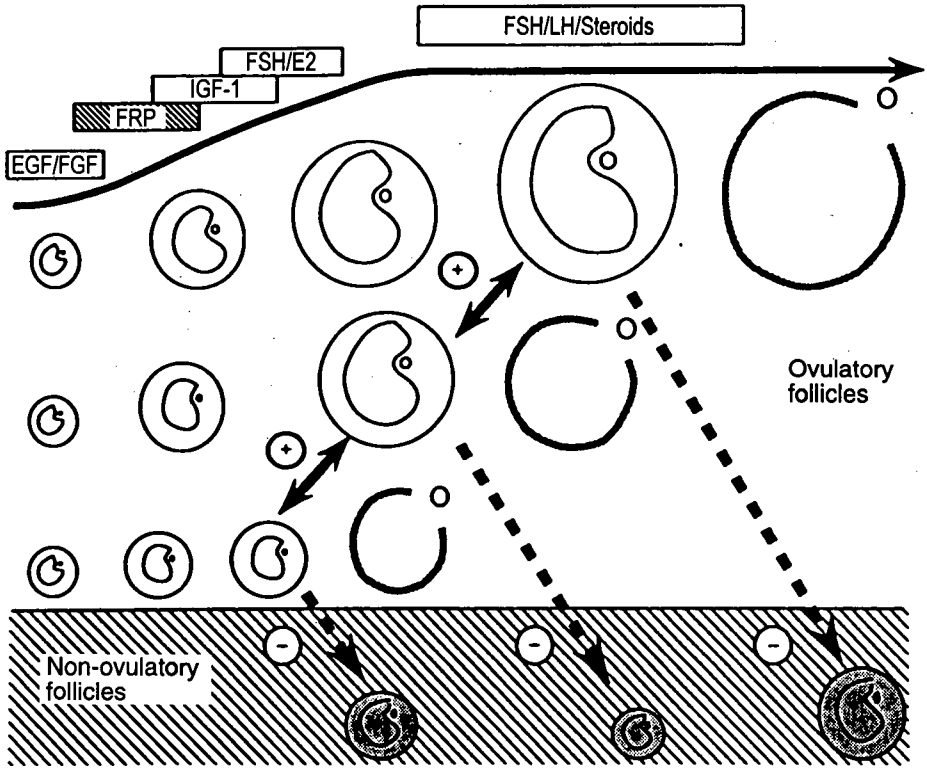

Figure 4. *Diagrammatic representation of follicle development in the pig. The paracrine factors that are likely to coordinate follicular maturation and/or atresia are indicated and the stage of development at which they may be important. Atresia is indicated by the hatched area and potential interfollicular interactions are indicated by arrows and by either + (supportive) or - (inhibitory) modes of action. (EGF/FGF: epidermal growth factor/fibroblast growth factor; FRP: follicle regulatory protein; IGF-1: insulin-like growth factor-1; FSH: follicle stimulating hormone; E2: oestradiol; LH: luteinizing hormone.) (From Hunter et al., 1992).*

Utero-ovarian changes from farrowing to breeding

At the time of parturition the ovary has a population of apparently healthy follicles which, with the establishment and continuation of suckling inhibition, will become atretic. Thereafter, early lactation is associated with an initial intense suppression of ovarian follicular development. However, in concert with endocrine parameters, the ovary progressively escapes from suckling-induced inhibition and limited follicular development ensues. Sows, however, generally remain anoestrus while suckling continues. Indeed, the ovulatory response to exogenous gonadotrophins is initially poor during early lactation (Guthrie *et al.*, 1978; Kirkwood and Thacker, 1990) and a consistent ovulatory response is unlikely before 25 d of lactation. It is, therefore, reasonable to suppose that following short lactation lengths, weaning occurs at a time of relatively profound suppression of the reproductive axis. Follicular development is potentially compromised when compared with that following longer lactation lengths. Since follicular development and follicular oestrogen production ultimately controls the weaning to oestrus interval, it is not surprising that early weaned sows will have a longer and more variable weaning to oestrus interval.

At term, the sows' uterus is greatly distended and has undergone histological changes to accommodate her litter. Following parturition, the uterus gradually returns to its non-pregnant condition, this being the process of involution. While reductions in size and weight are initially rapid, involution is not thought to be complete until about 21 d postpartum (Hughes and Varley, 1980). It has also been documented that involution occurs faster in sows which suckle than in those weaned after short lactation lengths (Hughes and Varley, 1980), which may suggest a role for oxytocin in uterine involution. It is therefore possible that early weaning results in a reduced rate of post-weaning involution because of the early removal of the suckling/oxytocin stimulation. While complete involution is not essential for the establishment of the next pregnancy, incomplete involution may contribute to poorer embryo survival.

Nutrition of the early-weaned sow

Excellent current reviews are available which provide details of the nutrient requirements and feeding management of sows (e.g., Close and Mullan, 1996; Neil, 1996). However, few if any, controlled studies have specifically addressed nutrition of the early weaned sow. Therefore, as with conventionally weaned sows, it is probable that metabolic influences on the fertility of the early weaned sow are greatly determined by nutrient intake during lactation. However, it is also true for the early weaned sow that nutrition during gestation and the weaning to oestrus interval can interact with nutrition throughout lactational to affect fertility.

Gestation

The objectives of nutrition during gestation are to allow feto-placental development and also (depending on sow parity) growth of the sow, without compromising nutrient intake during lactation. There is little evidence to suggest that nutrition during gestation of the early weaned sow will differ from that of conventionally weaned sows. However, given the predisposition of early weaned sows to reduced fertility, it is important that nutritional targets be determined and adhered to during gestation. If conformation must be used as a measure of metabolic status, the achievement of a target P2 backfat depth of 8-20 mm at farrowing in all sows, regardless of parity and lactation length is suggested. This is an arbitrary figure based on experience with PIC genetics and will be greatly determined by genotype and management of individual operations. Regarding pattern of feed intake, avoid overfeeding during the first 72 h after mating as this may depress blood progesterone concentrations and so reduce embryo survival (Jindal *et al.*, 1996). Regarding the end of gestation, Close *et al.* (1985) showed that sows fed 20.9 MJ DE/d mobilized fat from day 87 of pregnancy resulting in a loss of up to 20% of the sows fat reserves by the time of farrowing. Noblet *et al.* (1990) suggested that the daily energy intake required

to prevent this mobilization of body fat in late gestation is 30.5 MJ DE/d, a figure which will partially depend on environmental conditions.

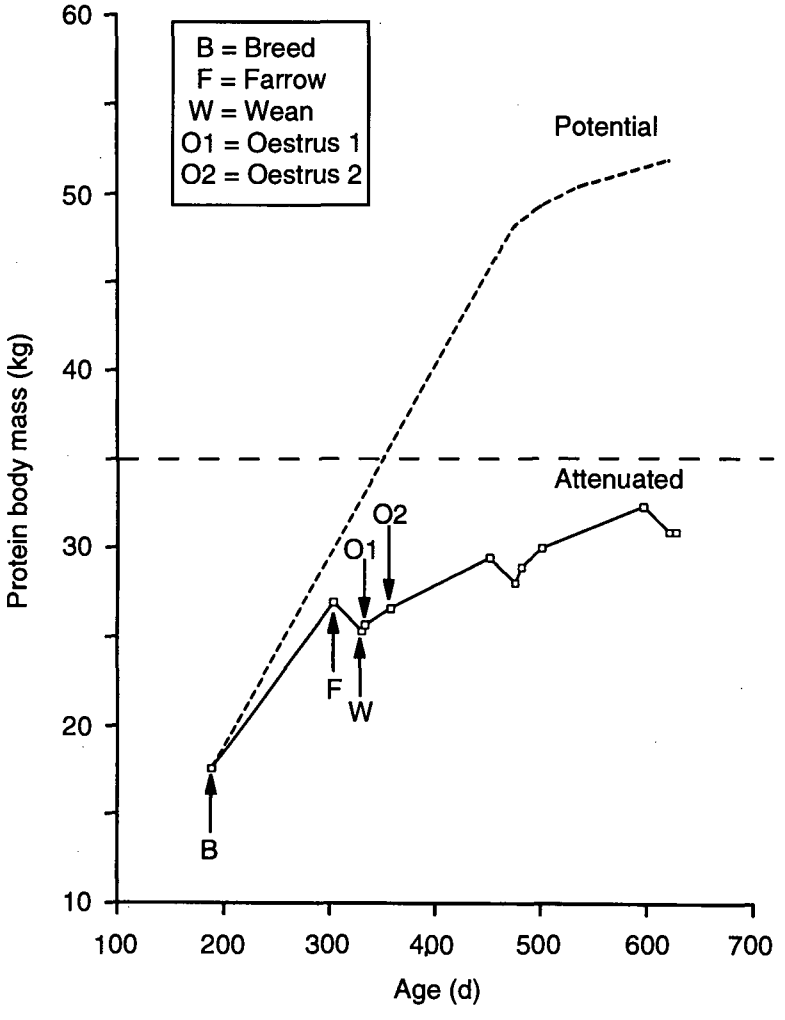

Figure 5. *Estimated maximal lean tissue growth potential (Potential) and the actual attenuated lean tissue curve estimated for the sows in the study of Clowes et al. (1994). (From Foxcroft et al., 1996).*

In addition to energy, protein requirements also increase with advancing pregnancy (Everts 1994), and the estimated nitrogen retention in the *conceptus* and udder have been reported to increase from 2 g/d in mid pregnancy to 14 g/d in late gestation (Noblet *et al.*, 1990). Therefore, gestation diets should be formulated to provide a minimum of 14 g/d utilizable nitrogen in addition to that required for sow maintenance. Feeding 2.3 or 3.9 kg feed/d to sows from day 100 of gestation until farrowing did not significantly affect feed intake during lactation, piglet growth rate, weaning to oestrus interval or subsequent litter size (Miller, 1996). Several studies report no effect of increased late gestation feed intake on feed intake during lactation (Sterling and Cline, 1986; Cromwell *et al.*, 1989), although one study did associate increased incidence of mastitis, metritis or agalactia with elevated feed intake in late gestation (Goransson,1989). Neil (1996) reported that either feeding sows *ad libitum* from day 111 of gestation until weaning, or from farrowing to

weaning, did not adversely affect sow or litter performance. Additionally this approach resulted in a significantly greater daily feed intake than did a system of feed restricting sows before farrowing and in early lactation. Some field evidence may exist that certain dam lines are prone to develop a congested udder unless subjected to a degree of feed restriction for 24 to 48 h before farrowing, with associated ramifications for piglet and sow nutrition. Whether this is a potential problem must be determined at the farm level.

Lactation

The voluntary feed intake of modern primiparous sows is low and generally results in nutrient output exceeding nutrient input (Aherne and Williams, 1992). Higher parity sows may more closely meet the metabolic demands of lactation by increased feed intake and, consequently reduce tissue catabolism. Changes in plasma concentrations or fluxes of metabolic hormones and substrates, as suggested above, almost certainly mediate the activity of the reproductive axis and regulate subsequent reproductive function of the sow.

Everts (1994) has suggested that breeding sows are subject to a physiological impetus to attain a protein body mass of at least 35 kg, whilst there may be no such 'drive' for a target body fatness. While this figure potentially refers only to the genotype studied, the concept of achieving a mature protein mass and that the physiological and biochemical mechanisms involved may impinge on subsequent reproduction is useful. If this hypothesis is accurate, reproductive efficiency would be expected to increase as the animal approaches its target protein mass. The pattern of protein accretion leading to its attainment is generally made up of a series of gestation gains and lactation losses (Figure 5). Currently, gilts are bred with a protein body mass of 20-25 kg and gain about 3-5 kg protein/parity. Thus, sows will reach a protein mass of 35 kg at the end of their third or fourth parity. The intensity of this 'drive' to accrete protein in the sow will, therefore, tend to decrease with increasing parity. It has been suggested that increases in reproductive efficiency with parity are associated with a larger maternal protein mass (Clowes *et al.*, 1994). The improvement in reproductive performance seen in young sows bred at their second, rather than first, post-weaning oestrus may be due to their improved metabolic status 26 d after weaning compared with 5 d after weaning (Clowes *et al.*, 1994). After weaning, first and second parity sows selectively partition nutrients into renewed lean tissue growth at the expense of fat. Sows of low parity have reduced feed intakes, are still growing and may, therefore, be compromised in achieving an adequate rate of protein accretion until the second post-weaning oestrus.

King (1987) and Britt *et al.* (1988) have suggested that the loss of protein during lactation may be of greater relevance to subsequent reproductive performance than loss of fat. Skeletal muscle accounts for about 45% of body protein, of which 40-50% is labile. These reserves support the free amino acid pools of the body and enable the sow to maintain metabolic functions, such as milk production, during periods of prolonged nutritional stress. How much of this labile protein can be mobilized before sow performance is impaired is not known. However, there is considerable evidence that if excessive maternal protein reserves are mobilized during lactation to supply amino acids for milk synthesis, sows will support inferior piglet growth rates and have reduced subsequent reproductive performance (Aherne *et al.*, 1995; Neil, 1996; Close and Mullan, 1996; Whittemore, 1996). Whittemore (1996) has suggested that a lipid to protein ratio in primiparous sows of less than 1:1 may indicate potentially impaired reproductive function. Subsequent weaning to oestrus interval in primiparous sows is positively related to the estimated loss of protein during lactation, as a percentage of total body protein at parturition, while a negative relationship exists between the estimated size of the maternal protein body mass at weaning and the weaning to oestrus interval. These relationships appear to be curvilinear above a 10% loss of total body protein, as depicted in Figures 6a and 6b. Reproductive performance may potentially be affected by factors that integrate both degree or rate of tissue (especially protein) loss at parturition and loss during lactation. Consequently, protein intake during lactation may have consequences for subsequent fertility (Figure 6c; personal communication, E.J. Clowes.).

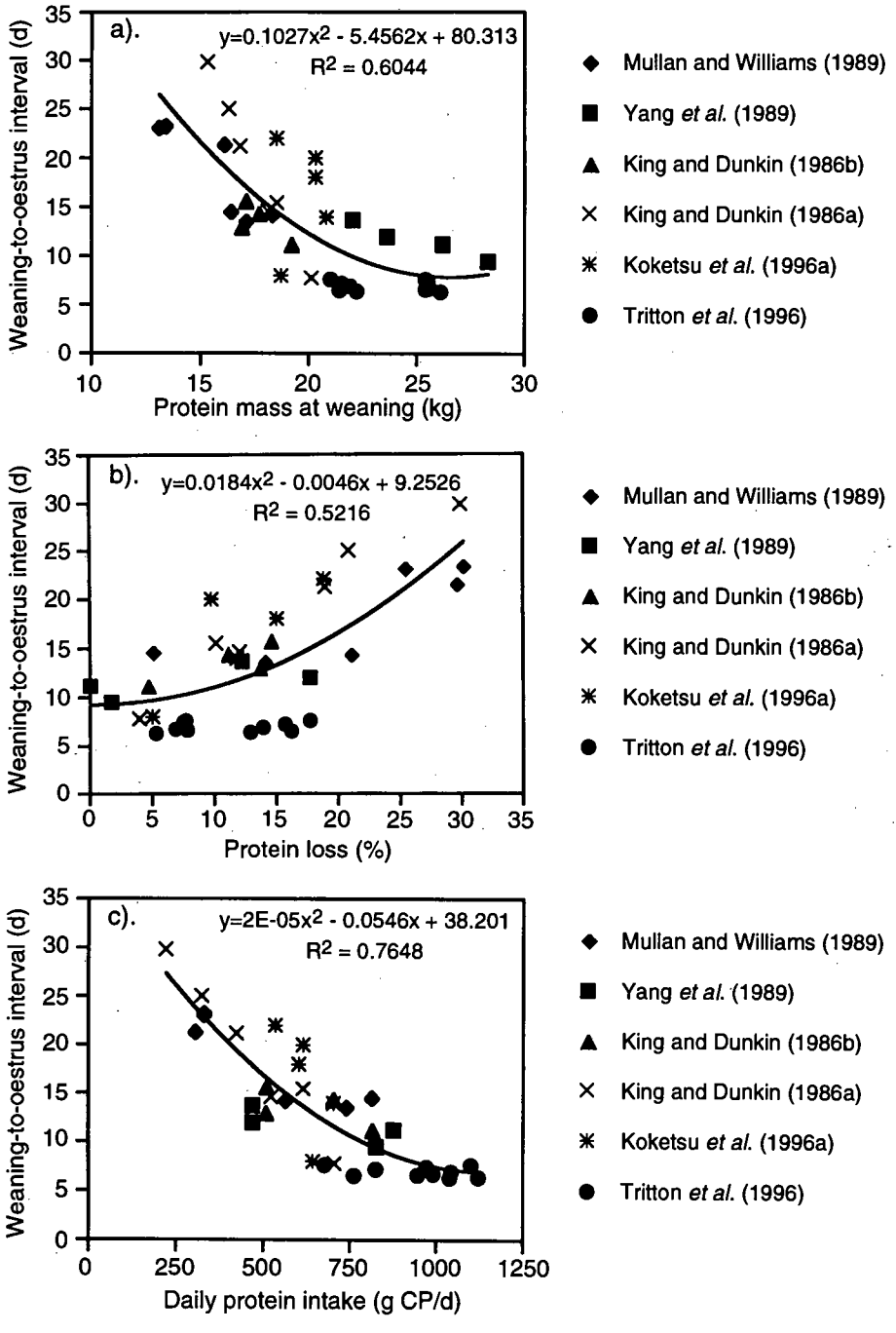

Figure 6. *Cumulative regression equations and lines for weaning to oestrus interval against a). predicted maternal protein mass at weaning, b). predicted maternal protein loss during lactation as a percentage of protein at farrowing and c). daily protein intake during lactation, modelled from data contained in cited studies.*

There is some evidence that increased fatness of the sow at parturition is associated with decreased feed intake in lactation (Whittemore *et al.*, 1988; Matzat *et al.*, 1990; Mullan and Williams, 1989; see Revell and Williams, 1993 for review). Until recently, a candidate metabolite or hormone linking 'fatness' to the reproductive axis has proven to be elusive. Leptin [a product of the obese (ob) gene] is synthesized by adipocytes (Campfield *et al.*, 1995), within the hypothalamus (Lee *et al.*, 1996) and ovary (Zamorano *et al.*, 1997) of rodents, and has been implicated as a potential mediator of reproductive cyclicity in humans (Chehab *et al.*, 1996).

Leptin receptors are present in the hypothalamus (Lee *et al.*, 1996; Zamorano *et al.*, 1997) and leptin has been reported to interact with actions of both neuropeptide Y (Schwartz *et al.*, 1996) and insulin (Kolaczynski *et al.*, 1996). The possibility of a role for leptin in modulating metabolic/somatic effects on the reproductive axis of the sow is currently under investigation. However, the physiological and biochemical consequences of fat accretion may not be significant until reaching an extreme, as indicated by P2 backfat thickness in the order 25 mm or greater. The inverse relationship between gestational and lactational feed intake appears greatest in early lactation and in primiparous sows (Noblet *et al.*, 1990; Young *et al.*, 1990), although the relatively poor appetites of young sows and sows in early lactation may mask this relationship. Whittemore *et al.* (1988) suggested that factors other than level of fatness are involved because they observed that when sows were fed the same amount in lactation the fatter sows at farrowing mobilized more body reserves. Therefore, the reduced feed intake in lactation associated with over feeding in gestation may also be partly caused by increased insulin resistance (Weldon *et al.*, 1994) or reduced insulin secretion (Xue *et al.*, 1996) in the sows after farrowing.

Primiparous sows usually consume 15% less food during lactation than do multiparous sows (NRC 1986). Because sows increase their voluntary feed intake gradually or are restricted in feed intake over the first two weeks of lactation, longer lactation lengths usually result in higher average daily feed intakes. The average daily feed intake of sows weaned after a 10 d lactation may be 25% lower than that of sows with a 28 d lactation. In a large scale survey, Koketsu *et al.* (1996c) reported that 38% of lactating sows showed a major dip (reduced feed intake of ≥1.8 kg/d for more than 2 d) in feed intake during lactation. A further 28% showed a minor dip in feed intake. The major dips in feed intake were associated with an increased occurrence of reproductive failure after weaning. They suggested that the more important risk factors affecting the reductions in feed intake were lower parity, thicker backfat and higher temperatures in the farrowing barn.

With the trend toward shorter lactation lengths it is interesting to note that the productivity of sows with different lactation lengths is influenced by feed intake. The effect of short lactation length (<18 d) on weaning to service interval was much more pronounced in sows eating less than 4.2 kg/d throughout a 19 d lactation than it was for sows eating more than 4.2 kg/d (Dial *et al.*, 1996b). Sows eating in excess of 5.7 kg/d were protected from the negative effects of shorter lactation lengths on weaning to service interval, subsequent litter size, and farrowing rate (Dial *et al.*, 1996b). Neil (1996) reviewed the data from 14 experiments and found that the correlation between daily energy intake during lactation and the interval from weaning to oestrus was high (r=-0.71).

Post weaning

The major objectives of nutrition during the postweaning period are to shorten the interval to effective mating, synchronise the onset of oestrus, and maximize ovulation and conception rates. From a review of the literature, King and Martin (1989) suggested that approximately 50% of first-litter sows fail to exhibit oestrus within 7 d of weaning, whereas almost 80% of older sows were mated within the same period. Increasing the level of feed intake after weaning has been reported to shorten the interval to service in primiparous sows and to increase the number of such sows exhibiting oestrus within 10 d of weaning, but it has no such effect on more mature sows in good body condition (Neil 1996). Thus, the response of sows to increased postweaning feed intake is determined by

parity, the amount of weight or condition lost during lactation and sow weight and body condition at time of weaning. In fact, weight loss during lactation has a clear effect on weaning to oestrus interval in first and second parity sows. Vesseur *et al.* (1996) reported that greater than 7.5% weight loss during lactation increased weaning to oestrus interval in first and second parity sows, respectively. The effect of of this scale of weight loss was less pronounced in second parity sows. However, the majority of experiments have failed to show any effects of postweaning feed intake on weaning to oestrus interval, particularly when the interval was short.

King (1987) reported that there is a lack of agreement on the effects of feed intake between weaning and mating on subsequent ovulation rate and litter size. Baidoo *et al.* (1992a) showed that levels of feeding after weaning had no significant effect on ovulation rate and pregnancy rate. Embryo survival was significantly lower for sows fed restricted levels of feed both in lactation and postweaning. These data suggest that if sows lose an excessive amount of body weight and backfat during lactation, then a high level of feeding after weaning may improve embryo survival.

Management options for early weaned sows

From the foregoing discussion and from the accumulated experience of the authors and their colleagues, it is evident that different genotypes may respond differently to early weaning. Furthermore, within genotype, fertility responses may differ on different farms, reflecting genotypic sensitivity to nutritional management. If early weaning is found to result in an unacceptable reduction in fertility, the simplest management option available is to increase lactation length to a point where the specific genotype, under a particular management regime, suffers an acceptable loss of fertility. Field experience with individual producers and anecdotal evidence from colleagues has suggested that some genotypes exhibit unacceptable levels of fertility (especially a prolonged and unpredictable remating interval) if weaned at less than 17 or 18 d of lactation while others do not show an appreciable drop in fertility unless weaning age is less than 14 d. Most, if not all, genotypes show depressed fertility if weaning age is less than 10 d. On occasion, it has been noted that a small shift in weaning age can have a large effect on fertility (authors' observation). One farm reported farrowing rates of 91% following lactation lengths of 16 to 18 d but, if lactation length was reduced to 14 or 15 d, farrowing rates of 70% were reported. The message from field evidence is clear; the fertility response to short lactation lengths must be evaluated on the individual farms. Industry averages are useful guides but are by no means an accurate reflection of likely specific fertility outcomes.

As discussed in the introduction, the choice of lactation length may be dictated by a particular herd health protocol, e.g., the control of *Mycoplasma* in multiplier herds, and so changes in lactation length are not an option. In this case, an intervention strategy may be necessary. Currently, the available protocols are to use exogenous gonadotrophins to stimulate a rapid post weaning oestrus or to delay breeding to allow recovery of metabolic status and so the reproductive axis. The latter implies the use of skip-a-heat breeding or the use of pharmacological agents to predictably suppress the post-weaning oestrus.

Table 1. The effect of PG600 at weaning, following a 12 d lactation, on reproductive performance of primiparous sows (LS means ± sem) (Kirkwood *et al.*, 1998).

	Control	PG600	P≤
Number of sows	62	56	-
Number of sows bred by 7 d post-partum	36 (58.1%)	54 (96.4%)	0.0001
Number of sows bred by 7 d post-partum	53 (85.5%)	56 (100%)	0.002
Weaning-oestrus (d)	7.7 ± 0.6	4.7 ± 0.3	0.0001
Farrowing rate	81.1	78.6	0.50
Second litter size: total born	10.1 ± 0.5	9.0 ± 0.4	0.09
Second litter size: born alive	9.7 ± 0.5	8.5 ± 0.5	0.06
Pigs weaned per sow	4.76	6.82	-

As mentioned earlier, variance in weaner output is largely driven by the ability to meet breeding targets. Constraints to the achievement of breeding targets are the duration and variance in the weaning to oestrus interval. A recent study of the effect of injection of the gonadotrophin preparation PG600 into early weaned (12 d) primiparous sows suggested that treated sows had a more prompt and synchronous return to oestrus (Kirkwood *et al.*, 1998) (Table 1). There was a small depression in litter size for PG600-treated sows but pigs born per sow weaned (percent sows bred x farrowing rate x litter size) was greater for the treated group. Other work from the USA, also working with sows weaned at 12 d, confirmed the beneficial effect of PG600 on returns to oestrus but indicated no effect on subsequent litter size (Yeske, unpublished observation). Based on available data, the use of PG600 to induce the post weaning oestrus is an economically viable option for early-weaned sows.

The second option is to delay breeding, either by skip-a-heat or oestrus suppression. Based on papers of Morrow *et al.* (1989) and Clowes *et al.* (1994) and field evidence, skip-a-heat will likely yield an extra 1.5 pigs per litter. Recent approaches to management of facilities in North America have emphasized 'productive' and 'non-productive' days of a sows life. A figure of 24 pigs/sow/year equates to 0.066 pigs/sow/d or 1.4 pigs per 21 d oestrous cycle. Therefore, the incurred extra non-productive sow days and, therefore problems of pig flow, indicate that skip-a-heat is unlikely to be economically justified. The option of using the orally active progestagen, allyl trenbolone (Regumate), to effect a shorter, controlled and predictable extension of the remating interval has been reported previously (see review by Martinat-Botté *et al.*, 1985), and may be associated with increased litter sizes (Kirkwood *et al.*, 1986; Morrow *et al.*, 1989). Allyl trenbolone can be fed at 15 mg/d from weaning until 5 d before sows are required to be bred and will effectively block oestrus and result in synchrony of oestrus after cessation of feeding. Problems have been noted in the field but this may be due to poor dosing management. Sows respond well to 15 to 30 mg/d but cystic ovaries are likely if the dose falls to 10 mg/d. Although a potentially useful product, the current cost of Regumate makes it economically unattractive.

An alternative to Regumate treatment is to induce the sow to ovulate at the time of farrowing. As discussed earlier, at the time of parturition the ovary has a population of large follicles. Induced final maturation and ovulation of these follicles by an injection of 1000 IU hCG is currently being investigated by workers in North Carolina (J.H. Britt, personal communication). The resultant, artificially induced corpora lutea, undergoing "normal" regression, should result in sows returning to oestrus 21 d after hCG injection. This timing would not be altered by weaning at, e.g., 14 d of lactation. The North Carolina group are suggesting an 80% success rate in inducing ovulation. However, studies in Alberta yielded only about 50% of sows ovulating. This technology does have potential but needs more research. It is possible that the timing of hCG injection relative to the end of farrowing is critical, the injection being necessary within the first 12 h after farrowing.

A final possibility is the use of split weaning. It is known that in conventionally weaned sows, removing the heaviest 50% of the litter 2 to 3 d prior to final weaning results in augmented basal LH release, improved ovarian follicular development at weaning and a more rapid return to oestrus (Cox *et al.*, 1982; Grant, 1989). Although the authors are unaware of any controlled studies using this technology in early weaning systems, its potential should not be ignored. Most large early weaning systems wean twice weekly and split weaning would lend itself to this system.

Conclusion

Whilst segregated early weaning undoubtedly optimizes the usage of facilities and possesses the potential to markedly reduce endemic and transient disease cycles, overall sow productivity may be compromised following its adoption without due consideration for genotype and current management approaches. The precise mechanisms underlying reductions in subsequent fertility following shortened lactation lengths remain poorly understood. Increasingly, however, the role of nutrition in manipulating the metabolic status of the sow (particularly the primiparous animal) from late gestation through lactation, appears to be important. Catabolism has consequences for the entire

hypothalamic-pituitary-ovarian axis and this review has indicated the potential impact of excessive protein mobilization on gross measures of fertility, e.g., weaning to oestrus interval. The more subtle but equally important ramifications for follicular/oocyte maturation and resultant ovulation rate and embryo survival cannot be ignored. In summary, therefore, adoption of SEW strategies must depend on the genotype and potentially parity distribution of the herd in question. The precise lactation lengths adopted will depend on a variety of factors and artificial manipulation of ovarian stimulation *post-partum* and post weaning (by either management practices or pharmacological intervention) may offer some means of overcoming difficulties. Ultimately, however, management recommendations must be determined by the herd in question considering genotype and economic considerations of the operation concerned.

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THE EFFECT OF INCREASED FEED INTAKE DURING GESTATION ON FERTILITY, LITTER SIZE AND LACTATION PERFORMANCE OF PRIMIPAROUS SOWS

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Commercial gestation feeding levels are generally low due in part to the belief that high intakes adversely affect litter size (Hughes, 1993). Restricting intakes in gestation may however compromise maternal gain in genetically lean primiparous sows (Everts and Decker, 1995) affecting breeding longevity. This experiment investigated the effect of feed intake on the reproductive and lactation performance of primiparous sows.

Five hundred and thirteen Large White x Landrace F1 gilts were mated at 32 weeks of age and weighed 123 ± 0.30 kg (mean ± SE) with a fat thickness at P2 of 15 ± 0.14 mm at the start of the experiment. Sows were allocated to one of three treatments based on a low (L) or high (H) level of feeding (2 or 3 kg/d) during three treatment feeding periods of gestation; 24 h post-mating-35 d; 35-85 d; 85 d-farrowing. All sows were fed a common diet during gestation (13.5 MJ DE/kg; 0.85% lysine) and were offered a lactation diet (14 MJ DE/kg; 1.2% lysine) *ad libitum* from farrowing for 27 ± 0.10 d. Litters were equalised to 9.6 ± 0.05 piglets within 2 d of birth.

There was no significant effect of feeding level during gestation on the proportion of sows which returned to oestrus after mating. The majority of these sows (75%) displayed oestrus 18-23 d post mating. There was no significant treatment effect on sows removed for abortion, death or not maintaining pregnancy, however there was a significantly higher proportion of sows ($P<0.05$) removed due to leg weakness from the H-H-H group (2.4%) compared to the L-L-L group (0%) and the H-L-H group (0.8%). Post-partum sow weight and fat thickness at P2 increased ($P<0.01$) with feed level during gestation: 165 kg, 17 mm; 181 kg, 19 mm; 183 kg, 20 mm for L-L-L, H-L-H and H-H-H sows respectively.

Table 1. The effect of feeding level during gestation on farrowing rate, litter size, birth weight and piglet performance during lactation.

Gestation feeding level (No. of sows)	Sows farrowed (%)	Total born	Average birth-weight (kg)	Litter weight at 2 d (kg)	Litter gain 2 d-weaning (kg/day)	Average weaning weight (kg)
L-L-L (165)	89.1	9.7	1.3[a]	14.9[a]	1.86	6.6[a]
H-L-H (155)	88.0	9.9	1.4[b]	15.6[b]	1.91	7.0[b]
H-H-H (191)	86.1	10.2	1.4[b]	15.5[b]	1.89	6.8[b]
SED	3.21	0.30	0.03	0.37	0.06	0.12

[a,b,c]Values within a column with different superscripts differ significantly ($P<0.05$).

Feeding level during gestation had no adverse effect on farrowing rate or litter size (Table 1). The higher nutrient intake significantly increased birth weight and litter weight at weaning. Litter gain from day 2 of lactation to weaning was similar among all treatments and was not affected by gestation intake. These results suggest that low feeding levels during gestation may restrict the supply of nutrients to the developing foetus in primiparous sows, reducing piglet birth weight and weaning weight.

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INCREASED SOW WEANING WEIGHT IMPROVES FERTILITY BUT NOT SUBSEQUENT LITTER SIZE OF FIRST-LITTER SOWS

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Reproductive performance of first-litter sows is affected by feed intake and mobilisation of body reserves during lactation, and the amount of reserves at weaning for subsequent reproductive puposes (Whittemore, 1996). However, there is conjecture as to the relative importance of each factor separately, or in combination, on subsequent fertility and litter size. This experiment tests the hypothesis that greater body reserves at weaning increases subsequent fertility and litter size in first-litter sows.

Four hundred and thirteen first-litter sows fed during their first gestation either 2 kg/d (L) or 3 kg/d (H) from mating to day 35; day 35 to 85; or day 85 to farrowing (Smits *et al.*, 1997) were weaned after 27 d of lactation. Sows were moved to the boar shed and offered a common diet (13 MJ DE/kg; 0.6% total lysine) *ad libitum* until mating. During the subsequent gestation, all sows were fed 2.4 kg/d.

Table 1. The effect of lactation intake, loss of body reserves during lactation and body reserves at weaning on subsequent fertility and litter size.

Gestation feeding level (No. of sows)	Lactation intake (kg/d)	Lactation loss Weight (kg)	Lactation loss P2 (mm)	At weaning Weight (kg)	At weaning P2 (mm)	Mated by 5 d (%)	Subsequent fertility Farrowed (%)	Subsequent fertility Total born
L-L-L (134)	5.1[a]	4.7[a]	1.3[a]	160[a]	16[a]	69[a]	89[ab]	9.8
H-L-H (144)	4.7[b]	13.3[b]	2.5[b]	168[b]	17[b]	77[ab]	83[a]	9.9
H-H-H (135)	4.6[b]	12.1[b]	2.9[b]	171[b]	17[b]	84[b]	95[b]	10.4
SED	0.10	1.38	0.43	1.61	0.51	5.34	3.98	0.36

[a,b,c]Values within a column with different superscripts differ significantly ($P<0.05$).

Increasing the level of feeding during gestation increased maternal body reserves at weaning even though H-H-H and H-L-H sows lost more live weight and backfat during lactation (Table 1). These heavier sows exhibited oestrus sooner than lighter sows whilst eating less during lactation and mobilising more body reserves. However there were no effects of body reserves at weaning, intake during lactation or live weight and fat loss during lactation on subsequent litter size. Although the main effect of lactation intake on fertility was not significant there was a significant positive interaction between feed intake in gestation and lactation on the proportion of sows mated within 5 d and farrowing rate ($P<0.05$). The H-L-H sows which consumed less than 4.3 kg/d during lactation had a lower farrowing rate (74%) than H-H-H sows (95%) ($P<0.01$). Both groups of sows had similar live weight and backfat P2 after farrowing than at weaning. Improving body reserves at first weaning by feeding more during gestation and offering diets *ad libitum* during lactation improved subsequent fertility, but did not affect subsequent litter size. The results also indicate that increasing maternal reserves prior to lactation may overcome poor fertility associated with low intakes during lactation.

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PIGS WEANED AT 14 D REACH SLAUGHTER WEIGHT AT THE SAME TIME AS PIGS WEANED AT 28 D BUT ARE FATTER

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Pigs are commonly weaned at 23-27 days of age. However, sow milk production peaks at 10-14 days of lactation after which it is only sufficient for pigs to attain about 50% of their growth potential (Dunshea *et al.*, 1995). By weaning earlier and providing pigs with a suitable diet, it may be possible to capitalize on their potential for rapid growth. The present study was designed to determine the interrelationships between sex, weaning age and weaning weight on subsequent growth performance.

Forty eight Large White x Landrace pigs were used in a factorial experiment with the respective factors being; age at weaning (14 or 28 d), weight at weaning (heavy (H) or light (L)) and sex (male or female). Pigs were weaned into individual pens and given a diet containing 15.5 MJ DE/kg and 0.95 g available lysine/MJ DE *ad libitum*. From 3 weeks post weaning, pigs were penned in groups and fed commercial rations.

Table 1. Effect of sex (S) and age (A) and weight (W) at weaning on pig performance.

	Male				Female					
	28 d		14 d		28 d		14 d			
	H[1]	L[1]	H	L	H	L	H	L	sed	Significance[2]
Live weight (kg)										
Birth	1.9	1.4	1.8	1.5	1.9	1.3	1.9	1.4	0.15	W***
2 weeks	5.4	3.3	5.4	3.3	4.8	3.3	5.3	3.4	0.29	W***
4 weeks	9.9	5.9	8.2	4.4	8.6	5.7	8.1	5.7	0.47	A**,W***,SxW**SxA**
7 weeks	19.7	13.4	16.2	12.7	18.9	14.3	16.9	14.0	1.0	A**,W***, SxA**
15 weeks	62.2	52.7	60.5	52.3	58.5	53.3	58.8	53.4	2.5	W***
19 weeks	85.3	71.7	84.8	71.5	79.2	72.1	79.7	71.7	2.8	W***, SxW*
23 weeks	109.6	94.4	112.9	91.5	102.9	95.2	104.4	93.2	5.3	W***
P2 backfat (mm)										
23 weeks	10.6	11.2	15.0	12.8	10.7	11.1	12.8	11.6	1.65	W**

[1]Weight at weaning, H=Heavy; L=Light. [2] *P<0.05; **P<0.01; ***P<0.001.

At 4 and 7 weeks, pigs weaned at 14 d were lighter than those weaned at 28 d. By 15 weeks, these differences were no longer apparent. Pigs which were heavier at weaning were also heavier at every age, but by 19 weeks, there was a significant SxW interaction which tended to be still apparent at 23 weeks (P=0.11). Thus, at 23 weeks H boars were heavier than H gilts (110.4 vs 103.8 kg, P=0.027) whereas this was not the case for L boars and gilts (94.3 vs 94.0 kg, P=0.96). It is hypothesised that the greater number of muscle fibres present in H pigs at birth (Dyer *et al.*, 1993) may allow androgenic benefits to be expressed later in boars. While there were no effects of sex or weight at weaning on fatness, pigs weaned at 14 d had a greater P2 backfat depth at 23 weeks than pigs weaned at 28 d (13.1 vs 10.9 mm, P=0.009). While not measured, it is suggested that increased feed intake during the finisher phase may have contributed to the increase in fatness. Although pigs can be weaned at 14 d and grow well, there is a potential for increased fatness.

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EMERGENCE OF "NEW" DISEASES RELATED TO SEGREGATED EARLY WEANING AND ALL-IN ALL-OUT PRODUCTION SYSTEMS

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Prevention of disease has been a consideration in profitable pork production for many years. In recent years, the economic importance of health status has been highlighted because of the positive production effects associated with all-in all-out (AIAO) and segregated early weaning (SEW) production systems. When applied correctly, these health-based production systems are effective in controlling or eradicating a number of important diseases, particularly those caused by *Actinobacillus pleuropneumoniae* (App) and *Mycoplasma hyopneumoniae.*

On the other hand, in association with the use of management systems that emphasize health status, a number of unrecognized or relatively minor disease causing organisms have emerged. The most common and important of these organisms include *Actinobacillus suis, Streptococcus suis, Haemophilus parasuis,* and porcine colonic spirochaetosis (*Serpulina pilosicoli*). Following the adoption of SEW and AIAO production systems by Australian producers, increases in these diseases can be expected.

The objective of this study was to provide a factual basis to explain the occurrence of previously unrecognized diseases (particularly those caused by *A. suis* and *H. parasuis*) in high health status herds. Six herds infected with App, *A. suis* and *H. parasuis* were examined in detail. The serologic, clinical disease, and organism isolation rates were followed prior to, and after, shifting from continuous flow production (>21 d old weaning) to SEW production (<16 d old weaning).

Prior to adoption of SEW production methods post-weaning mortality and treatment rates averaged 13 and 27% respectively, due almost exclusively to App. On average, 15% of the pigs were serologically positive for App by 2 months of age and 78% were positive by 6 months of age. In contrast, approximately 80% of the pigs were serologically positive for *A. suis* at both 2 and 6 months of age. *Actinobacillus suis* and *H. parasuis* were isolated from approximately 65% of the healthy pigs examined at 2 months of age, yet no clinical disease related to these organisms was recognized.

Following adoption of SEW production methods post-weaning mortality and treatment rate declined significantly ($P<0.05$) to an average of 3 and 7%, respectively. In contrast to the previous experience, all recognized disease was due to either *A. suis* and *H. parasuis.* Interestingly these organisms could now be isolated from only 32% of the healthy 2 month old pigs examined ($P<0.05$). There was also a significant shift in *A. suis* serologic status in that only 37% of the pigs were serologically positive at 10 weeks of age. On the other hand, the serological status to *A. suis* at 6 months had not changed (average of 78% positive). No App was isolated and all pigs were serologically negative for App at 2 and 6 months of age.

In conclusion, the impact of those diseases that emerge in association with AIAO or SEW systems on the economic performance of a herd can be significant. Nevertheless, in all cases where comparative data is available, these losses are more than compensated for by gains in production associated with improved utilization of facilities and the control of other more economically significant diseases (e.g., App). The current data indicates that shifts in the immunological status of the herd associated with SEW production methods underlie the emergence of diseases caused by *A. suis* and *H. parasuis.* Control of these diseases by changes in management practices while at the same time retaining the benefits associated with SEW and AIAO does not, at this time, seem practical. Fortunately, the sporadic nature of the disease outbreaks caused by these organisms and the ability to reliably control clinical disease through vaccination helps to limit their economic impact.

STIMULATION OF CONTINUED OESTROUS CYCLICITY IN THE POST-PUBERTAL GILT

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The stimulation of the attainment of early puberty in gilts using daily boar contact has become standard practice in commercial pig production. However, many commercial pig producers encounter problems with apparently post-pubertal gilts failing to return to oestrus and mate. This experiment was conducted to determine whether continuous boar contact is necessary for the maintenance of regular oestrous cyclic activity in the post-pubertal gilt.

Sixty four Large White × Landrace gilts were used in this study. The study was conducted in two replicates, 16 gilts being allocated to each of two treatment groups in each replicate. The treatments involved were either continued boar contact (BC) or no boar contact (NC) on attaining puberty. Four mature boars were used in each replicate on a rotational basis, with one boar being exposed to the BC treatment groups for 15 min daily. All gilts were checked daily for signs of behavioural oestrus and changes to the appearance of the vulva. The gilts were slaughtered after their third oestrous cycle and their reproductive tracts examined for corpora lutea and corpora albicans to confirm their reproductive status.

All gilts, irrespective of treatment, appeared to demonstrate a normal pubertal oestrous cycle. While there was greater variation in oestrous cycle length within the NC group, no significant difference was detected between treatments. However, a significant difference was observed in the proportion of oestrous gilts detected between BC and NC treatment groups over the three cycles (see Table 1).

Table 1. The effects of boar contact (BC) or no boar contact (NC) on oestrous cyclicity in gilts (mean ± SEM)

	Treatment	
	BC	NC
Number of gilts	32	31
Age of gilts at puberty (d)	186.9 ± 12.18	186.4 ± 3.98
Proportion detected cycling (over 3 cycles)	0.97[a]	0.66[b]
Length of oestrous cycle (d)	20.5 ± 0.36	20.0 ± 2.34

[a,b]Within rows means with different superscripts are significantly different ($P<0.05$).

The results appear to support earlier, unpublished results from this laboratory which suggested that boar contact was required in the post-pubertal period to maintain normal oestrous cyclicity in gilts. The observed benefit of improved oestrus detection through the use of boar contact could be due to either impaired ovarian activity in physiologically immature gilts due to the absence of boars, or the positive effect of boar contact on the expression of oestrus. Hemsworth and Barnett (1990) have clearly demonstrated that oestrus detection rates fall dramatically if boars are used incorrectly or not at all. Thus further work is required to determine the actual reason for the observed benefit of improved oestrus detection through the use of boars.

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DOES DIETARY FAT INFLUENCE OVARIAN DEVELOPMENT?

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Diets high in fat have been shown to increase cholesterol synthesis and serum insulin concentrations in cows. These increases enhance ovarian function by increasing the number of follicles (Ryan *et al.*, 1992). The results suggest that high fat diets may improve fertility. The effect of total fat intake and the type of fat on reproductive efficiency has been investigated in pregnant and lactating sows and does not appear to improve fertility (e.g., Weeden *et al.*, 1994). The objective of the present study was to investigate ovarian activity in gilts fed diets containing different levels and types of fats (polyunsaturated or saturated).

Thirty-six Large White gilts were used in this study. Following selection at 56 days of age they were individually fed and housed. Littermates were randomly selected within a litter of 10 piglets, of which at least five were females. Each litter was sired by a different boar. Littermates were then randomly assigned to one of the following dietary treatments: LL, gilts were offered *ad libitum* a low fat/low cholesterol diet (37 g/100 g and 7.8 mg/100 g respectively); HH, gilts were offered *ad libitum* a high fat/high cholesterol diet (61 g/100g and 44 mg/100g); LM, gilts were offered a low fat/medium cholesterol diet (37 g/100g and 28 mg/100g), at 90 % of the *ad libitum* intakes of LL and HH gilts for the first 21 d, and thereafter *ad libitum*. Blood samples (10 ml, via jugular puncture) were collected every 3 d until the gilts cycled. Puberty was induced by exposure to a mature boar for 10 min each day from 160 days of age. The gilts were slaughtered 7 d after attainment of puberty.

There were no dietary effects on live weight (119.7 ± 1.70 kg; mean ± SE) or backfat (20.8 ± 1.25 mm) at slaughter. The HH gilts consumed 81% more cholesterol than LL gilts, and 36% more than LM gilts. Mean daily feed intakes (and DE intake) for the LL, HH and LM gilts were 2.34 kg (35.6 MJ), 2.03 kg (31.7 MJ) and 1.92 kg (29.4 MJ) respectively. Reproductive parameters were not affected by treatment (Table 1), and all gilts attained puberty.

Table 1. Mean reproductive tract weight, ovarian weight, number of follicles, follicle size, number of CL's and age at puberty (d).

	Dietary Treatment			Significance	
	LL	HH	LM	SEM	Diet
Reproductive tract weight (g)	299.7	301.4	298.5	20.1	NS
Ovarian weight (g)	8.8	9.5	8.9	0.5	NS
No. follicles	16.7	18.7	18.6	0.82	NS
Follicles size (mm)	4.5	3.9	4.5	0.25	NS
No. CL's	6.9	7.6	8.0	0.73	NS
Age at puberty (d)	173.8	173.4	174.7	3.17	NS

These data suggest that neither the level of fat intake nor the type of fats used in the study had significant effects on the measured reproductive parameters. Total individual energy intakes were high enough to ensure that there was no negative dietary response, and therefore no negative influence on reproductive development. The long term effects of different dietary levels of fats and oils on sow health needs to be studied.

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SOW PREFERENCE FOR FARROWING SITE ORIENTATION

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To achieve high piglet survival rates in loose farrowing systems, it is important that sows farrow in an appropriate place, e.g., a specially provided 'nest'. Specific features to promote neonate survival can then be incorporated in the design of the 'nest', such as a small 'nest' area, protected perimeter zones, heater and bedding. A recent development in loose housing for individual, farrowing/lactating sows is the Werribee Farrowing Pen (WFP). The WFP consists of 2 compartments, a 'nest' for farrowing and a 'non-nest' area for feeding and dunging. One important consideration by industry affecting the adoption of this pen as a practical alternative to farrowing crates is that sows choose to farrow in the 'nest' rather than the 'non-nest' area. The aims of this experiment were to determine the effects of (1) the orientation of the nest entrance relative to, and (2) the distance of the nest from, the human activity area, on the sow's choice of farrowing site.

The experiment utilized four adjacent test pens (8.8 m deep × 2.7 m wide) with non-experimental pens at either end and a row of eight farrowing crates opposite the front of the pens. The stockpeople fed and checked the sows from the front of the pens and human activity at the rear was minimized. Each test pen contained two identical 'nests' (2.3 m deep × 1.75 m wide), based on the design of the WFP 'nest' area, and included a heater in the creep area and rice hull bedding on the solid concrete floor. The floor outside the 'nests' consisted of concrete slats. One 'nest' was at the front (near nest) and the other at the rear (far nest) of each test pen. Across the four pens, the entrance to each pair of 'nests' was oriented either opening towards the front or rear of the pen. Particularly while the sow was in a lying posture, her view of the shed 'activity area' was reduced in nests with entrances oriented to the rear of the pen. Eight replicates of 4 primiparous Large White × Landrace sows were observed in the experiment.

Of the 32 litters, 24 were born exclusively in one of the two available 'nests', five were born in a mixture of locations and three were born entirely outside the 'nests' (Table 1). Of the sows in the treatment pens where both nest entrances were oriented in the same direction, i.e., both to the front vs both to the rear, there was a difference in the preference of sows to farrow (exclusively) in one nest (8/8 vs 4/8 respectively; Fisher Exact Probability Test, P=0.038). There was no apparent preference for distance of the farrowing 'nest' relative to the stockperson activity area, i.e., near vs far position of the nest in the pen, by the 24 sows that farrowed exclusively in one 'nest' (11 vs 13 litters, respectively). There was a tendency for the litters born in far nests to be born in nests oriented to the front rather than to the rear (9/13 vs 4/13, respectively; Fisher Exact Probability Test, P=0.133).

Table 1. Number of litters born in the different locations (in ± outside nests) in test pens with different combinations of the orientation of the nest entrance and position of the nest within the pen. There were eight sows per treatment.

Nest entrance orientation		Location where piglets born			
Near nest	Far nest	Near nest	Far nest	Mixed	Outside nest
Front	Front	3	5	0	0
Front	Rear	2	3	3	0
Rear	Front	3	4	1	0
Rear	Rear	3	1	1	3

The results suggest that some sows had a preference for a 'nest' orientation that provided a view of the activity area in the shed. Thus, in the design of loose farrowing accommodation, the orientation of 'nests' relative to the shed activity area may be relevant in influencing the sow's choice of farrowing site.

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DOES PARTURITION STIMULATE METABOLIC ADAPTATION TO EXTRA-UTERINE LIFE?

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Birth is a stressful event and accounts for a large proportion of the perinatal losses in swine herds (Randall, 1992). However, birth may also stimulate adaptation to life *ex utero*. The present study examines if the process of birth stimulates some physiological functions in neonatal pigs, and whether the magnitude of responses are related to fetal age at birth. The concentrations of serum proteins (e.g., haemoglobin, α-fetoprotein, albumin, α_2-macroglobulin s), liver glycogen and liver glucose-6-phosphatase (G-6P) activity were used to assess the adaptation to extrauterine life. The above all show distinct maturational changes around the time of birth (Weström *et al.*, 1982; Randall, 1992). Pigs were delivered either prematurely or at normal term, and either by elective caesarean section (ECS) or induced vaginal birth (IVB).

Six pregnant sows (Danish Landrace × Large White, 106 or 113 d gestation) were anaesthetized (pentobarbitone, 10 mg/kg live weight; i.v.) and fetuses in one uterine horn were removed. After the operation, parturition was induced (200 μg cloprostenol, i.m.) and the remaining fetuses were delivered vaginally 30-45 h later. Samples of cord blood and liver tissue were collected at birth. The results of the biochemical analyses are shown in Table 1. Within each fetal age at delivery (premature or mature) differences between means were tested by Student's *t* test.

Table 1. Blood pH and haemoglobin, cortisol and proteins in plasma, and liver glycogen and glucose-6-phosphatase (G-6P) activity in premature or term pigs (mean ± SEM, n=9-25).

	Premature newborn pigs		Mature newborn pigs	
	(ECS)	(IVB)	(ECS)	(IVB)
Blood pH	7.36 ± 0.02	7.18 ± 0.02*	7.39 ± 0.01	7.26 ± 0.01*
Haemoglobin (g%)	8.5 ± 0.4	11.0 ± 0.5*	10.8 ± 0.2	13.2 ± 0.4*
Cortisol (ng/mL)	55 ± 4	115 ± 7*	80 ± 8	161 ± 10*
α-fetoprotein (mg/mL)	1.09 ± 0.03	1.20 ± 0.03*	1.03 ± 0.03	0.92 ± 0.03*
Albumin (mg/mL)	1.01 ± 0.12	1.38 ± 0.10*	1.55 ± 0.16	1.76 ± 0.23
α_2-macroglobulin s (%)	5.8 ± 0.6	8.0 ± 0.7*	8.5 ± 0.7	10.2 ± 1.6*
Liver glycogen (mg/g)	58 ± 4	57 ± 9	93 ± 3	87 ± 12
Liver G-6P (U/g)	45 ± 8	141 ± 21*	66 ± 10	96 ± 6*

*Means for IVB pigs differ significantly ($P<0.05$) from corresponding means for ECS pigs.

The stress of vaginal birth is indicated by the increased blood acidity and plasma cortisol concentrations in IVB pigs compared with corresponding ECS pigs. Across delivery methods, haemoglobin, cortisol, albumin, α_2-macroglobulin s, glycogen and G-6P concentrations were higher for mature pigs than for premature pigs ($P<0.05$; pooled values from the ECS and IVB groups). Within each fetal age at delivery (premature or mature), these parameters (except liver glycogen) were higher in IVB pigs than in ECS pigs. Finally, α-fetoprotein levels were increased in premature IVB pigs, but lowered in mature IVB pigs. Vaginal birth is associated with changes in serum protein concentrations and liver G-6P activity which reflect the normal developmental changes in these parameters around birth (Weström *et al.*, 1982; Randall, 1992; Svendsen, 1992). This effect of vaginal birth may be related to a parturition-associated rise in fetal cortisol production.

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EFFECTS OF DIETARY SUPPLEMENTATION OF INSULIN ON INTESTINAL ENZYME ACTIVITIES IN NEONATAL PIGS

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A high level of insulin has been detected in the milk of several species including the pig (Westrom *et al.*, 1987; Wang and Xu, 1996). It has been speculated that milk-borne insulin may play a role in regulating postnatal development in neonatal sucking animals (Xu, 1996). To test this hypothesis, the present study examined the effects of dietary supplementation with insulin on small intestinal tissue growth and digestive enzyme activities in neonatal pigs.

A total of 15 newborn unsuckled piglets obtained from five litters of the Chinese Meishan breed were bottle-fed for 3 d at 3-hourly intervals with one of three diets at the rate of 30 ml/kg body weight. One piglet from each litter received artificial milk, one received artificial milk supplemented with 60 mIU/ml insulin (Novo Industri, Denmark), and one received pre-hydrolyzed milk. The artificial milk was prepared using whole milk powder (Dutchlady, The Netherlands) and contained 3% protein, 3.5% fat and 4.2% lactose. The pre-hydrolyzed milk was prepared by trypsin and chymotrypsin digestion as described by Wang and Xu, (1996). At the end of the experiment all animals were euthanased, the entire small intestine was removed and the mucosa was collected for biochemical analyses following procedures described by Wang and Xu (1996).

Table 1. Effects of dietary treatment on small intestinal tissue weight, total mucosal protein and total DNA content and total mucosal hydrolytic enzyme activities in newborn pigs (mean ± SEM).

	Milk (n=5)	Milk + Insulin (n=5)	Pre-hydrolyzed milk (n=5)
Birth weight (g)	686 ± 15	732 ± 36	728 ± 31
Final body weight (g)	640 ± 32	666 ± 44	660 ± 29
Small intestinal weight (g)	14.6 ± 1.1	16.3 ± 0.9	15.2 ± 1.4
Mucosal protein (mg)	330 ± 43	491 ± 27*	394 ± 53
Mucosal DNA (mg)	16.3 ± 2.3	19.2 ± 0.5	17.1 ± 1.9
Lactase (mM/min)	6.2 ± 1.5	12.2 ± 1.7*	17.0 ± 3.7**
Maltase (mM/min)	15.3 ± 2.6	29.2 ± 2.9**	17.3 ± 2.7
Alkaline phosphatase (mM/min)	24.4 ± 5.3	82.7 ± 9.2**	52.3 ± 14.9*
Aminopeptidase (mM/min)	13.3 ± 1.6	19.0 ± 1.0**	14.3 ± 1.2

Different from the Milk group: *P<0.05; **P<0.01.

Dietary supplementation of insulin significantly increased small intestinal mucosal protein content, and mucosal hydrolytic enzyme activities (Table 1). Although the dosage of insulin used in the present study was higher than that found in porcine colostrum (60 mIU/ml vs 400 μIU/ml), the results do suggest that orally-administered insulin can stimulate intestinal enzyme maturation. Feeding pre-hydrolyzed milk had no effect on the mass of intestinal tissue or the amounts of intestinal mucosal protein and DNA, but it significantly increased intestinal mucosal lactase and alkaline phosphatase activities. How the feeding of pre-hydrolyzed milk affected intestinal enzyme activities is not clear, but it may be through changes in the concentrations of circulating gastrointestinal regulatory peptides. Further studies have been planned to address this question.

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WEIGHT AND AGE AT WEANING INFLUENCE PANCREATIC SIZE AND ENZYMATIC CAPACITY

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Many pig producers are moving towards early weaning in an effort to minimise disease transfer from sow to piglet. However, it is possible that the digestive function of early-weaned pigs may be immature and incapable of digesting many feedstuffs. The present study was designed to examine some of the factors that might influence pancreatic enzymic function in the weaned pig.

Forty-eight pigs (Large White x Landrace) were allocated to a 2 x 2 x 2 x 3 factorial experiment with the respective factors being; age at weaning (14 or 28 d), weight at weaning (heavy (H) or light (L)), sex (boar or gilt) and days post-weaning (1, 7 or 14 d). Sixteen pigs (two per treatment group) were weaned and fasted for 24 h prior to euthanasia. The remaining pigs were weaned into individual pens and offered a diet containing 15.5 MJ DE and 16.1 g lysine/kg *ad libitum*. Sixteen pigs were euthanased on day 7 and the remaining 16 on day 14 post weaning after a 24 h fast. Pancreatic glands were weighed and the activities of trypsin, amylase, colipase and lipase in pancreatic tissue were determined.

Table 1. Effect of sex (S), age (A) and weight (W) at weaning and days post weaning (D) on pancreas weight and units of pancreatic enzyme activity (U).

	Sex		Age		Weight			Day				
	Boar	Gilt	28d	14d	H	L	sed[1]	1	7	14	sed[2]	Significance
Pancreas (g)	10.9	12.6	14.8	8.7	13.7	9.8	1.6	8.0	12.0	15.4	1.9	A***,W**,D***
U per g of pancreas												
Trypsin[3]	11.5	15.3	20.2	8.6	15.7	11.1	0.06	9.0	14.4	17.9	0.08	A***,W*,D**
Amylase[4]	8.18	9.86	10.3	7.77	9.74	8.30	1.42	7.42	9.83	9.82	1.71	
Colipase[3,4]	11.6	15.8	14.2	12.3	15.5	11.8	0.08	22.1	10.2	11.0	0.10	D**
Lipase[3,4]	3.61	4.70	4.73	3.58	5.13	3.31	0.09	6.10	3.40	3.38	0.11	
U per pancreas												
Trypsin[3]	116	167	278	70.0	198	97.9	0.08	68.1	158	248	0.10	A***,W***,D***
Amylase[3,4]	71.3	91.4	118	55.2	110	58.9	0.09	47.3	90.8	122	0.11	A**,D**,W**
Colipase[3,4]	117	173	195	104	195	104	0.09	167	114	152	0.11	A**,W**
Lipase[3,4]	36.6	51.4	65.0	29.0	64.6	29.2	0.10	46.0	38.0	46.8	0.12	A**,W**

[1]sed for main effects of sex, age and weight. [2]sed for main effect of time. [3]Data log-transformed for analyses, presented as geometric means and log-transformed sed. [4]x10[3].

Trypsin activity per g of pancreas was greater in pigs that were larger and older at weaning and tended to be greater in gilts than boars (P=0.055). Total trypsin activity was also greater in pigs that were older (four-fold) and larger (two-fold) at weaning and increased more than three-fold between weaning and 2 weeks post weaning. Amylase activity per g of pancreas was relatively unaffected by sex or age and weight at weaning, and so differences in total activity mainly reflect differences in pancreas weight. Colipase activity per g of pancreas was highest at weaning, possibly due to the high fat content of sows milk, and tended to be higher (P=0.097) in gilts than in boars. Likewise, lipase activity per g of pancreas tended to be highest at weaning (P=0.052). In conclusion, total pancreatic activity of most enzymes was greater in heavy and older pigs and tended to be greater in gilts than in boars, and may explain the differences in post-weaning performance observed in these classes of pigs.

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SEX AND AGE AT WEANING AFFECT SMALL INTESTINAL HISTOLOGY AND ENZYMATIC CAPACITY

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While pigs are commonly weaned at 23-27 days of age, sow milk production peaks at 10-14 days of lactation after which it limits growth. By weaning earlier and providing pigs with a suitable diet, it may be possible to increase growth of the young pig. However, it is possible that the digestive function of early-weaned pigs may be immature and incapable of digesting many feedstuffs. The present study was designed to examine some of the factors that might influence small intestinal function in the weaned pig.

Forty-eight pigs were used in a 2 x 2 x 2 x 3 factorial experiment with the respective factors being; age at weaning (14 or 28 d), weight at weaning (heavy or light), sex (boar or gilt) and day post weaning (1, 7 or 14 d). Sixteen pigs were weaned and fasted for 24 h before being euthanased. The remaining pigs were weaned into individual pens and given a diet containing 15.5 MJ DE/kg and 0.95 g available lysine/MJ DE *ad libitum*. Sixteen pigs were euthanased on day 7 and the remaining 16 on day 14 post weaning after a 24 h fast. Samples of the small intestine were taken at 25, 50 and 75% and 10, 25, 50, 75 and 90% of the length from the duodenum to the ileum for histology and enzymology, respectively.

Table 1. Effect of sex (S) and age (A) and weight (W) at weaning and day after weaning (D) on small intestinal (SI) weight, histology and enzymatic activity.

	Sex		Age		Weight			Day				
SI	Boar	Gilt	28d	14d	H	L	sed[1]	1	7	14	sed[2]	Significance
Weight (g)	277	277	337	218	316	239	21	188	256	388	26	A***,D***,W***
Villous height (μm)	369	406	392	382	386	389	14	480	329	356	17	S*,D***
Crypt depth (μm)	177	174	177	174	175	176	4.8	143	182	201	5.9	D***
Maltase[3]	0.85	1.09	1.28	0.66	1.04	0.90	0.09	0.44	0.97	1.50	0.09	S**A***, D***
GLAase[3,4]	1.59	1.62	2.19	1.03	1.69	1.53	0.18	0.71	1.75	2.36	0.18	A***,D***

[1]sed for main effects of sex, age and weight. [2]sed for main effect of time. [3]μM glucose/min per g mucosa. [4]Glucoamylase.

Mean villous height was greater in gilts than boars but was not different between pigs weaned at 14 and 28 d or heavy and light pigs. Villous height was greatest immediately after weaning, then decreased and remained low whereas crypt depth increased with time after weaning. Mean maltase activity was greater in gilts than in boars and in pigs weaned at 28 d compared to 14 d and furthermore tended to be greater in the heavier pigs (P=0.081). Mean glucoamylase activity was greater in pigs weaned at 28 d rather than 14 d. It appears that the carbohydrate digestive capacity is lower in early-weaned pigs, particularly if they are small for age. On the other hand, absorptive capacity as indicated by villous height may be similar between age and weight groups. Gilts may be better prepared to handle the transition at weaning as indicated by their greater villous height and maltase activity. Split sex weaning practices may allow for separate diets matched to digestive function to be used for newly weaned boars and gilts.

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SUPPLEMENTAL MILK AROUND WEANING CAN INCREASE LIVE WEIGHT AT 120 DAYS OF AGE

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Sow milk yield peaks at 10-14 days of lactation after which it may limit piglet growth. By providing additional milk to sucking pigs it is possible to capitalize on their potential for rapid growth. It may also be possible to alleviate the post-weaning growth check by providing extra milk around the time of weaning. This study was designed to determine the effects of supplemental milk before and after weaning on the growth of pigs to 120 days of age.

Twelve Large White x Landrace sows nursing litters of six boars and six gilts were used in this study. Six litters were suckled the sow only, whereas the other six litters received supplemental skim milk (20% DM) *ad libitum* from 10-20 d. At 20 d the four heaviest pigs of each sex were allocated to two pairs and were weaned. Each pair was offered either pelleted or pelleted plus liquid feed. For the first 2 d post-weaning each pair of liquid-supplemented pigs received 2 l of skim milk (25% DM). At 23 d pelleted feed was added to the milk. Subsequently the volume of liquid was reduced and pelleted feed increased until at 28 d pigs were provided with pelleted feed only. Pigs remained in pairs until 41 d when they were housed in groups according to live weight and age. Pre-weaning data are presented elsewhere (Dunshea *et al.* 1997).

Table 1. Effects of supplemental milk during lactation (L), after weaning (W) and sex (S) on live weight (kg) of pigs to 120 days of age.

L	Suckled				Suckled + milk					
W	Solid		Solid + liquid		Solid		Solid + liquid			
S	Boar	Gilt	Boar	Gilt	Boar	Gilt	Boar	Gilt	sed	Significance[1]
Age										
20 d	6.73	6.31	6.87	6.30	7.33	7.44	7.01	7.36	0.34	L*
22 d	6.45	5.98	6.89	6.65	7.00	7.17	7.72	7.98	0.35	L**,W***
27 d	6.88	6.50	7.47	7.28	7.82	8.07	8.67	8.94	0.40	L***,W***
41 d	11.88	11.21	13.09	12.58	14.14	13.87	14.77	15.94	0.93	L**,W**
80 d	31.93	29.80	33.05	30.10	35.24	34.63	34.91	36.26	2.24	L***
120 d	61.02	57.23	64.75	59.59	65.41	62.06	67.06	63.38	3.69	L*,S*

[1] *P<0.05; **P<0.01; ***P<0.001

Pigs supplemented with milk during lactation grew more quickly than pigs suckled by the sow only and these differences were still evident at 41 d (14.7 vs 12.2 kg, P<0.001) and 120 d (64.5 vs 60.6 kg, P=0.047). Pigs weaned on to skim milk ate more (257 vs 30 g DM/d, P<0.001) and grew better (213 vs -151 g/d, P<0.001) over the first 2 d post weaning than pigs weaned on to pellets. Pigs provided with liquid feed after weaning continued to grow faster beyond 22 d resulting in the benefit of weaning on to liquid feed being maintained until 41 d (14.1 vs 12.8 kg, P<0.001), although it was diminished by 120 d (63.7 vs 61.4 kg, P=0.20). Gilts ate more (156 vs 132 g/d, P=0.024) and tended to grow faster (47 vs 15 g/d, P=0.11) than boars over the first 2 d post weaning although these differences soon disappeared. By 120 d, pigs that received extra milk before and after weaning were 10% heavier than pigs that were suckled by the sow only and weaned on to dry pellets (65.2 vs 59.1 kg, P=0.021). Much of this improvement was due to the extra nutrient intake from supplemental milk prior to and immediately after weaning. In conclusion, milk feeding for a short period around weaning can improve growth to 120 d.

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SUPPLEMENTAL MILK DURING LACTATION CAN INCREASE WEANING WEIGHT

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Sow milk production peaks during the second week of lactation after which time it may limit piglet growth (Toner *et al.* 1996). In addition the low protein to fat ratio of sow's milk also limits pig growth. By providing supplemental skim milk (which has a relatively high protein to fat ratio) to sucking pigs it may be possible to capitalize on their potential for rapid growth. The present study was designed to determine the effect of supplemental skim milk during lactation on growth of pigs until weaning.

Twelve Large White x Landrace sows nursing litters of six boars and six gilts were used in this study. Six litters were suckled by the sow only whereas the other six litters received, in addition, supplemental skim milk (20% DM) *ad libitum* from day 10 until weaning at day 20 of lactation. Milk was provided by a gravity fed trough constructed from 900 mm x 90 mm internal diameter storm water pipe, capped at each end, and with three 70 mm diameter feeder holes made into the pipe.

Table 1. Effects of supplemental skim milk during lactation (L), and sex (S) on growth of pigs to 20 days of age.

	Suckled		Suckled+milk			Significance		
	Boar	Gilt	Boar	Gilt	sed	L	S	LxS
Live weight (kg)								
Birth	1.74	1.63	1.71	1.63	0.13	0.908	0.046	0.838
10 d	3.96	3.81	3.75	3.91	0.24	0.804	0.989	0.100
20 d	6.26	6.00	6.53	6.94	0.31	0.038	0.674	0.086
Live weight gain (g/d)								
Birth - 10 d	223	218	204	223	16.8	0.855	0.217	0.073
Birth - 20 d	225	218	241	265	15.6	0.037	0.315	0.093
10 -20 d	228	217	278	304	14.7	<0.001	0.542	0.161

Boars were heavier at birth than gilts (1.72 vs 1.63 kg, P=0.046), but these differences disappeared by 10 d when the milk supplementation began. Milk supplementation increased live weight at 20 d (6.13 vs 6.74 kg, P=0.038). Supplemental milk increased growth by an average of almost 70 g/d between days 10-20 of lactation (223 vs 291 g/d, P<0.001). Milk intake increased linearly over the 10 d of supplementation from 2.37 to 7.22 kg per litter. Mean (±SE) milk intake was 388 (± 21) g/d per pig (97 g DM/d). Supplemental milk feeding did not alter either sow live weight change (-31.9 vs -30.3 kg for sows nursing litters with and without supplementation, respectively, P=0.894) or change in P2 backfat thickness (-5.3 vs -4.2 mm, P=0.279) between farrowing and day 20 of lactation. The sow performance data suggests that sow milk production was relatively unaffected when supplemental milk is provided to their litters. In conclusion, pig growth is limited by sow milk production and providing supplemental milk can readily improve the growth performance of sucking pigs.

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PORCINE SOMATOTROPIN TREATMENT OF SUCKING PIGS HAS LITTLE EFFECT ON PRE-WEANING GROWTH RATE

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The growth rate of the young pig is generally less than half its potential and may be constrained by endocrine status, specifically insulin-like growth factor-I (IGF-I) (Dunshea and Walton, 1995). Although porcine somatotropin (pST) increases plasma IGF-I and growth of grower/finisher pigs, the response to pST is less in younger pigs (Dunshea and Walton, 1995). Recently, it has been shown that artificially-reared pigs exhibit metabolic responses to pST as early as 19 days of age (Harrell *et al.*, 1994). The aim of this study was to determine whether pST could increase growth in sucking pigs.

Fourteen Large White x Landrace sows nursing litters of six (n=7) or 12 (n=7) pigs were used in this study. On day 4 of lactation the two median weight boars from each litter were randomly allocated to daily injections of either saline or pST (60 μg/kg per d, Bunge Scientific) until weaning at 31 d. Pigs were bled at 4, 13, 22 and 31 d and plasma analysed for IGF-I, IGF-II and IGF binding protein-3 (IGFBP-3). Pigs were weaned with the rest of the herd and live weight recorded at 63, 91 and 119 d.

Table 1. Effect of litter size (L) and daily pST injection (H) from 9-27 days of age on growth performance (live weight, kg).

Litter size	Six		Twelve			Significance		
Treatment	Saline	pST	Saline	pST	sed	L	H	LxH
Age								
Birth	1.52	1.59	1.55	1.52	0.13	0.894	0.630	0.306
4 d	2.19	2.27	2.12	2.14	0.22	0.612	0.391	0.607
31 d	10.16	10.36	8.23	7.96	0.79	0.014	0.866	0.309
63 d	25.07	27.00	22.14	22.64	1.87	0.037	0.264	0.504
91 d	48.14	51.93	45.43	46.14	4.03	0.277	0.166	0.334
119 d	71.57	71.29	67.29	68.40	5.61	0.512	0.825	0.703

Pre-weaning growth rates were greater for pigs from litters of six compared to pigs from litters of 12 (281 vs 213 g/d, P=0.011) with the former being 2.2 kg heavier at weaning (10.3 vs 8.13 kg, P=0.014). By 63 d, pigs from litters of six were 3.5 kg heavier (26.0 vs 22.5 kg, P=0.037). While the magnitude of this difference was maintained until 119 d, the difference failed to reach significance. Although there was no difference in daily gain between days 4 and 31 of lactation in pigs injected with either saline or pST (260 vs 258 g/d, P=0.684), pigs treated with pST grew more quickly over the last 3 days of lactation (241 vs 294 g/d, P=0.010). Daily pST injections had no effect on plasma IGF-I (182 vs 195 ng/ml, P=0.451), IGF-II (340 vs 328 ng/ml, P=0.478) or insulin-like growth factor binding protein-3 (IGFBP-3; 0.93 vs 0.94 μg/ml, P=0.848). While there was an indication that pigs previously treated with pST grew faster post weaning (623 vs 587 g/d, P=0.155) and were heavier at 91 d (49.0 vs 46.8 kg, P=0.166), there was no difference in live weight at 119 d (69.8 vs 69.4 kg, P=0.825). It is concluded that pST has little effect on growth of sucking pigs until day 28 of lactation even when nutrient intake and consequently individual growth rate is relatively high.

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THE EFFECT OF PIG WEANING WEIGHT ON POST-WEANING PERFORMANCE AND CARCASS TRAITS

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The severity of the growth check and the nutrient requirements of the weaned pig are influenced by age and weight at weaning. Mahan and Lepine (1991) found that pigs weaned at 4.5-5.5 kg live weight (LW) took 10-15 d longer to reach market weight than pigs weaned at greater than 8 kg LW. The objective of the present experiment was to quantify the effect of weaning weight on performance from weaning to slaughter and its effect on some carcass traits.

Twenty-four litters of sucking pigs (Large White x Landrace) were used in this experiment. From each litter at weaning two heavy (one male and one female) and two light pigs (one male and one female) were chosen. Pigs were selected from the heaviest 30% and lightest 30% of each litter. Sex was evenly distributed across weight categories. Pigs were weaned at 24-28 days of age. Animals were individually housed from weaning until they were transferred at approximately 40 kg LW to the finisher house where animals were group-housed. In the finisher house individual feed intake was recorded using the Hunday FIRE feeding system (Hunday Electronics, Newcastle-upon-Tyne, United Kingdom). Pigs were weighed on a regular basis from weaning to slaughter at 85-88 kg LW. Data on percentage lean, (Hennessey Grading Probe) and carcass weight were collected for each of the 96 pigs.

Table 1. Effect of weaning weight on post-weaning weight and carcass traits.

Category	Heavy	Light	SE	P value
Weight at weaning (kg)				
Mean	8.9	7.5	0.12	
Range	7.3-10.5	6.0-9.0		
54 day post-weaning weight (kg)	41.3	38.0	0.53	0.01
Slaughter weight (kg)	88.4	85.9	0.93	0.09
Slaughter age (d)	133	135	0.40	0.01
Average daily gain (g)				
Weaning to 26 d	491	460	11.10	0.09
Finisher (day 54 to slaughter)	857	838	12.30	0.34
Weaning to slaughter	750	726	9.40	0.12
Carcass weight (kg)	68.2	66.3	0.80	0.12
Carcass lean (%)	55.4	55.4	0.40	0.78
Dressing (%)	77.2	77.3	0.31	0.87

Heavier pigs at weaning (Table 1) were 2.5 kg heavier than lighter pigs at slaughter and took 2 d less to reach market weight. The results of the experiment suggest that heavier pigs at weaning perform better up to slaughter. However, lighter pigs performed equally well in the finisher stage. There was no evidence of a sex X weight interaction. Regression analysis showed that weaning weight explained 42, 8, 8, 7 and 7% of the variation in pig weight at 26 d and 54 d post weaning, slaughter weight, feed intake (weaning to slaughter) and daily live weight gain (weaning to slaughter) respectively.

In conclusion, while weaning weight explained a considerable amount of the variation in pig weight at 26 d post weaning, its effect on the variation in other traits such as slaughter weight, daily gain, daily feed intake and carcass traits was minimal.

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PREVENTION AND CONTROL OF *ACTINOBACILLUS PLEUROPNEUMONIAE* BY SEGREGATED EARLY WEANING AND ALL-IN ALL-OUT PRODUCTION

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Years of research on *Actinobacillus pleuropneumoniae* (App) have resulted in the development of numerous vaccines and the use of several antibiotics in an attempt to limit the economic impact of infection. Nevertheless, the disease continues to present a significant threat to profitable pork production throughout the world, including Australia. As a consequence, a number of novel production systems have been developed in the past few years to gain the production benefits related to preventing both clinical and subclinical infections. Prominent among these are segregated early weaning (SEW) and all-in all-out (AIAO) production. The objective of this study was to evaluate the factors that help assure success of these production systems, and to document the circumstances which increase the potential of failure as related to porcine pleuropneumonia.

Swine herds (n=12) with documented App infections (serotypes 1 and/or 5) were selected because of recent changes in production systems from one-location continuous-flow to either AIAO or SEW production methods. Health status were assessed by comparisons of treatment frequency, mortality rate, slaughter check records, and serologic status. When clinical disease or subclinical infections were found, attempts were made to determine the basis of these failures.

Regardless of production system, greater than 90% of the App herds (n=11/12) that weaned pigs at 21 d or older had evidence of infection as demonstrated by slaughter checks and/or positive serologic results. While shifting to AIAO production without changing the weaning age failed to reliably prevent App transmission as measured serologically by ELISA or Hemolysin Neutralization Test, there was a marked reduction in clinical disease and improvement of lung scores on slaughter checks ($P<0.05$) as compared to when the continuous-flow production systems were used.

The ability of SEW production schemes to reliably prevent transmission of App was dependent on weaning age, which in turn was dependent on the degree and uniformity of sow herd immunity. In three of the eight herds that changed to SEW, transmission of App could be reliably prevented if weaning occurred prior to 16 d of age. Weaning at less than 11 d prevented transmission in the remaining five herds. As with AIAO production, the occurrence of clinical disease was reduced or eliminated even in those cases where a few pigs had become infected (e.g., serologically positive).

Gilt replacement rate was determined to be a risk factor for the transmission of App as well as the occurrence of clinical disease in the SEW systems. As the percentage of serologically negative replacement gilts increases, the weaning age must be reduced in order to prevent App transmission. Exceptions to this finding were noted when replacement gilts were serologically positive at the time they were added to the breeding herd or when they were selected and raised in close proximity to the sows for several months prior to breeding. Interestingly, the influence of shed type and environmental conditions could not be demonstrated as being significant risk factors for the occurrence of clinical disease due to App.

The results presented here indicate that when applied correctly, both SEW and AIAO reliably control App. In SEW programs where pigs are weaned at the appropriate age for the immunologic status of the herd, transmission of App from the sows to piglets is reliably prevented. Some SEW and AIAO production systems do not totally prevent App transmission, yet the transmission frequency is reduced and accordingly the occurrence of clinical disease declines.

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EFFECT OF GROWTH VARIABILITY ON FINISHING PERIOD UNDER ALL-IN, ALL-OUT AND CONTINUOUS PRODUCTION

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To calculate the production costs per kg of pigmeat it is necessary to know (a) the annual cost of accommodation per pig space and (b) the time the space is occupied. Calculating the length of occupation from daily live weight gain (DLWG), and start and finish weights is straightforward when all pigs in a group grow at the same rate. However, in practice, DLWG varies within a group. The length of occupation will therefore depend on a producer's choice of marketing strategy. The extreme options are (a) to sell all pigs in a group at the same time (pure all-in, all-out (AIAO) system), or (b) to sell each pig at the optimum sale weight. Both approaches will have costs and benefits, such as price penalties, selection and marketing costs and understocked accommodation. A modified AIAO would involve selling pigs in two or three drafts per group.

Data for 1186 commercial hybrid pigs in eight groups (four groups of males, four groups of females; approximately 150 pigs per group) housed at an average weight of 22.4 kg in low-cost shelters in Western Australia (Payne, pers. comm.) were analysed to establish the distribution of DLWG. The DLWG was calculated for the first 84 d of the trial and expressed as a percentage of the group average (Table 1).

Non-optimising marketing rules (Bent and Coleman, 1997) were modelled for groups of pigs to determine the average weight and age and length of occupation of accommodation for groups with specified target weights (Tables 1 and 2).

Table 1. DLWG as % of group average

Decile	DLWG as % of average observed	model
1	84.6	84.0
2	90.8	90.0
3	93.9	93.8
4	96.5	96.4
5	98.8	98.9
6	101.1	101.1
7	103.7	103.6
8	106.4	106.2
9	110.0	110.0
10	116.3	116.0

Although average finishing time for AIAO was less than under continuous production because of higher DLWG, the length of occupancy under the modified AIAO was similar. Much lighter average weights needed to be produced under a pure AIAO system to avoid price penalties.

A full economic analysis showed that the modified AIAO had the lowest cost of production per kg in spite of the understocked accommodation shown in Table 2. Production costs per kg in continuous production were estimated to be 3-5% higher than in AIAO systems (Bent and Coleman, 1997).

Table 2. Average sale weight, finishing time and length of occupancy (target weight=95 kg)

	Sale weight (kg)	Finishing time (d)	Length of occupancy (d)
Pure all-in, all-out	88.78	84.0	84.0
Modified all-in, all-out	93.80	90.3	98.0
Continuous flow	93.10	98.0	98.0

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THE INTERRELATIONSHIP BETWEEN PARITY AND LITTER WEIGHT ON THE MILK PRODUCTION OF SOWS

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There are many factors which influence sow milk yield, e.g., piglet size, piglet sucking demand and litter size. There is also a strong positive relationship between piglet body weight, milk consumption and sow milk production (King *et al.*, 1997). However, the extent to which the relative milk production capacity of young and older sows is influenced by maternal and piglet factors remains to be established. The present experiment investigated the effects of both parity and piglet weight on the lactational performance of sows.

Twenty-seven first-litter sows (P1) and 30 third parity sows (P3) of a lean genotype suckled either heavy (H) or light (L) litters which had been standardised to 10 piglets at 2 days of age using fostered piglets from mixed parity sows. Piglets were individually weighed and transferred to sows between 6-24 h post partum. Average individual piglet weights at day 2, as allocated to H and L sows, were 1.9 ± 0.02 kg and 1.5 ± 0.04 kg respectively. During a 26 d lactation, all sows were offered a single diet (14.0 MJ DE/kg and 1.2% lysine) *ad libitum*. Piglets were weighed at day 2, 14 and 26, and milk yield was estimated using the relationship between milk consumption and growth rate published by King *et al.* (1989).

Table 1. Parity and litter weight effects on the lactational performance and estimated milk production of sows.

	Main effects				SED	Probability (P)	
	Parity		Litter weight (kg)				
	1	3	L	H		P[1]	LW[2]
Litter weight day 2 (kg)	17.2	17.6	15.1	19.7	0.850	0.675	<0.001
Litter gain 2-14 d (kg/d)	1.96	2.04	1.74	2.25	0.142	0.569	0.001
Litter gain 14-26 d (kg/d)	2.23	2.43	2.13	2.53	0.132	0.131	0.003
Litter gain 2-26 d (kg/d)	2.09	2.23	1.93	2.39	0.123	0.249	<0.001
Estimated milk yield (kg/d)	10.12	11.08	9.31	11.89	0.693	0.161	<0.001

[1]Parity. [2]Litter weight.

The P3 sows ate significantly more food (6.5 ± 0.09 kg/d; P<0.01) during lactation than the P1 sows (5.6 ± 0.08 kg/d). There was no effect of parity on piglet or litter growth rate during any period of lactation (Table 1). In contrast, piglet and litter growth rates were significantly higher for heavier litters. Sows nursing heavier litters lost more weight during lactation (14.0 ± 1.09 kg) compared to those nursing light litters (5.5 ± 1.63 kg), however there was no difference between P1 and P3 sows in loss of live weight during lactation. The results suggest that litter, and in particular birth weight, rather than age/weight of the dam, is the major factor affecting milk production and the pre-weaning performance of pigs. The development of technologies which improve birth weight have the potential to maximise milk output from the sow herd and consequently weaning weight.

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AUTOMATED OESTRUS DETECTION OF INDIVIDUALLY HOUSED SOWS BY MONITORING BODY ACTIVITY

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Financial losses within a 200-sow herd related to non-productive days may vary from 12,000 to 70,000 US$ per year (Polson *et al.*, 1990). A key issue is accurate oestrus detection, particularly the onset of oestrus so that the optimum time for insemination may be determined. Therefore, automated oestrus detection may assist the pig farmer in making day-to-day management decisions about individual sows. Increased body activity at the time of oestrus was reported by Altman (1949), who used a pedometer attached to the trunk of the sow. In the present study, a method of remote oestrus detection, based on the measurement of increased body activity with an IR-sensor, has been investigated for application with individually housed sows.

Eighty-five multiparous hybrid sows (Landrace type) were housed individually in stalls (2 x 0.65 m) on partially slatted concrete floors. Temperature conditions were around the lower critical temperature. Feed and water were restricted according to the general practice prevalent in Belgium. Lighting was provided naturally and artificially (0800 to 2000 hours). Body activity of the sows was monitored continuously with an IR-sensor, mounted about 50 cm above the shoulder area of each sow. Changes to the reflected IR-waves were translated into electrical signals and digitalized. Every 5 min a mean value was calculated and recorded for further analysis. Oestrus was also detected by the standing reflex before the boar, which was carried out each morning. Ovulation was checked by measuring the progesterone content of saliva samples using the method described by Moriyoshi *et al.* (1996). Descriptive statistics were calculated to investigate the distribution of data and potential rhythmicities using an SAS programme. Based on these results some algorithms have been tested to facilitate oestrus detection.

Sows were classified according to their mean daily activity level and the standing reflex before the boar: (1) standing, increase in activity (true positive); (2) standing, no increase in activity (false negative); (3) no standing, no increase in activity (true negative); (4) no standing, increase in activity (false positive) (Table 1). The increase in mean daily activity had to be significantly different from zero.

Table 1. Detection of oestrus in individually housed sows using an IR-sensor to detect behavioural differences compared with the standing reflex in the presence of a boar.

Classes	(1) true positive	(2) false negative	(3) true negative	(4) false positive
% of sows	52.0	13.5	28.5	6.0

Based on the detection of changes in daily mean body movement alone 80.5% of the sows were classified correctly. When the peak value per day was added to the algorithm, positive and false positive sows could be distinguished with a probability of 93%. However, for the method to be useful under commercial conditions it needs to be more precise. Further research using a larger number of sows, measurement of body temperature (Geers *et al.*, 1995), and other algorithms will concentrate on detecting the false negatives.

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OESTROUS BEHAVIOUR: CAN IT BE USED TO PREDICT OVULATION?

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The optimum time for insemination to achieve high reproductive performance is related to the timing of ovulation. The present study aimed to identify specific changes in behaviour during oestrus that may be useful in predicting ovulation.

Twenty-three 11-month old gilts (Large White x Landrace) were studied. The oestrous period can be divided into three consecutive behavioural stages; "B1, I and B2". The B stage is defined as the period in which the standing response (defined as standing immobile for 10 sec) is displayed to the back-pressure test (BPT) by the stockperson in the presence of the boar, while in the I stage the standing response is also displayed in the absence of the boar. If repeatedly tested with the BPT in the presence of boars, oestrous females may temporarily become refractory to the BPT (called the "refractory stage"). Furthermore, oestrous females may display the standing response near the boar but in the absence of the BPT (defined as the spontaneous or "S" stage). Over a 21-day period, the behavioural responses of all gilts were observed twice daily in the following sequential tests: (1) an area adjacent to a mature boar for the S stage, (2) at 0 and 15 minutes in an arena surrounded by 6 mature boars for the B stage and refractory response respectively, and (3) in their home pens in the absence of boars (at 5 minutes after detection of the B response in Test 2) for the I stage.

All gilts displayed a standing response in Test 2 at 0 minutes in at least one test (B stage), but 57% (13 gilts) showed the refractory response at 15 minutes after showing the B stage for the first time in Test 2. Seventy percent of the gilts displayed the S and I stages. Significant correlations between these responses are listed in Table 1.

Table 1 Correlation coefficients between the duration (number of test sessions) of some behavioural responses of oestrous gilts.

Variables	Oestrus	I stage
Onset of oestrus to S stage	+0.55	-0.08
B1 stage	+0.26	-0.69
Refractory stage	+0.58	-0.52
Non-refractory stage	+0.59	-0.54
B1 + B2 stages	+0.42	-0.84

Critical values for r, df=21: 0.413, $P<0.05$; 0.526, $P<0.01$.

These temporal relationships indicate the possibility of predicting the duration of either oestrus or the I stage using some of the behavioural changes that occur early in the oestrous period. Since there is evidence that ovulation occurs at about two-thirds of the duration of oestrus, observation of behavioural change early in oestrus may allow insemination to be timed relative to the occurrence of ovulation. Therefore, further research is required to examine the temporal relationships between these behavioural changes during oestrus, particularly early in the oestrous period, and the occurrence of ovulation.

A SEASONAL INFLUENCE ON PLASMA GROWTH HORMONE IN THE DOMESTIC PIG

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A seasonal pattern in the plasma concentration of growth hormone (GH) in the pig was suggested by observations in mature boars by Trudeau *et al.* (1988) who found that plasma GH was lower during the summer compared to other times of the year. An experiment was designed to define more clearly the relationship between season and the plasma GH concentration in the pig.

Forty Large White x Landrace prepubertal gilts (20 weeks of age) were randomly selected each month from the finisher herd of a commercial piggery located in NSW (34°S latitude). All gilts were group housed and were offered a finisher diet *ad libitum*. At monthly intervals, for a period of one year, single blood samples were taken by jugular venipuncture, while the animals were restrained with the aid of a nose snare. The sampling procedure was completed within 1 min for each animal. Plasma was analysed for GH using the method described by Chung *et al.* (1985). Outliers, considered to be associated with a secretory pulse, were identified as those which lay above two standard deviations of the monthly mean and were excluded from analysis. The monthly mean GH concentrations together with the mean daily photoperiod for each month are presented in Figure 1. The data were statistically analysed by analysis of variance.

The concentration of GH in the plasma of prepubertal gilts was distinctly seasonal. Plasma GH was significantly lower during the late summer - early autumn when compared to the remainder of the year ($P<0.001$). The lowest average concentration of GH was recorded during March (2.7 ng/ml) and the highest concentration in June (6.7 ng/ml). Daylength and GH concentration appeared to be inversely related, with a lag period of approximately 2 months providing the best alignment between the two variables.

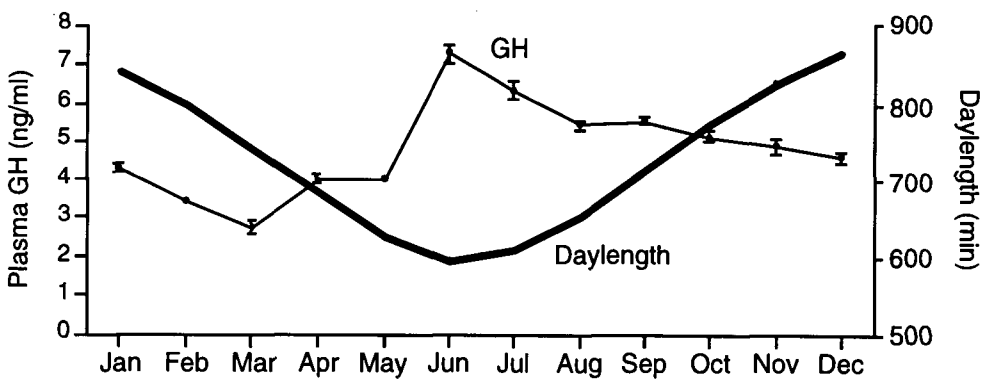

Figure 1. *Mean plasma GH concentration for prepubertal gilts plotted with the corresponding mean daylength for each month.*

Plasma GH concentration in the domestic prepubertal gilt is significantly affected by season, in a manner similar to that suggested by Trudeau *et al.* (1988). Growth hormone influences a wide variety of physiological functions which in turn play a role in determining an animal's productivity. The seasonal differences in plasma GH concentrations which have been shown to exist in the prepubertal gilt may explain some of the variation in production and reproduction which occurs throughout the year in the pig.

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INFLUENCE OF SEASON AND HOUSING METHOD ON PLASMA GROWTH HORMONE IN THE EARLY-PREGNANT GILT

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It is well established that farrowing rate (the proportion of mated sows which farrow) is reduced during the summer and autumn. Maintaining sows in individual stalls rather than in groups dramatically reduces the adverse effect of season on fertility (Love *et al.*, 1995). Growth hormone (GH) is increasingly recognised as having a critical role in reproduction in a variety of species. In the rat low endogenous secretion of GH delays puberty, an effect which can be overcome with GH administration (Advis *et al.*, 1981).

The following study was undertaken to investigate changes in plasma GH with season and housing method in pigs. Twenty-four gilts were selected at mating during autumn (March; n=12) and spring (September; n=12) and randomly placed into either individual stalls (n=6) or a group pen of six pigs (n=1). Logistical constrictions prevented the replication of group pens. At 15 d after mating the gilts were fitted with an indwelling jugular catheter using a non surgical technique. Blood samples were collected at 15 min intervals for 12 h on the day following cannulation. The concentration of GH was measured using a RIA validated for use with porcine plasma (Chung *et al.*, 1985). Basal GH was calculated as the lowest 25% of values for each pig over the 12-hour sampling period. Pulse amplitude was defined as the percentage increase of a pulse from the preceding basal level (Cluster © v5). All data were analysed using ANOVA and least significant differences.

Plasma concentration of GH was affected by season, with lower basal and average levels in the autumn relative to spring. The influence of season was significant in the group housed gilts. Group housing was associated with lower basal and average concentrations of GH, compared to individual housing, this effect being significant during autumn. There was no significant effect of either season or housing method on GH pulse frequency or amplitude in these gilts.

Table 1. Basal, average, pulse frequency and amplitude for plasma GH from gilts in individual and group housing during autumn and spring (mean ± SEM).

	Autumn		Spring	
	Individual	Group	Individual	Group
Basal (ng/ml)	5.0 ± 0.38^a	3.2 ± 0.25^b	5.8 ± 0.54^a	4.8 ± 0.35^a
Average (ng/ml)	6.1 ± 0.36^a	4.2 ± 0.33^b	6.8 ± 0.62^a	5.7 ± 0.33^a
Frequency (min)	255 ± 60.7	156.9 ± 16.3	167.0 ± 19.7	242.5 ± 47.4
Amplitude (% increase)	127.1 ± 14.2	116.2 ± 1.36	114.5 ± 2.44	114.4 ± 1.33

^{a,b}Treatments with different superscripts within rows are significantly different ($P<0.05$).

Group housing during autumn, previously associated with lower fertility (Love *et al.*, 1995) also resulted in lower plasma concentrations of GH in pregnant gilts. These results complement those obtained in the prepubertal gilt, where plasma GH was significantly lower in the summer-autumn period compared to winter (Telfser and Love 1997). The results from this experiment provide preliminary evidence for an association between GH and seasonal infertility in the pig. Whether the lower plasma GH observed in this experiment is responsible for lower fertility during summer and autumn remains to be established.

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RELATIONSHIPS BETWEEN REPRODUCTIVE PERFORMANCE AND SEROLOGICAL TITRES TO LEPTOSPIRAL SEROVARS hurstbridge AND bratislava IN A VICTORIAN PIG HERD

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Many Australian pig herds have in recent years experienced reproductive difficulties consistent with leptospirosis. Infection of many Australian pigs with *Leptospira inadai* serovar hurstbridge or *L. interrogans* serovar bratislava is recognized (Chappel *et al.*, 1992, 1996). Serovar bratislava has been associated with pig reproductive losses overseas, and serovar hurstbridge was discovered in Australia in 1994. A study is presented here of the relationships between reproductive performance and titres to these serovars in a Victorian herd.

A total of 165 mixed parity Large White x Landrace sows was randomly selected as they entered the farrowing shed. Sera from blood samples collected between 1 week before and 1 week after farrowing were subjected to the microscopic agglutination test (MAT). Titres of ≥64 to serovar hurstbridge were given by 41 sera (25%), and to bratislava also by 41 sera.

Table 1. Relationship between MAT titres to serovar hurstbridge and percentage of foetal deaths.

MAT titre hurstbridge	<32	32	64	128
Mean % foetal deaths	3.5	2.1	5.3	25.0
Number of sows	53	35	24	4

There were no significant relationships between titres to either serovar and the total number of piglets born alive, or the percentage of stillbirths. Foetal deaths *in utero* (10 d or more before full term) were more frequent with higher titres to serovar hurstbridge (Table 1), and the relationship approached statistical significance ($P<0.075$). The interval from weaning to first mating was longer with higher titres to either serovar ($P<0.01$). Table 2 shows the mean increases in weaning-to-first-mating interval associated with titres of ≥64 to each serovar.

Table 2. Increased weaning-to-mating interval associated with MAT titres of ≥64 to serovars hurstbridge and bratislava.

Serovar	hurstbridge		bratislava	
MAT titre	<64	≥64	<64	≥64
Weaning to mating (d)	5.0	9.0	5.7	7.1

These results suggest that leptospiral serovars hurstbridge and bratislava may both be reproductive pathogens in Australian pigs.

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CHARACTERIZATION OF COLD-SHOCK IN EXTENDED BOAR SEMEN: DIRECT EFFECTS ON SPERMATOZOA AND FERTILITY

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It is commonly assumed that irreversible damage (e.g., cold-shock) occurs if boar semen drops below 15°C in a prepared medium (i.e., semen extender). The proposed 15°C lower critical temperature appears to have originated from work performed by Ito *et al.* (1948) who studied storage temperatures for unextended, raw semen. Therefore, the aims of the present study were to: 1) identify the cold-shock critical temperature and time for extended boar semen as assessed by sperm viability, motility and morphology; and 2) determine the effects on fertility of using extended porcine semen exposed to the critical temperature and time identified in the first objective.

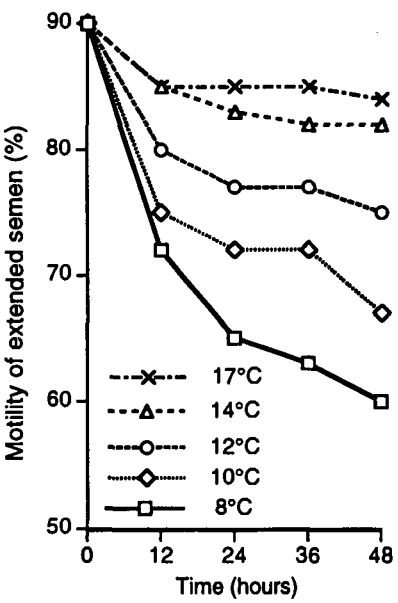

Figure 1. *Effects of time and temperature on sperm motility*

Objective 1: Ejaculates from 19 AI adult boars (>160 kg live weight, LW; PIC Line 326) were collected, analyzed and diluted with Androhep extender to 50 x 10^6 sperm/ml. Doses (4 x 10^9 sperm/dose) from each ejaculate were held at each of 5 storage temperatures (8, 10, 12, 14 and 17°C). Sperm viability, motility and morphology (including acrosome integrity) were assessed on the raw ejaculate immediately following collection (Althouse, 1997), and at all temperatures at 12 h intervals for 48 h. Results were compared using polynomial regression analyses. Both sperm viability and motility (Figure 1) had similar decreases over time at each temperature. Minor decreases in viability and motility occurred by 12 h at 12, 14 and 17°C, but remained constant thereafter. Large decreases ($P<0.05$) in viability and motility occurred in semen stored at 8 and 10°C for 12 h, and continued to decrease throughout the 48 h period. No changes in abnormal sperm morphology occurred at any temperature or time.

Objective 2: Ejaculates collected from 9 adult boars (>160 kg LW; PIC Line 326) were processed, and extended doses divided into treated (12°C for 60 h) and control (17°C for 60 h). A total of 103 sows (PIC Cambrough 15's and 22's) were bred twice by AI using either treated (n = 50) or control (n = 53) semen. All 50 sows bred with treated semen and 51 of 53 sows bred with control semen were diagnosed as being pregnant using real-time ultrasonography. Litter size data is not yet available.

This study demonstrated no differences in the *in vitro* quality of extended boar semen when stored at 12-17°C for up to 48 h (Figure 1). Extended semen, however, is sensitive to temperatures at or below 10°C, with detrimental effects apparent within 12 h of exposure. Storing semen at 12°C for up to 60 h had no negative effect on pregnancy rate. The results of this study thus far support the need to re-establish critical temperature guidelines for storing extended porcine semen.

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A SYMPOSIUM - STRATEGIES TO IMPROVE CONSISTENCY OF PORK QUALITY - TOWARDS 2010

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Introduction

In the production of pork for human consumption, the aim is to provide a lean product that is safe, of consistently acceptable quality, desirable economic value and grown and slaughtered under acceptably humane conditions. The quality of pork is defined by the consumer-desirable traits of attractive appearance in retail display and satisfying palatability when consumed. Undesirable traits in the appearance of fresh pork include meat which is too pale or too dark, exudes fluid from the surface, and the lean or the fat appears soft in texture. Undesirable traits in palatability include off-flavours or odours, lack of juiciness, mushy texture and toughness. Factors that contribute to these undesirable variations in appearance and eating quality are often beyond the abattoir's or retailer's control. Factors which influence changes in muscle glycogen content pre-slaughter and the rate of glycolysis post-slaughter can have significant effects on pork quality and can be controlled through modifications to the genetic, nutritional and handling management of the pig. Boar taint causes off-flavours and odours in pork which are unacceptable to the consumer. Research in this area suggests potential for control of taint through manipulation of genetics, nutrition and stressors pre-slaughter.

It is being recognized that there is a minimum fat level required for acceptable juiciness of pork. Control of fat quality and content (marbling) through nutritional and genetic manipulation is an area of increasing emphasis and interest in the pork industry. Pork is particularly susceptible to developing off-flavours due to the onset of oxidative rancidity and this can be controlled through nutrition.

Other factors that define a high quality pork carcass include the absence of blood splash or bruising in the meat and the absence of blemishes on the skin. These can be controlled by designing systems to manage the pig from farm to slaughter so that the animal's welfare is maximised. In the future, attention to the welfare of the pig will be an integral and essential component determining consumer acceptability of pork products. Consumers in Europe are starting to demand certain minimum standards for welfare when they buy meat and it is almost guaranteed that Australian consumers will follow.

Recent research has clearly shown that best practice procedures to improve pork quality must encompass all sectors of the production and marketing chain. The National Pork Quality Improvement Program has demonstrated that the occurrence of Pale, Soft, Exudative (PSE) meat can be reduced by 40% if abattoirs implement comparatively low cost changes to pre-slaughter management practices and chiller management and efficiency.

The aims of this symposium are to examine the current knowledge of the major factors that may influence pork quality, to discuss opportunities to manipulate these factors and predict the important areas for future development.

Symposium continued on next page

GENETIC INFLUENCES ON PORK QUALITY

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Abstract

The genetic influences on pork quality including that of the major genes, the halothane gene and the RN gene, are discussed in this paper. Breed differences and genetic parameters for meat quality traits are summarise. In addition, genetic influences on boar taint and further developments in genetic improvement of meat quality are discussed. The effect of the halothane gene on meat quality traits and the relationship between this gene and muscle morphology is discussed. In addition, the RN gene is described which has a high frequency in Hampshire and marker information to this gene is presented. Indications for further major genes influencing meat quality traits are summarised. Breed differences in meat quality are due to different levels of the halothane gene as well as different levels of leanness. Special reference is given to Durocs, the breed with a higher intramuscular fat content. The summary of genetic parameters shows moderate heritabilities for meat quality traits pertaining to PSE and dark, firm, dry (DFD) while intramuscular fat content is highly heritable. Genetic correlations between meat quality traits reflect characteristics of PSE and DFD meat. Components of boar taint differ between breeds. In addition, these components are highly heritable and might be influenced by major genes. Currently, DNA tests exist for the halothane and RN gene. It is most likely that further major genes or marker related to quantitative trait loci (QTL) will be detected. Meat quality traits should be incorporated into selection decisions in order to prevent further deterioration in these traits as a consequence of selection for higher lean meat growth.

Introduction

The phenotypic performance of an animal is dependent on its genotype as well as environmental influences. For meat quality traits, environmental influences include feeding, housing, handling and slaughter practices. In order to achieve improvements in pork quality, improvements of management practices have to be accompanied by genetic improvement. Other contributions in this symposium concentrate on environmental influences, while in this paper genetic influences on pork quality will be discussed. Genetic influences on pork quality comprise differences among breeds as well as differences among animals within breed. These differences can be caused by a large number of genes with small effects - polygenic effects, or can be due to a few genes with large effects - major genes. Examples of major genes are presented firstly followed by a description of breed differences and a summary of genetic parameters for meat quality traits.

Pork quality

Pork quality is a very broad term and different people will answer the question "what is good quality pork" differently. In order to define the term "quality", Hofmann (1994) distinguished between quality in the meaning of "goodness" and in the meaning of "condition". The latter definition of quality implies that it can be described through objective measurements of the properties and characteristics of a product. These objective measurements include pH measurements, colour and drip loss percentage which Hofmann (1986) described as technological properties of pork (hereafter called meat quality). The pork quality deficiencies pale, soft and exudative meat (PSE) and dark, firm and dry meat (DFD) are defined by these meat quality traits. Other pork quality characteristics include tenderness, juiciness and flavour which can be used to describe the eating quality of pork. However, the methods used to measure these traits are labour

intensive and costly. Indirect improvement of eating quality through selection for higher intramuscular fat content, which is related to enhanced eating quality (Bejerholm and Barton-Gade, 1986; de Vol et al., 1988), might be an alternative to direct selection for eating quality traits. In addition, for pork to be of good eating quality, it has to be free of any undesirable flavours such as boar taint. Because of the trend to increase carcase weight in Australia, the problem of boar taint is also increasing.

Aspects of meat quality reviewed here will include technological traits (pH, colour, drip loss) along with intramuscular fat content since these traits are most likely to be used in genetic improvement programs. In addition, an examination of genetic influences on components causing boar taint will give an indication whether it is possible to reduce boar taint through genetic selection programs.

Major genes

Halothane gene

Depending on the reaction to halothane anaesthesia, pigs are classified as halothane-positive or halothane-negative. The effect of these phenotypes on meat quality traits has been analysed frequently over the last 20 years. However, the halothane test does not distinguish between homozygous non-carriers and heterozygotes. Since Fujii *et al.* (1991) and Otsu *et al.* (1991) developed a gene probe to distinguish between these two halothane genotypes, it has been possible to analyse the performance of the three genotypes. Results are summarised in Table 1 for pH recorded 45 min after slaughter (pH45), colour of the *M. longissimus dorsi* and drip loss percentage. The halothane gene does not influence ultimate pH (Guéblez *et al.*, 1995; McPhee and Trout, 1995; Peschke *et al.*, 1993; Wittmann *et al.*, 1995). In contrast, pH45 was lower for homozygous halothane pigs, with the mean being below 6.0, and therefore the majority of carcases from pigs in this group exhibited PSE meat. Although heterozygotes were intermediate in meat quality traits relative to the homozygous genotypes, their performance is generally more similar to homozygous non carriers for pH45 and colour. Based on a few studies only, colour and drip loss percentage were inferior for homozygous halothane pigs in most analyses.

The differences between the homozygous halothane free group and heterozygotes (NN-Nn) are also listed in Table 1. Comparing different breeds and selection lines, for pH45 the difference between the halothane free and heterozygotes is largest for the Pietrain. In the study by McPhee and Trout (1995) the difference between halothane free and heterozygotes is larger in the line that had been selected for increased lean meat content for all three meat quality traits than for the control line. These differences between breeds and selection lines might indicate an interaction of the halothane gene with changes in muscle morphology at different levels of animal leanness.

Relationship between halothane gene and muscle morphology

The effects of the halothane gene on stress susceptibility and meat quality are influenced by muscle hypertrophy and associated changes in muscle fibre size and type, and these were summarized by Schmitten (1993) and are illustrated in Figure 1. The effects of the halothane gene are due to a change in the amino acid sequence of the sarcoplasmic reticulum Ca^{2+} - release-channel-membrane protein (the ryanodine-receptor protein). As a consequence, when pigs carrying the halothane gene are exposed to stress, Ca^{2+} release from the sarcoplasmic reticulum is increased due to a reduced release-limit of Ca^{2+} from the sarcoplasmic reticulum (Knudson *et al.*, 1990). Therefore, Ca^{2+} concentrations are elevated in muscles of stress susceptible pigs.

The ryanodine-receptor protein, located in the surface membrane of the sarcoplasmic reticulum, determines the rate of release of Ca^{2+} and is altered in all of muscles of the carcass of pigs carrying the halothane gene. However, the negative influences of the halothane gene on meat quality occurs mainly in muscles with a higher muscle mass, which are generally those exhibiting muscle hypertrophy with the associated properties shown in Figure 1.

Table 1. Meat quality traits for homozygous non-carriers (NN), heterozygotes (Nn) and homozygous carriers (nn) of the halothane gene along with the difference between homozygous non-carriers and heterozygotes (NN - Nn).

Study and trait	Breed	NN	Nn	nn	NN-Nn
pH45					
Wittmann *et al.* (1993)	German Landrace	6.43	6.18	5.64	0.25
Schmitten (1993)	Seven hybrid origin	6.55	6.20	5.66	0.35
Peschke *et al.* (1993)	Pietrain	6.52	5.94	5.54	0.58
Guéblez *et al.* (1995)	Large White/Pietrain	6.46	6.24	5.77	0.22
McPhee and Trout (1995)	Control line	6.40	6.23	5.96	0.17
McPhee and Trout (1995)	Selected line	6.56	6.29		0.27
Colour (L-value)					
Guéblez *et al.* (1995)	Large White/Pietrain	55.9	56.0	59.4	-0.1
McPhee and Trout (1995)	Control line	45.4	45.9	47.9	-0.4
McPhee and Trout (1995)	Selected line	42.4	44.7		-2.3
Luxford (1995)	Large White	52	55		-3.0
Luxford (1995)	Landrace	53	55		-2.0
Drip loss percentage (%)					
Guéblez *et al.* (1995)	Large White/Pietrain	5.20[1]	6.00	6.40	
McPhee and Trout (1995)	Control line	3.62	3.76	2.73	-0.14
McPhee and Trout (1995)	Selected line	2.28	3.62		1.34
Luxford (1995)	Large White	1.35	2.18		0.83
Luxford (1995)	Landrace	1.57	1.90		0.33

[1]Drip loss after 5 d storage.

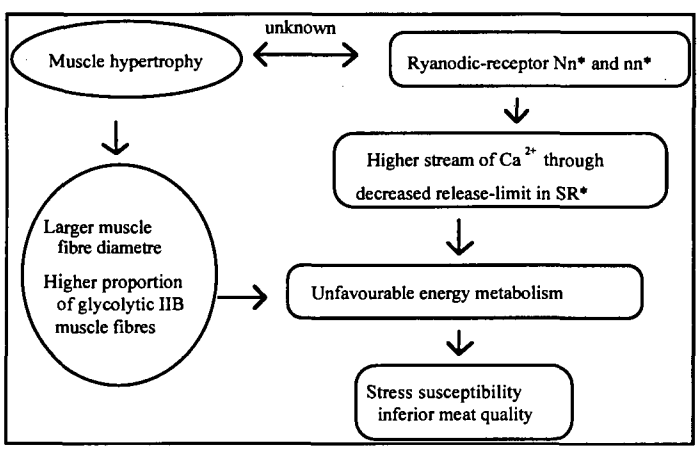

Figure 1. *Relationships between effects of halothane gene and muscle hypertrophy (Schmitten, 1993). *Nn: heterozygotes for the halothane gene; nn: homozygous carrier for the halothane gene; SR: sarcoplasmic reticulum in the muscle cell.*

Muscle fibres are classified into two major classes, muscle fibre type I (red, oxidative) and muscle fibre type II (white, glycolytic); the latter are subdivided into type IIA, IIB and IIC (Essen-Gustavsson, 1993). The proportion of each muscle fibre type differs among muscles. For example the *M. longissimus dorsi* (loin muscle) has 80-90% type IIB fibres (Essen-Gustavsson *et al.*, 1992; Karlsson *et al.*, 1994) while the *M. vastus intermedius* has 70-80% type I fibres (Essen-Gustavsson, 1993). As discussed in the subsequent paper, the proportion of different fibre types in a muscle determines its physiology, metabolism and subsequent meat quality. Higher muscle mass caused by muscle hypertrophy post-parturition is generally achieved by increases in muscle fibre size

and in the number of glycolytic muscle fibres (type IIB). In the studies of Solomon *et al.* (1990) and Wegner and Ende (1990) the higher glycolytic metabolism associated with an increase in muscle fibre size resulted in a higher incidence of PSE meat.

Rendement Napole (RN) -Gene

"Napole" technological yield was described by Naveau *et al.* (1985) and measures the yield of a muscle after brine injection and cooking. A major gene, called the Rendement Napole (RN) gene, was found in two commercial pig lines in France and Naveau (1986) postulated that "Napole" yield is influenced by the presence of the gene. Le Roy *et al.* (1990) used segregation analysis of the two commercial lines to confirm that the RN gene influences "Napole" yield. They found that the unfavourable RN gene is completely dominant, and an allele frequency of 60% was estimated for both lines. This allele frequency implies that 36% of the animals in the two lines were homozygous for this gene and 48% were heterozygotes. The RN gene causes a 70% increase in the glycogen content of glycolytic muscles (Estrade *et al.*, 1993). Lundström *et al.* (1996) and Enfält *et al.* (1997) used the known differences in muscle glycogen content to classify pigs into RN genotypes and found that the RN gene detrimentally affects the water-holding capacity of fresh and processed meat. For heterozygous RN pigs drip loss and cooking loss were 21% and 12% greater respectively, while the Napole yield was 7% lower compared with the homozygous non-carriers (Lundström *et al.* 1996). The drip loss, cooking loss and Napole yield for RN free pigs was 4.8%, 29.4% and 89.9% respectively. It was suggested that the detrimental effects of the RN gene on meat quality traits are a result of an increase in muscle glycogen and a decrease in protein content, together with a lower ultimate pH value.

The RN gene is found at a high frequency in Hampshire pigs which explains their inferior meat quality. Compared with other breeds (e.g., Large White, Pietrain, Large White x Landrace and Yorkshire) meat from Hampshires is characterised by having a lower ultimate pH, together with a higher cooking loss (Monin and Sellier, 1985), a higher water content, a lower protein content (Fjelkner-Modig and Tornberg, 1986; Wassmuth *et al.*, 1991), and a higher glycogen content (Monin and Sellier, 1985).

The molecular background of the RN gene has been investigated in France (Milan *et al.*, 1996), Sweden (Mariani *et al.*, 1995) and Germany (Rudat *et al.*, 1995). These studies have agreed that the location of the RN gene is on chromosome 15 in swine. Milan *et al.* (1995,1996) reported the specific location of the RN locus to be between markers Sw120 and Sw936 at a distance of 2cM from marker Sw936. Based on this knowledge, a DNA-test for detecting the RN gene has been developed and validated by de Vries *et al.* (1997). This DNA-test predicts large differences in ultimate pH and phosphate-free processing yield.

Indications for major genes for intramuscular fat content

Evidence for the presence of a major gene for intramuscular fat content was presented by Janss *et al.* (1994) based on a segregation analysis of F2 crosses between Meishan and Dutch pig strains. Gerbens *et al.* (1996) investigated the heart fatty acid-binding protein (H-FABP) as a candidate gene for intramuscular fat content since H-FABP is involved in intracellular fatty acid transport expressed predominantly in muscle cells. The H-FABP gene was located on porcine chromosome six and first results showed that it accounts for 10% of the variation in intramuscular fat content.

Breed differences

Differences in the rate and extent of postmortem pH fall explain a large proportion of the variation in quality of pork. The variation in rate of pH fall (pH_1) and extent of pH fall (pH_u) among different pig breeds is illustrated in Figure 2. Large White and Duroc, which are both halothane free breeds show a slow rate of postmortem pH fall and a normal ultimate pH. Consequently a low incidence of meat quality defects is observed in these breeds. Sellier (1988) explained that pork quality in Landrace pigs is dependent on

the frequency of the halothane gene in the considered population. Landrace populations with a high frequency of the halothane gene are characterised by a rapid fall in postmortem pH and thus high levels of PSE meat. However, in populations with a low frequency of the halothane gene, as is now the case in most Landrace populations, the rate of postmortem pH decline is reduced and pork quality is similar to that of Large White pigs. Pietrain and Belgian Landrace pigs have a high frequency of the halothane gene and are characterized by a fast rate of pH fall. However, in Belgian Landrace the unfavourable effects of a rapid pH fall on pork quality are somewhat counterbalanced by the high ultimate pH.

Breed differences in meat quality are not only due to a different frequency of the halothane gene but can also arise from different levels of leanness. Pork from breeds which have been selected for higher lean meat content is less firm, has a poorer water holding capacity and is paler in colour (Warris *et al.*, 1996). The Pietrain breed, which represents the leanest breed had by far the poorest meat quality while traditional breeds like Tamworth, Large Black and Berkshire produced meat with the best meat quality. In a US study, meat quality was best in Berkshire and Spotted, the breeds with the highest fat content (Johnson and Goodwin, 1995).

Durocs are known for their higher intramuscular fat content, as demonstrated by Sellier (1988). For the Duroc breed, the average intramuscular fat content in the *M. longissimus dorsi* is higher (3.9%) than the Hampshire breed average of 2.0% and well above the intramuscular fat content of Large White, Landrace, Pietrain and Belgian Landrace breeds (1.4%-1.6%). In comparison, Duroc pigs in Australia have an average intramuscular fat content of 2.5% in *M. longissimus dorsi*. Australian Large White and Landrace have a lower intramuscular fat content than Durocs with means of 1.6% and 1.7%, respectively (Hermesch *et al.*, 1997). In order to improve eating quality, de Vol *et al.* (1988) suggested a minimum level for intramuscular fat content of 2.5% which is only achieved in Duroc populations.

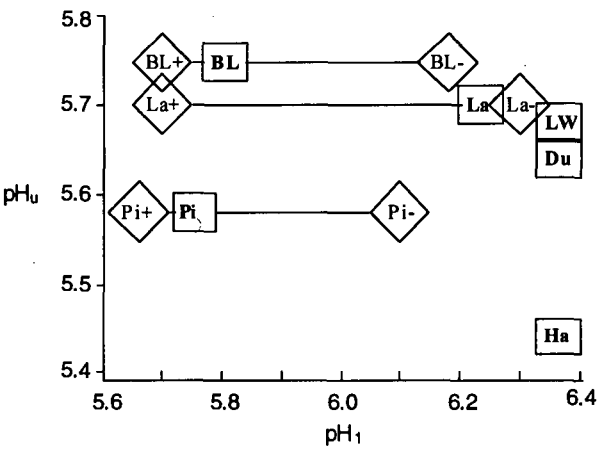

Figure 2. *Mean values of muscle pH shortly after slaughter (pH_1) and ultimate pH (pH_u) in 6 pure porcine breeds (after Sellier, 1988).*
Note: These values refer to a white muscle of the ham, in case of moderate slaughter stress; pH_1:pH measured 45 minutes after slaughter; pHu: ultimate pH (pH 24 h after slaughter); BL: Belgian Landrace; La: Landrace; Pi: Pietrain; LW: Large White; Du: Duroc; Ha: Hampshire; +: halothane positive; -: halothane negative;
Hypothesis on frequency of halothane sensitivity: 90% in Pietrain, 80% in Belgian Landrace; 5% in Landrace and 0% in the other breeds.

Cameron *et al.* (1990) and Oliver *et al.* (1993) found that Duroc pigs have a darker meat colour associated with a higher ultimate pH compared to other breeds. Edwards *et al.* (1992) reported similar muscle reflectance and pH values between Durocs and other

breeds whereas McGloughlin *et al.* (1988) found Duroc meat to be slightly paler than that from Large White and Landrace pigs. McGloughlin *et al.* (1988) argued that there was considerable variation between the Duroc populations investigated in the various studies and therefore, some variation in the reported relative merit of the Durocs would be expected. The average intramuscular fat content differed substantially among studies ranging from 1.6% (Cameron *et al.*, 1990) to 2.9% (McGloughlin *et al.*, 1988; Oliver *et al.*, 1993) but the level of intramuscular fat content was consistently higher in Durocs than in the compared breeds. Although the studies have shown that Duroc pigs consistently have a higher intramuscular fat content than other breeds, the meat quality characteristics were not consistently better or worse for Durocs then for the other breeds investigated (McGloughlin *et al.*, 1988; Cameron *et al.*, 1990; Edwards *et al.*, 1992; Oliver *et al.*, 1993). It can therefore be concluded that a higher intramuscular fat content in Duroc populations is not phenotypically associated with improved meat quality.

Genetic parameters for meat quality traits

Besides differences among breeds, variation in meat quality traits also exists among animals within a breed. The proportion of variation that is explained by genetic differences among animals is expressed as the heritability of a trait, while genetic correlations describe the genetic relationship between traits. Mean estimates of these genetic parameters, as summarised by Hermesch (1996), are presented in Table 2. On average, meat quality traits pertaining to PSE and DFD meat are 20% heritable with estimates ranging from 0.10-0.35. Intramuscular fat content is highly heritable with a mean estimate of 0.45. Genetic correlations between pH measurements, colour and drip loss percentage reflect characteristics of PSE and DFD meat. Low pH measurements are genetically related to a light colour of the meat with a high drip loss percentage. Selection for higher intramuscular fat content will mostly improve other meat quality traits in regard to PSE. A higher intramuscular fat content is genetically related to a higher pH45, a slightly darker colour and a reduced drip loss percentage.

Table 2. Mean literature values of heritability values and genetic correlations (r_g) for meat quality traits along with genetic correlations between meat quality traits and growth rate and backfat (Hermesch, 1996).

	pH 45 min pm	pH 24 h pm	Colour[1]	Drip loss %	Intra-muscular fat
Mean heritability	0.20	0.17	0.24	0.17	0.45
Range	0.04/0.29	0.07/0.35	0.11/0.37	0.07/0.20	0.36/0.61
No. of studies	3	8	5	2	8
r_g with pH45		0.28	-0.59	-0.32	0.25
Range		0.10/0.49	-0.70/-0.38	-0.55/0.01	-0.04/0.36
r_g with pH24			-0.33	-0.52	0.11
Range			-0.73/-0.08	-0.99/-0.30	-0.18/0.39
r_g with colour				0.67	-0.08
Range				-0.06/0.81	-0.33/0.07
r_g with drip loss					-0.15
Range					-0.23/-0.07
r_g with growth rate	0.05	-0.01	0.25	0.18	0.10
Range	-0.02/0.14	-0.17/0.26	0.04/0.50	-0.55/0.48	-0.16/0.36
r_g with backfat	0.26	0.05	-0.09	-0.07	0.42
Range	-	-0.22/0.22	-0.48/0.05	-0.20/0.06	0.05/0.60

[1]High values represent a light colour.

Currently, genetic improvement is mainly based on selection for growth rate and against backfat. This selection has an indirect influence on meat quality traits (Table 2). Selection for higher growth rate will lead to a lighter colour, a higher drip loss percentage and a slightly higher intramuscular fat content. A decrease in backfat has the strongest influence on intramuscular fat content leading to a further decline in this trait. Although genetic correlations with other meat quality traits are mostly low, further selection for increased leanness will also increase the incidence of PSE if these unfavourable relationships are not taken into account in selection decisions.

Boar taint

Breed differences in boar taint

The concentration of the two main components of boar taint, androstenone and skatole, seem to differ among breeds. Bonneau *et al.* (1979) found a higher concentration of androstenone in the fat of Pietrain pigs compared with that in Belgian Landrace pigs. In a review of European studies, Willeke (1993) concluded that androstenone concentrations in fat are higher in Large White breeds than in Landrace breeds. This was recently confirmed by Xue *et al.* (1996) who found that Landrace had the lowest average concentration of steroids and skatole. The highest average concentration of steroids and skatole were found in Duroc and Hampshire pigs in comparison to Landrace and Large Whites.

Indications for major genes

Applying segregation analyses Fouilloux *et al.* (1997) found indications of a major gene that affects androstenone concentration in fat. The "low androstenone" allele (A) was completely dominant over the "high androstenone" allele (B). The sexual maturity of pigs is indicated by the thickness of the bulbo-urethal glands, and Fouilloux *et al.* (1997) have preliminary evidence indicating that the average thickness of the glands is influenced by a major gene. They suggest that both traits might be influenced by a single major gene.

Canadian work (Squires, 1996) is now focusing on the development of genetic markers for high concentrations of cytochrome P402E and low concentrations of cytochrome b5 in the liver. This is based on the knowledge that males with high concentrations of skatole in fat were found to have low concentrations of cytochrome P450 in the liver and that pigs with low concentrations of androstenone have a low concentration of cytochrome b5 in the liver (Squires, 1996).

Genetic variation in boar taint

Willeke (1993) reviewed heritability estimates of androstenone concentrations in fat samples obtained from pigs of the Danish Landrace, Large White and German Landrace breeds. Estimates were moderate to high ranging from 0.25 to 0.87. These high estimates were in agreement with the findings of Fouilloux *et al.* (1997) who presented a heritability estimate of 0.55. Although no information was given on the actual additive genetic variance, which determines possible genetic response, these high heritabilities would suggest that selection against androstenone is possible.

Selection for reduced boar taint?

Genetic improvement has to consider all aspects of pig production. Some genes can influence a number of traits and this has to be taken into account when selecting against androstenone for the purposes of reducing boar taint since the reproductive traits of gilts and boars may also be affected. In an experiment in which selection was for high and low concentrations of androstenone, Willeke *et al.* (1987) showed that gilts from the high androstenone line had their first oestrus 14 d earlier than gilts from the low androstenone line. In addition, males from the high androstenone line had higher concentrations of testosterone and conjugate oestrogen than males from the low androstenone line. In

addition, Bonneau and Sellier (1986) suggested that selection for reduced androstenone content could adversely affect testicular growth and the development of the genital system in the male pig. Although it is possible to select against components of boar taint the effects on herd performance and their consequences need to be carefully considered.

Future developments

Major genes

In the section on genetic maps in the review by Nicholas (1997) there is a comprehensive description of the nature of genetic markers. Marker information can be used in two ways in breeding programs, namely by using marker assisted selection (MAS) and marker assisted introgression (MAI). The additional information provided by markers in MAS is most beneficial for traits that are costly to measure or cannot be measured in the breeding animal itself. This is the case for meat quality traits and MAS potentially might become a valuable tool to improve pork quality. In addition, marker information can be used to introduce favourable genes into another population (MAI). An example might be the major gene influencing intramuscular fat content.

Halothane gene

The utilization of the molecular genetic test for the halothane genotype permits the elimination of the halothane gene from breeding herds. Although there seems to be no doubt that the halothane gene should be eliminated from maternal lines in order to avoid homozygous halothane-gene-positive pigs at slaughter, opinions differ whether the halothane gene should be totally eliminated from terminal sire lines. In a comparison of the effects of the halothane gene and level of leanness on the occurrence of PSE meat, Rempel *et al.* (1995) and de Smet *et al.* (1996) found that the effect of the halothane genotype was the predominant factor determining meat quality. Therefore, if meat quality is the only consideration, elimination of the halothane gene from breeding populations is desirable. However, the halothane gene has also a major influence on lean meat content (Rempel, *et al.* 1995) and development of a halothane free terminal sire line might substantially reduce the production efficiency and associated economic advantages associated with lean, heavily muscled carcasses. De Vries *et al.* (1995) suggested therefore to use homozygous halothane positive terminal sire lines with halothane free maternal lines in order to produce heterozygous slaughter pigs. In contrast, Glodek (1996) proposed to develop a halothane free Pietrain line arguing that the increased level of PSE meat is unacceptable. Furthermore, in western societies where animal welfare issues are of high importance it might become ethically unacceptable to use a stress susceptible terminal sire line. Therefore, the benefits and disadvantages of the halothane gene have to be evaluated within each market and breeders must make their decisions accordingly. However, given the availability of the molecular gene test the level of the halothane gene in a breed can be now controlled.

RN gene

Given the availability of a commercial DNA-test (de Vries *et al.*, 1997) the RN gene could also be controlled and eliminated from a population as was done with the halothane gene in some instances. However, advantages in better meat quality attributes would have to be compared with possible losses in growth rate and carcase traits as Enfält *et al.* (1997) found a higher growth rate, a higher lean meat content and a larger proportion of ham for carriers of the RN gene.

Further major genes

There are indications based on segregation analyses that single genes influence cooking loss, drip loss, pH measurements, intramuscular fat content, shear force and backfat thickness (Janss, 1996). Currently, genes are mapped for growth, carcase and meat quality traits targeting chromosome four, six, eight and 15 (Nezer *et al.*, 1996) while the Swedish group found significant effects on meat quality traits on chromosomes two

and 12 (Andersson-Eklund *et al.*, 1996). It is therefore most likely that further major genes or markers that are linked to quantitative trait loci (QTL) will be found in the future.

Selection for meat quality traits within a breed

Ignoring meat quality traits in breeding programs will lead to a further decline in these traits. Consequently, meat quality traits should be included in selection decisions. For meat quality traits to be included in a selection index, knowledge of genetic parameters for meat quality traits as well as genetic correlations with other production and carcase traits is required. Such a set of genetic parameters has been obtained for Australian pigs (Hermesch, 1996). However, meat quality traits are only measured within the abattoir on relatives of breeding animals. Even if it is possible to record meat quality traits in commercial abattoirs and return the information to breeders, information on meat quality traits that is available when animals are selected is still limited thus inhibiting possible genetic progress. These drawbacks would be overcome by the availability of a measurement of meat quality on the live animal, enabling measurements of meat quality on the breeding animal itself. Real time ultrasound in combination with integrated image analysis might be a possibility to obtain some information.

Today's consumers not only want pork of high quality but they also want this high quality consistently. In the future, it will therefore not only be necessary to improve meat quality but also to decrease variation in pork quality. Given that meat quality traits are also influenced to a large extent by environmental effects (Hermesch, 1996) the joint efforts of genetic improvement and improvement of all other aspects of pork production influencing meat quality are required.

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Symposium continued on next page

NUTRITIONAL MANIPULATION OF MEAT QUALITY

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Abstract

Pork quality is still evolving as an area of intense interest as consumer demand for tender, juicy and tasty meat strongly influences the focus of the industry towards the quality of its products. The metabolism of glycogen is highlighted as a key determinant of pork quality through its influence on the rate and extent of pH decline post-slaughter. There is considerable genetic and nutritional scope for increasing muscle glycogen concentration pre-slaughter so as to prevent dark, firm, dry (DFD) pork but the resulting associated risk of increased pale, soft, exudative (PSE) pork would suggest that increasing muscle glycogen is not beneficial. However dietary manipulation to reduce the rate of glycolysis in muscle pre- and post-slaughter is of special interest since it will be associated with a reduced incidence of PSE pork. The role of dietary tryptophan and its effects on brain serotonin levels is discussed as a way of 'calming' pigs pre-slaughter. An additional approach is to utilize dietary magnesium as a means of reducing the secretion of catecholamines and so reduce the rate of post-slaughter glycolysis and PSE pork. The regulation of fat texture at both the subcutaneous and intramuscular sites is then discussed. The modern pig tends to show low rates of lipogenesis *de novo* and so relatively small changes in the ratio of saturated/unsaturated fatty acids in the diet can influence fat texture with decreases in the ratio being associated with softer fat. While the consumer associates more unsaturated fat with the perception of improved health, unsaturated fat has a softer texture and it is less desirable for the meat processing sector. This dichotomy needs to be addressed. Finally the role of vitamin E in promoting meat colour and reducing the incidence of PSE is discussed. Vitamin E supplementation is associated with improved meat colour during storage and reduced lipid oxidation, however the incidence of PSE is not reduced.

Introduction

Meat quality is a complex term involving many attributes that affect the technological and sensory quality of pork. This review focuses on the potential for nutritional modification of meat quality. The regulation of glycogen metabolism and the potential for dietary control is first discussed. It is followed by considering the dietary factors which can modify fat texture and quality.

Glycogen and pH

Regulation of glycogen metabolism

The rate and extent of post-slaughter change in the pH of pork is considered the single most important cause of variation in pork quality (Bendall and Swatland, 1988). The rate of pH and temperature decline in meat post slaughter influences protein denaturation, and when the rate of muscle pH decline is rapid while the carcass temperature is still high, then pale, soft, exudative (PSE) pork eventuates. When the decline in pH is not sufficient the meat becomes dark, firm and dry (DFD), and when the decline is too extensive the meat becomes pale and loses water resulting in lower cooking yield ('acid' pork; Sellier and Monin, 1994). The post-slaughter change in pH is largely based on the degradation of glycogen to lactic acid by glycogenolysis and glycolysis.

Glycogen represents the store of body carbohydrate with the quantitatively most important reserves found in the liver and skeletal muscle. The role of hepatic glycogen is primarily for the maintenance of blood glucose while the glycogen in skeletal muscle represents an energy reserve that can be rapidly mobilised. Typically glycogen in muscle

is mobilised during exercise, especially when the exercise calls upon a significant level of anaerobic metabolism.

Glycogen is a large ($MW=10^7$) branched polymer of glucose with each glycogen molecule associated with a protein primer and the enzymes of glycogen metabolism. The protein primer, glycogenin is required to form the template for initial synthesis of glycogen. The physiological role of glycogenin in the regulation of glycogen concentrations is poorly understood. However there is potential for regulation of glycogen concentrations at this step since it is the number of glycogenin primer molecules that will determine the number of glycogen granules (Alonso *et al.*, 1995). The maximum size of each granule is thought to be limited due to inhibition of glycogen synthase as the glycogen molecule increases in size.

The relative balance between glycogen biosynthesis and breakdown is controlled by regulation of glycogen phosphorylase and glycogen synthase. Anaerobic breakdown of glycogen is designed to allow for a very rapid acceleration (<5 secs to attain Vmax) and high final activity (high Vmax) (Sahlin, 1986). In contrast, synthesis is a more chronic process lasting hours to days.

Glycogen synthesis in skeletal muscle typically requires blood glucose as the substrate and is classically thought to be regulated at two steps; firstly, by the entry of glucose into the cell, which is regulated by the transport protein GLUT4, and secondly by the activity of glycogen synthase (Sugden and Holness, 1997). There is evidence for regulation at both levels with glucose availability and insulin concentration in the blood being key positive influences. Hormones such as the catecholamines strongly inhibit glycogen synthesis as a result of 3',5'-cyclic adenosine monophosphate (cAMP) induced phosphorylation of glycogen synthase. In addition, there is a more novel pathway for glycogen resynthesis in muscle which has been shown to exist in the type IIb muscle fibres of the rat after high intensity (sprint) exercise. During the resting phase glycogen resynthesis occurs within the muscle fibre via a reversal of glycolysis which can become thermodynamically favourable as a result of very high lactic acid accumulation (Palmer and Fournier, 1997).

Regulation of glycogenolysis is the most relevant metabolic pathway affecting pork quality, since this determines the rate and extent of pH decline. Glycogenolysis involves activation of glycogen phosphorylase which breaks down glycogen to release glucose-1-P for entry into glycolysis. The regulation of this step in skeletal muscle is by three primary mechanisms (Murray *et al.*, 1996). The classical pathway for activation of glycogen phosphorylase is by the cAMP dependant cascade resulting in phosphorylation of the enzyme. Catecholamine hormones and/or neurotransmitters are thought to be the primary agents initiating this process, and accordingly physical activity and/or stress is sufficient to elevate catecholamines concentrations which initiate glycogenolysis (Sahlin, 1986). This could lead to glycogen depletion pre-slaughter and associated DFD meat. Alternatively, acute stress before or at stunning may result in an accelerated muscle pH decline while the carcass temperature is still high, resulting in PSE pork. The mechanisms for this are not entirely clear but may be related to changes in calcium metabolism. Calcium is a potent activator of muscle contraction and glycogenolysis and the resultant effect is for the rate of muscle glycolysis and pH decline to increase. Stunning procedures such as CO_2 are associated with lower rates of PSE (Barton Gade, 1997) and this is likely related to reduced catecholamine and calcium release at and after stunning.

Muscle fibre type

Skeletal muscle is not a uniform tissue but instead consists of several different fibre types. Type I fibres are slow contracting, while type II are fast contracting. The type II fibres are split further into type IIa and type IIb. Type IIa fibres are fast contracting but also have good aerobic activity and give muscle a red colour (along with type I fibres). The metabolism of glycogen is different in the various fibres - type I fibres have low levels of glycogen. Type IIa fibres have high levels of glycogen, a high rate of glycogen resynthesis and loss of glycogen is least affected by acute stress. Type IIb fibres have lower glycogen levels, slower rates of glycogen synthesis and are the most susceptible to acute stress-induced glycogen depletion (Monin, 1981; Holness *et al.*, 1997). The metabolic differences between muscle fibres can be largely explained by the different

enzyme complement of each fibre type (Table 3). The very high activity of glycogen phosphorylase in combination with low activities of glycogen synthase and hexokinase mean that type IIb muscle fibres rapidly deplete and slowly replete glycogen levels.

Table 3. Enzyme activities for carbohydrate metabolism in rat skeletal muscle (Adapted from Saltin and Gollnick (1983).

	Enzyme activity for each fibre type (μmol of glucose converted/min/g of muscle)		
	Type I	Type IIa	Type IIb
Enzyme			
Glycogen phosphorylase	14	115	171
Glycogen synthase	6	10	5
Hexokinase	2	2	0.8

The skeletal muscle of the pig generally has much higher levels of type IIb fibres than the 'red meat' animals such as sheep and cattle. For example the *m. longissimus dorsi* of ruminants has a ratio of type I:IIa:IIb muscle fibres of 50:40:10 (Suzuki, 1971; Aalhus and Price, 1991) while in the pig the ratio is 8:8:84 (Karlsson *et al.*, 1994). Theoretically this would make the pig susceptible to (i) loss of glycogen from muscle pre-slaughter (i.e., DFD) and (ii) to an accelerated rate of glycogenolysis post-slaughter (i.e., PSE). In practice the incidence of DFD in pigs is not different to those rates reported in ruminants (Barton Gade, 1997; Fabiansson *et al.*, 1989). This may be attributed to the pig having a reduced sensitivity to catecholamines when compared to the ruminant (Pethick and Dunshea, 1996). In addition the decline in muscle glycogen during fasting is slower in type II fibres compared to type 1 (Fernandez *et al.*, 1995).

Fasting and sugar feeding

Fasting of pigs for up to 72 h pre-slaughter, in the absence of other stressors, has minimal effect on muscle glycogen content, certainly insufficient to cause DFD meat (Fernandez and Tornberg, 1991). It is well-known that the stressful events that occur between farm and slaughter, including loading and unloading, trucking, unfamiliar environments, mixing and fighting in lairage can induce muscle glycogen depletion and cause the occurrence of DFD pork (Fernandez and Tornberg, 1991). Activation of the sympathetic nervous system is enhanced in rats undergoing food deprivation (Weick *et al.*, 1983) and this may also occur for pigs although no evidence has been produced to date.

A long waiting time at the abattoir is known to increase the occurrence of DFD pork (Fernandez and Tornberg, 1991), thus sugar feeding to prevent the depletion of muscle glycogen has been investigated. Diet composition does not generally affect the muscle glycogen content of pigs if conventional energy sources are used. Sugar feeding in the last few days prior to slaughter can increase muscle glycogen content (Sayre *et al.*, 1963). Compared to controls, pigs provided with glucose in the water overnight during lairage have higher muscle glycogen concentrations. This has the effect of reducing the ultimate pH (pHu) of the meat from the high levels seen in DFD pork (pHu > 6.0) to the pHu of normal pork (5.5-5.9), and thus prevents the occurrence of DFD pork (Fernandez *et al.*, 1979; Gallwey and Tarrant, 1979; Gardner and Cooper, 1979). It is not clear from these studies whether muscle glycogen depletion was prevented or if repletion was enhanced. The mechanisms need to be understood so that sugar feeding can be used optimally under the range of situations which occur in commercial practice, and to determine if the sugar feeding should occur on the farm or at the abattoir. The advantage of sugar feeding is that it increases muscle glycogen content but by decreasing the propensity for the occurrence of DFD, the propensity for PSE may be increased. The occurrence of DFD pork has negative welfare implications as well as problems with reduced shelf life and potential problems with food safety. Thus DFD is as undesirable as PSE and both need to be prevented. The tendency towards producing PSE with sugar feeding would particularly be the case for stress susceptible animals or abattoir systems where pre-

slaughter stress is high. Also, it is clear that the occurrence of PSE pork results in an economic loss to the pig industry as it is unacceptable to the consumer due to its poor appearance and unacceptable palatability. However, the palatability of DFD is highly acceptable to the consumer as the meat is very tender and juicy due to its high water-holding capacity (Warner, 1994), and it is preferred for sausage manufacture. Thus a decrease in the occurrence of DFD at the expense of a possible increase in the occurrence of PSE pork is probably undesirable.

Exercise

Physical training is known to increase glycogen concentrations of skeletal muscle of the pig (Essen-Gustavsson *et al.*, 1988). The mechanism is poorly understood but in part relates to changes in fibre type. The pig is usually housed intensively and so this mode of control is probably of little practical importance.

Genetic influences

The discovery of the RN gene points to some level of genetic control of glycogen concentrations in skeletal muscle . This gene is associated with an 80% increase in glycogen level of muscle and a lower ultimate pH (pHu) of meat. The rate of muscle pH decline in pigs carrying the RN gene is similar to genetically normal animals (Monin and Sellier, 1985; Hermesch, 1997) suggesting that PSE pork is not associated with elevated glycogen in muscle at slaughter. The high drip loss and poor cooking yield in pigs carrying the RN gene is thought to be directly related to the lower than normal pHu (Lundström *et al.*, 1996). It is thought that the elevated glycogen levels drives the pHu lower than normal. However the relationship between the pHu of meat and glycogen levels of the muscle in RN gene carriers is not strong (Lundström *et al.*, 1996) suggesting that factors in addition to glycogen levels are involved.

The report of positive effects of the RN gene on taste and aroma intensity of cooked pork is of interest and may be related to the low pHu (Lundström *et al.*, 1996). However it is possible that flavour- and aroma-facilitating compounds arise from the interactions between protein and the 'extra' carbohydrate during cooking (i.e., the Maillard reaction; Farmer, 1992). On this basis, elevated residual glycogen levels in meat may be associated with improved flavours upon cooking.

Given the current level of understanding producers need to make a decision on managing glycogen levels. Options include procedures for manipulation of glycogen levels or utilising nutritional tools for slowing glycogen breakdown. The most appropriate 'post farm' gate procedures will depend upon the financial incentives offered by processors.

Dietary regulation of glycogen breakdown

Tryptophan

The main hormonal responses to sudden stress are the release of neurotransmitters in the brain which results in stimulation of the nervous system, and the release of stress hormones into the blood stream which results in stimulation of muscle metabolism. The relationship between stress and neurotransmitters in the brain may indicate ways of reducing the response of pigs to stress and thus reduce glycogen breakdown pre- and post-slaughter. Dietary tryptophan has been used to alleviate 'hysteria' in laying hens (Laycock and Ball, unpublished), reduce the stress response of horses to transport, and reduce pain sensitivity in mice and rats. Ball (1988) reported a relationship between brain serotonin and stress susceptibility in market pigs and subsequently showed that adding 5g of tryptophan/kg to the diet of finisher pigs for 5 d pre-slaughter resulted in increased concentrations of serotonin in the hypothalamus and a reduction in the incidence and severity of PSE (Ball *et al.*, 1988). Warner *et al.* (1990) fed 5g of tryptophan/kg of feed to finisher pigs for 5 d pre-slaughter and monitored their behaviour in pens on the farm and at the abattoir. There was no difference in behaviour on the farm between treated and untreated groups of pigs. When the pigs were exposed

to the unfamiliar environment of the abattoir and mixed in lairage pens (within treatments), the tryptophan-treated pigs were markedly less aggressive (see Figure 3) and exhibited less mounting activity in the lairage pens than the control pigs. The tryptophan-treated carcasses also had less blemishes but there was no difference in the meat quality.

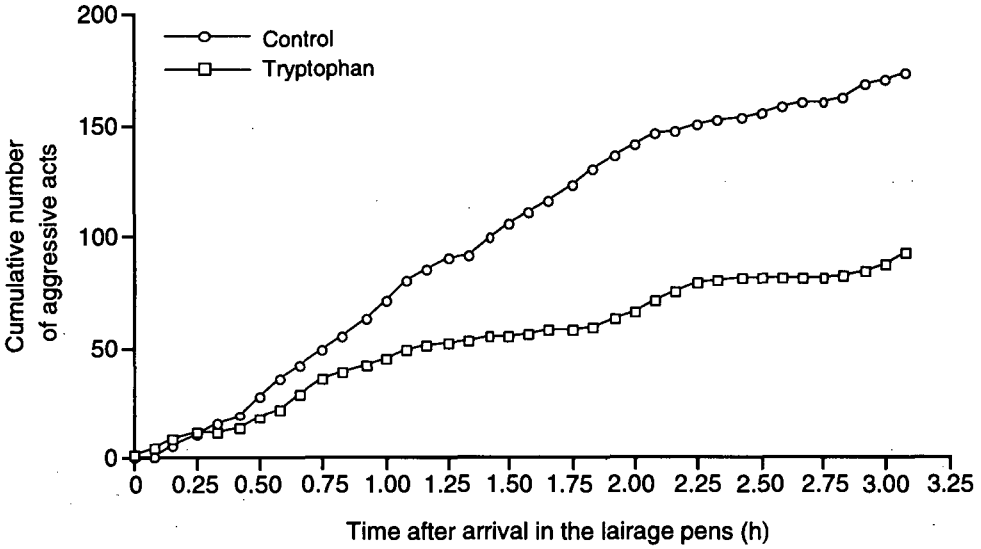

Figure 3. *Cumulative number of aggressive acts of pigs in separate lairage pens for the control and tryptophan-treated pigs during the 3 h in lairage subsequent to arrival at the abattoir.*

Magnesium Compounds

Niemack *et al.* (1979) and Kietzman and Jablonski (1985) have shown that dietary magnesium supplementation is effective in reducing the effect of stress in pigs by reducing plasma cortisol, noradrenaline, adrenaline and dopamine concentrations. This has led to the suggestion that magnesium supplementation may be a viable option for reducing glycolysis pre- and post-slaughter and therefore improving meat quality (Kuhn *et al.*, 1981) and reducing the incidence of PSE (Otten *et al.*, 1992; Schaefer *et al.*, 1993). Magnesium is an essential cofactor for numerous metabolic and enzymatic pathways (Stryer, 1988). Magnesium directly depresses skeletal muscle activity by antagonising calcium at the site of the voltage gated channels in the pre-synaptic terminal which prevents migration and exocytosis of vesicles containing the neurotransmitters to the surface of the pre-synaptic junction. Thus magnesium causes a reduction in the secretion of acetylcholine by motor-nerve impulses, which in turn reduces neuromuscular stimulation (Hubbard, 1973; Hagiwara *et al.*, 1974). Magnesium may be similarly involved in reducing the release of catecholamines (noradrenaline and adrenaline) from both nerve terminals and the adrenal glands (Classen *et al.*, 1983; Herman and Brown, 1983; Kuhn *et al.*, 1981; Kietzmann and Jablonski, 1985).

Otten *et al.* (1992) have reported that long-term (from grower to slaughter weights) dietary magnesium fumarate supplementation (10 and 20 g magnesium fumarate/kg diet; 30-100 kg live weight) in pigs resulted in higher initial muscle pH and conductivity values and less pale meat compared to pigs which were fed a standard finisher diet. Schaefer *et al.* (1993) also reported that meat from pigs fed short-term dietary supplementation of magnesium aspartate (40 g magnesium aspartate-HCl/pig per d for 5 d) displayed reduced muscle temperatures at 45 min post-slaughter and a reduced % drip loss. Dietary supplementation of pigs with magnesium aspartate at 40 g/pig per d for 5 d increased plasma magnesium concentrations by 6%, reduced plasma noradrenaline concentrations and resulted in lower % drip loss and less pale meat compared to pigs fed

the control diet (D'Souza *et al.*, 1997; see Table 4). While negative handling significantly increased the % drip loss (2.4 %) in pigs from the control diet, there was no difference in % drip loss in pigs fed the magnesium aspartate supplemented diet irrespective of the handling treatment. The results presented in Table 4 demonstrate that the use dietary magnesium aspartate supplementation in pigs can be used to reduce the effects of pre-slaughter 'stress' in pigs and hence improve meat quality and reduce the incidence of PSE meat.

Table 4. The effect of dietary magnesium aspartate (Mg Asp) supplementation and pre-slaughter handling (minimum or negative) on plasma noradrenaline and adrenaline concentrations, muscle glycogen and lactic acid concentrations in the *Longissimus thoracis* at slaughter, and muscle pH decline and meat quality indicators in the *Longissimus thoracis* at 24 h post-slaughter.

Diet (D)	Control		Mg Asp			P - values		
Handling (H)	Minimum	Negative	Minimum	Negative	sed	D	H	DxH
Noradrenaline[1] (nmol/ml)	1.79	1.24	0.89	1.05	0.380	0.048	0.470	0.194
Adrenaline[1] (nmol/ml)	0.40	0.43	0.32	0.33	0.085	0.150	0.729	0.945
Glycogen[1] (mg/g)	8.4	6.9	9.6	9.4	0.818	0.003	0.136	0.292
Lactic acid[1] (mg/g)	3.8	4.2	3.2	3.5	0.420	0.036	0.229	0.671
pH at 40 min post-slaughter	6.60	6.59	6.79	6.69	0.074	0.007	0.285	0.431
Ultimate pH[2]	5.48	5.51	5.61	5.57	0.045	0.004	0.864	0.224
% Drip Loss[2]	4.0	6.4	3.5	3.5	0.824	0.006	0.047	0.047
Lightness-L*[2]	48.7	49.1	45.2	47.4	1.109	0.002	0.115	0.247
% PSE[2,3]	8	33	0	0	-	0.050	0.280	0.093

[1]Measured at slaughter. [2]Measured at 24 h post-slaughter. [3]Exact contingency table test used.

Recently reported compounds

The addition of high levels of L-carnitine (Vitamin Bt, up to 300mg/kg) has been reported to improve the in vitro digestibility of muscle (Bonomi, 1995; as cited by Mordenti and Marchetti, 1996) and reduce the paleness of pork (Sardi *et al.*, 1996; as cited by Mordenti and Marchetti, 1996). The addition of up to 150 mg/kg of niacin (Vitamin PP) in the diet for 7 d pre-slaughter may increase muscle glycogen content although the effects on meat quality would need to be investigated in more detail (Piva *et al.*, 1995; as cited by Mordenti and Marchetti, 1996).

Acid and alkaline salts administered in the drinking water for 4 d pre-slaughter have been found to influence pork quality; oral loading with 8g/l of ammonium chloride detrimentally affected pork quality whereas 12.6g/l of sodium bicarbonate tended to improve pork quality (Boles *et al.*, 1993; 1994).

Dietary and other regulation of fat quality

Dietary fat and fat texture

Genetic selection and nutritional manipulation over the last 15 years have focused on reducing the amount of subcutaneous fat with a corresponding increase in feed conversion efficiency by the animal. However, anecdotal and scientific reports suggest

that these putative improvements have been to the detriment of eating or processing quality (Wood, 1993). Many of the flavour components (both positive and negative) are found in fat and the reductions in fat content of the pig can lead to alterations in eating quality. Although from a productive efficiency point of view it is desirable to reduce excessive deposition of fat subcutaneously it may be advantageous to ensure that intramuscular fat is not reduced to levels that compromise meat quality. Coupled to this is the desire by consumers and health authorities to reduce the consumption of saturated fatty acids while increasing the consumption of some of the n-3-polyunsaturated fatty acids. Thus, many in the butchering and processing sector would prefer fat rich in saturated fatty acids whereas the marketing sector and the health professionals would prefer unsaturated fatty acids.

From a processors perspective poor fat quality manifests itself as soft fat and lean/fat separation. With the trend towards producing leaner carcasses through either genetic selection, use of intact males or dietary and hormonal manipulation there has been an increase in the incidence of soft fat. In a review of the genetic effects on fat quality in the growing pig, Metz (1985) concluded that the prevalence of soft fat in lean pigs was due to decreased *de novo* lipogenesis and increased reliance on dietary fatty acids rather than to any genetic predisposition towards preferentially depositing unsaturated fatty acids. Subcutaneous adipose tissue triglyceride fatty acids are derived from either *de novo* lipogenesis or are incorporated directly from dietary fatty acids. The principle fatty acids produced *de novo* in pigs fed a fat free diet are the saturated fatty acids, palmitic acid and stearic acid and the mono-unsaturated fatty acid, oleic acid (Leat *et al.*, 1964; Metz and Decker, 1981). However, inclusion of vegetable oils in the diet dramatically increases the unsaturated fatty acid content of adipose tissue triglyceride (Leat *et al.*, 1964; Metz and Decker, 1981; Marchello *et al.*, 1983; St John *et al.*, 1987; Leskanich *et al.*, 1997). Fat firmness is largely governed by the degree of saturation of the triglyceride fatty acids with soft fat being associated with unsaturated fatty acids. Therefore, there is potential for dietary fatty acids to influence carcass fat quality. For example, in pigs fed an isocaloric diet but differing widely in source and type of fat, there were high correlations between individual fatty acids in the diet and in carcass fat (Hertzman *et al.*, 1988) with diets high in unsaturated fatty acids resulting in softer fat. However, carcass fat can be influenced by even moderate changes in dietary fat. Leskanich *et al.* (1997) recently conducted an experiment with a relatively lean genotype where changing the added dietary fat from 3% tallow: soybean oil (4:1) with 3% canola: fish oil (2:1) resulted in softer subcutaneous fat, particularly over the shoulder.

In many parts of the world high dietary copper levels are used to promote growth but high dietary copper has also been shown to increase the ratio of oleic to stearic acid in the backfat of pigs through stimulation of desaturase activity (Moore *et al.*, 1969). However, the effects upon backfat softness are more profound than can be attributed to gross fatty acid composition alone and other factors need to be considered. For example, Moore *et al.* (1969) selected some back fat samples with similar fatty acid composition but widely different melting points. After chemical randomisation of the position of the fatty acids on the triglyceride molecule *in vitro*, the melting points of the backfat were more uniform. Although individual fatty acids are preferentially incorporated into specific positions in the triglyceride molecule, considerable scope does exist for positional isomers (Brockerhoffe *et al.*, 1966). The synthesis of positional isomers in turn may be related to whether the fatty acid is synthesised *de novo* or of dietary source. Therefore, in addition to fatty acid composition, position of the fatty acids within the triglyceride molecule can be an important determinant of fat quality. Leskanich *et al.* (1997) cites work from his PhD dissertation where inclusion of dietary polyunsaturated fatty acids actually increased backfat firmness in the presence of high levels of linoleic acid, presumably through specific changes in the distribution of triglyceride molecular species (Leskanich, 1995).

Biotin is an important vitamin involved in the formation of malonyl CoA, a rate limiting step in *de novo* fatty acid synthesis and chain elongation of linoleic and linolenic acids. Therefore, lipid obtained from the subcutaneous adipose tissue of biotin deficient pigs has low levels of the major saturated fatty acids synthesised *de novo* (palmitic and stearic acids) while linoleic acid accumulates (Glattli *et al.*, 1973). Increasing the level of

biotin reduces the ratio of unsaturated to saturated fatty acids and so can have marked effects upon fat quality.

While there is little evidence of genetic control over the deposition of polyunsaturated fatty acids in subcutaneous fat (Metz, 1985), there are quite marked genetic influences on the amount and type of fat deposited intramuscularly (Hermesch, 1997). There is also potential to alter the composition of intramuscular fat by dietary means although not to the same extent as subcutaneous fat (Marchello *et al.*, 1983). For example, inclusion of sunflower oil in the diet increased the linoleic acid and decreased the oleic acid content of intramuscular fat of pigs (Marchello *et al.*, 1983). Leskanich *et al.* (1997) were able to use dietary manipulation with canola and fish meal to increase the levels of desirable unsaturated fatty acids in intramuscular fat. However, changes in the fatty acid profile were not at the expense of saturated fatty acids nor was total intramuscular fat altered. Rather, monounsaturated fatty acids were decreased. Many fish meals are also rich in oils containing n-3 fatty acids and fishmeal is often used as a protein supplement in pig diets. A problem with this approach is that meat can become tainted with a "fishy flavour" if fishmeal is included at too high a level or for extended periods of time. However, recent research has suggested that feeding 20% Porcmega fishmeal for 6 to 10 weeks followed by a one week withdrawal can increase intramuscular n-3 fatty acids without compromising handling and processing quality (Howe *et al.*, 1996).

A potential novel method of altering fat deposition for the future may be through the dietary inclusion of conjugated linoleic acid (CLA). Conjugated linoleic acid has one double bond in the *cis* and the other in the *trans* configuration with no methylene interruption probably giving it a shape more like oleic acid. The fatty acid is found in appreciable levels in dairy products and has been shown to increase feed efficiency in rats, mice and chickens (Chin *et al.*, 1994), and to decrease carcass fat content in mice (Albright *et al.*, 1996). It is possible that this fatty acid can be used to manipulate the amount and type of fat deposited in pigs. It is worth noting that CLA itself is a potent antioxidant and anticarcinogen. The former characteristic may ensure some protection against oxidative rancidity while the health and marketing benefits of any anticarcinogen effects are obvious.

Vitamin E

The beneficial effects of dietary supplementation with Vitamin E on aspects of meat quality in all major meat-producing species has been reported by various investigators (see Mitsumoto *et al.*, 1993 for review). The improvement in lipid and colour stability during retail display of beef from cattle supplemented with Vitamin E (Mitsumoto *et al.*, 1991) and in the colour and lipid stability of pork from pigs supplemented with Vitamin E (Monahan *et al.*, 1992) can be attributed to the antioxidant effect preventing both lipid and myoglobin oxidation. Dietary vitamin E has been shown to reduce drip loss of thawed pork (Asghar *et al.*, 1991; Monahan *et al.*, 1994) and fresh beef muscle (Mitsumoto *et al.*, 1995). It has been suggested that the basic mechanism reducing drip loss in muscles from carcasses of vitamin E-fed pigs is alterations in muscle cell membrane permeability to calcium. This is postulated to result in the reduction in rate of muscle glycolysis at slaughter. Rapid rates of glycolysis post-slaughter result in the PSE defect, particularly in pigs di-mutant for the *ryr1* gene (previously called the 'halothane' gene) (Duthie *et al.*, 1989, 1992; Cheah and Cheah, 1985). Pigs which are di-mutant for the *ryr*1 gene have increased requirement for anti-oxidant defence mechanisms in the cells which results in sustained oxidative stress. This oxidative stress that *ryr1* pigs undergo is reported to be alleviated by supplementation of the diet with Vitamin E (Duthie *et al.*, 1989). However, Asghar *et al.* (1991) and Monahan *et al.* (1994) attribute the reduced drip loss in the meat of Vitamin E-fed pigs to a reduction in lipid oxidation in cell membranes. Conceivably, this could reduce the movement of water across the muscle cell membrane post-slaughter. Warner *et al.* (1995) found that feeding pigs di-mutant for the *ryr*1 gene 200 IU of Vitamin E/kg of feed for 40 d pre-slaughter did not prevent the PSE condition and had no major effects on water-holding capacity. However dietary supplementation with vitamin E prevented lipid oxidation until 9 d post-slaughter whereas lipid oxidation in control

samples increased to unacceptably high levels after 2 d post-slaughter and continued to increase (Figure 4).

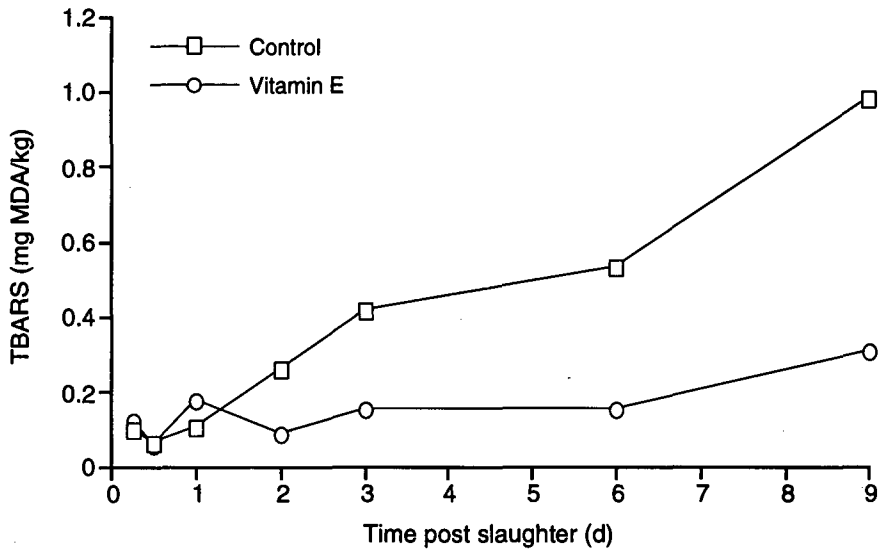

Figure 4. *Changes in lipid oxidation (TBARS value, mg malonaldehyde/kg of meat) for each treatment with days of suspension post slaughter. Each point is a least squares mean of 3 (control; SE ± 0.093) or 5 (vitamin E; SE ± 0.072) pigs.*

Conclusion

Given the relative intensive nature of modern pig production and the increasing use of specific diets, there is considerable potential to improve meat quality through nutritional manipulation. The key role of glycogen and its metabolites in determining final meat quality suggest that manipulation of muscle glycogen depletion and repletion is an area to target. There is a risk that increasing muscle glycogen may result in an increase in PSE pork. On the other hand, reducing the rate of glycolysis in muscle pre- and post-slaughter through inclusion of dietary magnesium may be useful. The relatively low rate of *de novo* lipogenesis in the modern pig means that deposited fat reflects dietary fat and so can be easily manipulated. The difficulty here is balancing the requirements of the processor with that of the consumer. Another means of manipulating meat quality is through the inclusion of Vitamin E which has been shown to improve meat colour and reduce lipid oxidation during storage.

Symposium continued on next page

THE EFFECT OF PRE-SLAUGHTER HANDLING ON MEAT QUALITY IN PIGS

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Abstract

Pre-slaughter handling affects the incidence of PSE and DFD meat, bruising, blood splashing and other damage, and in addition, animal welfare. There are three implicated parties in pre-slaughter handling: the farmer, the haulier and abattoir personnel and all three must collaborate to get the best possible result. For the incidence of PSE and DFD it is the interaction between genotype and energy reserves in muscle at slaughter that is particularly important - at least until 24 h after leaving the farm. Over longer periods energy levels can be built up again from body fat reserves with consequences for meat quality. However, the few meat quality results that are available for long pre-slaughter periods are conflicting. On-farm factors such as group size in pens and rearing pigs outdoors affects behaviour at the abattoir, particularly aggressiveness, and hence the incidence of DFD and unacceptable skin blemish. Stunning method affects the incidence of PSE meat with electrical stunning leading to more PSE than CO_2-stunning. Stunning method is also important for the incidence of fractures and blood splashing. Stunning with CO_2 does not cause fractures and gives a lower incidence of blood splashing compared to electrical stunning. Better welfare during the pre-slaughter period reduces trauma and mortality during transport and lairage, and the incidence of DFD. The emphasis of research in recent years has mainly been on improving welfare. Loading and off-loading have been found to be the most stressful parts of transportation, and optimisation of these processes reduces stress and trauma. Optimal stocking densities during transport are still a matter of debate but will vary depending on transport time, genotype and climate. At the abattoir keeping pigs in small groups of 15 in the lairage reduces aggression and promotes resting behaviour. Elimination of the lining up process for stunning can remove the necessity for force completely, other than guidance from moving gates. There are advantages and disadvantages in both electrical and CO_2-stunning in terms of welfare. In the future the industry must expect increasing demands both with respect to meat quality and welfare, and customer requirements will be the driving force behind future implementation of new developments.

Introduction

Denmark has always concentrated on quality rather than quantity in pig meat production. Nearly 80% of the 20 million pigs produced annually are exported and this export is important for the Danish economy, amounting to about 8% of the total export earnings and employing about 6% of the working population.

Seen in a worldwide perspective, however, Danish pig meat exports amount to only 3% of the total export. This fact, together with higher production costs in the industry, means that the country can never compete on quantity alone but must concentrate on producing quality. Previously, quality was mainly defined in relation to the export of bacon sides, but in recent years quality aspects have become much more diversified to accommodate the requirements of different customer groups.

Denmark's strength lies in the degree of integration within the industry. Most producers are members of co-operative slaughterhouses. These in turn can have processing facilities, sometimes in the slaughter plant itself, sometimes centralised in processing factories. Members receive a bonus at the end of the financial year that depends on the factory's economy. Thus, in contrast to most other countries factory economy does have consequences for Danish producers.

All producers are paid according to warm carcass weight and an objectively measured lean meat percentage for both slaughter pigs and sows. They mainly buy

breeding animals - or use semen - from the nationally approved system run by the National Committee for Pig Breeding and Production.

There is a close collaboration between research institutions with respect to breeding and disease prevention, and the Danish Meat Research Institute, which is owned and financed by the industry, ensures that the latest developments in meat science, meat processing and plant design are available to the whole Danish industry. Finally, a national network of pig advisers ensures that the latest knowledge in breeding, feeding and management reaches producers directly.

This integration means that quality goals are national and that quality aspects, which do not have direct economic consequences for producers via the payment system can, if necessary, be taken into account.

The pre-slaughter handling period can be divided into three main phases with three implicated parties:

- farmer: preparation of pigs for slaughter
- haulier: loading, transport, off-loading
- abattoir personnel: resting period, moving to stunning, stunning and sticking.

All three of these areas must be optimised to get the best result. The highly integrated nature of Danish pig production has meant that there is the possibility of improving all three concomitantly and the export orientation of the country has given the impetus for this to occur.

The objectives of this review are to describe Danish experience in optimising pre-slaughter handling as well as relevant results from the literature to give an update on knowledge of its effect on meat quality and welfare, and to look into the next 15 years or so to try to predict coming trends in this area.

What factors are affected by pre-slaughter handling?

Not all meat quality characteristics are affected by pre-slaughter handling, so that this review will be restricted to the following:

- the incidence of pale, soft and exudative (PSE) and dark, firm, dry (DFD) meat
- transport and lairage mortality
- bruising, trauma and blood splashing
- ethical aspects

Previously, ethical aspects were not mentioned as a separate area, although they have always been taken into account. They have been included in the term "meat quality" as, all things being equal, better welfare leads to better meat quality. Today, welfare is a quality factor in its own right, irrespective of whether a better treatment leads to better meat quality or not, and hence must be taken into account when selling pig meat to customers emphasising welfare. Emphasis on welfare is not yet universal and at present is mainly a Northern European phenomenon, and although good welfare is difficult to define (specifications vary with different customers), it nevertheless is a factor of increasing importance.

Great efforts have been made to get a definition of good welfare from customers with a consensus that good welfare is equivalent to a treatment which "looks good". Exactly what is meant by this is then laid down in specifications that must be fulfilled for that particular customer. Thus, observations of pig behaviour during pre-slaughter handling are necessary to take ethical aspects into account. Pigs that move willingly through a given system will "look better" than squealing pigs forced forward using goads.

Effect of pre-slaughter handling on the incidence of PSE and DFD

Much research has been carried out over the years on the effect of transport and handling procedures on the incidence of PSE and DFD in pig carcasses. This research has shown clearly that the effect of any pre-slaughter handling will be dependent on the

genetic predisposition of the pigs concerned. Pre-slaughter handling is a chain of interacting events that must be considered as a whole and treatment on-farm during fattening and at the abattoir after slaughter can affect the incidence of DFD and/or PSE. Identical experiments carried out in different countries can therefore give quite different results, as such factors are seldom similar.

The Danish Meat Research Institute has worked extensively in the field of transport and handling of pigs in the practical situation. The results of this work will be used to illustrate important factors influencing meat quality. Experiments have shown that for all practical purposes only those factors which affect energy reserves in the muscle at the point of slaughter are important. Nielsen (1981) proposed the following scheme for the relationship between genotype, energy reserves and meat quality:

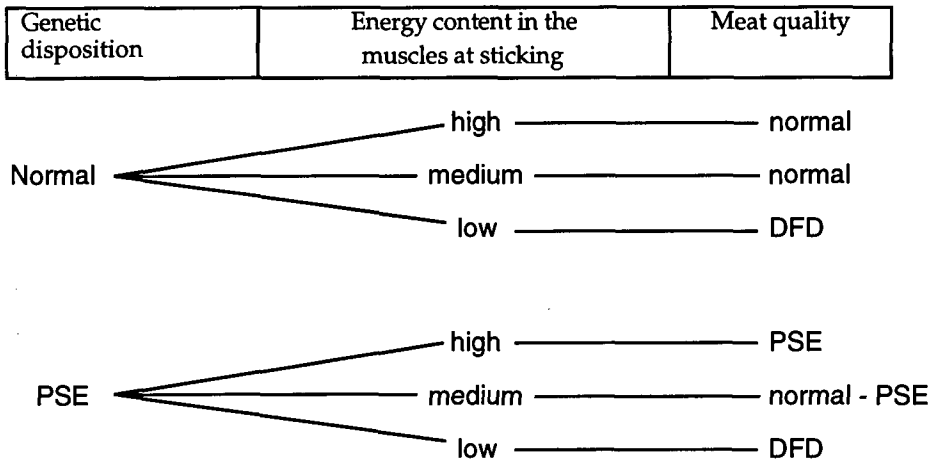

Figure 5. *Meat quality in relation to genotype and energy content in muscle at slaughter*

Genetic disposition for PSE is, of course, highly (but not completely) dependent on the halothane gene (Hovenier *et al.*, 1992), and the energy content in muscles at the time of slaughter by the presence of the RN2 gene (see review by Sellier and Monin, 1994). However, the scheme does point to the factors that must be changed to get the required quality.

The genotype of any group of pigs is fixed, so that it is only by regulating the energy reserves in muscles that meat quality can be influenced in the practical situation. Various conditions are important for the energy content of muscles (Figure 6).

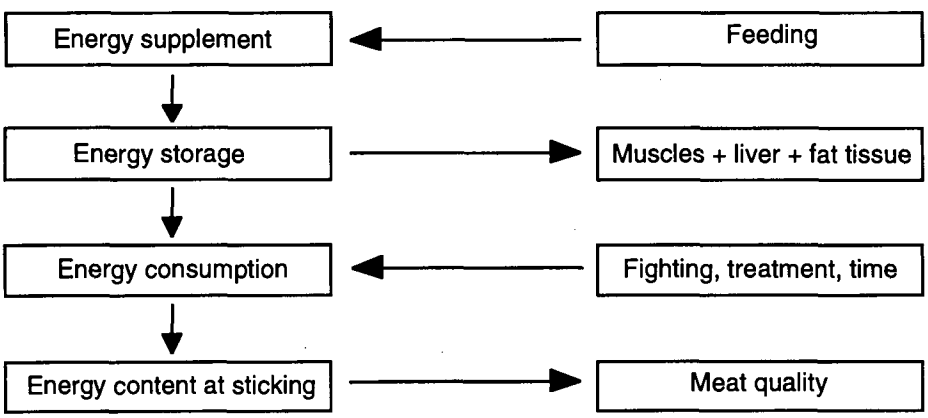

Figure 6. Factors affecting energy levels in muscle at slaughter

Through their daily intake of food pigs are supplied with sources of energy for their growth and maintenance. Surplus energy is stored in the liver, muscles and fat tissue. The longer the time between feeding and collection, the less is the available energy for withstanding the rigours of transport and lairage.

During transport and lairage the energy consumption of pigs will be dependent on the treatment they receive. Energy consumption will be quite different from pig to pig. Some pigs will consume large amounts of energy - especially if they fight, other will have a low consumption. Pigs genetically pre-disposed to the PSE-condition have a more rapid energy turnover than pigs not so disposed. The duration of the transport and holding period also affects energy consumption, the longer the time, the higher the energy consumption. Weather conditions during transport also have an effect - hot weather leads to a greater PSE-frequency, while extremely cold weather leads to a greater energy consumption, and hence a higher DFD-frequency (Barton Gade, 1971, 1974).

Using the above information it would be expected that the PSE-frequency of any group of pigs will increase with feeding on the day of slaughter, short transport and lairage times and hot weather, while DFD-frequency, will increase with no feeding for prolonged periods before slaughter, long transport and lairage times, fighting and/or inconsiderate treatment and finally very cold weather conditions. This is indeed what is found.

Nielsen (1981) compared pigs that had not been fed on the day of slaughter with pigs that had been fed in the morning of the day of delivery for varying holding periods in the lairage. The results showed higher percentages of PSE-meat in pigs which had been fed and which had a short holding period at the factory, than was the case from pigs with no feeding and with a long holding period. Carcasses from pigs which had not been fed had the most DFD-meat and furthermore the DFD-frequency increased during the first couple of hours of the holding period due to fighting.

Table 5. Incidence of PSE and DFD in relation to holding period for pigs fed or not fed on the day of slaughter.

Meat quality	Holding period (h)	No. of pigs	Unfed (%)	Fed (%)
% PSE[1]	0	204	7.8	13.1
	2	206	5.8	7.5
	4	205	2.9	4.0
	24	104	1.9	2.5
% DFD[2]	0	175	2.9	3.4
	2	174	17.0	10.3
	4	177	12.2	6.2
	24	81	20.2	7.4

[1]PSE was subjectively evaluated in *M. gluteus medius* and *M. semimembranosus.*
[2]Pigs were considered DFD when ultimate pH in at least one muscle >6.5 or in at least two muscles >6.1.

The pigs in the experiment of Nielsen (1981) were Danish Landrace, i.e., did not contain the RN2 gene. In pigs with this gene it would be expected that PSE-incidence would remain higher for longer and that DFD-incidence would remain lower for longer. Fernandez *et al.* (1992) confirmed that pH_u did not increase with overnight lairage, irrespective of whether or not pigs were held in mixed groups, when Hampshire crossbred pigs (with a high frequency of RN2 but no Hal^n gene) were used. On the other hand Martoccia *et al.* (1995) and Warriss *et al.* (1996) showed that respectively longer transport and longer lairage times increased pH_u relative to short transport and lairage times in pigs that were more or less free of the RN2 gene. The latter work also showed an increasing frequency of pigs with unacceptable skin damage which has previously been shown to be related to ultimate pH and DFD meat (Warriss and Brown, 1985).

The general scheme shown in Figure 5 is certainly valid at least up to 24 h after leaving the farm. Over longer periods - such as with transport over long distances and/or prolonged fasting periods, which can be with or without access to water - energy reserves

in muscle can be built up again from fat depots with consequences for meat quality. The published data (Lambooij, 1983, 1988; Lambooij and Engel, 1991) give conflicting results with respect to meat quality, although the effects on loss of body weight are quite consistent. Extensive British work in this field has shown that body weight loss begins 9-18 h after the last feed and continues thereafter at 0.1% per h at least up to 48 h (Warriss, 1982; Warriss *et al.*, 1983).

The scheme does not imply that increased stress levels at slaughter lead to a higher incidence of PSE in pigs. Some work does seem to show that severe short term stress, particularly just before slaughter, does increase the incidence of PSE in pigs with high energy reserves (Klingbiel and Naudé, 1976; Barton Gade, 1984), while Nielsen's (1981) and other unpublished Danish work seem to point to the fact that any increase in stress in animals that have already been subjected to many unfamiliar situations will only lead to a greater degree of exhaustion and hence a higher DFD-frequency.

Some on farm factors have been found to affect pig behaviour at abattoirs and hence energy consumption before slaughter. Rearing pigs in groups of 12 led to more aggression during 1 h of mixing in the lairage than pigs reared in groups of 36 and this increased ultimate pH levels but decreased reflectance values (Hansen *et al.*, 1989). Pigs raised outdoors do not show the same exploratory behaviour and lay down to rest more quickly in the lairage than pigs raised intensively indoors. Fighting, as such, did not occur in the lairage (Barton Gade and Blaabjerg, 1989). Meat from pigs raised outdoors had lower pH_u and a low incidence of DFD-meat but also had a tendency to higher internal reflectance values. Free range pigs must also be expected to have a better physical condition than intensively raised pigs. Essen-Gustavsson *et al.* (1988) showed that moderate exercise increased glycogen levels and oxidative capacity in muscles before slaughter, thus leading to the possibility of lower ultimate pH-values and less DFD-meat.

Two other factors not associated with the above scheme affect the incidence of PSE-meat: the method of stunning and the chilling regime. Only stunning method will be considered here. Danish experiments have shown quite consistently that CO_2-stunning systems, irrespective of type of equipment, give less PSE than electrical stunning systems (Table 6).

Table 6. Incidence of PSE in relation to stunning system: Overview (Barton Gade, 1996).

			L. dorsi			Biceps femoris		
	Stunning	No. of	Probe value			Probe value		
Factory	Method	pigs	Ave	s	% PSE[1]	Ave	s	% PSE[1]
1	CO_2-oval	3464	67	25.2	5.5	77	18.7	2.1
2	CO_2-oval	1696	68	22.5	3.8	79	18.3	2.5
3	CO_2-compact	3012	60	20.7	2.7	72	17.0	0.8
4	CO_2-compact	524	63	20.1	2.3	66	18.4	2.3
5	El-restrainer, 70V	255	81	26.9	12.2	76	18.2	2.0
6	El-restrainer, 70V	1612	77	35.2	10.5	81	20.7	4.0
	CO_2-compact	1760	65	20.9	3.1	78	19.3	2.6
7	El-restrainer, 300V	2639	82	33.3	18.5	84	28.8	15.1
	CO_2-compact	1587	66	21.9	4.0	71	17.5	1.4
8	El-restrainer, 700V	1174	83	30.0	15.1	85	23.3	9.0

[1]PSE was evaluated using a probe that measures internal reflectance values in the near infrared range; values ≥120 = PSE meat.

The experiments in Table 6 were carried out under practical conditions on pigs with a relatively low incidence of the halothane gene. In pig populations with a high incidence of the gene, no difference between stunning methods has been found, as the genotype dominates (Hölscher *et al.*, 1989).

One controlled experiment, which casts further light on the effect of stunning systems in relation to meat quality has been carried out in Denmark (Barton Gade, 1984). The aim of this experiment was not directly to investigate stunning systems as such. Its aim was to investigate the effect of a stressful treatment immediately pre-slaughter on pigs of known halothane genotype. However, different stress levels were attained by using different stunning systems. All other variables up to the point of driving to the stunning were the same ,i.e., breed, feeding/management, transport and lairage. Transport was short (40 min) and pigs went to stunning directly from the vehicle, so that variations in lairage conditions were eliminated. Treatment up to the point of driving to stunning was extremely considerate and no force, such as electrical goads, was used to move the pigs.

Some pigs were then slaughtered at abattoir A, where low voltage (70V) manual stunning in a restrainer was used. Conditions at the entrance to the race were less than optimal at this factory, so that a considerable amount of stress was unavoidable at this point. Passage through the race, which was about 15 m long and contained a sharp bend, also required force as did the transition between the race and restrainer. Slaughter speed was 190 pigs per h.

The other pigs were slaughtered at abattoir B either using low voltage (70V) electrical stunning on the floor or CO_2-stunning in the compact equipment. Conditions in abattoir B, which was used for training apprentices (slaughter speed 40-50 pigs per h) were relaxed, and stress before stunning was minimal, e.g., no electrical goads were used. With CO_2-stunning pigs were driven singly or in small groups into a short (ca. 5 m) race before entering the stunning equipment itself. The slaughter process was similar at both factories and both used traditional batch chilling.

The results, which are summarized in Table 7, indicate that halothane susceptible nn-pigs were relatively insensitive to changes in pre-slaughter treatment nearly always showing PSE-meat irrespective of stunning method. On the other hand heterozygotes (Nn-pigs) and pigs free of the halothane gene (NN-pigs) were highly affected by pre-slaughter treatment. Electrical stunning in a restrainer thus gave 53% PSE in Nn-pigs as compared to 25% for electrical stunning on the floor and 13% for CO_2-stunning. The corresponding figures for NN-pigs were 41%, 8% and 0%. Rigor development and pH-fall after slaughter were fastest with electrical stunning in a restrainer and slowest with CO_2-stunning.

Table 7. Meat quality in pigs of known halothane genotype in relation to pre-slaughter stress (Barton Gade, 1996).

Genotype	nn-pigs			Nn-pigs			NN-pigs		
Abattoir	A	B		A	B		A	B	
Stunning method	el-restr.	el	CO_2	el-restr.	el	CO_2	el-restr.	el	CO_2
% in full rigor [2]	63	48	19	31	17	0	8	0	0
% pH<5.9[2] l.dorsi	100	97	95	62	30	41	25	15	0
% pH<5.9[2] semimem.	69	69	62	17	25	6	25	0	0
% PSE[3]	100	93	90	53	25	13	41	8	0
% DFD[4]	0	0	0	2	0	0	0	0	0

[1]Handling at abattoir A was stressful; at abattoir B stress was minimal.
[2]Rigor and pH were measured objectively in *M. semimembranosus* 45 min after slaughter.
[3]Pigs were considered PSE if the soluble sarcoplasmic and myofibrillar proteins in *M. longissimus dorsi* and/or biceps femoris was <0.150 units.
[4]Pigs were considered DFD if at least 5 of the 7 muscles measured were higher than normal.

It should be stressed that the treatment in abattoir B in this experiment is much more considerate than that possible under normal abattoir conditions where slaughter speeds and stress levels are much higher. However, the results do supplement those found with the previous random sampling very well (Table 6).

There is no doubt that the higher PSE-frequency with electrical stunning is due to the stimulation of the carcass as the electrical effect is applied. Electrical stunning can therefore be considered as a form of electrical stimulation, which is used in other situations to accelerate post-slaughter processes. The clonic convulsions often seen after electrical stunning also contribute to a faster pH-fall (McGloughlin and Davidson, 1966; Overstreet *et al.*, 1975) and the use of cardiac arrest, which reduces these convulsions considerably, has been shown to reduce PSE-incidence in German pigs (Fehrenburg *et al.*, 1991). Similarly, Troeger and Woltersdorf, (1991) showed that convulsions during the excitation phase of CO_2-stunning increased when low (60%) CO_2-concentrations relative to high (≥80%) concentrations were used and this had a negative effect on pH-fall after slaughter in both halothane positive and halothane negative pigs.

Effect of pre-slaughter handling on mortality during transport and lairage

Mortality during transport and lairage is highly affected by the frequency of the halothane gene in a pig population. A comparison of transport mortality in seven European countries carried out in 1993 showed clearly that mortality was highest in countries where a significant proportion of the population was halothane sensitive (Table 8).

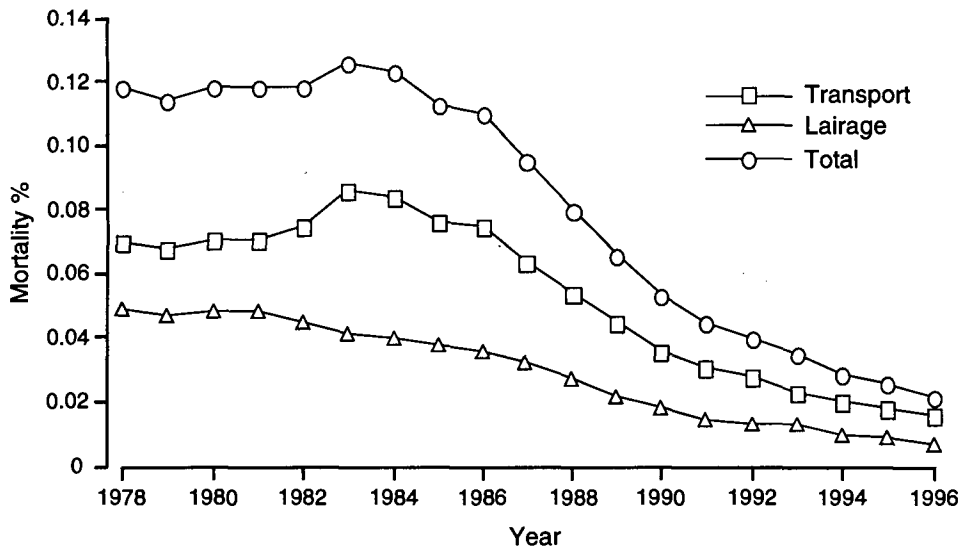

Figure 7. *Transport and lairage mortality statistics for Danish pigs (Baltzer, 1997; unpublished material).*

In Denmark efforts to eliminate the gene from the breeding pig population were started in 1986 and the effect of this can clearly be seen in the annual mortality statistics in all slaughter pigs (Figure 7). In 1996 transport mortality reached a new low of 0.015% and lairage mortality 0.006%.

Within any one genotype transport and lairage conditions do have an effect on mortality. Nielsen (1981) showed that mechanical ventilation in single decked transport vehicles reduced transport mortality by almost 50% compared to transport in similar vehicles using natural ventilation only. Christensen and Barton Gade, (1996, unpublished material) showed that transport deaths occurred only in the front compartment of the lower tier in a double-decked vehicle optimised for pig comfort, i.e., in the compartment

with the least effective ventilation. Group size in lairage pens affects lairage mortality, and keeping pigs in groups of 15 corresponding to compartment sizes on most Danish vehicles reduced mortality by more than half (Christensen, 1991, unpublished material). Small group sizes reduce fighting and hasten resting behaviour (Barton Gade *et al.*, 1992) and this result would seem to indicate that lower stress levels do reduce mortality in lairage. Extreme stress, which often occurs at the entrance to or in races can increase heart rates so much that the heart goes into fibrillation. Deaths occurring in the race area are undoubtedly due to heart failure.

Table 8. Transport mortality in relation to halothane genotype (Christensen et al., 1994).

Country	Pig population	Transport mortality
Portugal	mixed	0.16%
Italy	halothane negative	0.10%
Belgium	halothane positive[1]	0.30%
Germany	halothane positive	0.50%
Holland	halothane negative	0.16%
UK	halothane negative	0.09%
Denmark	halothane negative	0.03%

[1]Tranquillizers used during transport

Trauma, bruising and blood splashing

Trauma, bruising and blood splashing are mainly caused by pre-slaughter handling, whereas genotype and on-farm treatment play only minor roles.

Bruising and broken bones with massive haemorrhaging are due to inappropriate handling procedures, where excessive force has been used or to poorly maintained facilities, such as gaps in walls, where pigs can become trapped, or gates that come down on pigs with too much force. Forcing pigs down steep slopes also increases the risk of broken bones. Slippery flooring, whether in the transport vehicle or at the abattoir, can cause hip dislocation.

Forcing large pigs through small races causes bruising on the back and sides of the animals, and race profiles that prevent animals getting under and over one another in the race will reduce bruising in loins. The design proposed by Grandin (1982/83) is a good example of this.

The use of the V-belt restrainer where pigs are held, feet off the floor, and automatically moved to electrical stunning has also been implicated in increasing skin damage and bruising especially for pigs that do not enter the restrainer willingly and those that do not lie quietly during restraint. Similarly, the restrainer in the compact equipment, where the floor falls away as the gondola descends into the pit, has been shown to increase skin damage compared to CO_2-stunning with no race system or restraint (Blaabjerg *et al.*, 1990, unpublished material). The new development of a band restrainer for pigs (Lambooij *et al.*, 1992) is said to reduce this problem for electrical stunning and in the present CO_2-equipment, at least in Europe, no restraint is allowed.

Superficial skin damage, which in the worst instances also affects underlying tissue, is mainly due to aggression when unfamiliar pigs are mixed or, in the event of entire males being produced, by animals riding one another.

Trauma, bruising and blood splashing can be affected to some extent by on-farm factors. Some pigs are excitable and difficult to handle which will increase the need for force to move animals forward and hence the risk of bruising and other damage. The reason for this excitability is complex and there are probably both genetic and management components. Grandin (1986) showed that regular positive handling and the use of toys such as chains and pieces of rubber hose in farm pens to relieve boredom made pigs easier to handle in general. Positive handling during fattening has also been shown to affect a pig's response to other humans and the behavioural response of a pig to

one handler is likely to be extended to other handlers (Hemsworth *et al.*, 1994, 1996a, 1996b).

Skin damage is also affected by on-farm factors. Group sizes on farms and whether pigs are raised outdoors or raised intensively indoors have been previously mentioned. Entire males are known to be more aggressive than females and castrates. In addition, some pig breeds have been found to be more aggressive than others. A survey of purebred animals, which had had little opportunity for aggression (no lairage), showed that unacceptable skin damage was particularly associated with the Large White breed (Table 9) and it has been commonly observed that crossbreeds containing Duroc and/or Hampshire are less aggressive than crossbreeds containing Landrace and Large White only. However, there is a wide variation in aggressiveness within a breed or crossbreed, which may indicate a genetic component.

Table 9. Incidence of unacceptable skin damage in relation to breed (Barton Gade, 1984; unpublished material).

Breed	Landrace	Large White	Duroc	Hampshire
No. of pigs	1142	1553	565	148
Skin damage, %	1.0	4.9	0.2	0

At present the aetiology of blood splashing is not known completely. Two types are distinguished, the so-called petechial haemorrhages which are circular up to pea-sized (0.5 cm) and the so-called diffuse haemorrhages which are patches of varying shapes up to 5 cm in diameter (Warrington, 1974). Blood splashing depends on the type of stunning process used; electrical stunning leads to more blood splashing than CO_2-stunning (Table 10).

Table 10. Grams of blood splashed meat per shoulder in relation to stunning system (Nielsen, 1976; unpublished material).

			El-restrainer			CO_2		Pre-slaughter
Expt[1]	Abattoir	El-floor 70V	70V	Manual 300 V	Autom. 700V	Oval	Comp	treatment
1	A		201					1-2 h transport 1 h lairage
2	A		278					Unknown
	B		193					
	C	123						
3	D		194			37		2-3 h transport 1-6 h lairage
4	E					20		2-3 h transport
	F						5	1 h lairage
5	G			145				Unknown
	H				59			
	I						8	

[1]Pigs were randomly chosen at different abattoirs, normally there was only one stunning method per abattoir. The number of animals per experimental group was above 500 (Expts 1-4) or above 145 (Expt 5).

Diffuse haemorrhages occur in specific parts of the carcass with the shoulder area being particularly affected. Blood pressure increases much more with electrical stunning than with CO_2 and it is likely that the differences between the stunning methods with respect to blood splashing are caused by the violent contraction that occurs as the electrical current is applied in combination with the higher blood pressure.

Fractures without massive bleeding, sometimes without bleeding at all, are found exclusively with electrical stunning, again as a result of the initial application of electrical current. Klovborg Larsen (1982) showed that high voltage stunning led to fractures in over 1% of the pigs and it is well known that electrical stunning on the floor, irrespective of voltage used, can give blade bone fractures, if stunning personnel do not follow the pig down properly. Electrode placement is extremely important for just exactly where fractures occur. Automatic electrode placement behind the ear mainly leads to spinal fractures but, if head to back stunning is used, they can occur in the hip area and even in the leg depending on the position of the rear electrode. Wotton *et al.* (1992) confirmed the importance of electrode placement for fracture positions.

Effect of pre-slaughter handling on welfare

The previous sections have considered many of the aspects that are important for pig welfare pre-slaughter, since treatment that leads to low incidences of DFD (and perhaps PSE) meat, skin damage and trauma as well as transport and lairage mortality implies better welfare. However, such a conclusion does not take the psychological factor into account. Moving pigs at the high speeds used on many abattoirs today is visibly quite stressful to pigs as noise levels are high, especially on transfer from lairage pens and during the lining up process immediately before stunning. Many such pigs will give good meat quality if genotype is optimal and the pig avoids damage, so that welfare aspects are insufficiently addressed using these measurements. Apart from behavioural studies and registration of noise levels physiological measurements have been used for this purpose.

Blood and saliva cortisol concentrations or heart rate are often used as a measure of psychological stress, and blood lactate or creatine kinase (CK) concentrations as a measure of physical stress (Broom, 1996; Grandin 1997). Lysine vasopressin measured in plasma is used as a measure of travel sickness during transport (Broom, 1996). Physiological measurements can give information on differences among given treatments but they still have the disadvantage that absolute values corresponding to unacceptable or optimal welfare are not known exactly. Moreover, other factors than the ones being investigated may affect levels, e.g., the halothane genotype has been associated with higher blood CK concentrations. Lactate concentrations can be affected by the degree of fighting to which a pig has been subjected. One possible approach is to use resting concentrations as a basis for comparison but this presupposes that pigs should not be stressed at all during the pre-slaughter handling period. In practice, of course, all animals will be stressed in some way on leaving the home pen, being exposed to unfamiliar individuals and unfamiliar situations such as transport and lairage. The important thing is to ensure that stress levels are as low as possible throughout the handling period. The decision as to exactly what constitutes good welfare in the last instance is a moral one reflecting the ethics of society (Broom, 1988).

It is generally accepted that loading and off-loading are the most stressful parts of transport and many studies have shown that heart rate increases at loading and then gradually falls as the pig becomes accustomed to the transport, only to rise again at off-loading (Schütte *et al.*, 1996; Christensen and Barton Gade, 1996). Moreover, careful loading/off-loading lowered heart rates compared to a more conventional treatment, but only slightly. Bradshaw *et al.* (1996a) showed that plasma cortisol concentrations increased in pigs after loading and remained higher for longer for rough rather than smooth journeys. Plasma concentrations of lysine vasopressin coincided with behavioural observations of travel sickness. The quality of the transport vehicle, in particular its vibration characteristics, have been found to be extremely important for pig comfort during transport (Randall *et al.*, 1996) and the vehicle used in this work was not optimal in that respect. Bradshaw *et al.* (1996b) also investigated the effect of mixing unfamiliar pigs during transport to slaughter in a commercial livestock vehicle for 1.5 h. Plasma cortisol was highest in the mixed groups.

Similarly, it is generally accepted that ventilation in transport vehicles should be sufficient for the climatic conditions encountered and ventilation requirements have been based on practical experience rather than scientific research. Hence, transport vehicles in

Finland, where outside temperatures can vary from -30°C in winter to +30°C in summer, are enclosed with forced ventilation, whereas vehicles in Spain and Portugal, where summer temperatures can reach +40°C are open with natural ventilation only. Danish research into optimal ventilation openings and tier heights (Barton Gade and Christensen, 1996, and unpublished material) showed relatively little effect of the openings and tier heights used on blood cortisol, lactate or CK concentrations for transport in a two-tiered vehicle with optimal vibration characteristics (Randall *et al.*, 1996) driven over good roads. However, extremes of temperature were not encountered in this work, the temperature range being mainly between +5°C and +25°C. Natural ventilation only was used in these experiments to reflect current practice but the vehicle's forced ventilation was used in the routine transports when temperatures were above 25°C, as was the showering (misting) system, which was used intermittently. Present recommendations are to use forced ventilation and intermittent misting to cool pigs at temperatures above 25°C but the effects of these procedures have not yet been scientifically investigated.

Optimal stocking densities during transport are still a matter of debate. The survey of transport conditions in seven European countries (Christensen *et al.*, 1994) showed that the stocking density lay between 0.35-0.39 m^2/100 kg pig for the majority of journeys (Table 11).

Table 11. Stocking density during transport in seven European countries.

Country	Stocking density m^2/100 kg pig
Portugal	0.36 - 0.38
Italy	0.37 - 0.40
Belgium	0.38 - 0.39
Germany	0.36 - 0.46
Holland	0.32 - 0.35
UK	0.37 - 0.49
Denmark	0.34 - 0.36

Lambooij *et al.* (1985) suggested a figure of 0.425 m^2/100 kg pig as a suitable compromise between welfare, meat quality and transport economy for journeys of long duration (2 d). Guise and Penny (1989) compared 0.3 and >0.4 m^2/100 kg pig for a 200 km journey and concluded that welfare was compromised at the highest stocking density. Skin blemish scores were higher and fibre optic probe values lower with 0.3 m^2/100 kg pig, probably as a result of higher ultimate pH values. Guise and Warriss (1989) compared 0.3 and 0.4 m^2/100 kg pig for a 192 km journey and found no significant effect on meat quality, as did Nanni Costa *et al.* (1996) in a comparison of <0.4 and >0.6 m^2/100 kg pig. Christensen and Barton Gade (1997) compared four different stocking densities, 0.35, 0.39, 0.42 and 0.50 m^2/100 kg pig for journey times of 2-3 h and found that plasma cortisol and lactate were unaffected but that 0.50 m^2/100 kg pig resulted in lower CK concentrations. This was not, however, accompanied by differences in ultimate pH levels. Of the meat quality characteristics investigated significant effects were only found for skin damage, being least with 0.35 m^2 /100 kg pig and most with 0.42 m^2/100 kg pig. However, for most characteristics measured there was a significant interaction with day of experiment, indicating that factors other than stocking density have affected the results. Pigs could support one another at 0.35 and 0.39 m^2 /100 kg pig but had difficulty maintaining balance on cornering or on rough roads at 0.42 and 0.50 m^2/100 kg pig. More pigs sat and lay down at the higher stocking densities in this work. Follow-up behavioural studies have confirmed this pattern for journeys of up to 2.75 h, and shown moreover that pigs will fight during transport if given the space to do so. Fighting during transport was also seen by Bradshaw *et al.* (1996) when pigs were mixed. Optimal stocking densities will undoubtedly vary with transport time, genotype and climate, so that it is not possible to give standard values which are applicable for all types of journeys.

Investigations of treatment at the abattoir itself have mainly concentrated on optimal lairage times and stress levels on movement to, and through, stunning systems.

Lairage times vary widely in practice, from zero, when pig supply is outstripped by pig slaughter, to overnight, when a reserve is needed to start-up next morning. In Europe average lairage times are usually short. Geverink *et al.* (1996) showed that average lairage times in five Dutch abattoirs varied from 1-2 h, and in four Belgian abattoirs the average was about 1.5 h. In Danish abattoirs the average is about 1 h. Holding pigs for such short periods does not allow them sufficient time to recover from the stresses of transport. Warriss *et al.* (1992) showed that 2-3 h were necessary to reduce cortisol concentrations significantly. Optimal lairage times are, of course, dependent on environmental conditions and Santos *et al.* (1996) showed that at temperatures above 35°C and 85% relative humidity pigs should be slaughtered within 30 min of arrival. However, this conclusion was based on meat quality rather than physiological measurements.

In lairage, stocking densities of about 0.55 to 0.67 m^2/100 kg pig have been recommended (Warriss, 1994). In Danish experience 0.50 m^2/100 kg pig is sufficient when pigs only need access to water. Group sizes in lairage pens are generally large - 40 to 60 individuals and this has been found to reduce resting behaviour, as animals which fight disturb others that are resting (Barton Gade *et al.*, 1992; Geverink *et al.*, 1996). Fighting increases when unfamiliar pigs are mixed (Guise and Penny, 1989; Karlsson and Lundström, 1992; Warriss *et al.*, 1992), and Warriss and Brown (1985) showed that lactate increases significantly in pigs that had been fighting before slaughter. Dividing lairage strings into pens of 15 has been found to reduce aggression and promote resting behaviour in Danish pigs, despite the fact that the group could be composed of pigs from different farms or from different pens on the same farm. Using a group size of 15 corresponds to compartment sizes in most Danish vehicles, i.e., there is no further mixing of individuals at the abattoir. The ideal would, of course, be no mixing at all during transport and lairage but this is generally not possible in practice.

The use of water sprays or misting systems to cool pigs in hot environmental conditions affects pig behaviour in lairage pens, especially when this is used intermittently to maximise evaporative cooling (Weeding *et al.*, 1993). Cooling reduces surface and rectal temperatures and respiratory rate in pigs. Heart rates are also reduced and the effect was observed at environmental temperatures as low as 10°C in German pigs (Schütte *et al.*, 1996).

Movement of pigs from the lairage to the stunning area, particularly the lining up process immediately before stunning is very stressful for pigs, and stress levels increase with higher slaughter rates. Warriss *et al.* (1994) showed that sound levels immediately before stunning increased at higher slaughter rates, and that blood lactate and CK levels were elevated in pigs subjectively assessed as stressed. Line speed varied between 100 and 300 pigs per h in this work, i.e., still relatively moderate. Weeding *et al.* (1993) showed that there were differences between abattoirs with respect to CK and cortisol concentrations in blood and that the difference was due to a combination of system design and personnel experience at the factories concerned. There is much published work on optimal design of this area (Grandin, 1982/83). All authors stress the need to adapt plant design to pig behaviour to facilitate forward movement and this aspect becomes increasingly important as slaughter rates increase.

Adapting plant design to pig behaviour has been extensively researched in Denmark during the last 10 years, both in the lairage itself and in the area immediately before stunning. In this work it has been the aim to adapt lairage design to accommodate pig behaviour rather than the reverse. If systems are adjusted so that they facilitate forward movement of pigs, then high slaughter rates can be achieved without undue use of force. In fact, the aim of the research was to eliminate the use of force entirely, including that of electrical goads, but with the exception of guidance from push hoist gates. Such gates must clearly be finely adjusted to prevent animals becoming trapped in the system and generally all gates are adjusted so that they stop when a counterpressure of 100 kg is attained.

It proved impossible to move large groups of pigs from lairage pens without undue use of force, simply because it was impossible for personnel to reach pigs causing a blockage. Thus, keeping pigs in smaller groups was the only option. A system was therefore developed that divided lairage strings into groups of 15 pigs using a series of flap gates

and a push hoist gate for moving pigs into and out of compartments (Barton Gade *et al.*, 1992; Christensen and Barton Gade, 1997). Filling this system presented no problems whatsoever. Pigs moved calmly and noise levels were equivalent to background level. Emptying the small pen system is also easy. The push hoist gate moves above the heads of the resting pigs as the flap gates open, thus alerting them, so that many stand up and move along of their own free will. Correct lighting facilitates this forward movement. Both filling and emptying pens is thus carried out without human intervention and with a minimum of force being used, and is a major improvement in pig welfare. Heart rate measurements could reach low levels (90 bpm) in resting pigs. The fully automatic system has now been running since 1989 at one Danish factory, where pig movement out of the lairage is presently carried out at a rate of 800 pigs per h. At lower speeds fully automatic systems can be replaced by manually operated systems, that retain the welfare level provided that design and utilisation is optimal.

Solving the problems in the lairage highlighted the difficulties that occur in the lining up process and this formed the basis for a final development - how to get pigs automatically from the small pen system to and through stunning with no use of force other than guidance from moving gates. This precludes the use of a race completely. The system was also designed to eliminate two other known stressors: isolation and restraint. Pigs are social animals and do not like to be separated from one another. Restraint, such as that seen in V-belt restrainers, is known to increase adrenaline and noradrenaline concentrations relative to that in pigs electrically stunned standing freely on the floor (Troeger and Woltersdorf, 1989). Moreover, post-slaughter glycolysis is accelerated (Hunter *et al.*, 1994). The alternative rail restrainer (Lambooij *et al.*, 1992) is probably an improvement to the V-belt restrainer, as pigs are mainly calm on the rail. The newly developed system assumes therefore that stunning occurs in groups of pigs that are not restrained in any way. Thus, CO_2-stunning is used.

The total system, which has been tested in practice with 400 pigs per h but with a potential of 800 pigs per hour, is comprised of three main elements:

- an area where groups of 15 pigs divide themselves into smaller groups
- automatic transfer of these smaller groups to and through the stunning equipment
- a system for presenting the stunned animals for shackling and sticking.

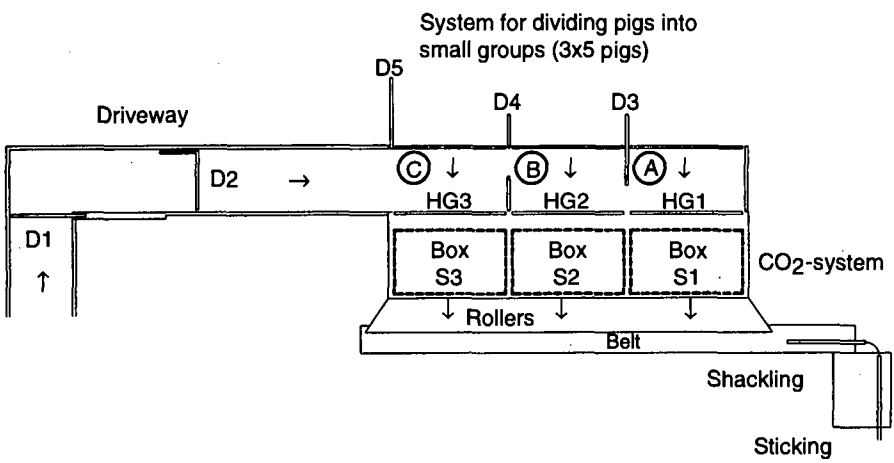

Figure 8. *Drawing of the experimental system (Christensen and Barton Gade, 1997).*

Pigs are driven manually with a pig board in groups of 15 into the system and a sliding door, D1, is activated to close off the system. Pigs advance into areas A, B and C and at the same time push hoist gate, D2, moves behind the pigs. Video cameras are mounted above areas A and B. The number of animals are counted and when 5 have entered A the sliding gate D3 closes and similarly for area B and gate D4. The remaining pigs are brought up to area C by D2, and D5 is closed.

In any group of 15 pigs there are 2-3 leaders, a number of followers and 2-3 timid pigs and it is important that the leaders do not lose contact with the followers or the flow will be interrupted. The labyrinth design slows down the lead pigs and keeps the group together. Push hoist gate, D2, ensures that timid pigs enter area C. Again all moving gates/parts are designed to stop at a maximum counter pressure of 100 kg so that pigs are not forced forward or trapped in the system.

After subdivision, hoist gates HG1, HG2 and HG3 are raised and the opposite walls in the dividing area guide the pigs into the stunning boxes, S1, S2 and S3. When the walls reach their final position, the box wall is raised from the floor to close off each box and the boxes are lowered sequentially into CO_2. Pigs are rapidly exposed to high concentrations of CO_2 (90% within 15 secs) and the through time is 120-140 secs. This length of exposure allows a long stun-stick interval to be used, i.e., up to 60 seconds, without pigs regaining consciousness before death intervenes via exanguination. This long stun-stick interval was necessary in the experimental set-up, as the pigs are not orientated when they are tipped out of the system and those from boxes B and C have a certain distance to travel before reaching the point of shackling. When the box regains the top position with the stunned pigs, they are tipped out on to rollers, where they glide down on to a rapidly moving belt, which moves them along to the shackling point. Pigs are then shackled and stuck.

Pigs moved through the system easily without apparent stress, no vocalisation and no intervention from slaughterhouse personnel. Noise level was on average 85 dB(A), i.e., background noise. Heart rates actually fall as pigs move through the system from an average of 155 bpm as they leave the lairage pens to 149 bpm after division into stunning groups to 136 bpm on transfer to the stunning box.

The welfare aspects of stunning are still under debate. Here, an optimal process is one that leads to a rapid loss of consciousness and a degree of unconsciousness that ensures that the animal never regains consciousness before death intervenes via de-bleeding. Two methods are in use for pigs: electrical stunning and CO_2-stunning.

In theory the induction of unconsciousness is very rapid with electrical stunning, although some concerns have been voiced as to how fast animals actually lose consciousness (Gregory and Wotton, 1985; Anil, 1991). This presupposes, however, that sufficient current passes through the brain immediately on application of the tongs. In the real world, tongs are sometimes placed incorrectly, insufficient current is often used, there can be repeat stunning and contact to the skin can be poor. Pain before loss of consciousness and in the worst instances paralysis without loss of consciousness can be the result. The use of automatic systems and restraining devices eliminate many of these problems but introduce a new stress factor - restraint. There is a conflict between requirements for a correct stun and requirements for a low stress environment immediately pre-stun that have not yet been solved for electrical stunning. The new rail restrainer (Lambooij *et al.*, 1992) is a step in the right direction.

Fast stun to stick intervals are necessary with electrical stunning (within 15-20 seconds) to prevent return of consciousness before death intervenes via exanguination. The use of cardiac arrest eliminates this problem.

CO_2-stunning suffers from the disadvantage that loss of consciousness is not immediate. There are three phases in CO_2-stunning:

- a phase of analgesia, where the pig progressively loses the ability to feel pain
- a phase of excitation, which sets in immediately after the animal loses consciousness, where there is uncoordinated movement and vocalisation from some animals
- a phase of anaesthesia, where the animal is deeply unconscious.

The first phase can be as short as 10-12 seconds, the second as short as 6-8 seconds but depends on the CO_2-concentration used. In paternoster systems with CO_2 concentration of 85% at the bottom of the pit the first phase is typically between 20-25 seconds. Research carried out in Sweden and Germany has clearly shown that pigs become unconscious immediately before the phase of excitation begins (Forslid, 1987; Ring *et al.*, 1988), so that welfare concerns are restricted to the first phase, where pigs increasingly lose their ability to feel pain.

In humans high concentrations of CO_2 are pungent and cause breathlessness (Gregory, cited in Lambooij, 1990). Research carried out under laboratory conditions has shown that the majority of pigs will avoid an atmosphere of high concentrations of CO_2 if given the choice to do so (Raj and Gregory, 1995). Raj and Gregory (1996) have interpreted the increase and depth of respiration as respiratory discomfort. The same authors also reported that some of the pigs exhibited escape attempts during exposure to CO_2. On the other hand research carried out under both laboratory and practical conditions in Germany, Sweden and Denmark show that pigs react very little to exposure to high CO_2-concentrations provided that exposure is rapid (Ring *et al.*, 1997; Barfod, Ring and Erhardt, Barton Gade - cited in Lambooij, 1990; Troeger and Woltersdorf, 1991; Barton Gade, 1996). These workers consider the faster and deeper respiration as an advantage because it facilitates the uptake of CO_2 and shortens the time to loss of consciousness. The lack of reaction under optimal conditions could be due to fast induction of analgesia so that, in effect, pigs do not feel discomfort during this phase. However, this has yet to be confirmed via investigation.

Alternatives to high concentrations of CO_2 have been suggested (Raj and Gregory, 1995, 1996). 90% argon in air or a mixture of 60% argon and 30% CO_2 in air eliminate oxygen and there is no reaction from the pig during the phase before loss of consciousness occurs. However, killing rather than reversible stunning is recommended with these gases, as return to consciousness can be rapid, and the time needed to ensure killing means that existing CO_2 stunning systems cannot be used. Moreover, the convulsions that occur after loss of consciousness are extreme and may affect meat quality.

Codes of Practice for pre-slaughter handling of pigs

Any code of practice for the pre-slaughter handling of pigs must emphasise a considerate treatment, which at the same time leads to a certain decrease in energy reserves. Fighting among pigs should be minimised at all times. Improvements in handling must be accompanied by genetic improvement via breeding programmes to keep the number of susceptible pigs in the population at an acceptable level for the maximum effect to be obtained.

In Denmark the knowledge gained by the Danish Meat Research Institute during many years of research has been assembled in a 13 point programme which was introduced at the beginning of the 1980's. This programme lays down guidelines for producers, hauliers and abattoir personnel which ensure the following:

- good welfare
- good, uniform meat quality
- low transport and lairage mortality
- delivery ensuring protection of a herd's health
- rational collection and transport.

Producers must slap-mark the pigs before the day of collection. The use of dye is recommended as this means that the tattoo number remains visible and slap-marking can be carried out at feeding or weighing pigs at a time convenient to the producer. The pigs should not be fed in the 10-12 h before collection but have access to water. They should be held away from the main herd in such a way as to prevent fighting and there should be good access to the loading area.

Hauliers must provide a well-equipped vehicle with good loading facilities (normally a tail-gate lift), adequate ventilation and non-slip flooring. There should be at least one partition but preferably two. All lorries must be cleaned before leaving the factory and they must not be overloaded. Driving should be careful with no unnecessary stops on the way. Treatment should be considerate at all times.

Factories should have good off-loading facilities such as an adjustable ramp. Electric goads should not be used to get the pigs off the lorry or when moving them from the off-loading ramp to the lairage pens. Pens should have good ventilation, solid walls and watering facilities. The holding period before slaughter should be at least 2-3 h. Pigs should be driven to the race considerately by allowing them as far as possible to move by themselves. At the entrance to the race the use of an electric goad may be necessary for

some pigs but with a well-designed facility at least 70% should enter of their own volition. Stunning should be with CO_2, preferably in the Combi og Jumbo equipment, i.e., systems which hold more than one pig in the gondola. Finally, there should be a person at the factory who is responsible for the implementation of the programme. Management commitment to good handling is absolutely essential for the success of the programme.

This Code of Practice reflects Danish collection, transport and abattoir conditions and modifications will be necessary for use in other countries.

Future developments in pre-slaughter handling

What developments can be expected during the next 12 years or so considering that the meat industry is rather conservative and that generation interval in lairage equipment at abattoirs is of the order of 20-30 years. Several trends affecting welfare and meat quality are to be expected.

Farms are increasing in size and integrated systems where the farmers have their own genetic programmes, multiplication units, feed mills and production units, sometimes directly affiliated to abattoirs, are becoming more common. These developments mean that tailormade transport vehicles that ensure pig comfort to the greatest possible extent become more economically viable and integrated systems allow the production of a pig with the right meat quality characteristics for customers. Abattoir slaughter rates must be expected to increase in general with negative consequences for welfare and meat quality unless systems adapted to pig behaviour are installed.

Ordinary consumers will place increasing emphasis on welfare and demand certain minimum standards, when they buy meat. It is not the actual buyer in the shop who makes the specifications but the supermarket chains, who take care of consumer requirements. This development can be seen in full swing in Northern Europe, where the various supermarket chains compete for consumers with a proliferation of "welfare" productions. These can range from minor changes on farm to the production of organic, free range pigmeat. Most of these concentrate mainly on on-farm conditions but there is now an increasing emphasis on welfare during transport and at the abattoir with factors such as no mixing of unfamiliar animals, holding in small groups at the abattoir and no use of electric goads at all, being emphasised. How fast the new developments described in this paper will be implemented will depend mainly on consumer pressure in this area, although abattoirs renovating or building lairage pens will incorporate the latest developments to a greater or lesser extent.

A similar development will take place with meat quality where industrial buyers will make increasingly detailed demands regarding the quality of the meat that they buy, especially that for further processing. Emphasis here is on final yields and quality of processed product. Different markets have different requirements depending on the processed products they manufacture, but most will emphasise low levels of PSE and blood splashing/bruising as well as a certain ultimate pH-level. Here developments will depend mainly on the willingness of buyers to pay a premium on meat produced according to specification.

Conclusions

Pre-slaughter handling procedures have a profound effect on pig welfare and meat quality factors such as the incidence of PSE and DFD, bruising and blood splashing. The interaction between pig genotype and energy reserves at slaughter is found to be the deciding factor for the incidence of PSE and DFD in a pig population - at least until 24 h after leaving the farm, whereas stunning method seems to be most important for blood splashing, least with CO_2-stunning.

The quality of transport vehicles, particularly the ventilation and vibration characteristics are important for pig welfare during transport and systems at abattoirs that reduce aggression and promote resting behaviour and that are adapted to pig behaviour promote welfare at the abattoir. Welfare is highly affected by slaughter rate with more force being necessary as rates increase. Systems are now being developed that are adapted to pig behaviour and that run at high slaughter rates and are thus an option for the future.

SYMPOSIUM CONCLUSIONS

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The most important factor that will drive improvements in pork quality will be value based marketing - payment for quality. Processors in the USA have used routine measurements of the quality of incoming product to convey dissatisfaction with product quality to abattoirs. This approach was successful in driving changes in abattoir practices and resulted in a reduction in quality defects. The satisfaction of the end-user with the product obviously determines the long-term viability of the industry. There is increasing pressure in the pig industry to improve quality and the definition of best practice has identified a number of gaps in our knowledge.

Hermesch (1997) described genetic influences on pork quality including the halothane and RN genes and breed differences in intramuscular fat content and boar taint. The use of marker assisted selection is recommended as a valuable tool for the future. Ignoring meat quality traits in breeding programs will lead to a further decline in these traits. It would be a short-sighted industry which chose to exclude meat quality from selection indices as the inevitable result would be a decline in pork consumption.

Pethick *et al.* (1997) described the role of nutrition in determining meat quality and the potential to strategically feed compounds to maximise quality. The control of glycogen metabolism in relation to the occurrence of PSE and DFD is discussed including the use of therapeutic substances such as tryptophan and magnesium to alter the stress response and reduce glycogen depletion. The reduction in subcutaneous fat levels in the pig industry has been to the detriment of eating and processing quality. Many of the flavour components reside in the fat and the composition of the fat determines the acceptability in appearance and the healthiness to the consumer. Carcass fat can be influenced by even moderate changes in dietary fat because in the lean pig, subcutaneous adipose tissue is principally derived directly from dietary fatty acids. Dietary copper and biotin influence the type of fatty acids which accumulate in the subcutaneous fat stores. Dietary fat source has less effect on the composition of fatty acids in intramuscular fat compared to subcutaneous fat. The potential for altering fat deposition and fat quality through dietary inclusion of conjugated linoleic acid or Vitamin E are also discussed. There is potential in the pig industry to use dietary manipulation to produce novel pigmeat products for niche markets and this should be further explored. The paper highlights the limitations in our present knowledge and stimulates a lateral-thinking approach to the relationship between nutrition and pork quality.

Pig meat production in Denmark has emphasised quality as well as quantity for a number of years. Barton Gade (1997) discusses the change in definition of quality to accommodate the requirements of different customer groups. The strength of the pig industry in Denmark is linked to the degree of integration with close links between research and industry. Barton Gade (1997) includes ethical aspects of pork production as an essential component of quality to the consumer. The importance of transport, pre-slaughter conditions and stunning methods in influencing meat quality is discussed. Good welfare of the pig equates to excellent meat quality. Thus in Denmark, the occurrence of poor quality is a direct indication of unacceptable practices. The insistence by consumers on welfare in meat production in Denmark and other European countries will inevitably occur in Australia. Barton Gade (1997) also discusses aggression, blood splash, skin damage and trauma during the marketing process and highlights the detrimental indications for welfare. The need to adapt the design of abattoir handling facilities to pig behaviour to facilitate forward movement is highlighted. The new system for pig movement from pen to slaughter is presented which is fully automatic, does not require human intervention and maximises pig welfare. Barton Gade (1997) emphasises that the commitment of management to good handling is essential for the success of the program. Future developments are predicted to include tailormade transport vehicles, negative consequences of increased slaughter rates on pork quality, increasing consumer demand for welfare and increasing intolerance of poor quality.

In the year 2010, the pig industry will look back in amazement to 1997 and realise how its perceptions and definition of quality have changed. The industry will be measuring quality and the variation in quality will be minimal. There will be a precise definition of the critical control points between conception and consumption which influence quality using a 'Palatability Analysis of Critical Control Points' approach. Our domestic and export markets will demand nutritious healthy pork which is produced under clean and green condition. This will be achieved through continuous advancements in the manipulation of genetics, nutrition and pre-slaughter handling to maintain a prosperous pork industry.

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EFFECT OF STUNNING METHOD ON PIGMEAT QUALITY

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Improved methods for stunning of pigs have the potential to improve carcass and meat quality, consistency of product and animal welfare. In this study the effects of carbon dioxide (CO_2) stunning on carcass and meat quality factors in comparison to both head only (HO) and head to brisket (HBR) electrical stunning were examined. A total of 30 Large White x Landrace entire male pigs were randomly allocated immediately prior to slaughter to one of three stunning treatments: carbon dioxide (90% CO_2); HO (1.3 amps for 4 seconds) electrical stunning; HBR (1.3 amps for 4 seconds) electrical stunning. Pigs were placed in a V-restrainer immediately prior to electrical stunning. Muscle pH and temperature decline post slaughter were measured at 40 min (pHi), 90 min, 3 h, 6 h and 24 h (pHu) post slaughter. Drip loss, tenderness (Warner-Bratzler (WB) shear force, kg) and surface lightness (L* value) were measured on the *M. longissimus thoracis et lumborum* (LTL) at 24 h post slaughter. The rate constant (k) for pH decline was determined by fitting pH data to an exponential decay equation. All carcasses were dissected 24 h post slaughter and the amount of meat affected with ecchymosis (blood splash) in shoulder primals was removed and weighed.

Table 1. Effect of stunning method on meat and carcass quality traits.

	Meat quality attributes (LTL)						Carcass quality defects
Stunning method	k	pHi	pHu	L* value	Drip loss (%)	WB shear force (kg)	Ecchymosis (g meat/shoulder)
CO_2	0.21^b	6.62^b	5.60	49.73^b	2.78^b	7.49^{ab}	8^b
HO	0.42^{ab}	6.57^b	5.61	47.29^b	2.93^b	8.12^b	102^a
HBR	0.69^a	6.23^a	5.60	50.47^a	4.51^a	6.57^a	117^a
s.e.d.	0.15	0.08	0.03	1.14	0.40	0.49	
P	P<0.05	P<0.001	NS	P<0.05	P<0.001	P<0.001	P<0.05[1]

[a,b]Means within columns with different superscripts are significantly different (P<0.05).
[1]Chi-square analysis for equality of means.

Muscles from HBR stunned pigs had a faster rate of pH decline compared with CO_2 stunned pigs; HO stunned pigs were intermediate (Table 1). Muscle pH measured at 40 min post slaughter was also lower in HBR stunned pigs compared with CO_2 and HO stunned pigs. Muscle from HBR stunned pigs was paler (higher L* value), had a higher drip loss and was more tender compared with muscles from HO stunned pigs. The pHu of muscles from pigs in all three stunning treatments were similar.

Differences in meat quality due to stunning treatment were probably directly related to the rate of muscle pH decline during the early post mortem period. As a faster rate of pH decline post-slaughter was found in LTL muscles from HBR stunned pigs, HBR stunning may stimulate LTL muscles to a greater extent compared to those in HO and CO_2 stunned pigs, which may in turn influence drip loss. Carcass quality was improved by stunning with CO_2 as less meat was affected by ecchymosis in shoulder primals compared with electrically stunned pigs. In conclusion, for processing plants which utilise electrical stunning systems, the use of head only tongs may be better than head to brisket tongs as the rate of pH decline post-slaughter is slower, resulting in lower levels of drip loss.

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STUNNING OF PIGS: EFFECT OF METHOD, CURRENT LEVEL AND DURATION ON CARCASS AND MEAT QUALITY OF PIGS

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Carbon dioxide (CO_2) and electrical stunning (using different equipment configurations and handpieces) are two methods used commercially in Australia to stun pigs. Stunning of pigs using head to back (HB) stunning electrodes is considered to be more humane, as this method induces cardiac fibrillation, compared with head only (HO) stunning. Head to back stunning may result in broken vertebrae if the voltage applied is too high, whilst muscle contraction, resulting from the application of electrical current may contribute to an increased incidence of ecchymosis (blood splash). The aim of this study was to determine the effect of stun duration, level of electrical current applied and electrical stunning method used on carcass and meat quality of pork compared with that of pigs stunned with CO_2.

A total of 48 Large White x Landrace entire male pigs were randomly allocated immediately prior to slaughter to one of six stunning treatments: carbon dioxide (90% CO_2, n=12); HO (1.3 amps for 4 seconds, n=8); HB (0.9 amps for 10 seconds, n=7); HB (1.3 amps for 4 seconds, n=7); HB (1.3 amps for 10 seconds, n=7); HB (2.0 amps for 4 seconds, n=7)). The *M. longissimus thoracis et lumborum* (LTL) was used for measurements of muscle pH and temperature at 40 min (pHi), 90 min, 3 h, 6 h and 24 h (pHu) post slaughter, and drip loss and surface lightness (L* value) at 24 h post slaughter. Carcasses were dissected into shoulder, middle and leg primals. All bones were removed and assessed for bone fractures, and the amount of meat in pork shoulders affected with ecchymosis was weighed and recorded.

Table 1. Effect of stunning method on meat and carcass quality traits.

	Meat quality attributes (LTL)				Carcass quality defects	
Stunning method	pHi	pHu	Drip loss (%)	L* value	Bone fractures (% of carcasses)	Ecchymosis (g/shoulder)
CO_2	6.67[b]	5.62	2.39[a]	48.34	0[a]	0[a]
HO 1.3 Amp, 4 sec.	6.59[b]	5.59	4.70[b]	50.53	25[b]	314[b]
HB 0.9 Amp, 10 sec.	6.51[b]	5.52	4.70[b]	49.51	28[b]	357[b]
HB 1.3 Amp, 4 sec.	6.28[a]	5.49	4.96[b]	50.17	56[b]	378[b]
HB 1.3 Amp, 10 sec	6.50[b]	5.60	4.53[b]	47.83	28[b]	496[b]
HB 2.0 Amp, 4 sec	6.34[ab]	5.51	5.70[b]	49.34	28[b]	351[b]
s.e.d.	0.09	0.09	1.00	1.72		
P	P<0.001	NS	P<0.01	NS	P<0.05[1]	P<0.05[1]

[a,b]Means within columns with different superscripts are significantly different (P<0.05).
[1]Chi-square analysis for equality of means.

Drip loss was higher in LTL muscles from electrically stunned pigs compared with pigs stunned with CO_2, regardless of stun duration or current level applied (Table 1). No carcasses from CO_2 stunned pigs had ecchymosis-affected meat in the shoulder or any bone fractures in contrast to carcasses from HB and HO stunned pigs. A higher incidence of vertebral fractures occurred with HB stunning compared to HO stunning, regardless of stun duration or level of current applied. In conclusion, both carcass and meat quality were markedly improved when pigs were stunned using CO_2 compared to either of the electrical stunning systems, irrespective of current level applied or stun duration.

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EFFECT OF HALOTHANE GENOTYPE, PRE-SLAUGHTER HANDLING AND STUNNING METHOD ON MEAT QUALITY OF PIGS

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The effect of stunning method on pork quality has been determined using pigs of different halothane genotypes (Barton-Gade, 1984), however the combined effects of halothane genotype, pre-slaughter handling and stunning method of pigs in the same abattoir has not been investigated. The aim of this experiment was to determine the effects of halothane genotype, pre-slaughter handling and stunning method on meat quality attributes of pigs, using a 2 x 2 x 2 factorial design. Seventy-six Landrace and four Large White x Landrace pigs of known halothane status were transported 330 km and slaughtered after overnight lairage. The treatments were: (G) Halothane genotype - normal (NN) or carrier (Nn); (H) handling pre-slaughter - minimal or negative (an electric goad applied 15 times 5 min prior to slaughter); (S) Stunning method - carbon dioxide (90% CO_2) or electrical (1.3 amp applied using head only tongs for 4 seconds). Data from five erroneously allocated pigs were excluded (see Table 1 for numbers (n) per treatment). Meat quality analyses were conducted on the *M. longissimus thoracis et lumborum* (LTL). The rate of muscle pH decline post-slaughter (k) was determined by measuring muscle pH at 40 min (pHi), 90 min, 3 h, 6 h and 24 h (pHu) post slaughter and fitting data to an exponential decay equation. Drip loss and surface lightness (L* value) were determined at 24 h post slaughter. Pale, soft and exudative (PSE) pork was classified as having a drip loss ≥5% and a L* value >50.

Table 1. Effect of genotype (G), pre-slaughter handling (H) and stunning method (S) on meat quality attributes of pork.

Genotype	Normal				Carrier					
Handling	Minimal		Negative		Minimal		Negative			
Stunning	CO_2	E	CO_2	E	CO_2	E	CO_2	E	sed	Significance
n	9	10	9	9	10	10	9	9		
k	0.21	0.18	0.47	0.34	0.50	0.91	0.74	1.29	0.33	G**
pHi	6.68	6.61	6.54	6.56	6.29	6.14	6.05	5.91	0.13	G***,H*
pHu	5.47	5.52	5.58	5.45	5.65	5.39	5.38	5.40	0.09	GxHxS*
Drip loss	4.87	4.81	4.47	5.60	5.01	8.57	8.86	10.14	1.11	S**, GxH*
L* value	48.43	47.12	47.86	48.57	45.91	50.41	52.21	52.63	1.50	GxHxS*
PSE (%)[1]	33	10	11	11	20	50	78	100		P<0.05

[1]Chi-square analysis for equality of the eight means. *P<0.05, **P<0.01, ***P<0.001.

The rate of muscle pH decline post-slaughter was higher and the pHi was lower in muscles from carrier pigs than normal pigs (Table 1). Muscle from negatively handled carrier pigs had a higher drip loss, was paler and had a higher incidence of PSE meat compared with pigs in other treatments. Meat from electrically stunned pigs had a higher drip loss compared with that from pigs stunned with CO_2. Overall, genotype was the major factor influencing meat quality; carrier pigs were more likely to produce PSE pork than normal pigs. In conclusion, carrier pigs should be identified on-farm and the abattoirs notified accordingly so that appropriate pre- and post-slaughter management systems can be implemented to minimise the incidence of poor meat quality.

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MANIPULATING MUSCLE pH FALL DURING POST MORTEM

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The rate and the extent of the decrease in muscle pH post mortem (pm) is important for many meat quality characteristics (Monin, 1989). As the course of pH fall is determined by metabolic factors at the time of slaughter, experiments including bouts of physical exercise, adrenaline administration and fasting were conducted to manipulate and standardize physiological states of the live muscle, mainly the concentration of glycogen and creatine phosphate (Henckel *et al.*, 1997). In this study, three of the models were used to investigate the effects on the pm pH fall in *M. longissimus dorsi* (LD) in pigs.

Thirty female, halothane-free crossbred pigs from 10 litters were used (Duroc sires X Danish Landrace and Large White dams). The pigs had free access to standard feed from 30-100 kg live weight (LW). All pigs were reared in stables near the experimental abattoir. One of the three littermates was slaughtered under minimal stress conditions (Model A), the second was subjected to 10 min of treadmill exercise at a speed of 4 km/h immediately before slaughter (Model B), and the third received 0.3 mg adrenaline/kg LW subcutanously 15 h before slaughter, and was subjected to 5 min of exercise prior to slaughter (Model C). All animals were stunned with CO_2. Muscle biopsies were obtained from the LD muscle 15 h before slaughter to determine resting levels of glycogen, and immediately before and after stunning. Muscle pH was measured at 1, 15, 30 and 45 min, and at 1, 3, 6, 24 h pm.

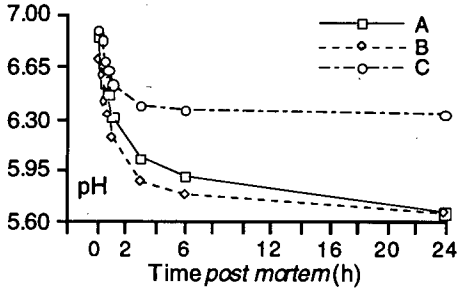

Figure 1. *Changes in post-mortem muscle pH in Models A, B and C.*

Table 1. Glycogen concentrations (μmol/g wet weight) in the LD muscle of pigs at rest (15 h before), and immediately before (pre mort.) and after stunning, together with pH immediately after stunning.

Model	A	B	C
Glycogen at rest	78.21	77.68	76.00
Glycogen pre mort.	65.80[a]	67.85[a]	26.10[b]
Glycogen 1min pm	62.20[a]	66.87[a]	21.15[b]
pH 1 min pm	6.86[a]	6.69[b]	6.89[a]

[a,b]Values in rows with different superscripts are significantly different, P<0.05.

The three models induced significantly different time-courses of pH fall in the 24 h pm period. Muscle pH 1 min pm was lower in Model B (P<0.001) than in Models A and C. No difference in muscle pH at 24 h pm (pH_u) was observed in Models A and B whereas pH_u in Model C was higher (P<0.001, Figure 1). Muscle pH measured within 1 h pm did not show any significant correlations with pH_u.

There were no differences in resting glycogen concentrations among the models (Table 1). Glycogen concentrations following the mild pre-slaughter handling in Model A were 15.6% lower than that at rest (P=0.12). Adrenaline administration (Model C) caused a reduction of 67.4% in glycogen concentration compared to that at rest (P<0.001).

The muscle glycogen concentration prior to stunning was closely correlated with pH_u (r=-0.86, P<0.001). It is concluded that the muscle glycogen concentration prior to stunning is important for the ultimate pH, and that a low pH within 1 h pm does not signify a faster rate of pH fall but a lower pH at exsanguination.

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THE AVERSIVENESS OF CARBON DIOXIDE STUNNING IN PIGS

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Carbon dioxide stunning of pigs is widely used in Europe. However, there are some concerns about the effects on animal welfare, particularly due to the pungent nature of CO_2 gas and the induction of breathlessness. The objective of this study was to examine the aversiveness of CO_2 to pigs in comparison to an electric prodder (negative control).

Sixty Large White x Landrace 4-month-old boars were used in two experiments, with two replicates in each. The pigs were trained to enter the CO_2 stunner crate over two consecutive days, using a standardized route, where they were rewarded with pieces of apple. After the training period, on the third day, pigs were assigned to three treatments. Time to enter the crate during six sessions after the treatments were applied was used to measure the aversiveness of the treatments. The treatments, which were applied once, were: 1) Control, the crate descended either to the bottom of the CO_2 pit (Experiment 1) or halfway down the CO_2 pit (Experiment 2), remained stationary for 10 sec and ascended. 2) Electric Prodder, as for treatment 1, but during the stationary period the pigs were remotely given two electric shocks with a cattle prodder. The electric prodder was used as a negative control as its use is perceived by some sections of the community as an 'unacceptable' practice, although it is used in the industry. 3) Exposure to CO_2, as for control treatment but without a stationary period, with the pit filled with CO_2 giving a concentration of 90% CO_2 at the bottom of the pit (Experiment 1) and 60% halfway down the pit (Experiment 2).

As shown in Table 1, there were significant differences ($P<0.05$) between the electric prodder and the two other treatments during sessions 4 and 5 (Experiment 1) and 3 and 4 (Experiment 2). There were no significant differences between the CO_2 and Control treatments in either experiment.

Table 1. Average times (sec) to enter the stunning unit on the day of treatment, with the treatment applied during session 0 (C=Control; EP=Electric Prodder).

	Experiment 1				Experiment 2			
Session	C	90% CO_2	EP	SEM	C	60% CO_2	EP	SEM
0	23.6	23.9	23.6		30.6	30.2	27.0	
1	22.0	27.0	25.9	3.34	31.9	37.3	46.3	11.18
2	21.4^{a}	25.8	32.9^{b}	3.79	31.6	37.2	50.7	9.42
3	18.2^{p}	22.8	28.6^{q}	2.80	32.2^{a}	24.4^{a}	55.9^{b}	9.11
4	18.8^{x}	22.3^{a}	34.7^{yb}	3.83	28.4^{a}	26.6^{a}	48.4^{b}	8.01
5	18.4^{a}	23.1^{a}	31.8^{b}	5.28	24.8	21.1	34.9	6.54
6	18.5^{a}	20.3^{a}	31.9^{a}	4.04	23.5	22.0	28.4	5.40

^{a,b,p,q,x,y}Means in the same row (within the same experiment) with different superscripts differ significantly: $^{a,b}P<0.05$; $^{p,q}P<0.01$; $^{x,y}P<0.001$.

The results of Experiment 1 suggest that 90% CO_2 was considerably less aversive than an electric shock with a prodder. However, during this exposure to CO_2 all pigs lost consciousness which may have affected their memory of the procedure. In Experiment 2 pigs mildly hyperventilated, but did not lose posture. As in experiment 1, CO_2 was considerably less aversive than an electric prodder. Further work is required to determine if CO_2 becomes more aversive with longer exposure.

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THE EFFECT OF TEMPERATURE CONDITIONING ON MEAT QUALITY OF PORK AFTER ACCELERATED PROCESSING

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Accelerated processing or hot boning, has many economic advantages compared to conventional processing but careful control of chilling is necessary to prevent cold shortening. Improvements in meat quality can be achieved in meat which undergoes accelerated processing by controlling the rate of temperature decline prior to *rigor mortis*. The objective of the present experiment was to determine the optimum pre-rigor conditioning temperature for *M. longissimus thoracis et lumborum* (LTL) to maximize meat quality. At thirty minutes post slaughter, the LTL was removed from both sides of the carcasses of eight Large White x Landrace finisher pigs (carcass weight, 72 kg; P2, 22 mm) and split into two. The four sections of LTL from each carcass were randomly allocated to conditioning temperatures of 0, 7, 14 or 21°C in temperature controlled water baths and held until 1 h after *rigor mortis*, as defined by pH≤5.7. At this time the samples were assessed for surface lightness (CIE-L*), cooking loss, sarcomere length, tenderness (Warner-Bratzler peak shear force) and drip loss (fresh). After 4 d of ageing post slaughter (aged), the samples were again assessed for the above measurements and purge levels (moisture lost during storage) were also determined.

Temperature conditioning at 0°C resulted in increased drip loss, cooking loss and decreased sarcomere length in the fresh samples relative to muscle held at 7, 14 or 21°C (data analysed by analysis of variance). After ageing, muscles held at 0°C had a darker colour, decreased tenderness, shorter sarcomere length, and increased purge relative to muscle held at 7, 14 and 21°C (Table 1). Although improvements in tenderness were seen, all muscle samples would still be considered slightly tough. A shear force value of <5 kg is considered to be acceptably tender by the consumer. Overall, pork which undergoes accelerated processing at conditioning temperatures of 14 and 21°C will produce the most tender meat after 4 d ageing. In addition drip loss is reduced, although the surface lightness is paler relative to the LTL muscle held at 0°C.

Table 1. Treatment means for meat quality measurements for accelerated-processed pork *M. longissimus thoracis et lumborum* after temperature conditioning at 0, 7, 14 or 21°C.

	Conditioning temperature				Significance	
	0°C	7°C	14°C	21°C	P	SED
Drip loss (%)	3.94^{a}	2.61^{b}	2.21^{b}	2.95^{b}	P<0.01	0.447
CIE-L* fresh[1]	42.41	43.49	44.40	44.52	NS[3]	0.861
CIE-L* aged[2]	47.54^{a}	49.14^{b}	50.69^{c}	50.14^{c}	P<0.001	0.496
Shear force (kg) fresh	9.94	9.35	9.31	8.08	NS	0.933
Shear force (kg) aged	10.51^{a}	8.08^{c}	6.55^{b}	7.21^{bc}	P<0.001	0.597
Sarcomere length (μm) fresh	1.30^{a}	1.60^{b}	1.58^{b}	1.47^{b}	P<0.01	0.077
Sarcomere length (μm) aged	1.45^{a}	1.67^{b}	1.69^{b}	1.65^{b}	P<0.001	0.049
Cooking loss (%) fresh	33.19^{a}	29.87^{bc}	30.48^{b}	27.98^{c}	P<0.001	1.124
Cooking loss (%) aged	36.10	36.54	36.72	37.16	NS	0.568
Purge (%) aged	6.27^{a}	4.83^{b}	3.50^{b}	3.48^{b}	P<0.01	0.857

^{a,b,c}Within rows, means with different superscripts are significantly different (P<0.05). [1]fresh = 60 min post rigor. [2]aged = 4 d post slaughter. [3]NS = not significant.

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CLEARANCE OF SKATOLE FROM PIG FAT AFTER EITHER IMMUNOCASTRATION OR DIETARY INULIN SUPPLEMENTS

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Boar taint is an unpleasant odour perceived by consumers when pork from certain boar carcasses is cooked and eaten. This odour has been correlated with high levels of skatole (SKA) and androstenone. Skatole is produced by bacterial fermentation of tryptophan in the hind-gut and a proportion is deposited in fat tissue. Skatole tends to be higher in boars, and levels that are perceived as tainted (>0.2 μg/g fat), are almost exclusive to them. High protein turnover in the hind-gut favours high SKA in the carcass but Claus *et al.* (1994) has shown that short term supplementation with inulin, an indigestible, but fermentable carbohydrate, leads to a sharp decline in the concentration of SKA in gut, plasma and fat. In addition, castration (surgical or immunological) reduces SKA compared to entire boars. The aim of this experiment was to determine the changes in SKA concentrations following immunocastration and dietary inulin supplementation.

Twenty-four boars (slaughtered at 24 weeks) were assigned to one of three treatments: Immunocastration (IC) with a Gonadotrophin Releasing Factor (GnRF)-based antigen, vaccination took place 8 and 4 weeks before slaughter; inulin supplementation (IS) of the diet (2.5% by weight) for the last 14 d before slaughter; and control (C), pigs were not vaccinated and were fed a standard finisher ration. Shot biopsy samples from the P2 region were taken at 28, 21, 12, 7 d before slaughter and equivalent samples were taken at slaughter. Skatole in fat was measured by HPLC using fluorescence detection. Results were analysed by ANOVA corrected for repeated measures.

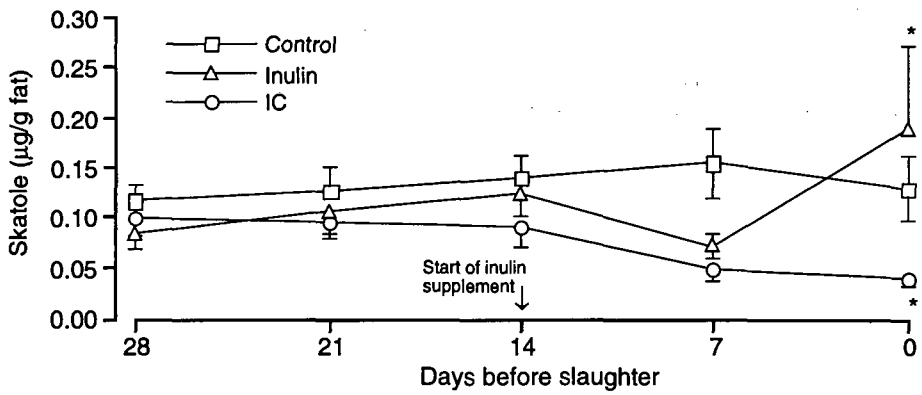

Figure 1. *Effect of IC and IS on skatole in fat. The IC boars were given the second dose of vaccine at 28 d before slaughter. Mean values (± SEM) indicated by (*) are significantly different to values at the start of the trial, for that treatment (P<0.05).*

Overall, concentrations of skatole in the pigs were quite low but increased slightly in the control treatment over the study period. In the IC group skatole concentrations declined after a boost dose of the vaccine, and at slaughter were significantly lower than at the start of treatment (Figure 1). Skatole declined 2-5 d after the change to an inulin-supplemented diet, but rebounded to be significantly higher than pre-treatment concentrations at slaughter. A similar pattern was noted by Claus *et al.* (1994), although the mechanism is unknown. Even low concentrations of skatole in fat can be reduced by immunocastration although it takes up to 4 weeks to produce a significant decline. Inulin has a prompt action in reducing skatole but timing is important if the elevated concentrations which occur after 7 d of dietary supplementation are to be avoided.

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EVALUATION OF THE ELECTRONIC NOSE TO DETERMINE TAINT IN PORK FAT

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Boar taint is detected as an unpleasant odour in meat or fat from some entire boars. Skatole (Sk) and 5α-androstenone (5α) are thought to be the major compounds contributing to the taint. There is an industry requirement for an objective, rapid method for the detection of boar taint. One recent approach for odour detection is to use an Electronic nose (E-Nose™) which utilizes multi electronic sensors to develop an odour fingerprint. The objective of this preliminary study was to determine if an electronic nose could discriminate among the odour of fat from females, castrates and entire males.

Samples of bellyfat were collected at slaughter from the carcasses of 12 Large White x Landrace pigs (70-110 kg body weight). The pigs were from a commercial piggery where they had been offered a finisher diet *ad libitum*, and had been fasted for 24 h prior to slaughter. The concentrations of 5α and Sk were measured in the fat according to the HPLC method of Hansen-Møeller (1994). The samples were also analysed using the E-nose 4000™ (Neotronics Scientific). Fat samples (20g) were cut into five pieces and left at room temperature for 30 min prior to analysis. Each of the 20 g samples was placed in the E-nose™ chamber which was purged and equilibrated with ultra high purity nitrogen to develop a headspace The sensors were then exposed to the odour of each sample twice and odour profiles were developed.

Results of chemical analysis by HPLC showed that fat from female (n=3) and castrate (n=3) pigs had 5α concentration <0.20 μg/g and Sk concentrations <0.06 μg/g. Fat from entire boars (n=6) had 5α concentrations between 0.20-2.77 μg/g and Sk concentrations between 0.03-0.67 μg/g. The output from the E-nose™ sensors was analysed by multi-discriminant analysis using Unistat. The results are illustrated in Figure 1 and indicate that the odours from the fat of females and castrates are in a similar category, whereas the odours from the fat of entire males were in a separate category. Within the boar group there was an indication of segregation according to the concentration of taint compounds. This separation however was not complete possibly due to the small number of samples and the limited range in the concentration of taint compounds. Further work is currently underway to determine whether the E-Nose™ can distinguish between high and low levels of taint.

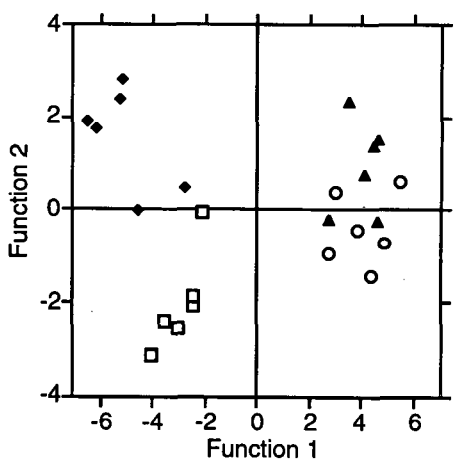

Figure 1. *Multi-discriminate analysis on the E-nose data set from fat samples from females* (□), *castrates* (◆), *boars with low taint* (○), *and boars with high taint concentrations* (▲).

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PREDICTING THE CRUDE FAT CONTENT OF PIG CARCASES USING NEAR INFRA-RED SPECTROPHOTOMETRY (NIRS)

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Crude fat content is routinely measured in pig carcases as part of nutritional and physiological experiments. Traditional wet chemistry methods used to determine crude fat content (ether extracts) are expensive and time consuming. The aim of this study was to examine the potential for near infra-red spectrophotometry (NIRS) to be used as a rapid and inexpensive technique for the accurate prediction of crude fat in ground freeze-dried (GFD) or wet (GW) samples of pig carcases.

The GW samples (n=58) were from carcases (head, trotters, tail and hair off) split longitudinally down the midline, ground through a commercial butchers mincer and subsampled prior to analysis. The GFD samples (n=79) were from carcases (head off, trotters, tail and hair on) prepared as above and freeze-dried prior to analysis. All samples were analysed for crude fat (AOAC, 1984). Reflectance spectra were recorded on an NIRSystems Model 6500 NIR Spectrophotometer over the wavelength range 1100-2500 nm at 2 nm intervals. Equation development and statistical analysis were performed using ISI-NIRS 3.00 software. Calibrations were developed using 69 GFD samples (CF (mean ± SD) =478 ± 99 g/kg dry matter (DM); range 243-630 g/kg), and 48 GW samples (CF=122 ± 28 g/kg as received (AR); range 71-180 g/kg). The remainder of the samples were used as validation sample sets (GFD: CF=426 ± 89 g/kg DM, range 273-580 g/kg; GW: CF=118 ± 15 g/kg AR, range 99-148 g/kg).

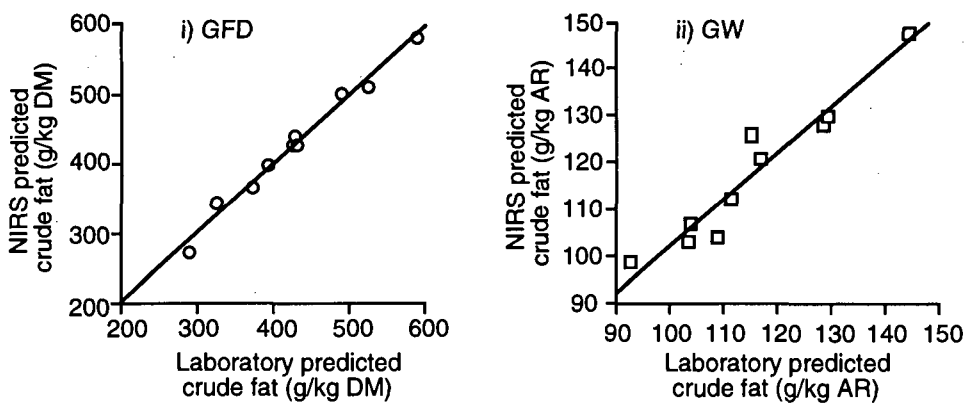

Figure 1. *Scatter plot of NIRS predicted crude fat values vs laboratory predicted crude fat values in i) ground freeze-dried (GFD) and ii) ground wet (GW) pig carcase samples.*

The coefficients of multiple determination (R^2) and standard errors of cross validation (SECV) of the prediction equations were R^2=0.98, SECV=1.80 and R^2=0.96, SECV=0.65 for GFD and GW samples, respectively. Evaluation of the prediction equations using separate validation sample sets demonstrated high correlation coefficients (r^2) and low standard errors of prediction (SEP) between NIRS and wet chemistry methods of determining crude fat, for both GFD (r^2=0.98, SEP=0.997, SD/SEP=8.9) and GW (r^2=0.92, SEP=0.462, SD/SEP=3.2) carcase samples (Figure 1).

These preliminary results demonstrate potential for using NIRS as a rapid method for the accurate determination of crude fat in samples from pig carcases.

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SHELF-LIFE OF AUSTRALIAN PIG CARCASES AND MEAT

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The microbiological shelf-life of Australian pork was investigated as part of the Pig Meat Hygiene Project. In order to promote consumer confidence and maximize storage time this survey was undertaken to determine the shelf-life of fresh pork in retail outlets and give an indication of shelf-life of pork carcases at the abattoir. The prospective shelf-life of meat is indicated by the numbers of *Pseudomonas* spp. and the Total Viable Count at 4°C (TVC 4°C). Estimates of shelf-life (days) of fresh meat were determined using the Predictor™ software (predictive model for *Pseudomonas* growth).

Ten paired butterfly steaks were purchased within 1 hour of boning from 10 different retail outlets in each Australian capital city on two occasions. Samples were purchased from supermarkets and butchers, at a ratio of 7:3 which reflects current retail trends, and were kept at 4°C until processed in the laboratories (VETLAB and VIAS). An area of 2 x 20cm^2 of fresh meat was swabbed (Kitchell *et al.*, 1975) and the swabs were placed in 30mL of buffered peptone water. A total of 680 carcase swabs was collected from randomly selected carcases (pre-chilled) at 18 abattoirs over a 12 month period. Each sample consisted of 3 x 20cm^2 swabs, from the jowl, flank and rump, in 30mL buffered peptone water using the wet/dry swabbing method (Kitchell *et al.*, 1975). Samples from meat and carcases were processed by Australian Standard methods and results are presented in Tables 1 and 2.

Table 1. Mean TVC 4°C and *Pseudomonas* numbers on fresh meat from retail outlets, and predicted shelf-life.

	Log TVC/cm^2		Log *Pseudomonas*/cm^2		Shelf-life in days @ 4°C	
	Supermarket	Butcher	Supermarket	Butcher	Supermarket	Butcher
Mean	3.62	3.95	3.19	3.51	4.53	4.28

Table 2. Mean TVC 4°C for carcasses from abattoirs.

	Minimum mean	Maximum mean	Overall works mean
Log count/cm^2	0.20	2.32	1.25

Total viable counts at 4°C are indicators of potential spoilage and *Pseudomonas* spp. are the most common spoilage organisms. *Pseudomonas* counts/cm^2 can be used to estimate shelf-life in predictive models. The mean TVC 4°C for fresh meat was 3.62 and 3.95 for supermarkets and butchers respectively; differences between types of retail outlet and among cities were not significant (Table 1). Also, differences in *Pseudomonas* counts between types of retail outlet and among cities were not significant. Based on these data the mean shelf-life for pork in Australian retail outlets is 4.3 to 4.5 d (Table 1). Mean TVC 4°C counts for the carcase swabs was 1.2 log/cm^2 (Table 2). The data reported here indicate that an increase in TVC 4°C of log 2.5 occurs between the pre-chiller stage at the abattoir and display in retail outlets. Other studies (Coates *et al.*, 1995) have shown that counts of spoilage organisms are low on carcases before chilling and increase during transport and storage. The increase in TVC 4°C reported here is consistent with an increase in psychrotrophic organisms during transport and storage at refrigeration temperatures. Recommended maximum limits for TVC 4°C on carcases are 5x10^5 (Silliker, 1980) therefore Australian pig meat and carcases are being produced well within limits which are acceptable world-wide. *Supported by the Pig Research and Development Corporation*

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IMPROVING THE RETAIL SHELF LIFE OF FRESH PORK

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The retail shelf life of fresh pork was increased by 1-2 d beyond the mean of 3 d by training retail meat managers with particular attention being paid to carcase and boning room hygiene (Coates *et al.*, 1995a). As meat retail stores operate on small profit margins, any improvement in shelf life would benefit the industry, by extending retail display time and/or increasing the flexibility of product management.

Between August and December 1996, work was conducted with a major retailer to train meat managers to improve the shelf life of fresh pork. Four retail stores (denoted A, B, C and D), different from each other in terms of turnover and age of the store, were nominated for the training programme. To establish a baseline level for each store, five packs of pork loin chops were purchased each day from the meat display case over a 5 d period. The number of spoilage organisms (*Pseudomonas* spp.) on the pork chops (per cm^2 surface area) was determined and the data processed using Predictor™, a software package based on a predictive microbiology model to estimate remaining shelf life at 4°C (Coates *et al.*, 1995b). Meat managers from each of the stores were then trained in basic hygiene, microbiology and strategies previously developed by Coates *et al.* (1995b) to improve shelf life. The strategies were designed to limit the transfer and growth of bacteria on meat surfaces. Following training, the same sampling procedure was repeated to determine after-training levels of *Pseudomonas* contamination and remaining predicted shelf life. Table 1 shows the shelf life and numbers of *Pseudomonas* before- (BT) and after-training (AT).

Table 1. The effect of training on the numbers of *Pseudomonas* (log_{10}) on the surface, and the remaining retail shelf life (h), of pork loin chops.

	Store A	Store B	Store C[1]	Store D[1]
log count/cm^2 (BT)[b]	2.05	2.29	1.93	2.06
log count/cm^2 (AT)[a]	1.37	1.89	2.46	2.60
Shelf life (BT)	94.7	90.2	97.1	94.7
Shelf life (AT)	107.8	98.2	87.1	84.3

[1]The managers of these two stores were changed after the training and the new strategies were therefore not implemented. [a]AT = after training. [b]BT = before training.

Results of the study show that in store A there was a decrease in the level of *Pseudomonas* contamination with a corresponding improvement in predicted shelf life of 13 h ($P<0.05$). Store B had the largest volume of throughput and thus less attention was paid to the strategies resulting in only minor improvement. The improvements in shelf life were less than those found by Coates *et al.* (1995b) and can be largely attributed to the lower initial baseline levels of contamination in the present study compared to the those in the study by Coates *et al.* (1995b). The numbers of *Pseudomonas* on pork chops from all stores were at acceptably low levels both before and after training indicating an initial high level of awareness of the need for strict hygiene. In conclusion, the implementation of improved strategies and the training of boning room staff has the potential to improve the retail shelf life of pork.

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A RISK ASSESSMENT APPROACH TO PIG MEAT HYGIENE IN AUSTRALIA

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The Pig Meat Hygiene Project (PMHP) was developed from industry workshops on meat hygiene and inspection conducted by the Pig Research and Development Corporation in January 1995. Scope for improvement in both efficiency and effectiveness of food safety procedures, including post-mortem inspection, was identified at the workshops. The international outlook for implementing change has been greatly enhanced by the World Trade Organisation's Sanitary and the Phytosanitary Agreement (WTO, SPS), which provides a trade environment bound by rules of scientific justification, risk assessment and equivalence. In this context the PMHP (Pointon 1997) is seen as an important preliminary step in the development of a sustainable export strategy.

The risk assessment being conducted (Hathaway *et al.*, 1988) requires that foodborne organisms associated with pig meat be identified, ranked according to their human disease importance, and that contamination with these organisms arising from different sources be quantified (Figure 1). These procedures provide a scientific bias for assessing the risk (i.e., the likelihood and severity) that consumers have of becoming infected with disease causing organisms. As the approach identifies (quantitatively) the major points of entry of "foodborne" organisms into the pig meat production/processing chain, it provides a sound basis for implementation of critical control points in quality assurance programs.

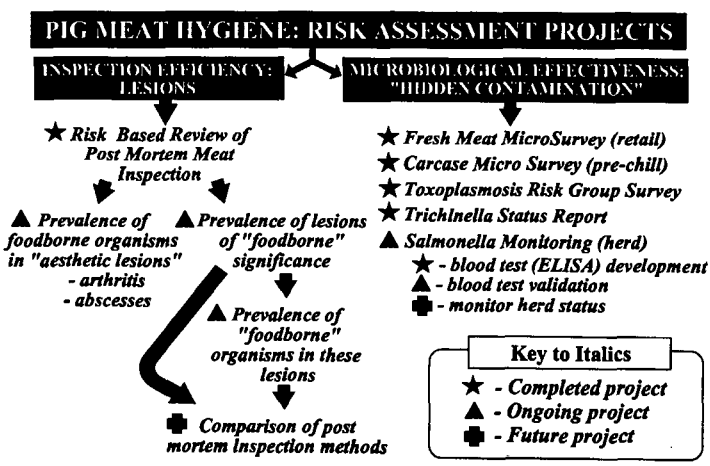

Figure 1. *Pig meat hygiene risk assessment investigations (Pointon 1997).*

The surveys will quantify the prevalence of the most important "foodborne" organisms through the pig meat production chain, thereby providing a focus for implementation of improved food safety procedures which meet WTO criteria. The Salmonella herd blood test will provide industry with a tool to gauge the infection status of the pig herd and to monitor the effectiveness of procedures conducted on farm to minimise infection. By comparing the residual level of carcase contamination with "foodborne"organisms at the time of post-mortem inspection, with contamination arising from other sources, a scientific basis will be provided for redirection of expenditure on food safety procedures to better protect consumers.

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RESTRICTION ENDONUCLEASE ANALYSIS, CAPSULAR TYPING AND *toxA* PHENOTYPE OF AUSTRALIAN PORCINE ISOLATES OF *PASTEURELLA MULTOCIDA*

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Capsular type A strains of *Pasteurella multocida* are most often recovered from infected lung tissue of pigs with *Pasteurella* pneumonia. In contrast, capsular type D strains, which produce a 145 kDa dermonecrotic toxin, are usually the primary cause of progressive atrophic rhinitis (PAR). Occasionally, toxigenic type A strains are associated with PAR. There are no studies comparing whole cell DNA fingerprints of pneumonic isolates of *P. multocida* recovered from sporadic outbreaks from different geographic locations, or a comparison of fingerprints of such isolates with toxigenic isolates recovered from herds with signs of PAR. Porcine isolates, identified as *P. multocida* by standard biochemical tests (Cowan, 1974), were examined by DNA fingerprinting using the restriction endonuclease *Cfo*I. Production of toxin was analysed in a cytotoxic assay using bovine turbinate cells, and capsular typing was based on acriflavine agglutination and hyaluronidase sensitivity (Eamens *et al* 1988). The toxigenic status of these isolates was assessed by amplification of a portion of the *toxA* gene by polymerase chain reaction (PCR) (Nagai *et al* 1994) and by Southern blotting using ^{32}P labelled *p*PmF3.5 (*p*KUN 1 containing a *Xba*I/*Sal*I fragment encoding a portion of the *toxA* gene; a gift from E. Kamp) (Table 1).

Table 1. Geographic location, capsular type, and toxigenic status of *P. multocida* isolates

Origin	Type	Syndrome	Source	No. isolates	Cyto-toxicity	toxA PCR	toxA blot
NSW(7), WA (1)	D	PAR	Nasal swab	8	8/8	8/8	6/6
NSW	A	PAR	Nasal swab	1	1/1	1/1	ND
WA, Vic.[1]	D	PAR	Nasal swab	2	0/2	0/2	0/2
WA	A	PAR	Nasal swab	1	0/1	0/1	0/1
WA (2), NSW(3)	A	Pneumonia	Lung	5	0/5	0/5	0/5
NSW	D	Pneumonia	Lung	5	0/5	0/5	0/5

[1] Isolate recovered from a herd where PAR was suspected and nasal discharge was present.

Toxigenic, capsular type D isolates of *P. multocida* displayed virtually identical *Cfo*I profiles whilst pneumonic, non-toxigenic isolates of either capsular type revealed greater genomic heterogeneity. Two *P. multocida* isolates, one a type D, toxigenic isolate and the other a type A, non- toxigenic isolate were recovered from nasal swabs from the same pig and displayed very different *Cfo*I profiles. The *toxA* gene was not detected amongst *P. multocida* isolates recovered from pneumonic lesions. Genomic *Cfo*I profiles resolved by polyacrylamide gel electrophoresis and stained with silver are highly discriminatory and show promise for future epidemiological studies of porcine *P. multocida* isolates.

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THE USE OF AN ISOLATED PERFUSED LUNG MODEL TO STUDY THE EARLY PATHOGENESIS OF PLEUROPNEUMONIA

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Irrespective of preventative measures, economic losses in the pig industry due to respiratory disease are high. Environmental factors influence lung conditions, however the mechanisms by which this occurs are unclear. The aim of this study was to establish whether an isolated perfused lung *(ex-vivo)* model is a valid tool for examination of the early pathogenesis of *Actinobacillus pleuropneumoniae* (App) infections.

Specific-pathogen-free pigs (Dutch Landrace x Yorkshire; both sexes; live weight 15-20 kg) were anaesthetised, intubated and mechanically ventilated with humidified room air. Each pig was heparinised and exsanguinated via the carotid artery. Blood was collected into a sterile blood sac placed in a water bath maintained at 39°C. The thoracic cavity was opened, and perfusion tubing was secured in the pulmonary artery and the left atrium. Blood was then re-perfused through the lungs at a flow rate of 50 ml/min by a roller pump. The heart and lungs were then removed from the thoracic cavity and placed inside a heated, humidified, air-tight chamber, with the perfusion tubing and endotracheal tube passing through holes in the chamber wall. The maximum time which elapsed from exsanguination to re-ventilation in the chamber was 30 min. The lungs were ventilated using negative pressure and the blood flow rate was increased to 180-200 ml/min to give a pulmonary artery pressure of 25 mm Hg. After the lungs had stabilised for an hour, the right lung was inoculated intrabronchially with 5 ml of either Eagles Minimum Essential Medium (EMEM) (n=6) or a suspension of 10^6 cell/ml of App (serotype 9) in EMEM (n=5). Ventilation of the lungs continued for approximately 3-4 h. Blood samples were taken pre-anaesthesia, at the time of inoculation, and 150 min post inoculation for a differential white blood cell count (WBC), and the results are shown in Table 1.

Table 1. White blood cells (x 10^9)/l of blood (mean ± SEM) for *ex-vivo* lungs inoculated with 5 ml of either a suspension of 10^6 cells/ml of *Actinobacillus pleuropneumonia* (serotype 9) in Eagles Minimum Essential Medium (App; n=5) or medium alone (EMEM; n=6).

Time	EMEM	App	Significance[1]
Pre-anaesthesia	11.30 ± 1.610	13.68 ± 1.713	NS
Inoculation	5.70 ± 1.392	6.28 ± 0.894	NS
150 min post inoculation	4.22 ± 0.658	1.93 ± 0.477	*

[1]NS, non significant, *P<0.005.

There were no significant differences in WBC between treatment groups both pre-anaesthesia and at the time of inoculation. The WBC in the App treated group was significantly reduced by 150 min post inoculation suggesting margination of the neutrophils and extravasation into the lung tissue. The results suggest that the *ex-vivo* lungs are responsive to bacterial challenge and that this model is a useful tool for examination of the pathogenesis of App and other respiratory diseases. The *ex-vivo* model also allows manipulation of conditions within the respiratory system and reduces the need for *in vivo* experiments.

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INITIATION OF APOPTOSIS IN PORCINE LEUKOCYTES BY ACTINOBACILLUS PLEUROPNEUMONIAE

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The ability to excrete cytotoxic haemolysins by pathogenic strains of *Actinobacillus pleuropneumoniae* (App) may be an important virulence strategy for inactivating inflammatory cells of the innate immune system *in vivo*. Such interactions can be studied *in vitro* by incubating porcine leukocytes with App or its purified products. Affected cells would show alterations in membrane structure as well as nuclear DNA fragmentation, two processes which are characteristic of programmed cell death or apoptosis (Mangan *et al.*, 1992). Membrane phosphatidyl serine molecules are inverted to the exterior of apoptotic cells and this is detected by flow cytometry as a green fluorescent signal (right-shift) after staining with fluorescein conjugated annexin V (FAV). Intercalation of DNA in apoptotic cells by propidium iodide (PI) generates a red hypodiploid left-shift that is also detectable by flow cytometry.

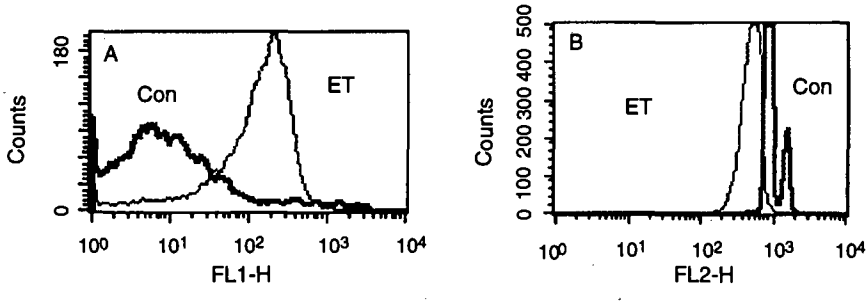

Figure 1. *Induction of apoptosis in porcine leukocytes. (A) Etp-treated apoptotic leukocytes (ET) display an increased fluorescence right-shift from 10^1 to 10^2 compared to unstained control (Con) cells. (B) Etp-treated leukocytes stained with PI show a hypodiploid peak shifted to the left of control non-apoptotic cells.*

The FAV staining pattern of porcine leukocytes after *in vitro* culture for 18 h in the presence or absence of an apoptosis inducing agent etoposide (Etp) is shown in Figure 1A. Untreated cells remained unstained while >95% Etp-induced apoptotic leukocytes were stained. Leukocytes incubated with App were also stained by FAV. Control porcine leukocytes did not display a hypodiploid peak when stained with PI whilst >95% of cells treated with Etp showed such a spectral shift (Fig. 1B). Similarly, leukocytes incubated with App also displayed a prominent hypodiploid peak. Increasing periods of incubation of leukocytes with App resulted in an increased frequency of staining by either FAV or PI procedures. These results demonstrate that flow cytometry can be used to assess apoptosis in porcine leukocytes and confirm the hypothesis that App is able to initiate programmed cell death in inflammatory cells. This may be an important virulence strategy used by App to inactivate the innate immune system.

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PREVALENCE OF GASTROESOPHAGEAL ULCERS IN GROWER-FINISHER PIGS IN NORTHERN PROVINCE, REPUBLIC OF SOUTH AFRICA

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Gastric ulceration, especially of the pars oesophagea, in grower-finisher pigs, is observed world-wide in all areas with intensive pig production. Haemorrhage from severe erosive lesions usually results in sporadic deaths that can be up to 1% or higher of the stock population. Previous abattoir studies revealed that erosions in the pars oesophagea may range from 32-65% (Ball *et al.*, 1996). The objectives of this survey were to establish the prevalence of gastric ulcers in abattoir pigs in Northern Province, Republic of South Africa, to determine the severity of the lesions in the pars oesophagea, and to compare the findings with those previously reported in the literature.

Pietersburg abattoir, that slaughters an average of 250 pigs every working day, was used for the survey. Random visits were made to the abattoir between July 1996 and February 1997. The abattoir receives pigs from intensive farms around the central region of the province. The time between loading the animals and arrival at the abattoir ranged from 20 min to 2 h which was also about the time between the last meal and slaughter. There were occasional exceptions when animals were kept at the abattoir overnight before slaughter as a result of technical or labour issues. The pigs for slaughter weighed between 60-150 kg live weight. The stomachs were recovered along the slaughter line and the time between slaughter and examination of the gastric mucosa was about 15 min. The mucosa was examined for gross pathological changes which were classified as hyperkeratotic or erosive or ulcerative. Tissue samples were collected for histopathological examination.

A total of 4320 pig stomachs was examined. The prevalence of gastroesophagic ulcer was 5.1%, erosion 15.2% and hyperkeratosis 18.9%. In the group with gastric ulcers, the lesions varied from acute with blood clots, to subacute, to chronic gastric ulceration with possible perforation. Histopathological examination revealed multifocal superficial lytic necrosis of the mucosa with haemorrhage, and exudation of fibrin and neutrophils. Other histopathological findings included coagulative necrosis, thrombi in blood vessels, leucocytic infiltration that varied from fairly heavy to light with neutrophils and macrophages as the main cell types. The necrosis was generally confined to the superficial layer of the gastric mucosa. Other observations were the presence of trichobezoar and plastic materials in the stomach.

The prevalence of gastric ulcers in pigs in Northern Province is low compared with similar abattoir surveys reported elsewhere, e.g., 17.7% in Slovenia (Senk, 1986) and 11% in Canada (Friendship *et al.*, 1996). Lower figures, however were reported in England, 2.3% by Penny and Hill (1973), and in Zimbabwe, 2.8% (Makinde and Obwolo, 1990). The low rate of gastric ulceration in pigs in Northern Province may be a reflection of the high management standard, which includes not using feed with fine particle size, on the farms that supply the abattoir. Most of the active ulceration observed in this study was superficial with no involvement of the submucosa. The lesions were fairly mild, as would be expected in pigs that survive to slaughter. It is difficult to assess the duration of the lesions, but as few of them showed evidence of healing by fibrosis most of them were regarded as acute.

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PRELIMINARY EVALUATION OF A SALMONELLA ELISA AS A PIG HERD MONITORING TEST

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A *Salmonella* ELISA developed to monitor the infection status of pig herds in Denmark (Nielsen *et al.*, 1995) was adapted for Australian conditions. Four major serotypes represent 99.7% of all *Salmonella* spp. recovered from pigs in Australia since 1988. Isolates representing these serotypes were used to extract lipopolysaccharide (LPS) which was used as antigen in the assay. These antigens were shown to be highly specific for homologous antisera. Negative reference serum comprised a pool of pig sera obtained from a piggery free of *Salmonella* infection. Sera from 52 pigs from two herds free of *Salmonella* infection were previously titrated in the ELISA to establish appropriate serum concentrations and cut-off values for the assay. These parameters were set such that all sera from these herds were negative in the ELISA.

The ELISA was evaluated using samples collected at abattoir from 20 animals from each of five piggeries suspected of being *Salmonella* positive on the basis of having management and housing attributes conducive to *Salmonella* infection. Twenty pigs per herd were sampled. Mesenteric lymph nodes (MLN) and caecal contents were tested for the presence of *Salmonella* spp. using Australian Standard Diagnostic Techniques for Animal Diseases (Murray and Barton, 1993).

On a herd basis, all herds were culturally positive for *Salmonella* from MLN or caecal contents, and were serologically positive by ELISA (Table 1).

Table 1. Proportion of pigs positive by culture and serology among the 20 pigs sampled on each of the five farms suspected to be positive for *Salmonella* infection.

Farm	Caecal culture +ve (%)	MLN culture +ve (%)	ELISA +ve (%)
A	20	10	30
B	25	10	40
C	5	0	25
D	0	5	35
E	65	15	25

The results indicate that the ELISA described is capable of detecting herds infected with *Salmonella*. Such an assay would provide a valuable tool for monitoring herd infections and the efficacy of control programs, with the ultimate aim to reduce carcase contamination. In control programs based on this assay (Nielsen *et al.*, 1996) different levels of management responses based on the proportion of pigs that are serologically positive within herds have been developed. A reduction in herd sero-prevalence in the Danish pig industry has led to a reduction in *Salmonella* contamination of pig meat (Mousing *et al.*, 1997). Further studies of the national pig herd will evaluate this assay as a herd monitoring tool.

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THE SEROLOGICAL PREVALENCE OF *TOXOPLASMA GONDII* IN THE AUSTRALIAN PIG HERD

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Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is recognised as the most widespread zoonotic disease in the world. Although largely asymptomatic in healthy humans, infection during pregnancy can seriously affect the foetus, and can be fatal in the immunocompromised (e.g., AIDS sufferers). Felines (definitive hosts) shed oocysts in their faeces. Intermediate hosts (other warm-blooded animals) become infected congenitally or by ingesting either the oocysts from cat faeces or tissue cysts from other intermediate hosts. Pigs are an important intermediate host. In the USA, where 50% of the human population are serologically positive to *T. gondii*, the within-herd prevalence on pig farms ranges from <1% to 69% (Dubey,1990), and it is believed that pork is the major meat source of human toxoplasmosis. Extensive surveys have been undertaken in overseas herds, but little is known about the prevalence of *Toxoplasma* in Australian pigs.

As part of the Pig Meat Hygiene Project, a pilot survey of the Australian pig herd was undertaken to determine the prevalence of *T. gondii*. The national herd was divided into four risk groups based on the known epidemiology of toxoplasmosis; these were finishers, sows kept inside, sows kept outside and feral pigs (Smith *et al.*, 1992). Samples were collected according to the pro rata state pig population (Ransley and Cleary, 1995), with the exception of feral pigs. Serum samples were examined for antibodies to *T gondii* using a commercial modified direct agglutination test (Table 1).

Table 1. Prevalence of *Toxoplasma gondii* antibodies in the Australian pig herd.

Test group	Pig population	Pigs positive (%) [1]		Herds positive (%) [1]	
Finishers	2838000	4/310	(1.3)	4/143	(2.8)
Inside sows	304000	16/139	(11.5)	14/119	(11.8)
Outside sows	15000	7/104	(6.7)	4/40	(10.0)
Feral	0.5 - 30 m	7/75	(9.3)	n/a	

[1]Modified direct agglutination test (bioMérieux): deemed positive at ≥ 1:4 as per kit instructions.

The prevalence in intensively-managed finisher pigs (1.3%) was similar to that found overseas, e.g., 2.1% in The Netherlands (Berends *et al.*, 1991). The prevalences in intensively-managed (11.5%) and extensively-managed (6.7%) sows and feral pigs (9.3%) were all at the lower end of the international range of 1-98% (Fayer, 1981). The use of these animals in fermented (uncooked) sausage or pork products that may be eaten undercooked, may however, represent a risk to Australian consumers. To properly evaluate that risk, more information is needed about the effects of the cured meat process (e.g., salting, drying, fermentation, smoking) on *T. gondii* cyst survival. Heating porcine tissue to 67°C for 3.6 min or freezing to -8.0°C for ≥ 3 d inactivates the parasite (Smith, 1992), and may represent simple and effective critical control points to minimise the risk. Alternatively, restricting the source of pigs for use in uncooked product to confirmed uninfected herds may be an option.

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IMPLEMENTING QUALITY STANDARDS IMPROVES PORK QUALITY IN VICTORIAN ABATTOIRS

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A major cause of economic loss to the Australian Pig Industry is drip loss which can be attributed to pale, soft, exudative pork (PSE) and reddish, soft, exudative pork (RSE). Recently, Eldridge *et al.* (1995) found that the incidence of soft, exudative pork (SE; PSE and RSE combined) in Australia was between 41-64% with an average of 51%.

The aim of the National Pork Quality Improvement Program was to reduce the incidence of PSE by 50% in participating abattoirs. Three abattoirs in Victoria were each audited three times during 1995/1996. Audits included observation of animal treatment from time of arrival, through lairage, slaughter and chilling until a deep butt temperature of <18°C was reached. The incidence of meat quality defects was measured and recommendations were made to reduce this incidence. Each audit was conducted over a period of 3 consecutive slaughter days with an average of 21.3% (6014 pigs) of the total number of pigs slaughtered being measured. Muscle pH was measured at two sites on the carcase 6-8 h post slaughter; the loin at the P2 site, and the exposed surface of the topside. Meat quality was described as normal if the pH was between 5.6 and 6.0, and dark, firm and dry (DFD) if the pH was >6.0. If the pH was ≤ 5.6, muscle paleness (L*) was measured using a Colormet probe. Meat quality was described as PSE if the pH was ≤5.6 and colour was ≥28, and RSE if the pH was ≤5.6 and colour was <28. Carcases were described as having an extensive quality defect if the condition was found in both the loin and ham, or localised if the condition was found in only one of the two sites. The incidence of meat quality defects initially (audit 1) and after implementation of the recommended changes (audit 3) at each abattoir is shown in Table 1. For the purpose of this paper the RSE and PSE categories have been combined into SE.

Table 1. The incidence (%) of meat quality defects in three Victorian abattoirs at the first and third audit.

	Abattoir 1		Abattoir 2		Abattoir 3		Weighted mean	
Audit series	1	3	1	3	1	3	1	3
n	666	833	493	653	639	804	1798	2290
Extensive SE	13.7	10.4	11.6	1.3	8.5	4.0	11.3	5.6
Localised SE	17.6	21.6	16.1	2.6	7.8	5.2	13.7	10.4
Normal	42.8	44.6	30.5	42.3	40.4	34.2	38.5	40.3
Localised DFD	14.8	17.3	23.1	24.7	17.2	22.6	18.0	21.3
Extensive DFD	11.1	6.1	18.7	29.1	26.1	34.0	18.5	22.4

Changes made in Abattoir 1 were minimal. Abattoir 3 made several changes and Abattoir 2 implemented all the recommended changes. The changes implemented included: Reduced use of electric goads, reduced double stunning, improved race design which allows better movement of pigs, and increased space between each carcase on the chiller rails to increase the rate of temperature decline. These changes appear to have resulted in an increase in the incidence of normal meat and a reduction of SE meat at abattoir 2. The reason for the increase in DFD in abattoirs 2 and 3 is unclear. In conclusion, abattoirs that implement the recommended changes to their operating procedures can have a positive effect on the production of quality meat which should result in economic benefits to their company and also to the pork industry.

Supported in part by the Pig Research and Development Corporation

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ELIMINATION OF BOAR TAINT: A BOAR TAINT VACCINE FOR MALE PIGS

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Male pigs accumulate substances (including androstenone and skatole) in their fatty tissue that are responsible for "boar taint" of the meat. In Australia, in recent years, the average slaughter weight of pigs has increased and continues to increase. The increase in weight is driven by the production and processing efficiencies associated with heavier pigs. However, as shown by Hennessy *et al.* (1997) the incidence of highly tainted carcasses rises with increasing sexual maturity.

A vaccine has been developed which reduces the production and accumulation of tainting substances, without adversely affecting growth. Any taint already present is progressively metabolised and the boar may be presented for slaughter, without boar taint, at a higher live weight.

The objective of this study (one of a series of studies to gather data for product registration) was to assess the efficacy of an anti-gonadotrophin releasing factor vaccine (BT Vaccine), in suppressing boar taint when the second dose was given 4 weeks prior to slaughter. The study was conducted on a commercial piggery and involved 300 cross-bred male pigs, split into three treatments and two ages at slaughter. One hundred and fifty pigs were slaughtered at 23 weeks of age with the other 150 slaughtered at 26 weeks of age. The treatments were entire boars vaccinated with either BT Vaccine or a placebo vaccine; or non-injected castrate boars. The vaccines were given as two, 2 mL sub-cutaneous doses, one dose given 8 weeks, and the second dose 4 weeks prior to slaughter.

There was no difference in the efficacy of BT Vaccine in reducing taint at either age thus results for the two ages groups have been pooled (Kruskal-Wallis non-parametric analysis of variance). All pigs treated with BT Vaccine showed a significant antibody response to vaccination. As a consequence testis function was inhibited (data not shown) and boar taint, as assessed by the concentration of both androstenone and skatole in sub-cutaneous fat (refer Hennessy *et al.*, 1997 for methods), was suppressed to low or non-detectable values in 100% of boars. None of the pigs treated with BT Vaccine had high concentrations of both androstenone (>1.0 μg/g) and skatole (>0.2 μg/g). The concentrations of both compounds in the boars treated with BT Vaccine were not significantly different to the concentrations in castrated boars. The mean concentrations of androstenone and skatole in controls and pigs treated with BT Vaccine are shown in Table 1.

Table 1. Androstenone and skatole concentrations (mean ± SD) in fat and the percentage (%) of pigs above the International thresholds of 1.0 μg/g for androstenone and 0.2 μg/g for skatole.

	Androstenone		Skatole		Both high
Treatment (n)	μg/g	>1.0 μg/g	μg/g	>0.2 μg/g	>1.0 & >0.2 μg/g
Placebo (93)	1.12 ± 0.77*	49.5%	0.11 ± 0.10*	10.6%	9.6%
BT Vaccine (94)	0.14 ± 0.15	0%	0.06 ± 0.03	1%	0%
Castrates (95)	0.11 ± 0.03	0%	0.05 ± 0.02	0%	0%

* Indicates significant differences between placebo and BT Vaccine and castrate groups at P<0.001, (Kruskal-Wallis non-parametric ANOVA). The BT Vaccine and castrate groups were not different.

It is concluded that vaccination with the boar taint vaccine, can be used to control and eliminate boar taint in male pigs prior to slaughter.

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BOAR TAINT IN AUSTRALIAN AND NEW ZEALAND PIGS

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There is growing concern and debate throughout the Australian pig industry as to the level and significance of boar taint. Some, with little or no evidence, claim that the level of taint does not pose a problem to Australian consumers. In contrast, there is much published literature and a growing list of anecdotal comments, particularly from Asian, European and North American visitors, that they find meat from entire male pigs smelly and objectionable. This paper reports the concentration of androstenone and skatole in boars from four piggeries in Australia and New Zealand (Sites A-D). The boars were the controls from a series of trials to develop a boar taint vaccine. Sub-cutaneous fat was sampled from a total of 369 boars. Androstenone and skatole were determined simultaneously using a HPLC assay based on the method of Hansen-Moller (1994). The results are presented in Table 1.

Table 1. Androstenone and skatole concentrations (mean ± SD) in fat and the percentage of pigs above the International thresholds of 1.0 μg/g for androstenone and 0.2 μg/g for skatole.

	Androstenone		Skatole		Both high	Live weight
Site (n)	μg/g	>1.0 μg/g	μg/g	>0.2 μg/g	>1.0 & >0.2 μg/g	(kg)
A (228)	0.89 ± 0.71	33.9%	0.10 ± 0.10	8.3%	6.1%	97 ± 17.4
B (43)	1.30 ± 0.93	55.8%	0.12 ± 0.13	14.0%	11.6%	103 ± 10.4
C (49)	0.72 ± 0.67	24.5%	0.22 ± 0.15	40.8%	18.4%	92 ± 10.1
D (49)	0.44 ± 0.35	10.2%	0.23 ± 0.15	42.9%	6.1%	103 ± 10.1

The testes were collected from each boar at slaughter and weighed. Testes weight, as an index of sexual maturity, was highly correlated with androstenone (r=0.40), but not skatole (r=0.07). In each study the Spearman rank correlation coefficient for testes weight versus androstenone was significant (P<0.05). However, testes size is not seen as a suitable screening test to detect high taint carcasses. Boars with "small" testes may still be highly tainted.

Research by Laing *et al.* (1995) showed that the consumer's sensitivity to androstenone was related to a general dislike of pork and pork flavours. Laing *et al.* (1995) demonstrated that Australian consumers preferred the meat from low taint male carcasses and female carcasses compared to the high taint carcasses. The research of these authors also suggests that for Australian consumers the androstenone concentration in pig meat is an important determinant of their overall liking of pig meat.

The major outcome of this paper is the clear demonstration of the high level of taint in boars slaughtered at approximately 85-110 kg live weight. In this study, there were differences in the incidence of taint among the four sites, with between 8.3-42.9% of boars having high skatole concentrations (>0.2 μg/g), while 10.2-55.8% had high androstenone concentrations (>1.0 μg/g). If the pig industry wants to improve the quality and consistency of pig meat flavour and perhaps increase (or at least maintain) domestic consumption, or if industry wants to significantly increase exports to the Asian region, then boar taint is an issue that should be addressed. Further significant increases in slaughter weight are likely to exacerbate the problem in both the domestic and export markets. Elsewhere in this proceedings a method for the effective control of boar taint by vaccination is described (Hennessy *et al.*, 1997).

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RELATIONSHIPS BETWEEN GROWTH PERFORMANCE AND CHEMICAL COMPOUNDS IN FAT FOR ENTIRE MALE PIGS

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Rearing entire male pigs instead of castrates is economically advantageous to pork producers. Entire males grow faster, use feed more efficiently and produce leaner carcasses. However, unpleasant odours can occur when the fat and meat of entire male pigs is cooked, and this represents a potential risk to consumer confidence in pork. Skatole and 5α androstenone are the main chemical compounds associated with unpleasant odours in pork, and there is a synergistic relationship between these two compounds in the development of unpleasant odours (Sather, 1995). The objective of this study was to investigate if traits routinely recorded at slaughter can be used for screening entire male pigs for skatole and 5α androstenone concentration.

Samples of adipose tissue (bellyfat) from the carcasses of 91 entire male pigs (Large White/Landrace/Duroc crosses), originating from seven farms in New Zealand, were collected and frozen. Fat was extracted from adipose tissue with hot acetone, removed from solution by low temperature (-20°C) crystallisation, cleaned up by aminopropyl column chromatography and the concentration of skatole (3-methylindole) and 5α androst-16-en-3-one determined by gas chromatography using a non-polar column.

Mean concentrations of skatole and 5α androstenone were 0.184 ppm (range 0.010-2.097) and 1.555 ppm (range 0.000-7.94), respectively. As the frequency distributions of skatole and 5α androstenone were skewed to the right, these data were $\log_{10}$ transformed. Age at slaughter (155.8 ± 16.3 d; mean ± SD), carcass weight (65.5 ± 6.8 kg) and backfat thickness (P2 measured with a Hennessy Grading Probe, 10.1 ± 2.3 mm) were recorded. Carcass growth rate (430 ± 62 g/d) and lean tissue growth rate (262 ± 37 g/d) were calculated from these data (Prince, 1996).

Skatole and 5α androstenone tissue concentrations were significantly correlated to each other (0.30, P<0.01), but not with any of the growth or carcass traits (Table 1).

Table 1. Correlations between skatole and 5α androstenone concentrations and age at slaughter (AGE), carcass weight (CW), backfat thickness (P2), carcass growth rate (CGR) and lean tissue growth rate (LTGR).

	Androstenone	AGE	CW	P2	CGR	LTGR
Skatole	0.30**	0.19	0.05	0.06	-0.12	-0.13
Androstenone		0.19	0.01	0.09	-0.13	-0.16

**P<0.01

A multivariate statistical analysis (canonical correlation) was also conducted to identify associations between carcass-related traits and chemical compound concentrations in fat. The two canonical correlation coefficients obtained were 0.27 and 0.05, and were not significantly different from zero. Overall, the results show that traits routinely recorded at slaughter (AGE, CW and P2) could not be used to predict the levels of chemical compounds associated with unpleasant cooking odours.

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EFFECT OF COOKING METHODS AND PROCESSING INTO SMALLGOODS ON PERCEPTION OF BOAR TAINT IN PORK

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Boar taint is an unpleasant odour and flavour perceived by consumers when pork from certain boars is cooked and eaten. The presence of taint has been correlated with two chemicals, skatole and androstenone. In Australian finisher-weight carcases, over 50% have levels of androstenone, and over 10% have levels of skatole, which would be detected as tainted by sensitive individuals (Salvatore *et al.*, 1995). Most studies on the perception of taint, as assessed by sensory panels, have focussed on dry roasted fresh pork. This study was conducted to test if different cooking methods, and processing into smallgoods could alter the perception of boar taint in pig meat.

Carcasses were obtained from gilts (n=9), boars with low concentrations of androstenone and skatole (low taint; n=9) and boars with concentrations above the taint threshold (high taint; n=9). Skatole and androstenone were measured by HPLC and gas chromatography respectively. Some meat from each carcass was processed into ham, bacon and salami. On each test day, fresh pork was dry roasted or moist roasted or roasted with a sweet and sour marinade in calibrated ovens; bacon was cooked.

Taste panellists (13 in total) were trained to identify boar taint. Products from the 27 pigs were evaluated by three panels (each comprising 10-12 of the 13 panellists). Each panel assessed nine products (three each from gilts, low-taint and high-taint boars) in blocks of three samples and rated boar odour and flavour on a line scale (Absent=0, Strong=10). The method of Residual Maximum Likelihood was used to analyse results from each panel and the significance of any variation was assessed with likelihood ratio tests. Differences between specific groups were examined using least significant difference tests. Analysis of the combined data from all panels (27 samples) was carried out using weighted least squares. Specific comparisons of pairs of means was carried out using approximate z-tests.

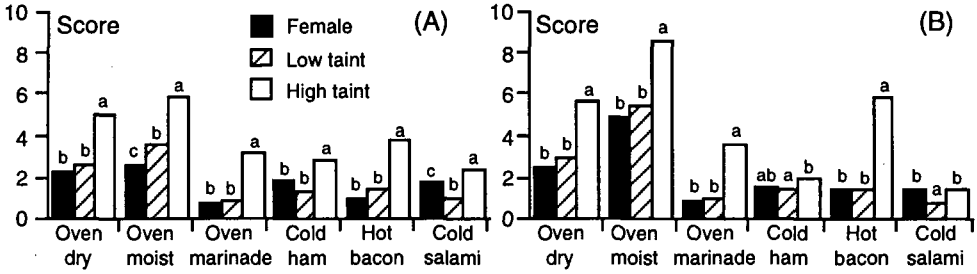

Figure 1. *Effect of cooking methods and processing on the taint score for (A) flavour and (B) odour of pork from gilts, high-taint and low-taint boars. Legend is common to both figures. Means within a treatment with different letters, differ significantly (P<0.05).*

Boar flavour and odour in tainted pork could be detected in all oven cooked meat. Boar taint could not be masked by marinading. For ham and salami, there was no difference in the detection of boar odour between high-taint boars and gilts. In contrast, bacon (cooked) from high-taint boars had larger taint scores than ham or salami (cold). The difference may be due to the lower amounts of fat in ham or reduced levels of volatile components that contribute to taint when ham and salami are consumed cold. This study indicates that neither cooking method nor processing can mask boar taint in hot pork products.

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HYGIENE LEVELS OF AUSTRALIAN PIG CARCASSES

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The Pig Meat Hygiene Program has been funded by the Pig Research and Development Corporation to monitor and improve the hygiene of Australian pig carcasses and meat. The work presented here forms part of the surveys of meat and carcasses undertaken in the program and was conducted to establish the microbiological quality of Australian pig carcasses (Pointon, 1997). The paper reports the number of *Escherichia coli* found on the surface of carcasses. Such counts are considered as indicators of contamination of carcasses with food-borne pathogens of faecal origin.

Twenty pig abattoirs with the highest output in Australia, which collectively represent 70% of the national kill, were selected for the survey. Of the eighteen abattoirs which participated twelve were licensed for domestic supply and six were licensed for export markets. A total of 580 carcasses were swabbed to evaluate numbers of *E. coli* on their surfaces. Samples were collected by swabbing (Kitchel *et al.*, 1975) from randomly selected carcasses at the pre-chiller stage in the abattoirs and were then transported at 4°C to the laboratories. Samples were composites of three swabbed 20 cm^2 areas from the butt, flank and jowl, in 30 ml of peptone water. *Escherichia coli* counts were performed on Petrifilms (3M, USA).

The *E. coli* data are presented as a frequency distribution in Table 1 (abattoir by abattoir). The data are presented in this way to allow comparison with the USDA/FSIS Pathogen Reduction Program 3-class sampling plan where n (number of carcasses sampled) =13; m (lower limit) =10 *E. coli*/cm^2; M (upper limit) =10^4 *E. coli*/cm^2; and c (number of samples between m and M) =3. Under this sampling regime no more than 3 samples in 13 are permitted to be between the lower limit of 10 and the upper limit of 10^4 and no samples are permitted to exceed the upper limit. None of the abattoirs had counts above M, or more than 3 counts in 13 above m.

Table 1. Frequency distribution of *Escherichia coli* counts (*E. coli*/cm^2) from pig carcasses for 18 abattoirs*.

Abattoir No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Counts (range)	Number of carcasses in each range																	
<5	44	10	42	42	19	30	61	20	16	34	59	15	48	19	20	30	20	18
5-10	1		2	10	1		4		2	1	1		1					6
10-10^2			1	2														
10^2-10^3			1															
10^3-10^4																		

*Abattoirs 7, 8, 11, 15, 16 and 17 are licensed for export.

There were no instances where an abattoir would fail the current USDA/FSIS Pathogen Reduction Program criteria, indicating that Australian pig carcasses were of a good standard of hygiene compared to the present USA standards.

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LEVEL OF FOOD-BORNE PATHOGENS ON AUSTRALIAN PIG MEAT AND CARCASSES

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This work forms part of the surveys of meat and carcasses undertaken in the Pig Meat Hygiene Program to establish the microbiological quality of Australian pig carcasses (Pointon, 1997). In this paper the prevalence of food-borne pathogens on Australian pig meat and carcasses are described.

Twenty pig abattoirs with the highest output in Australia, which collectively represent 70% of the national kill, were selected for the survey. Of the eighteen abattoirs which participated twelve were licensed for domestic supply and six were licensed for export markets. A total of 680 carcasses were evaluated at abattoirs for the presence of *Salmonella* spp., *Listeria monocytogenes*, coagulase positive staphylococci (CPS), *Campylobacter jejuni, Yersinia enterocolitica, Escherichia coli* O157 and *E.coli* O111. Samples were taken by swabbing (Kitchell *et al.*, 1975) randomly selected carcasses at the pre-chiller stage in the abattoirs and then transported at 4°C to the laboratories. Samples were composites of three 20 cm^2 swabbed areas from the butt, flank and jowl, in 30ml of peptone water. Culture was performed by Australian standard AS1766 or recommended methods (ICMSF, 1996). The presence of enterohaemorrhagic *E.coli* (EHEC) was detected by polymerase chain reaction (PCR) for shiga-like toxin (SLT) and by PCR for serotype O111. Meat was collected from retail display in 60 stores across Australia, transported at 4°C to the laboratories and sampled by swabbing 20 cm^2 areas and tested similarly.

Table 1. Prevalence of major food-borne pathogens on pig meat and carcasses in Australia.

	Number tested		Number positive		% Prevalence	
Organism	Carcasses	Meat	Carcasses	Meat	Carcasses	Meat
Salmonella sp.	680	120	7	0	1.0,	<2.0
Yersinia enterocolitica	680	120	1	0	0.15	<2.0
Coagulase +ve staphylococci	680	120	97	10	14.9*	8.3
Campylobacter jejuni	680	120	0	0	<0.5	<2.0
Listeria monocytogenes	680	120	0	3	<0.5	2.5
Escherichia. coli O157	680	120	0	0	<0.5	<2.0
PCR SLT[1] (PCR O111[2])	680	120	14(3)	2(0)	2.15 (0.4)	1.7 (0)

*Prevalence on carcasses of toxigenic strains is 7%. [1]Shiga-like toxin. [2]Serotype O111.

The presence of SLT as detected by PCR was used as an indicator of the likely presence of EHEC. A negative SLT PCR indicated that the presence of EHEC was unlikely as EHEC produce that toxin. A further PCR specific for O111 was undertaken on positive SLT samples. A positive PCR for O111 indicates the presence of a pathogenic serotype in the sample. In a similar study of the incidence of EHEC on meat it was shown that few of the SLT positive samples were O111 positive (M. Barton, personal communication). Other serotypes may be present but they are hard to detect using culture techniques and no molecular techniques were available for their detection at the commencement of the project. Organisms which are SLT positive but not EHEC may also be present.

Based on the data from this study, the incidence of pathogens in Australian pig carcasses and meat is low.

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A REVIEW - PIG GENETICS INTO THE 21ST CENTURY

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Abstract

The pig industry has a well deserved reputation for the practical application of modern genetics. In recent years, major advances in quantitative genetics have been applied in improvement programmes, through the use of restricted maximum likelihood (REML) procedures to estimate genetic and phenotypic parameters, and the use of best linear unbiased prediction (BLUP) to calculate estimated breeding values (EBVs). Even more powerful statistical tools are being developed, and in the future these will provide increasingly accurate EBVs of potential breeding animals for a greater range of economically important traits. Complementary to these developments in quantitative genetics, advances in molecular biology are also beginning to provide practical benefits to the pig industry. The most important contribution of molecular biology has been in providing the means of identifying genes that affect economically important traits. This is being achieved through the construction of a genetic map of the pig, and by the study of candidate genes (genes whose product is known to be involved with a particular trait). This work is providing markers that can be used as additional clues in the calculation of EBVs, and, in the longer term, will lead to the identification of the actual genes that affect important traits. Another contribution of molecular biology is in identifying the cause of, and the means of controlling, inherited disorders, such as malignant hyperthermia. In the 21st century, pig improvement will be exploiting both quantitative and molecular genetics. Advances in knowledge about the actual genes that determined economically important traits will open up new possibilities for the non-genetic control and manipulation of production.

Introduction

The pig industry has a long history of innovation - of applying ideas that have often arisen from scientific discoveries. The aims of this review are to summarise the present state of scientific knowledge in relation to pig genetics, and to speculate on how this knowledge will be expanded in the future, and how it will be utilised in practical pig breeding into the next century. Parts of this review have drawn on some of the material presented in the recent review by Nicholas and Webb (1996). For another speculative review, readers can consult Webb (1996).

Quantitative genetics

Quantitative genetics is sufficiently mathematical to strike fear into the hearts of many practical pig breeders. And yet, pig breeders round the world have been at the forefront of the practical application of quantitative genetics in pig improvement programs. This has been made possible by the provision of "black boxes" of specialised computer software that take raw data and process it into numbers that pig breeders can act upon, i.e., numbers that form the basis of selection of replacement breeders.

What is going on in these black boxes? There are two major things happening.

Estimation of phenotypic and genetic parameters

First, phenotypic and genetic parameters (variances and covariances - often expressed as heritabilities and genetic and phenotypic correlations) are being estimated. Breeders could be forgiven for thinking that this is an old-fashioned business that is no longer needed. Surely, it is argued, there are sufficient estimates of the heritability of growth rate. Well, maybe there are, for that particular trait. But there are many traits of major economic importance, such as carcass and reproductive traits, for which there are

only very inadequate estimates of genetic and phenotypic parameters. Also, there are quite strong arguments for having a good set of estimates for each population undergoing selection - genetic and phenotypic parameters are not the same in all populations. In addition, better methods of parameter estimation are continually being developed, especially for the estimation of covariances between traits, which is always a far greater challenge than estimation of variance for a single trait. The 1990s, for example, have been the decade of Derivative-Free Restricted Maximum Likelihood (DF REML; Graser *et al.*, 1987; Meyer, 1989, 1991), a very powerful method of parameter estimation that enables all available information from all known relatives to be included in the analysis, with just the correct degree of emphasis being placed on each item of information. Recent examples of parameters estimated using this approach on data collected from populations of Australian pigs have can be found in Bunter (1997), Hermesch (1996), Hermesch *et al.* (1995, 1997a, 1997b) and Tholen *et al.* (1996a, 1996b). An indication of the range of traits included in these studies is presented in Table 1.

The estimates in Table 1 represent the first comprehensive set of heritabilities for Australian pigs. They indicate, as expected, that there is substantial genetic variation on which selection can be exerted. Estimates for phenotypic and genetic covariance between most of the pair-wise combinations of the above traits have also been obtained, and are reported in the studies cited above. Space limitations in this review preclude the presentation of a summary table of these estimates; readers are referred to the original papers, and to Hermesch (1997) for details. In general, the parameter estimates indicate a favourable genetic relationship between meat quality (on the one hand) and growth rate, ham yield and intramuscular fat (on the other), but an unfavourable relationship between meat quality (on the one hand) and backfat thickness and bacon yield (on the other). Reproductive traits tend to have negligible correlations with other traits, which (in conjunction with the heritability estimates for reproductive traits) shows that selection for improved reproductive performance is practicable. Most importantly, these estimates provide the information that is essential for constructing multi-trait selection indices, which introduces the second type of calculation that goes on within the black box.

Estimating breeding values

Selection indices were developed many decades ago, and have been used in pig breeding for a long time. For each economically important trait, a selection index is used to calculate what is called an Estimated Breeding Value (EBV) for each of a group of animals that are available for selection as breeding replacements (so-called "candidates"). The calculation of an EBV of a candidate for a particular trait often combines the candidate's performance for that trait, with data on the performance of relatives of that candidate, for the same trait. In more complicated cases, performance of the candidate and/or relatives in relation to correlated traits is also included. These various "clues" to the candidate's true breeding value are combined into a single EBV that provides the best possible prediction of how well the progeny of that candidate will perform.

In the 1980s and early 1990s, a new statistical methodology, called Best Linear Unbiased Prediction (BLUP) was adapted for use in pig breeding (Hudson and Kennedy, 1985; Brandt *et al.*, 1988; Lofgren and Stewart, 1991; Stewart *et al.*, 1991; Bampton, 1992). This methodology has all the advantages of the selection-index approach; indeed, the selection index is just a particular case of BLUP. But BLUP has several additional features that have important practical implications. First, BLUP automatically takes account of the effects of identifiable non-genetic factors such as management regimes or years, thereby enabling each candidate to be compared with all other candidates, including those in different management regimes and/or years. Second, so long as the pedigree of each animal is recorded, BLUP automatically takes account of measurements made on all relatives of each candidate, thereby substantially increasing the accuracy of selection, and hence increasing response to selection. Third, BLUP automatically enables comparisons to be made between candidates for which there are differing quantities of information, and for which there has been different selection in the past. This feature of BLUP substantially increases the number of potential candidates, which in turn has the

Table 1. Estimates of phenotypic variance and heritability for traits in Australian pigs.

Trait	Phenotypic standard deviation	Heritability
Average daily gain[1] (g.)	192	0.13
Food intake[1]	0.44	0.27
Backfat[1] (mm.)	3.19	0.53
Colour of longissimus dorsi[1] (Minolta chromamometer)	4.77	0.27
pH 45 minutes after slaughter[1]	0.46	0.14
Drip loss[1] (%)	1.89	0.21
Ham yield[2](%)	2.54	0.11
Bacon yield[2](%)	3.20	0.06
Intramuscular fat[3] (%)	0.58	0.35
Weaning-conception interval for parities 1-2[4](days)	11.20	0.09
Weaning-conception interval for parities 2-3[4](days)	10.43	0.01
Weaning-conception interval for parities 3-4[4](days)	12.53	0.03
Farrowing interval, parities 1-2[4](days)	11.60	0.05
Farrowing interval, parities 2-3[4](days)	11.14	0.03
Farrowing interval, parities 3-4[4](days)	10.72	0.01
True stayability 1-2 (probability of the sow surviving in the herd from parity 1 to parity 2)[4]	-	0.03
True stayability 1-3 (probability of the sow surviving in the herd from parity 1 to parity 3)[4]	-	0.07
True stayability 1-4 (probability of the sow surviving in the herd from parity 1 to parity 4)[4]	-	0.09
Functional stayability 1-3 (true stayability 1-3 corrected for the average number of piglets born alive)[4]	-	0.08
Functional stayability 1-4 (true stayability 1-4 corrected for the average number of piglets born alive)[4]	-	0.08
Number born alive, parity 1[5]	2.61	0.11
Number born alive, parity 2[5]	2.72	0.12
Number born alive, parity 3[5]	2.68	0.13
Average birth weight (kg), parity 1[5]	0.34	0.23
Average birth weight (kg), parity 2[5]	0.36	0.20
Average birth weight (kg), parity 3[5]	0.32	0.25
21-day litter weight (kg), parity 1[5]	12.92	0.17
21-day litter weight (kg), parity 2[5]	15.14	0.12
21-day litter weight (kg), parity 3[5]	15.67	0.23
Age at first farrowing[6] (d)	21.4	0.13

[1]Hermesch *et al.* (1995)
[2]Hermesch *et al.* (1997a)
[3]Hermesch *et al.* (1997b)
[4]Tholen *et al.* (1996a) (averaged across two herds)
[5]Tholen *et al.* (1996b) (averaged across two herds)
[6]Bunter (1997)

effect of substantially increasing selection intensity, which increases response to selection. Finally, by automatically taking account of identifiable non-genetic factors, BLUP makes it possible to separate genetic changes from non-genetic changes over time, thereby providing a direct indication of how much genetic progress is being achieved in a herd (Southwood and Kennedy, 1991; Bejar *et al.*, 1993) - the so-called genetic trend.

In Australia, quantitative geneticists at the Animal Genetics and Breeding Unit (AGBU, Armidale, NSW) have developed a tailor-made, user-friendly software package for personal computers called PIGBLUP (Long *et al.*, 1991; Henzell, 1995). This package enables individual breeders to capture the many benefits of BLUP technology without having to understand the black box. Currently, PIGBLUP calculates EBVs for average daily gain, backfat and number born alive, for each animal whose performance has been recorded in a herd. By calculating the average EBV for all animals born in each year, for each trait, and then plotting these averages against year of birth, the package also shows the genetic trend for each trait in that herd. By then subtracting the average EBV from the average actual performance for each year, the package also shows the environmental trend, i.e., the extent to which changes in average performance are due to non-genetic factors that have changed over time. To be able to separate genetic from non-genetic factors in this way is a very useful aspect of the service provided by PIGBLUP and other packages using BLUP technology.

PIGBLUP also calculates what is called the $INDEX, which is an EBV for overall profitability. This is a particularly important practical application of the black box, because it solves the very practical problem of how to come up with a single ranking of candidates - how to compare a candidate that has a very high EBV for, say, average daily gain, but an ordinary EBV for, say, backfat, with another candidate that might be just a bit above average for both traits. The means of doing this in a rational way is very simple in principle: the EBV for overall profitability is the weighted sum of the EBV for each trait, where the weight for a particular trait is the increase in profitability that would be achieved from a unit increase in performance of that trait, all other traits remaining unchanged. These weights are called economic weights, and they can be calculated either from regional economic data (e.g., Stewart *et al.*, 1990) or from relevant financial details provided by an individual breeder, as in PIGBLUP. Despite the simplicity of the idea, the actual calculation of economic weights is very complex, and somewhat controversial - there has been much argument as to how one should proceed. For example, since selection decisions being made today will be reflected in pigs of the future, and since it is not desirable to be continually altering the weights, there is a strong argument that the weights used today should be based not on today's economic data, but on best guesses as to what will be economically important in the longer term future. But how can anyone predict what will be important in the future with any certainty? Some people have argued against the whole concept of economic weights on the basis that it is just not possible to calculate them in any rational way. But these people overlook the fact that whenever selection is based on more than one trait, economic weights have to be used in one form or another. In many cases, they are determined subjectively, and could therefore be very unreliable. Despite the many complications, the process of calculating economic weights at least has the virtue of forcing people to think about the extent to which each trait will contribute to overall profitability in the future.

In addition to the above features, PIGBLUP provides other services to breeders. Its Genetic Audit, for example, provides vital information about the major factors that determine the rate of genetic progress in a herd - intensity of selection, accuracy of selection, generation interval and inbreeding. Its Mate Selection Module, which is based on the work of Bunter (1995), provides invaluable assistance to breeders in deciding on matings among selected boars and sows, especially in relation to the inbreeding consequences.

What of the future for PIGBLUP and other similar programs in other countries? In the immediate future, the major advancements will involve the inclusion of additional traits for which EBVs can be calculated. Indeed, all of the effort summarized in Table 1 has been directed towards this purpose - the estimation of phenotypic and genetic parameters is the first essential step for being able to include a new trait in packages such as PIGBLUP. Much more work remains to be done in this area. For reproductive traits, in

particular, there are still many unanswered questions about exactly which traits should be included, how they should be measured, and how they should be adjusted to take account of identifiable sources of non-genetic variation. There is also a continual need for refinement of the calculation of economic weights - each new trait included in PIGBLUP presents a new set of challenges in this regard.

As stated above, PIGBLUP currently calculates EBVs for all members of a herd. These EBVs are directly comparable amongst all members of that herd, but are usually not comparable with EBVs calculated for another herd. In other words, PIGBLUP currently provides EBVs for selection within herds, but not for selection among herds. This limitation is not because of any limitation in BLUP technology; it is solely a reflection of the fact that there are relatively few herds that have sufficient connections (use of common breeding stock) to enable valid comparisons of EBVs among herds. The extent of connectedness amongst smaller, independent Australian seed-stock producers was recently investigated by Bunter and Macbeth (1997). They showed that about one-third of the 29 herds were sufficiently connected to justify across-herd evaluations, but that considerable caution would be needed in interpreting the result. Given that the genetic improvement of a large portion of the Australian pig herd is in the hands of a very small number of seed-stock providers, there is some merit in the idea of providing smaller, independent seed-stock producers with the means of directly comparing EBVs across their herds, thereby increasing their potential source of breeding replacements (McPhee *et al.*, 1995). A scheme providing just such a service, called the National Pig Improvement Program (NPIP) is currently being operated by the Queensland Department of Primary Industries (Macbeth, 1997).

Gene hunting: Prising open the black box of quantitative genetics

One of the major challenges facing pig geneticists today is to prise open the black box of quantitative genetics - to identify the actual genes that contribute to all that useful genetic variation that is summarised in Table 1. Nowadays, such genes are called quantitative trait loci (QTL). There are two basic ways in which the search for QTL is being approached - statistical and molecular.

Statistical approaches

Many years ago, Sewell Wright (1952) developed a statistical method for searching for QTL. However, it was not sufficiently powerful to be of any real use. In fact, it is only with the recent development of Markov Chain Monte Carlo (MCMC) methods of analysis of data, that potentially useful results have begun to emerge. As implied by the term "Monte Carlo", this method uses stochastic simulation to obtain estimates of parameters. Recently, Janss (1996) and colleagues have utilized the MCMC approach to search for QTL of large effect in the F2 of a cross between Chinese (Meishan) and Western (Landrace and Large White) pigs. The results reported by Janss *et al.* (1997a) suggest the presence of a QTL of large effect on pH of meat and cooking loss (loss of weight during cooking), and of another QTL of large effect on intramuscular fat and possibly shearforce (a measure of meat toughness) and drip loss. In both cases, the allele which increases the traits is recessive, and most likely originates from the Meishan parents. Janss *et al.* (1997a) named these two genes the Meishan cooking loss (MC) gene and the Meishan intramuscular fat (MI) gene. In a second study, Janss *et al.* (1997b) identified a dominant QTL allele of large effect for backfat, with a difference of 6 mm between the homozygotes (the high backfat allele being recessive); and a dominant QTL allele for litter size at first parity, with a difference of 5 to 6 piglets between homozygotes (the low litter-size allele being recessive). Thus both recessive alleles are unfavourable.

Statistical approaches like those just described can be very useful for having an initial look at a set of data to gain some feel for the likelihood of QTL of large effect being present. With the advent of molecular technology, however, it is now possible to use molecular tools to determine the chromosomal location of such QTL, and to eventually identify them at the DNA level.

Molecular approaches

Genetic maps

The 1990s has been the decade of the genetic map. In humans, in mice, and in all the major domesticated animal species, large-scale application of the tools of molecular biology have enabled, for the first time, the construction of maps of the entire genome of each species (Georges and Andersson, 1996). In pigs, the first round of this activity culminated with the publication of genetic maps arising from the United States Department of Agriculture (USDA) (Rohrer *et al.*, 1994) and the global PiGMaP consortium (Archibald *et al.*, 1995). Since these pioneering publications, the major task of pig gene mappers has been to combine the information of the two original maps, and to substantially increase the number of mapped markers. For a recent review of achievements in pig gene mapping, see Schook and Alexander (1997).

The construction of a genetic map is analogous to the construction of a geographical map by explorers. In the case of genetic maps, the explorers are molecular biologists, and the flags "planted" by these latter-day explorers are DNA markers. Some of these markers indicate actual genes, but the vast majority are anonymous, in the sense that they are not known to be part of a gene that encodes a peptide.

The most common form of anonymous DNA marker is called a microsatellite. Readers will recall that a chromosome consists of a very long strand of DNA, which in turn consists of a particular sequence of four nucleotides - A, T, G, and C. A microsatellite is a short segment of DNA consisting of a variable number of tandem repeats of one or a few bases, e.g., repeats of AC (i.e. ACACAC) or repeats of AGG (i.e., AGGAGGAGGAGG). Such tandem repeats occur throughout the genome of all species so far examined. Microsatellites can be detected easily by a laboratory procedure known as the polymerase chain reaction (PCR), in which a small fragment of DNA (up to several hundred bases in length, including the tandem repeats) is amplified more than a million-fold, in a chain reaction of DNA replication (2, 4, 8, 16, 32...) that is catalysed by the enzyme DNA polymerase. By designing PCR primers that correspond to the unique sequence that occurs on either side of a microsatellite, it is possible to amplify just one microsatellite with one pair of primers. When a microsatellite is amplified in a group of animals, it is found that there is usually substantial variation in the number of repeats - that there are many different "alleles" (different numbers of repeats) at this "locus". For example, in these proceedings Chen *et al.* (1997) report that for 14 microsatellite loci detected in Australian pigs, the number of alleles per locus varies from 3 to 10, with an average of 5.4. Because of this relatively large number of alleles per locus, a large proportion of pigs (usually between 0.5 and 0.6) are heterozygous at each microsatellite locus. This makes microsatellites very powerful DNA markers, since the usefulness of a marker is determined by its level of heterozygosity.

Thousands of microsatellite loci have been discovered in humans, in mice, and in all the major animal species. At the time of writing (September 1997) approximately 1400 microsatellite markers have been mapped in the pig, which represents considerable progress towards the goal of having a set of closely linked, evenly-spaced DNA markers covering the entire genome. Up-to-date details of all aspects of genetic maps in pigs are available at various sites on the world wide web. The best starting point is **http://www.ri.bbsrc.ac.uk/pigmap/pig_genome_mapping.html**, which provides integrated access to the maps compiled by the global PiGMaP project, the USDA, and the Scandinavians. Much of this material is available from a mirror site in the USA (**http://tetra.gig.usda.gov:8400/pigbase/manager.html**). Another site, in Japan (**http://ws4.niai.affrc.go.jp/dbsearch2/jgbase.html**), provides a different and very useful means for accessing the latest information on porcine genetic maps.

How are these microsatellites used in gene hunting? The idea is very simple. If every region of every chromosome has at least one DNA marker, then every gene will be "linked" to at least one marker. If the performance of a group of related animals is measured, and if these same animals are genotyped for each of a set of markers that cover the entire genome (a genome-wide scan), it is possible to identify which regions of which chromosomes contain genes that affect performance in the trait that was measured. Of

course, identifying a DNA marker for a QTL is still a long way from actually identifying the QTL (the gene) itself. However, it is a major first step.

The first study of this type to be reported for any domestic animal species was the analysis by Andersson *et al.* (1994), who showed that a single region of pig chromosome 4 accounted for a large part of the breed difference in growth rate, fatness, and length of the small intestine. Many similar studies are now underway throughout the world. In Australia, a PRDC-funded hunt for DNA markers for QTL is being conducted jointly by molecular geneticists at the University of Sydney (under the direction of Chris Moran) and quantitative geneticists at the Animal Breeding and Genetics Unit (AGBU) at the University of New England (under the direction of Mike Goddard).

This collaboration between molecular and quantitative geneticists is an essential component in any hunt for genes that affect quantitative traits: indeed, one of the major challenges facing quantitative genetics today is how to most effectively analyse the data being generated from animals that have been genotyped for DNA markers and measured for economically important traits. When molecular genetics first came on the scene in the mid-1970s, there were grave fears that the days of quantitative genetics were numbered; that molecular genetics would render all other forms of genetics redundant. In fact, the opposite is true: the only people who can determine how the advances in molecular genetics should be applied in practical pig breeding are quantitative geneticists. To return to the context presented earlier in this review, the tools of molecular genetics are prising open the black box of quantitative genetics - they are not throwing the box away.

Candidate genes

There is another approach to identifying QTL; the so-called candidate-gene approach, in which a gene whose product is known to be involved with a particular trait, is studied to see if any of the variation in the trait is due to variation at that gene. By far the best example in pigs is the work of Rothschild *et al.* (1994, 1996) with the gene for the oestrogen receptor, located on chromosome 1. Through its role in the action of oestrogen, the oestrogen receptor is obviously involved in reproduction. Noting this role, Rothschild *et al.* (1991) cloned the gene for this receptor, and identified a polymorphism. They then genotyped sows from various populations for this gene, and discovered that sows with one allele at this locus had a greater litter size than those with the other allele. Their initial results were obtained from only a small number of sows, and were not particularly convincing (Rothschild *et al.*, 1994). However, subsequent investigations of thousands of sows have supported the initial results, and have confirmed that allelic variation at this locus does affect litter size (Rothschild *et al.*, 1996). It is not yet clear what the exact nature of this effect could be, but it does seem to be real, and the DNA marker for the high-litter-size allele is now being introgressed into populations where it was absent.

The candidate-gene approach has a very low probability of success, because many genes that are known to be involved with a trait do not show any useful variation. However, when it does hit the jackpot, as in the case of the oestrogen receptor, it has a distinct advantage, in that the actual QTL has been identified. In other words, the candidate-gene approach has the very real advantage of completely bypassing the frustration of having identified a linked DNA marker to an unknown QTL, which still leaves researchers with the substantial task of identifying the QTL itself.

Obviously, in order to capitalise on the candidate-gene approach, it is necessary to have a list of genes known to be involved in the trait of interest. Knowledge in this area is still very scanty. The total number of genes in a typical mammalian genome is thought to be somewhere between 50,000 and 100,000. In pigs, only a handful of these have been identified, let alone characterised. However, knowledge of pig genes is increasing at an ever-increasing rate. For example, in 1996 (the most recent year for which 12-month data are available), approximately 300 papers on pig genes or peptides were published. There is no single list of all known pig genes, but the number is likely to be around 1000, and appears to be increasing at a rate of around 7 per month. An example of the type of knowledge that will be much more available in the future, and which will be so useful to researchers using the candidate-gene approach, is the catalogue of genes involved in pig ovarian follicular differentiation, compiled by Tosserklopp *et al.* (1997). The approach used by these workers provides a good illustration of the power of molecular biology. In

this particular case, Tosserklopp *et al.* (1997) isolated RNA from (ovarian) granulosa cells, knowing that this RNA would be representative of many of the genes that are involved in ovarian follicular differentiation. They then created a DNA copy of the RNA (using the enzyme reverse transcriptase), and then cloned and sequenced the so-called cDNA. Of the 136 unique sequences (genes) that they detected, four corresponded to known pig genes, 35 matched previously reported human genes, and 15 matched genes previously reported in other non-human mammals. The other 82 genes appear to be newly discovered pig genes. This is a powerful way to discover new genes that are involved in particular physiological functions, and holds great promise for the rapid expansion of the list of known genes in pigs and other species.

Comparative maps

One of the truly amazing things to emerge from molecular biological research in the last decade is the high degree of conservation of gene location among species. This picture is visible at the gross level of chromosomes, and at the level of individual genes. Evidence for the former has come from so-called zoo-FISH studies, in which all the DNA from a single chromosome of one species, e.g., human, is fluorescently labelled and then hybridised ("painted") on to a spread of chromosomes of another species, e.g., pig, by a process known as fluorescent in situ hybridisation (FISH). Those regions of pig chromosomes that are homologous to that particular human chromosome are lit up - ready for all to see. When this same exercise is done for DNA from each of the human chromosomes, a complete picture of chromosomal homology between the two species becomes clearly evident (Rettenburger *et al.*, 1995; Fronicke *et al.*, 1996). The results show that large sections of chromosomes (and, in some cases, whole chromosomes) in the two species are homologous. For example, pig chromosomes 8 and 11 appear to be homologous with human chromosomes 4 and 13 respectively; and pig chromosome 2 appears to be homologous to portions of human chromosomes 5 and 19.

The other line of evidence for strong homology across the mammalian spectrum is provided by comparisons of gene location among species. Consistent with the results from zoo-FISH, the picture that emerges here is of whole blocks of genes being linked together in the same order in both species (Wakefield and Graves, 1996).

What is the value to animal breeders of this homology across species? The main value is that if a particular gene has been mapped in one species, then its approximate chromosomal location in all other species can be predicted with confidence. This is of enormous benefit for hunters of genes in pigs and other domestic animal species - all of which have relatively sparse maps, compared with the far more dense maps of humans and mice, which have been studied so extensively. Thus, if a pig-gene hunter identifies a microsatellite marker linked to a QTL for, say, backfat, then this hunter can go immediately to the homologous regions of the mouse and humans maps, and examine the genes that are located in those regions, looking for genes that are likely to be relevant to the trait of interest.

Putting it all together: A renaissance for quantitative genetics

As mentioned earlier, far from being an outdated science, quantitative genetics is undergoing a renaissance. The new generation of quantitative geneticists are scientists who spend a lot of time talking with molecular biologists, advising on the design of experiments for the detection of QTL, and devising more effective means of analysing marker data in the search for QTL.

Once useful markers have been found, quantitative geneticists again have a major role to play - they are the only ones who can determine how best to make use of these markers in practical selection programs, i.e., in marker-assisted selection (MAS). There are still many unknowns about the most effective way to use MAS, and about how to decide when MAS will be worthwhile (e.g., Gibson, 1997). For traits that are readily measurable before the usual age of selection in both sexes, MAS probably has limited utility because traditional selection is so effective. Not surprisingly, much of the effort

currently devoted to detection of QTL is concentrating on other types of traits, e.g., reproductive traits, meat-quality traits, and various aspects of disease resistance.

Other issues

Inherited disorders and their control

At about the time John Webb was writing his review of pig genetics for the 1991 APSA conference (Webb, 1991), a discovery of major importance to the global pig industry was being announced: A Canadian research team led by David MacLennan reported the molecular isolation and sequencing of the coding sequence of the "halothane" gene (Fujii *et al.*, 1991). As most readers will know only too well, the gene was originally identified in terms of reaction to the anaesthetic halothane. As readers will also know, its importance lies in the very strong association between adverse reaction to halothane (called malignant hyperthermia syndrome, MHS) and two economically important defects: porcine stress syndrome (PSS), which often results in sudden death; and pale, soft, exudative (PSE) meat. In contrast to these undesirable features, the halothane gene also has a substantial advantage; it increases yield of lean meat. A summary of the effects of this gene is provided by Hermesch (1997).

By comparing the nucleotide sequence of the two alleles at the halothane locus (normal and reactor), Fujii *et al.* (1991) showed that the reactor allele differed from the normal allele by the simplest of all possible mutations; the substitution of a single nucleotide (thymine, T, for cytosine, C) at the 1843rd nucleotide position, which causes an amino-acid substitution (of arginine for cysteine) at the 615th position in the polypeptide chain. The importance of this discovery was that it immediately suggested a variety of straightforward molecular genotyping tests that could be conducted on a commercial scale, using PCR on any cells (usually white blood cells) that contain DNA.

The sequencing of the halothane gene enabled extensive testing to be conducted in populations throughout the world. In Australia alone, more than 10,000 animals have been genotyped by the National Hal Gene Testing Service run by the Animal Research Institute (Queensland Department of Primary Industries) at Yeerongpilly (Ouwerkerk, personal communication). Despite the obvious genetic differences among pig breeds and even within pig breeds in different countries, the mutation discovered by Fujii *et al.* (1991) appears to be responsible for MHS in all breeds throughout the world (for a recent review, see O'Brien, 1995). In humans, the picture is very different: in some families the same mutation as in pigs is associated with MHS, but in other families, MHS does not co-segregate with the halothane gene, indicating that there are other genes involved in MHS (for a recent review, see Mickelson and Louis, 1996). Given this information, it would not be surprising to see, sometime in the future, cases of MHS in pigs which are not associated with the halothane gene. Indeed, Ian Hughes (personal communication) already has some suggestive data that is consistent with this possibility.

In practice, however, genotyping for the C1843T mutation has been a great success around the world. Indeed, it represents the first widespread practical application of molecular biology in animal breeding. The genotype test has enabled thousands of pig breeders throughout the world to identify which of their phenotypically normal animals are carrying the reactor allele. Armed with this knowledge, it has then been possible in the short term, to plan matings so as to avoid the production of any reactor pigs; and in the longer term, it has enabled breeders to remove the reactor allele completely from their herds.

Other inherited disorders

There is an enormous number of papers on the halothane gene in pigs. There is also a substantial number of papers on other inherited disorders in pigs. However, until recently, this literature has remained scattered throughout libraries. After many wasted hours chasing up references in response to queries received regularly from veterinarians and breeders, the present author decided to compile a catalogue of inherited disorders in all the major species of domesticated animals, to regularly update this catalogue with the

latest published papers, and to make the catalogue freely available. This dream became a reality in May 1995, when Online Mendelian Inheritance in Animals (OMIA) was made available at: **http://www.angis.su.oz.au/Databases/BIRX/omia/** on the world wide web. The pig portion of this catalogue has been summarised by Nicholas (1998). This catalogue is modelled on, is complementary to, and is electronically linked to, the catalogue of human inherited disorders - Online Mendelian Inheritance in Man (OMIM) - developed by Victor McKusick at the Johns Hopkins University Hospital in Baltimore.

At the time of writing, the pig section of OMIA contains entries for 189 inherited disorders or traits, including 42 disorders/traits that are most likely caused by a single gene (Table 2). For 8 of these disorders/traits, the gene (or gene product) has been identified (Table 2); and in 2 cases (malignant hyperthermia and white coat colour), the molecular basis of the mutation has been determined. Among these disorders/traits, 17 are very similar to known disorders/traits in humans, and therefore provide useful animal models (indicated in Table 2 by the relevant identifier - MIM number - in the human catalogue). While the catalogue is far from complete, each entry for pigs contains a brief description of the disorder/trait, and a brief summary of the current state of knowledge. Whenever a new paper appears on a particular disorder, the entry for that disorder/trait is appropriately updated. Despite its incompleteness, OMIA already contains a total of 1616 references on pigs, including 665 on the halothane gene.

Table 2. Single-locus disorders/traits in pigs.

Disorder/trait	Gene	Homologous human disorder/trait[1]
Aplasia of tongue		
Arthrogryposis		208100
Ataxia, progressive		
Coat colour, dominant white spotting	Mast/stem cell growth factor receptor	164920
Dermatosis vegetans		
Dwarfism		
Epitheliogenesis imperfecta		107600, 107601, 136500, 207700, 207731, 302803, 600268, 600360
Gangliosidosis, GM2	Hexosaminidase B	268800
Heterochromia iridis		142500
Hind limb paralysis		
Hypotrichosis, recessive		146520, 146550, 250460, 211370, 146530, 183849, 246500, 241900, 278200, 600077
Hypotrichosis, dominant		146520, 146550, 250460, 211370, 146530, 183849, 246500, 241900, 278200, 600077
Legless		
Lymphosarcoma		
Malignant hyperthermia	Calcium release channel	145600, 180901
Meat quality		
Membranoproliferative glomerulonephritis type II	Factor H	134370
Motor neuron disease, lower		

Table 2 (continued)

Disorder/trait	Gene	Homologous human disorder/trait[1]
Neonatal diarrhoea, K88		
Nucleoside transport defect		
Oedema		
Oedema disease, resistance to		
Polydactyly with otocephalic monster		
Porphyria	Oroporphyrinogen III synthase	176000, 176200, 125270, 176010, 176090
Progressive myopathy		
Protamine-2 deficiency	Protamine 2	182890
Pseudo-vitamin D deficiency rickets	Renal 1-alpha-hydroxylase	264700
Pulawska factor		
Renal cysts		
Respiratory distress syndrome		
Syndactyly		212780
Three-legged		
Thrombopathia		185050
Tremor type A III, congenital		
Tremor type A IV, congenital		
Tremor, high-frequency		190200, 190300, 190310, 214380, 146500, 160500
Uterus aplasia		
von Willebrand disease	von Willebrand factor	193400, 177820, 231200, 277480, 314560
Wattles		
Wilms tumour		194070, 194071, 194090, 194080, 194072
Woolly hair		

[1]The six-digit numbers are the unique identifiers for disorders used in McKusick's (1997) *Mendelian Inheritance in Man* (**http://www.angis.org.au/Databases/BIRX/**).

For a comprehensive, up-to-date list of references arranged chronologically, readers are referred to the world-wide-web site for OMIA, given above.

Major genes

Apart from inherited disorders, there are several major genes that are directly relevant to pig production. These, too, are included in OMIA and in Table 2.

One such gene determines resistance to neonatal scours caused by strains of the bacterium *Escherichia coli* that carry the so-called K88 antigen on their surface. These bacteria cause fatal neonatal scours in young pigs that have a receptor for the K88 antigen on the surface of their intestine. Presence or absence of the receptor is due to alleles at an

autosomal locus on chromosome 15 of the pig. Absence of the receptor, which is a recessive trait, confers resistance to the K88 strains of bacteria, because the bacteria are unable to attach to the surface of the small intestine, and hence cannot proliferate and cause scours by excreting their toxins. Linked DNA markers are known for the K88 gene, but the actual gene has yet to be determined (Grange and Mouricout, 1996).

Also of interest is a gene affecting meat quality, called the RN (for Rendement Napole) discovered by LeRoy *et al.* (1990). Longissimus dorsi (LD) muscles from carcasses of carriers of this dominant gene have a lower pH, higher surface and internal reflectance values, lower protein extractability, lower water-holding capacity, lower Napole yield (yield after curing and cooking), and greater cooking loss. On the positive side, LD muscles have a lower shear force value, a stronger taste and smell, and greater acidity. The primary cause of these differences is that the mutant allele results in higher stored glycogen content in muscle. Linked DNA markers have been identified for this gene (Milan *et al.*, 1996).

The molecular revolution has also provided other research breakthroughs that are directly relevant to pig breeding. One notable recent example is the discovery of the molecular basis of white coat colour in pigs by Leif Andersson and colleagues in Sweden (Moller *et al.*, 1996). This is a truly fascinating story, which began in the mid-1980s with the discovery by Besmer *et al.* (1986) of a cancer-causing gene in cats, called (with appropriate recognition of the feline species) c-kit. This gene causes cancer only when it mutates to an allele that operates out of control. Other alleles at this locus control the proliferation and migration of certain cells (including melanosome precursor cells) from the embryonic backbone down either side of the developing embryo. White-spotting alleles at this locus exert their effect by limiting the number of cells produced, and also by limiting the extent of their migration. White coat colour is simply the absence of colour. In extreme cases, alleles at this locus produce so few melanosomes that animals are entirely white. After the initial discovery in cats, this work was pursued with great success in mice, a species in which there is an enormous number of papers on the genetics of coat colour. Once the murine equivalent of c-kit had been cloned and studied at the molecular level, it was a relatively straightforward matter (although still pioneering) to isolate the same gene in pigs, and to ask whether there was an allele at this locus in pigs that was associated with white coat colour in pigs. This is what Andersson and colleagues (Moller *et al.*, 1996) did so successfully. Many of the details of their discovery have not yet been published, but a genotyping test is now available commercially. This is the second example of how molecular discoveries are leading to practical advances in pig breeding. In the case of white spotting, the importance of the genotyping test is that it enables breeders to capitalise on the many merits of coloured pigs while at the same time avoiding the penalties associated with coloured skins.

Transgenics

Pigs have been to the forefront of transgenic research, both in an agricultural context, in which the aim has been to create strains of pigs that are more economically productive (Hammer *et al.*, 1985; Solomon *et al.*, 1994; Solomon *et al.*, 1997), and in a medical context, in which the aim has been to create "bioreactors" for the production of specific human proteins such as haemoglobin (Logan and Martin, 1994), or strains whose tissues lack those porcine antigens that are the major cause of tissue rejection following xenotransplantation (Greenstein and Sachs, 1997; Hancock, 1997).

In the agricultural area, despite a large and very expensive research effort, the results have been disappointing, and, to my knowledge, not one transgene is being utilised in commercial pig production anywhere in the world. Furthermore, if transgenesis is to be used solely as a means of obtaining extra improvement in economically important traits, the effects of the transgene need to be quite large before transgenesis is any better than conventional selection. The reason for this is that once a transgenic animal has been created, it takes several generations to introgress the transgene into a population, and to evaluate its effect in large numbers of animals. During this time, substantial additional gains will have been made by conventional selection. Consequently, by the time the transgenic line is ready for commercial release, its non-transgenic competitors will have

made substantial additional progress by conventional selection (Gama *et al.*, 1992). In view of these very important limitations, it seems likely that the greatest commercial benefits of transgenesis will come from the introduction of novel effects, such as the utilisation of novel feedstuffs, or overcoming problems associated with welfare (e.g., natural disease resistance) or pollution. However, even with developments such as those just listed, there could easily be a substantial public outcry at the whole process of transgenesis, especially if, as happened in Australia, the transgene contains some human DNA. The fact that the human DNA, in the Australian case, was non-coding, did not assuage the fears of the many members of the public who were appalled at the thought of eating something that is perceived as being part human. An educational programme aimed at explaining this technology would undoubtedly allay the fears of some members of the public. However, if work on animal transgenesis is continued in the future, it is to be hoped that those involved will confine themselves to using animal DNA.

Xenotransplantation

Although not directly related to pig production, there has been enormous scientific activity in investigating the use of pigs as the source of organs for transplanting into humans, and, as mentioned above, much effort and expense has been devoted to developing strains of transgenic pigs as donors of organs for transplantation into humans (Greenstein and Sachs, 1997; Hancock, 1997). However, the huge commercial investment in this research is now under threat because of the fear that porcine retroviruses (which are natural components of pig chromosomes) can infect and proliferate in human cells (Stoye and Coffin, 1995). Considerable fuel was added to the fire by Patience *et al.* (1997), who provided the first direct evidence that a porcine retrovirus can actually infect human cells. This has caused widespread concern in the medical community (e.g., Nasto, 1997), and has led to the banning of porcine xenotransplantation in some countries. However, if the choice is between the certainty of dying in the short term without a xenotransplantation, and the possibility of dying in the longer term as a result of a retroviral infection after xenotransplantation, it seems likely that pigs still have a future as a source of organs for human transplantation.

Pig genetics into the 21st century

Predicting the future is always a risky business. With this qualification firmly in mind, let us do a spot of gazing into the crystal ball. Selection within lines and crossing between lines will continue to be the mainstays of seed-stock producers. Advances in quantitative genetics will enable faster rates of response to selection. The tools of molecular genetics will provide long lists of pig genes, and shorter lists of regions of chromosomes that contain QTL. Developments in quantitative genetics will greatly increase the effectiveness of searches for markers for QTL, and will also enable the practical application of MAS for traits that can not be measured in both sexes prior to puberty. As more of the genes responsible for quantitative genetic variation in economically important traits are identified, understanding of the biochemistry and physiology of pig production will be greatly expanded. In turn, this knowledge will lead to novel non-genetic means of making pig production cleaner and greener. In fact, the great paradox of genetics is that the more that is learnt about the genetic basis of any trait, the more likely it is that non-genetic means of altering the performance of that trait will be devised. In this sense, MAS and transgenesis are likely to be only transient phenomena - technologies that will tide us over until the stage is reached where biochemists and physiologists rule supreme, giving rise to a truly acceptable and sustainable form of pig production.

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PREPARATION AND BANDING OF MEIOTIC PACHYTENE BIVALENTS OF PIGS FOR GENE MAPPING

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Gene mapping on somatic chromosomes is an important and effective way for analysing the genome of pigs. In the present paper, a new way for studying the chromosomes and physical gene mapping of pigs, banding and gene mapping on meiotic bivalents, is reported.

Well-spread meiotic pachytene bivalents were obtained by using prolonged hypotonic treatment combined with high-chloroform Carnoy's fixative solution on cells from the testes of pigs. Detailed G and C banding was performed, and a pachytene chromomere map of porcine spermatocyte autosomal bivalents was constructed. G-banding on bivalents basically corresponded to that on somatic cell chromosomes. A total of 317 bands including 139 positive bands and 178 negative bands were identified. Landmarks and regional divisions have been decided according to the Committee for the Standardised Karyotype of the domestic pig (Gustavsson, 1988). C-banding appears round or ellipsoid in shape and shows polymorphism. Bivalents 1, 4-6, 10, 13, 16-18 are large and dark staining; other bivalents are small and light staining. The sex bivalent is easy to recognise because it shows only partial synapsis.

Primed *in situ* DNA syntheses (PRINS) and multicolor-PRINS on the bivalents were performed according to the methods described by Koch *et al.* (1989), Hindkjr *et al.* (1994) and Koch *et al.* (1995). Microsatellites SW605 and SW60 were regionally localised on 12q15 and 12q13-q15 by PRINS combined with multiple banding before or after hybridisation. A chromosome framework map of the loci for growth hormone (GH), SW60 and SW605 on bivalent 12 was constructed by double-colour PRINS. The results are expressed in terms of relative distance between loci, where the whole chromosome has a length of 100. The average relative distance between GH and SW605, and between GH and SW60, is 80.5 and 67.3, respectively. From these data, the physical distances can be deduced as 59.6 and 49.9 Mbp on the basis of porcine chromosome 12 having a total length of 74 Mbp (Rohrer *et al.*, 1996).

There are advantages of pachytene bivalents compared with the standard mitotic chromosomes. First, this is an easy and simple method for obtaining large numbers of spreads compared with routine cell-culture techniques. Secondly, more precise physical mapping is possible due to the greater length of pachytene bivalents relative to mitotic chromosomes. Thirdly, there is improvement in the efficiency of detecting PRINS labelling due to the pairing of homologues and thus doubling of the DNA target. Finally, pachytene bivalents are more convenient for banding, either before or after PRINS. Therefore, this study of banding and gene mapping on porcine pachytene bivalents will lay the foundation for high-resolution physical gene mapping in the pig.

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CLONING, SEQUENCING AND POLYMORPHISM OF PORCINE MICROSATELLITES

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Microsatellites can be used in gene mapping, marker-assisted selection (MAS) and evaluation of genetic diversity, because of their abundance, uniform distribution throughout the genome, high polymorphism and convenience for genotyping. Two incomplete genomic libraries were constructed using M13mp18 and pBluescript SK+, and 23 AC-positive clones were selected using ^{32}P-labelled $(TG)_{10}$. Three clones have been sequenced using an ABI373 sequencer and the $(TG)_n$ repeats confirmed. The inheritance and polymorphism of these three microsatellites (provisionally called HAU01, HAU02, HAU03) has been studied by polymerase chain reaction (PCR), using the following respective primer pairs. The PCR products were resolved on 12% polyacrylamide gels stained with silver (Huang *et al.*, 1995; Neilan *et al.*,1994).

The sequence of primer pair HUA01 is:
5'-GAATGTCCGTCTGCATGAGTC-3', 5'-TCACACAGGAACAGCTATGACC-3'
The sequence of primer pair HUA02 is:
5'-CCACCTTCCTAGCATATCAGTTG-3', 5'-CCTGAGTAAGAATCCTGCGCTAG-3'
The sequence of primer pair HUA03 is:
5'-CCTATCAGTGGCTATGGTGTAGTT-3', 5'-GGAGTAGGATTCTGGCGTAGGAAT-3'

A Large White x Erhualian half-sib family (consisting of two full-sib families each with 7 offspring) confirmed simple Mendelian co-dominant inheritance patterns for all alleles at these loci. Allele frequencies in six breeds are shown in Table 1 with three, four and two alleles in HUA01, HUA02 and HUA03, respectively. Chi square homogeneity tests (X^2_{10}=47.5, P<0.001; X^2_{15}=27.9, P=0.024; X^2_5=16.8, P=0.005) on the allele data show highly significant differences among breeds, mainly due to frequency differences between the Erhualian and Tongcheng breeds versus the three European breeds. The Hubei White, which is a synthetic breed of Chinese and European descent, is more similar to the European breeds. The results confirm that the three microsatellites can be applied to reveal genetic diversity and they will eventually be used to screen for quantitative trait loci (QTL) of pigs.

Table 1. Allele frequencies of microsatellites in six pig breeds.

Breeds	n	Microsatellites								
		HAU01 (3 alleles, bp)			HAU02 (4 alleles, bp)				HAU03 (2 alleles, bp)	
		167	171	173	106	108	112	114	101	103
Landrace	12	0.42	0.50	0.08	0.12	0.67	0.17	0.04	0.42	0.58
Large White	14	0.54	0.39	0.07	0.11	0.43	0.36	0.10	0.32	0.68
Duroc	16	0.34	0.44	0.22	0.25	0.25	0.41	0.09	0.44	0.56
Hubei White	8	0.31	0.63	0.06	0.06	0.50	0.31	0.13	0.38	0.62
Erhualian	19	0.32	0.10	0.58	0.00	0.50	0.40	0.10	0.68	0.32
Tongcheng	16	0.37	0.13	0.50	0.00	0.63	0.25	0.12	0.72	0.28

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USE OF INSULIN-LIKE GROWTH FACTOR-I AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 AS INDIRECT SELECTION CRITERIA FOR AVERAGE DAILY GAIN, P2 AND FIVE WEEK WEIGHT

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Insulin-like Growth Factor-I (IGF)-I and Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) fulfil a number of criteria for potential inclusion as indirect selection measures in pigs. They can be accurately measured in the blood of young animals (Owens *et al.*, 1990) and have been found to be significantly related phenotypically to at least growth rate (Owens *et al.*, 1997). However there is little information on the genetic associations between IGF-I and the economically important traits. Outlined below are the results from an initial study designed to provide this information.

Thirty-six sires were mated to 258 dams to produce a total of 952 progeny. The pigs were either Large White or Landrace. The following traits were measured at approximately five weeks of age, body weight (WT5W), and plasma concentrations of IGF-I and IGFBP-3 (Owens *et al.*, 1990). Average daily gain from birth to 25 weeks of age (ADG) and fat depth 6.5 cm from the midline over the last rib (P2) at 25 weeks were also measured. The heritability and genetic correlations were estimated with a standard sib analysis using Harvey's Model 5 (Harvey, 1988). Fixed effects in the model included line, date of measurement and sex. For P2, final weight was included as a co-variate. The heritability, genetic and phenotypic correlations for all traits are presented in Table 1.

Table 1. Genetic parameters for IGF-I, IGFBP-3, WT5W ADG and P2. Heritability estimates are on the diagonal (in underlined bold type) and genetic and phenotypic correlations are above and below the diagonal respectively.

	IGF-I	IGFBP-3	WT5W	ADG	P2
IGF-I	**0.10**	0.87	0.96	*	*
IGFBP-3	0.48	**0.15**	0.85	0.39	0.97
WT5W	0.69	0.38	**0.56**	0.30	0.19
ADG	0.25	0.15	0.43	**0.15**	0.79
P2	0.15	0.11	0.09	0.48	**0.26**

*Genetic correlations are greater than 1.

The heritability estimate for IGF-I is similar to that reported in mice and sheep (Blair *et al.*, 1990). The most interesting result is the very high positive genetic association between at least IGFBP-3 and most probably IGF-I with P2. In other words to reduce P2 selection would be for lower concentrations of the hormones. Further work with larger population sizes is required to confirm this result and determine the genetic correlations with other traits such as feed efficiency.

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THE *OBESE* GENE AND VOLUNTARY FEED INTAKE IN PIGLETS

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Piglets are born with very little body fat, usually less than 2%, but accumulate it rapidly while sucking the sow. By the time they are 3 weeks of age and weigh 6 kg they have accumulated large amounts of lipid, around 16% of body weight. Coupled with this rapid fat accumulation is a food intake which is about half that of piglets reared artificially on cow's milk. Does this rapid accumulation of fat reduce food intake and, if so, what is the mechanism?

Leptin is a protein that circulates in the blood stream and acts directly on the central nervous system to control food intake (Pelleymounter *et al.* 1995). It is produced by the *obese*(*ob*) gene which itself is expressed only in fat tissue. There is interest in how the obese gene and its product, leptin, might control voluntary food intake in pigs. For this study newly-weaned piglets were chosen because of their capacity to eat large amounts of energy and their potential to accumulate body fat very rapidly. The working hypothesis is that the more fat a piglet accumulates the greater the expression of the *ob* gene in fat tissue and the greater the amount of circulating leptin which in turn will act on the central nervous system to reduce food intake.

This hypothesis was tested by manipulating the body composition of pigs to make them either fat or lean and then measuring the concentrations of leptin in blood and the expression of the *ob* gene using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Eight female piglets from two litters were selected at three weeks of age. Four piglets (two from each litter) were induced to deposit lean tissue by feeding them cow's milk, a protein-adequate diet (26% crude protein). The remaining four piglets were induced to deposit mainly fat tissue by feeding them a protein-deficient diet, cow's milk with added fat and glucose (8.5% crude protein). Blood samples were taken at the start of the experiment at 7.5 kg, 9.5 kg and 12.5 kg live weight (LW), and plasma was assayed for leptin using a human radioimmunoassay kit (Linco Research, Inc., MO, USA). The depth of subcutaneous fat was measured at 12.5 kg LW and a fat sample was taken for measurement of mRNA. Isolation of total RNA from 0.3-0.6 g of fat tissue was performed using a RNAzol™B kit. Reverse Transcriptase Polymerase Chain Reaction was carried out to detect and quantify the relative amounts of the *ob* mRNA using gel electrophoresis and a pair of nucleotide primers (OBF2 - CACCGGTTTGGACTTCATTC; OBX3R - TCAGCAGCCAGGGCTGAGGTCCAGC) yielding an expected 334 bp product.

At 12.5 kg LW as planned the pigs fed the protein-adequate diet were lean (2.5 mm) and those given the protein-deficient diet were fat (6.0 mm). But, when both groups of pigs were offered cow's milk *ad libitum* their voluntary food intake was the same (fat pigs consumed 0.77± 0.01 and lean pigs consumed 0.78 ± 0.02 Kg(DM)/day). There was a higher relative expression (assessed by band intensity of RT-PCR products) of the *ob* gene with fat pigs producing more *ob* mRNA than their lean counterparts. However, leptin was not detected in either group.

The hypothesis was not supported by the results. Despite a big difference in body fat between the groups, fat pigs had the same voluntary intake as lean pigs suggesting that body fatness exerts no control on food intake at this stage of the pig's life. But the *ob* gene is certainly expressed in the pig at this early stage of its life and it is related to the amount of body fat. It is possible that the *ob* gene has no control on voluntary intake at this stage of life because the mRNA is not translated to leptin. Unfortunately, this could not be confirmed because pig leptin was not detected by the RIA kit used. Alternatively, leptin may be present but ineffective because receptors in the brain are not switched on.

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PHENOTYPIC PERFORMANCE OF GILTS AND YOUNG BOARS CAN BE PREDICTED FROM THEIR ESTIMATED BREEDING VALUES FOR GROWTH RATE AND BACKFAT

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Estimated Breeding Values (EBVs) obtained from genetic evaluation programs such as PIGBLUP (Henzell, 1995) are used by breeders to select genetically superior animals. The PIGBLUP program uses information from all relatives, therefore an animal without records can obtain an EBV for a trait using information from relatives. Differences in EBVs represent the expected difference in phenotypic performance of these animals. The aim of this study was to obtain EBVs for average daily gain and backfat of gilts and young boars and to compare ranking on these EBVs with mean phenotypic performances.

A data set from Aztec Farms (Hermesch *et al.*, 1997) was used and comprised 16 years of data. Ten PIGBLUP evaluations were performed. Data were divided into six monthly intervals between January 1989 and July 1993 in order to obtain EBVs for gilts and young boars. In total 22,225 gilts and 22,450 boars with EBVs were subsequently recorded for average daily gain, while 5615 gilts and 3773 boars had backfat records. Animals were then grouped in ten percentile classes based on their EBV for each trait. The mean EBVs and mean phenotypic performances are presented for each percentile group in Figure 1.

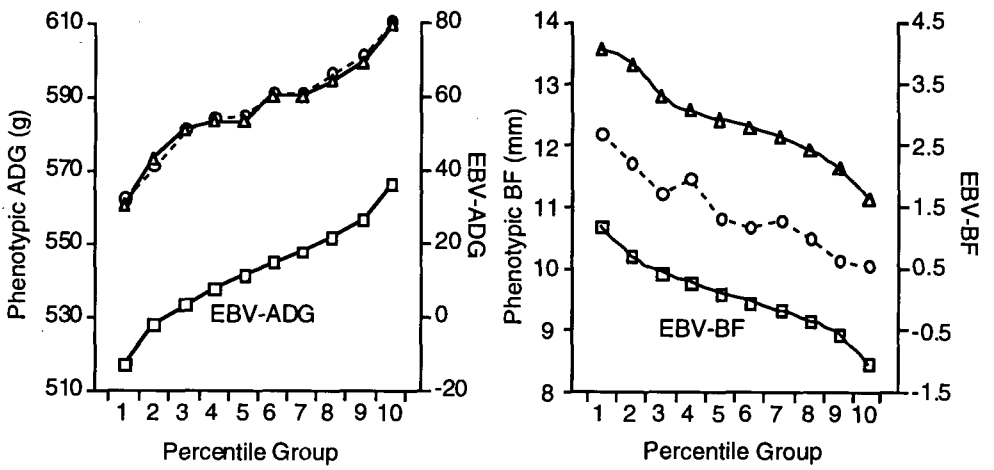

Figure 1. *Mean EBVs (□) for average daily gain (ADG) and backfat (BF) of gilts (Δ) and young boars (○), along with mean phenotypic performances for each ten percentile group.*

The mean EBVs in the top and bottom percentile groups differed by 49 g for growth rate and 2.20 mm for backfat. These differences are mirrored by differences in mean phenotypic performance for average daily gain of 49 g and 48 g and for backfat of 2.4 mm and 2.2 mm for gilts and boars respectively. In addition, the slope of mean EBVs over all groups is generally parallel with the slope of mean growth rate and backfat showing that EBVs predict real differences in phenotypic performance.

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GENETIC RELATIONSHIPS BETWEEN AGE AT FIRST FARROWING AND SOW REPRODUCTIVE TRAITS

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Although age at first farrowing (AF) is not a selection criterion, this trait significantly affects other economically important sow reproductive traits such as weaning to conception interval (WCI), number born alive (NBA), average piglet birth weight (ABW), 21 d litter weight (LW21), and sow stayability traits (STAY) (Tholen *et al.*, 1996a, 1996b). To optimise procedures for genetic evaluation, it is necessary to establish the basis (genetic and/or environmental) of the relationships between AF and other sow reproductive traits. Previous studies have shown AF to be heritable and uncorrelated with NBA (Rydhmer *et al.*, 1995). This study examined relationships between AF and other sow reproductive traits recorded under commercial conditions.

Data analysed were a subset of data from two large Australian piggeries, described by Tholen *et al.* (1996a, 1996b). Following editing, AF records were available for 3472 sows in Herd 1, and 5314 sows in Herd 2. Significant environmental effects for AF were season (Herd 1) or week (Herd 2) of birth, and date at end of performance test (both herds). Heritabilities for AF were low: 0.06 ± 0.02 in Herd 1 and 0.13 ± 0.02 in Herd 2. Genetic parameters for the remaining traits are presented in Tholen *et al.* (1996a, 1996b). Genetic and environmental correlations between AF and sow reproductive traits, averaged over parities where appropriate, are presented in Table 1.

Table 1: Genetic (r_g) and environmental (r_e) correlations between age at first farrowing and sow reproductive traits.

	Herd 1		Herd 2	
Trait	r_g	r_e	r_g	r_e
Number born alive (NBA)(parity 1)	-0.33	0.11	0.00	0.10
Number born alive (NBA) (later parities)	-0.34	0.13	-0.04	0.04
Average piglet birthweight (ABW)	-0.04	0.03	-0.03	0.01
21 d litter weight (LW21)	-0.11	0.05	na[1]	na
Weaning to conception interval (WCI) (1-2)	0.41	0.00	na	na
Stayability (STAY) (to parities 2, 3 and 4)	-0.01	-0.07	0.01	-0.04

[1]na = not available

In both herds, genetic correlations between AF and ABW and between AF and STAY traits were not significant. The favourable genetic correlations between AF and NBA and between AF and WCI found in Herd 1 were not apparent in Herd 2. Differences between herds may have been the result of different management practices, with mean AF of 312 d (Herd 1) vs 336 d (Herd 2). Overall, the results indicate that selection for sow reproductive traits will not have detrimental effects on AF. However, for traits other than STAY, environmental correlations between AF and sow reproductive traits were consistently low and unfavourable.

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ENVIRONMENTAL CORRELATIONS BETWEEN INSULIN-LIKE GROWTH FACTORS (IGFs) AND GROWTH RATE SHOW THAT ENDOCRINE IGFs ARE GROWTH REPORTERS, NOT DRIVERS

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Treatment with insulin-like growth factors (IGFs) promotes live weight gain in rats but not in pigs (Dunshea & Walton, 1995). Therefore, while IGF-I is secreted into blood to promote growth in rats, in pigs IGF-I may be secreted into blood to perform some other function. Correlations between blood IGF concentrations and growth rate were therefore examined in pigs to see if live weight gain is a consequence or a cause of blood concentrations of IGFs. Blood was collected serially between 5 and 20 weeks of age for analysis of plasma IGF-I and IGF-II from 76 Large White boars managed under a commercial weaner-grower environment until 16 weeks of age at which time they were individually penned.

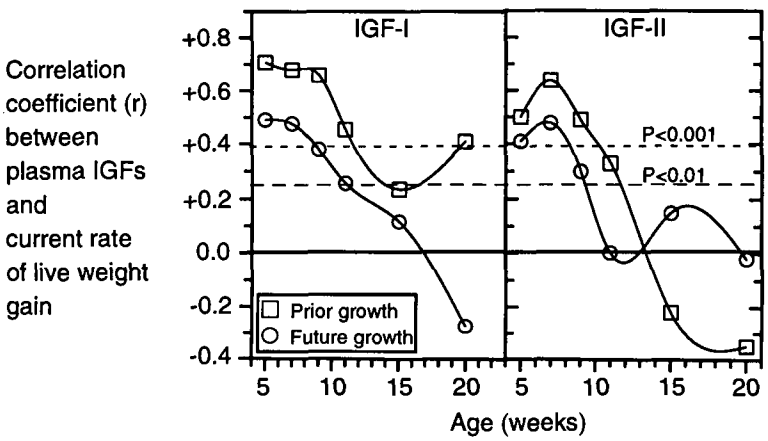

Figure 1. *Correlations between plasma concentrations of endogenous IGFs and growth rate in Large White boars (n=76).*

Plasma IGF concentrations were generally positively correlated with growth at the time of specimen collection. Associations were stronger for immediate prior, rather than future, rate of live weight gain. Between 5 and 9 weeks, the ratios of the correlation coefficients (prior/future, mean ± SEM) were 1.53 ± 0.12 for IGF-I and 1.44 ± 0.18 for IGF-II.

Plasma concentrations of IGFs are thus better 'reporters' (by ~50%) than 'predictors' of growth rates of grower boars. It is proposed that the endocrine function of IGFs in the blood of grower pigs is not to promote growth, but rather they are indicators of somatic growth. Their synthesis by tissues may have local anabolic actions but systemically they most likely function as insulin agonists to coordinate energy utilisation and storage with the demands of somatic growth.

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USE OF COMPARATIVE ANCHOR TAGGED SEQUENCE (CATS) MARKERS FROM HUMAN CHROMOSOMES 20 AND 22 IN PIG GENE MAPPING

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On the assumption that there are conserved chromosomal segments among species, animal geneticists can exploit the rapid advances in human and mouse molecular genetics. Heterologous chromosomal painting (ZOO-FISH), hybridization of human chromosome-specific DNA libraries on to pig metaphase spreads, has broadly established the relationship between pig (*Sus scrofa*, SSC) and human (*Homo sapiens*, HSA) chromosomes. Human chromosome 20 (HSA 20) is homologous with porcine chromosome 17 (SSC 17), and HSA 22 corresponds to two different porcine chromosome segments, on 5p and 14q (Frönicke *et al*, 1996). Lyons *et al.* (1997) showed that conserved sequences in expressed genes (Type I markers) can be used for making consensus PCR (Polymerase Chain Reaction) primers to amplify fragments of these genes from species in which they have not been previously studied. If these genes can be mapped, they provide anchors points for comparing the maps between species. Seventeen comparative anchor tagged sequence (CATS) primers, which are located on HSA 20 and 22, have been tested for amplification of homologous sequences in pig. The primers are derived from exon sequences conserved in at least two eutherian species.

Seven of the seventeen primer pairs gave single PCR products with adjustment of PCR conditions ($MgCl_2$ concentration and annealing temperature). Single banded PCR products in agarose gels (2%) were purified for sequence analysis using an ABI 373 automatic sequencer and dye terminator chemistry. Comparison of the resultant sequences with those from the relevant human genes confirmed that each of the seven primer pairs had amplified an actual gene, as shown in Table 1.

Table 1. Porcine CATS PCR products verified by sequencing.

Gene	Human location	PCR product length(bp)	% identity with human sequence
Adrenergic, alpha-1A-, recepter	20	450	68.2
Arylsulfatase A	22q13.31-ter	190	79.5
Guanine nucleotide binding protein (G protein), alpha stimulating activity polypeptide 1	20q13.2-q13.3	320	73.3
Guanine nucleotide binding protein (G protein), alpha z polypeptide	22q11.2	150	89.1
Immunoglobulin lambda gene cluster	22q11.2	190	80.2
Oxitocin, prepro- (neurophysin I)	20p13	600	79.3
Topoisomerase(DNA) I	20q11.2-q13.1	400	64.8

All seven verified CATS PCR product sequences in the porcine genome are located in coding sequences or at exon and intron boundaries. These verified PCR products will be used for further mapping in pigs to refine the maps of conserved synteny between human and pig and ultimately to allow pig geneticists to better exploit the huge advances being made in human molecular genetics.

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MAPPING OF QUANTITATIVE TRAIT LOCI ON PORCINE CHROMOSOMES 2 AND 5

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A major objective of applying molecular genetic techniques to animal improvement is to identify and clone genes, known as quantitative trait loci (QTL), which influence economic traits. Markers closely linked to the QTL can be used for improving the efficiency of selection, via marker assisted selection (MAS). The cloned QTL can in theory be utilised in gene transfer.

Recently genetic linkage maps for pigs have been developed, which cover the entire genome. The largest published linkage maps for pigs, namely the PiGMaP consortium (Archibald *et al.*, 1995) and USDA (Rohrer *et al.*, 1996) maps, have been typed on different reference families, but have numerous markers in common. By choosing highly informative markers uniformly distributed throughout the genome, and analysing their inheritance in suitable resource families, it is possible to detect the segregation of genes influencing economically relevant traits through statistical analysis.

After having aligned the PiGMaP and USDA maps of chromosomes 2 and 5 (Zhang, Haley and Moran, 1995), it was decided to search for QTLs for economically important traits on these same chromosomes using a resource family bred, and performance tested, at the University of Hohenheim. At this stage, two F2 families with a total of 605 animals, generated by crossing European Wild Boar and Pietrain (W x P) as well as Meishan and Pietrain (M x P) are being analysed. A third F2 family from Meishan and Wild Boar will be available shortly. A total of 49 performance traits were recorded according to the German breeding standards. The aim of this project is to genotype 20 marker loci from chromosomes 2 and 5.

Microsatellite markers were genotyped using an ABI 373 automatic genotyping system to detect fluorescently labelled products generated by polymerase chain reaction (PCR) with fluorescently labelled primers. The PCR reactions were multiplexed in groups of four, two and two and all eight products were loaded and analysed simultaneously. All data are stored and manipulated in the GEMMA version 3.06 database. Linkage analyses of the markers will be performed using CRIMAP version 2.4, and a Least-Squares method will be applied to simultaneously use all markers within a linkage group for detection of QTLs. Eventually data from all collaborating laboratories will be combined for a complete genome-wide analysis of quantitative effects.

So far microsatellite markers *S0141* [heterozygosity 0.63, number of alleles 5], *S0010* [0.74, 7], *Sw240* [0.76, 7] and *Sw395* [0.68, 5] have been genotyped for chromosome 2 on the first two F2 families. For chromosome 5, *S0005* [0.87, 11], *Sw967* [0.76, 7] and *Swr453* [0.73, 6] have also been genotyped on the same families. The average heterozygosity of F1 is 0.74 which means that these markers will be very useful for mapping. The overall exclusion probability for these markers in pedigree checking is more than 99%.

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GENETIC MAPPING OF A HEREDITARY HIGH-FREQUENCY TREMOR IN PIGS (CAMPUS SYNDROME, CPS) TO CHROMOSOME 7q1.5-2.1

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In Germany, a new hereditary movement disorder (Campus Syndrome, CPS) characterized by muscular weakness and a progressive high-frequency tremor in the legs in standing and walking was observed among offspring of the Pietrain boar "Campus" (Richter *et al.*, 1995; Schulze *et al.*,1996). First clinical symptoms are observed from 2-9 weeks postnatally. Affected piglets are very susceptible to stress and their life expectancy is reduced to between 3-18 months. Breeding studies indicate that the healthy boar Campus carries a germline mutation which is inherited as an autosomal dominant trait (Tammen *et al.*, unpublished).

A positional cloning study was carried out. Analysis of a subset of three animals with 254 microsatellite markers (kindly provided by Max Rothschild, US. Pig Genome Co-ordinator) suggested genes affecting CPS may be located on chromosome 7. Genotyping the whole pedigree (61 animals, including 33 affected pigs) revealed linkage between 14 markers on chromosome 7 and CPS (Table 1).

Table 1: Two-point Lod scores between CPS and 14 chromosome-7 markers.

	Marker													
	SO	S0	SW	SWR	SW	SW	SWR	SW	SW	SW	SW	SW	SW	SW
	102	078	1681	2036	1418	1614	2152	255	352	304	263	147	252	632
cM[a]	70.1	73.4	73.4	78.2	82.8	85.2	85.2	85.6	87.7	88.6	90.0	90.1	99.4	104.4
Lod score[b]	3.99	7.83	7.53	6.91	2.38	11.44	10.24	7.22	6.52	7.97	5.78	7.74	5.03	3.31
θ[b]	0.11	0.00	0.00	0.06	0.08	0.00	0.00	0.00	0.03	0.03	0.06	0.05	0.11	0.16

[a]Location of markers are in cM according to Rohrer *et al.* (1996); http://sol.marc.usda.gov.
[b]Lod scores and θ are calculated with CRI-MAP, version 2.4 (Green *et al.*, 1990).

Using the order of markers reported by Rohrer *et al.* (1996), multipoint analysis with CRI-MAP (Green et al., 1990) reveals that the gene affecting CPS is located between SW1418 and SW352. The interval spans 4.9 cM on 7q1.5-2.1. This part shows conserved synteny to a region of human chromosome 14 where in humans an unknown gene for dominant distal myopathy (MPD1) is located (Laing *et al.*, 1995). Distal myopathy bears clinical and histopathological similarities to CPS. The identification of the CPS gene will provide insights into the pathogenesis of this new inherited tremor disorder, and might provide useful tools for the mapping and cloning of the gene causing MPD1.

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MAPPING QUANTITATIVE TRAIT LOCI IN PIGS

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Most traits of economic importance, like growth rate, litter size, carcase yield and meat quality are continuously variable, and variation is determined by many genes with effects ranging from small to large. Identification and characterization of genes controlling quantitative traits, namely quantitative trait loci (QTL), may eventually improve the speed and efficiency of animal breeding (Haley, 1995). There are two ways to identify QTL. The candidate gene approach identifies genes likely to cause variation in a trait based on physiological, immunological or endocrine evidence. Polymorphism within that gene is typed in a performance tested resource population to determine its effect, as for the estrogen receptor gene which was shown to contribute to variation in reproductive performance (Rothschild *et al.*, 1994). However the chance of success is small. By contrast, genome scanning increases the possibility of systematic identification of all loci with even modest effects on economically important traits. The first step is to identify quantitative effects in regions demarcated with flanking markers. The position of the QTL can then be refined with additional markers from this region. In the short term, these markers can be used for marker assistant selection (MAS) and in the long term for cloning and characterising the gene. The aims of the present project are: 1) identification of chromosome regions containing putative QTL by genome scanning,; 2) fine mapping these regions and providing useful markers for MAS and possibly for positional cloning.

Reference family: A two-generation pedigreed population consisting of 563 animals (8 boars and 65 sows in the parental generation) was bred at Bunge Meat Industries Ltd in 1995. All animals are from commercial lines used at Bunge and can be broadly classified as Landrace or Large White. The DNA was extracted from blood (sows), semen (boars) or spleen tissue (offspring). All the offspring were recorded for growth performance, carcase and meat quality traits.

Markers: There are more than one thousand microsatellite markers available for pig genome mapping (Rohrer *et al.*, 1996). A subset of 150-200 evenly spaced markers is being genotyped in the reference family. Assuming a total porcine genome length of 2286 cM (Rohrer *et al.*, 1996), these markers will have an average intermarker-interval of about 13 cM. When regions containing putative QTL are detected, additional markers will be used to create a more detailed map of those regions. For each marker one primer was labelled with fluorescent dye. Reactions were multiplexed in sets of 3 or 4 using 100 ng template DNA, 2.0 mM dNTPs, 2.0 mM $MgCl_2$, 100 nM each primer and 0.2 U AmpliTaq DNA polymerase in 10 µl volume. The PCR-products were multiloaded with internal standard (Genescan-350 Tamara) in an ABI 373 sequencer.

Ninety-six markers have been evaluated for the reference family, 90 of which are informative for at least one of the boars. So far genotyping has been completed for 20 markers. For the first 14 loci, the average heterozygosity is 0.61, so these markers are highly informative even within breeds as opposed to the wide crosses employed in construction of the original maps. Although insufficient loci have been genotyped to permit QTL analyses, the markers have been demonstrated to be very efficient for parentage testing with an exclusion probability (Weir, 1990) of 0.9999. The aim is to complete genotyping by mid 1998 with statistical analyses and QTL mapping occurring in the following year.

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GENETIC RELATIONSHIPS BETWEEN pH45 AND LEAN GROWTH IN PIGS.

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Traditionally, pigs have been selected for carcass lean, average daily gain, and feed conversion ratio. However in some breeds or populations, unfavorable genetic relationships exist between these production traits and meat quality (Hovenier *et al.*, 1993). Therefore quality traits, such as meat pH, are often considered in the selection process. Alternative traits to improve lean growth in pigs have been proposed, e.g. lean tissue growth rate (Fowler *et al.*, 1976). Another possible selection criterion for lean growth is protein deposition rate. This is used by nutritionists to characterize genotypes when formulating diets. In the present study the genetic relationships between the alternative selection criteria, lean tissue growth rate (LTGR), protein deposition rate (Pd), and meat pH were investigated. Meat pH is an important quality trait because of its relationship with colour, water-holding capacity and tenderness.

The pH value (pH45) was measured in the loin at the level of the last rib 45 min after slaughter in Landrace pigs (LR, 200 carcasses) and Large White pigs (LW, 269 carcasses). Age at slaughter, carcass weight and backfat thickness (P2, measured with a Hennessy Grading Probe) were recorded and used to calculate LTGR (Ferguson *et al.*, 1994) and Pd (Morel *et al.*, 1994) from birth to slaughter. A multivariate animal model was fitted to the data using the program AIREML (D.L. Johnson, LIC, Hamilton, New Zealand). Sex and day of slaughter were included as fixed effects.

Table 1. Estimates (±SE) of genetic parameters for lean tissue growth rate (LTGR), protein deposition rate (Pd), and pH45 in Large White (LW) and Landrace (LR) pigs.

	Heritabilities			Genetic correlations		
Breed	pH45	Pd	LTGR	pH45-Pd	pH45-LTGR	Pd-LTGR
LW	0.28 ± 0.13	0.56 ± 0.16	0.29 ± 0.14	0.05 ± 0.32	-0.30 ± 0.35	0.94 ± 0.08
LR	0.18 ± 0.14	0.62 ± 0.20	0.57 ± 0.20	-0.43 ± 0.39	-0.48 ± 0.38	0.99 ± 0.03

High heritability values for LTGR and Pd were found in both breeds. The heritability values for LTGR are comparable with those found by Cameron and Curran (1994) for lean growth (0.34 and 0.28 for LW and LR pigs, respectively). A strong genetic antagonism exists between pH45 and LTGR and between pH45 and Pd for LR pigs, but not for LW pigs. This antagonism might be explained by the presence of the halothane gene segregating in the Landrace population. The halothane gene has a strong influence on both meat pH and lean development (Sellier, 1988). These results show that both Pd and LTGR are highly heritable and are worthwhile selection criteria for lean growth in pigs, and that meat quality traits should be taken into account during the selection.

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SEX-SORTING OF BOAR SPERM BY FLOW CYTOMETRY

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The only validated method of sex-sorting boar sperm is by fluorescence activated cell sorting (FACS; Johnson *et al.*, 1989). The techniques, fluorescence *in situ* hybridization (FISH) and polymerase chain reaction (PCR) were established to confirm the sex of FACS-separated porcine sperm *in vitro* and after *in vivo* insemination.

Sperm samples were tagged with the fluorescent DNA stain Hoechst 33342 and sorted into putative male and female enriched populations using an Epics V cytometer modified as described previously (Johnson & Pinkel, 1986). For FISH analysis, each sample was probed simultaneously with Dig-labelled chromosome Y-specific and biotin-labelled chromosome 1-specific probes (Kawarasaki *et al.*, 1996). Modifications to account for the low number of sperm available included the use of silica coated slides to minimise sample loss due to washes. Sealed CoverWells (Astral Scientific, NSW) were used in place of humidified chambers to decrease problems caused by evaporation. Slides were examined by dual band fluorescent microscopy. Those sperm showing positive hybridisation signals for the autosomal and Y probes were considered male, and those showing positive signals for the autosome only were considered female.

Ejaculates from two Large White × Landrace boars were individually sorted into male- and female-enriched fractions. Proportions of male sperm (percent purity as assessed by FISH) from the 2 putative male-enriched fractions were 268/360 (74.4%) and 1067/1400 (76.2%). Proportions of female sperm from the 2 putative female-enriched fractions were 431/500 (86.2%) and 1043/1231 (84.7%). The proportion of male sperm (percentage) in an unsorted control was 488/965 (50.5%). The purity of the male-enriched samples was lower than the female-enriched samples of the 2 boar samples combined (75.9% vs 85.2% respectively, $P<0.01$, chi-square).

Putative male or female sorted sperm were used in an *in vitro* fertilisation trial, according to the method of O'Brien *et al.* (1995). *In vitro* matured oocytes (N=269) were inseminated with 4×10^5/ml sorted or unsorted (control) sperm. Sorted sperm had similar oocyte penetration (20.5% vs 30.0%) and lower fertilisation (11.0% vs 25.0%, $P<0.05$, chi-square) rates than control sperm, indicating that sperm viability was compromised during the sorting process. To demonstrate that sorted sperm were capable of *in vivo* fertilisation and to confirm the sorting efficiency, oviducal insemination of four synchronised gilts was performed using 6×10^5 sorted sperm/gilt and 2 to 30-cell embryos were recovered at slaughter 3 or 4 d later. Sex of embryos was diagnosed using PCR with male-specific primers (Mileham *et al.*, 1988) and porcine chromosome 1-specific primers (Jansch *et al.*, 1990) as a positive control. Embryos of 2-cells and above provided enough DNA for accurate, reproducible results. Analysis of embryos by PCR analysis confirmed the sorting efficiency with 77% male embryos (n=13) produced from the male enriched sperm sample and 78% female embryos from the female sperm enriched sample (n=9).

These techniques confirmed the effectiveness of flow cytometry for separation of spermatozoa samples into X- and Y-chromosome bearing populations. The establishment of FISH in particular allows rapid confirmation of the sorting efficiency. The ratio of male:female sperm diagnosed by FISH also provides an indicator of the sex ratio of litters after insemination.

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HEAT PRODUCTION IN BOARS IN RESPONSE TO SHORT-TERM EXPOSURE TO HIGH AMBIENT TEMPERATURE

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Compared to other species, pigs have a poor heat tolerance because they have a low capacity to sweat and evaporate water from the respiratory tract. Pigs can evaporate exogenous water from the skin; however, when they are unable to wet the body, exposure to high ambient temperatures can be stressful, or even dangerous. The effects of a short period of exposure to very high temperatures, and the subsequent recovery when the temperature was decreased, on gas exchange (oxygen consumption and carbon dioxide production), heat production (HE) and respiration rate (RR) was studied in boars to evaluate the general response pattern and possible differences among breeds.

The effects of short-term exposure to high ambient temperatures were investigated in 17 boars (Danish Landrace (n=5); Duroc (n=8); Yorkshire (n=4)) at an approximate live weight of 100 kg by means of indirect calorimetry in an open-air circulation system. Following a 1 h rest in the respiration chamber at 17°C, gas exchange measurements commenced and were performed in five periods, each of 1 h duration. During the basal period the ambient temperature was kept at 17°C, and then maximum heating of the chamber commenced, resulting in a temperature of 35.0°C by the end of Period I, and increasing further to 39.7°C after 2 h heating (Period II). In Period III cooling of the chamber commenced, and by the end of this period the temperature had decreased to 21.8°C, and after the second hour of cooling (Period IV) the temperature was 18.2°C. The RR was recorded every 15 min.

Breed effects were evaluated, but in the measured parameters there were no significant differences among the three breeds in their response to the high ambient temperatures; the pooled results are presented in Table 1. The gas exchange and HE increased slowly during Period I and rapidly during Period II, followed by a decrease in these values in the two cooling periods. In Period IV the HE values were below those in the basal period (Table 1). The RR increased slightly in Period I, and was followed by a sharp increase during Period II reaching a maximum in Period III (Table 1). Rectal temperatures measured at the end of Period IV were normal, mean 38.9°C (SEM 0.06).

Table 1. Gas exchange (O_2, L/h, CO_2, L/h), heat production (HE, kJ/h) and respiration rate (RR) in boars in response to short-term exposure to high ambient temperature, and their recovery during cooling (mean ± SEM).

	Basal period		Period I		Period II		Period III		Period IV	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
O_2 (L/h)	36.1^{c}	0.98	38.4^{c}	1.42	49.4^{a}	1.41	36.7^{c}	1.38	30.5^{b}	1.04
CO_2 (L/h)	48.7^{cd}	1.19	51.3^{c}	1.53	61.5^{a}	1.72	46.0^{d}	1.40	37.6^{b}	1.14
HE (kJ/h)	822^{c}	21.2	872^{c}	30.0	1102^{a}	30.7	818^{c}	28.6	676^{b}	21.7
RR[1]	23.7^{c}	10.1	30.5^{c}	1.9	154.6^{a}	7.8	171.1^{a}	10.1	63.2^{b}	9.8

[1]Breaths per min. ^{a,b,c,d}Values within rows with different superscripts differ significantly ($P<0.01$).

The maximum value for HE, which was observed in Period II, was 34% above the value in the basal period, whereas minimum HE values 18% below those in the basal period were recorded in Period IV.

It was concluded that exposure to ambient temperatures close to 40°C induced a fast response in gas exchange, HE and RR, but once cooling had commenced, the animals recovered relatively quickly to have gas exchange rates and HE close to, or somewhat below, basal values.

DIETARY NON-STARCH POLYSACCHARIDES: INTERACTIONS WITH WEANER PIG GROWTH AND POST-WEANING COLIBACILLOSIS

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Growth setbacks and the occurrence of post-weaning colibacillosis in piglets in the first week post-weaning can have a major influence on the overall time taken to reach market weight. The inclusion of non-starch polysaccharides (NSP) in the diet has been advocated as a means of reducing the severity and incidence of post-weaning colibacillosis by accelerating large intestinal development (Bolduan *et al.*, 1988). The aim of this experiment was to study the effects of differences in post-weaning dietary NSP on pig growth, gut development, and intestinal proliferation of haemolytic *Escherichia coli*.

Large White-x pigs (*n=69*) weaned at 21-25 days of age (mean weight 7.2kg) were randomly assigned to 'uninfected' (*n=30*) or 'experimentally-infected' (*n=39*) groups, and offered *ad libitum* one of three diets differing in NSP content for a period of 7 d. A hammer-milled wheat and barley-based diet (commercial) served as a reference diet. The other diets were pregelatinised rice with an animal protein supplement (rice/AP) or the same rice diet with 10% guar gum added (rice/GG) to increase the NSP content. Rice/AP was low in total NSP (0.7g/100g DM), rice/GG was high in soluble NSP (6.4g/100g DM) but not insoluble NSP (0.2g/100g DM), and the commercial diet had some soluble NSP (2.2g/100g DM) but mostly insoluble NSP (7.6g/100g DM). The 'experimentally-infected' group was orally inoculated 48 h post-weaning with 50mls each of $10^{8.5}$/ml haemolytic *E. coli* serovar O8; K88; K87. One week after weaning, all pigs were reweighed and then euthanased for collection of intestinal samples for pH and bacterial counts.

Table 1. Growth rate, large intestinal weight and pH of proximal colon contents in uninfected weaner pigs, and small intestinal haemolytic *Escherichia coli* populations in experimentally-infected weaner pigs (mean ± SEM).

	Commercial	Rice/AP	Rice/GG	P value
Uninfected pigs				
Growth rate (g/d)	57 ± 30[a]	141 ± 21[b]	85 ± 15[a]	<0.05
Empty large intestine (g)	128 ± 39[a]	95 ± 4[b]	125 ± 9[a]	<0.05
pH proximal colon contents	5.97 ± 0.13[a]	6.25 ± 0.07[b]	5.33 ± 0.06[c]	<0.0001
Experimentally-infected pigs				
Escherichia coli (cfu/g)[1]	$4.1 \times 10^{4\,ab}$	$1.3 \times 10^{4\,a}$	$8.0 \times 10^{9\,b}$	<0.05
Haemolytic *E. coli* (% of total)	29.6 ± 16.1[a]	3.6 ± 1.4[b]	28.8 ± 8.93[a]	<0.05

[a,b,c]Mean values in the same row with different superscripts differ significantly. [1]Colony-forming units per g mucosal scraping.

Pigs fed the rice/AP diet were heavier, and had lighter large intestines and less fermentation in the large intestine (as indicated by the higher pH of colon contents) than pigs fed the rice/GG and commercial diets. The addition of guar gum to the base rice diet was associated with increased proliferation of haemolytic *E. coli* in the small intestine. Although the total number of haemolytic *E. coli* colonies did not differ significantly between pigs fed the commercial and rice/AP diets, there were significantly greater proportions of haemolytic *E. coli* cultured from pigs fed the commercial diet. Contrary to expectations, results from this study indicated that reducing concentrations of soluble NSP in the diet increased pig performance in the first week post-weaning, and reduced the extent of proliferation of haemolytic *E. coli* in the small intestine.

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THE EFFECTS OF EXTRUSION AND ENZYME ADDITION IN WHEAT BASED DIETS ON FERMENTATION IN THE LARGE INTESTINE AND EXPRESSION OF SWINE DYSENTERY

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Diets containing soluble non-starch polysaccharides (NSP) and resistant starch (RS) have been shown to increase both hindgut fermentation and the incidence of swine dysentery (SD) (Pluske *et al.*, 1996). The aim of this study was to investigate the effects of extrusion (to reduce RS) and exogenous enzyme addition (to reduce soluble NSP) to a wheat based diet, on the extent of fermentation in the large intestine and on expression of SD. Wheat/animal protein diets were prepared using extrusion of wheat and addition of an enzyme premix containing xylanase and protease activity, in a 2 x 2 factorial design. Specific pathogen free Large White x Landrace pigs (n=48), weaned at 21 d, were fed the diets for 4 weeks. Six pigs in each group were slaughtered for assessment of gut parameters (Table 1). The remaining six pigs were infected with *Serpulina hyodysenteriae*, fed the same diets for a further 4 weeks, and monitored for development of SD (clinical signs, rectal swabs, post-mortem lesions, *S. hyodysenteriae* isolated at post mortem).

Table 1. Growth rates and intestinal fermentation parameters in non-infected pigs and incidence of swine dysentery in infected pigs on different diets.

Parameters	Diet[a]				SED[b]	Significance[c]		
	RW	ExtW	RW/Enz	ExtW/Enz		Ext	Enz	Enz xExt
Growth rate (g/d)	427	430	489	423	33.8	NS	NS	*
Starch proximal colon (mg/g)	10.2	0.6	6.2	2.0	2.52	***	NS	*
Starch distal colon (mg/g)	7.2	0.2	2.1	0	2.97	**	NS	NS
pH proximal colon	5.7	6.1	5.7	6.0	0.34	NS	NS	NS
pH distal colon	6.1	6.6	6.6	6.8	0.30	**	*	NS
ATP proximal colon (nmol/g)	0.30	0.10	0.42	0.44	0.26	NS	*	NS
ATP distal colon (nmol/g)	0.18	0.14	0.17	0.23	0.18	NS	NS	NS
Swine dysentery (No. of pigs)	4	2	6	6		-	-	-

[a]RW- raw wheat; ExtW - extruded wheat; Enz - enzyme. [b]Standard error of difference between interaction means. [c]NS = non-significant; *$P<0.05$; **$P<0.01$; ***$P\leq0.001$.

Both extrusion and enzymatic hydrolysis of wheat NSP increased pre-caecal starch digestion as judged by reduced amounts of starch in the large intestine. Unexpectedly, significant treatment effects on pH values of digesta were only noted in the distal colon. Swine dysentery occurred in all treatment groups, although the incidence was lower in pigs receiving the extruded wheat diet (Table 1). The failure of combined extrusion and enzyme treatment effects to protect against SD might be related to the apparent increased fermentation in the proximal areas of the large intestine as judged by an increase in the level of bacterial ATP. An appropriate combination of grain processing and dietary enzyme inclusion to obtain full protection against SD is yet to be determined.

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ALTERING THE SITE OF FERMENTATION IN THE PIG: IMPLICATIONS FOR COLON CANCER RISK IN HUMANS

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In humans, most colonic tumours occur in the distal colon. Fermentation of carbohydrates such as resistant starch (RS) and non-starch polysaccharides (NSP) by the microflora in the distal colon may have implications for risk of colon cancer in humans. Fermentation of RS results in a number of beneficial effects including increasing concentrations of butyrate (an anti-tumour agent) and decreasing potentially toxic ammonia (Phillips *et al.*, 1995, Birkett *et al.*, 1996). However, RS is easily fermented and is often degraded before it reaches the distal colon. The aim of the present study was to determine whether wheat bran, a rich source of insoluble NSP, which is known to increase the rate of passage, could move RS to the distal colon and thus shift the favourable fermentation-dependent events distally.

Twenty-four Large White x Landrace, boars (60 kg ± 1.9 kg live weight) were fed for 21 d (40 MJ GE/d) one of four diets, differing only in the amounts of RS and insoluble NSP. Macronutrient composition and total fibre content was similar to human diets. The Control diet contained 40 g RS and 80 g NSP/40 MJ GE. High-amylose corn and wheat bran were used to increase the RS and NSP content, respectively, creating a diet high in RS (120 g RS/40 MJ GE), a diet high in NSP (140 g NSP/40 MJ GE) and a diet high in both. Celite® was added as an indigestible marker. Pigs were slaughtered on day 21 and intestinal contents sampled. Butyrate was measured by gas capillary chromatography and ammonia was measured spectrophotometrically. The disappearance of RS and NSP (g/d) from the proximal or distal regions of the colon, relative to the amounts of RS and NSP at the terminal ileum, were used as indicators of RS and NSP fermentation (Table 1).

Table 1: Disappearance of RS and NSP (g/d) and effects on butyrate and ammonia concentration in the proximal and distal regions of the colon (mean ± SE, n=6).

	Control diet	RS diet	NSP diet	RS+NSP diet
Caecum + Proximal colon				
RS fermented (g/d)	20 ± 2	57 ± 13*	15 ± 5	48 ± 7
NSP fermented (g/d)	19 ± 3	21 ± 5	34 ± 9	41 ± 6
Butyrate (mM)	10.7 ± 0.8	17.8 ± 1.8*	13.0 ± 1.5	14.1 ± 0.9
Ammonia (mM)	43 ± 3	35 ± 3*	45 ± 2	30 ± 4
Middle + Distal colon				
RS fermented (g/d)	5 ± 2	13 ± 2*	9 ± 4	21 ± 2
NSP fermented (g/d)	9 ± 2	5 ± 3	4 ± 9	5 ± 6
Butyrate (mM)	10.6 ± 0.8	12.9 ± 1.2	11.2 ± 2.1	17.1 ± 1.6#
Ammonia (mM)	46 ± 3	40 ± 2*	44 ± 5	34 ± 1#

*Main effect of RS or NSP from control group (one-way analysis of variance, $P<0.05$). #Significant ($P<0.05$) positive interaction between RS and NSP; effects of RS+NSP diet were not additive therefore results analysed using a two-way analysis of variance.

The results suggest that wheat bran was effective at shifting the fermentation of RS to the distal colon, thereby improving luminal conditions in the distal colon as indicated by higher butyrate and lower ammonia concentrations (Table 1). As tumours are most common in the distal colon, the combined intake of RS and insoluble NSP may contribute to the dietary modulation of colon cancer risk. The pig appears to be a good model to study the effects of dietary manipulation on luminal changes which are relevant to colon cancer risk in humans.

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VACCINATION AGAINST PROLIFERATIVE ENTEROPATHY IN PIGS

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Porcine Proliferative Enteropathy (PPE) is a commercially important intestinal infection of pigs caused by the obligate, intracellular, Gram-negative bacterium, *Lawsonia intracellularis* (previously known as *Campylobacter*-like organism and Ileal symbiont intracellularis) (McOrist *et al.*, 1995). The disease affects the ileum and can lead to either acute haemorrhagic enteritis and death, or severe growth retardation. Current control of PPE is based on the use of prophylactic antibacterial treatment. Alternative control strategies, such as vaccination, are being sought in order to reduce the costs associated with treatment, and the level of antibacterial residues in meat.

Two vaccines were tested in this study, a DNA vaccine, in which plasmid DNA encoding a *L. intracellularis* GroEL-like protein, is injected and the protein then expressed in cells of the vaccinated animal, and a formalin-killed, whole bacterial cell vaccine (bacterin). Sixteen weaned pigs (Landrace x Large White) were randomly assigned to four groups of four pigs each. Group 1, uninfected control pigs, received no treatment and were housed separately. Group 2 pigs were immunised intramuscularly (im) on day 7 and day 28 with a *L. intracellularis* bacterin, in incomplete Freunds adjuvant. Group 3 and Group 4 pigs were vaccinated im on day 0, day 14 and day 28 with 200µg of the DNA vaccine pCIGH-EL, and the empty vector construct, pCIGH, respectively. On days 40, 41 and 42, Group 2, 3 and 4 pigs were challenged by oral dosing with *L. intracellularis*. At the time this trial was conducted culture of this organism was not routinely possible, consequently both the challenge material and the bacterin were prepared from infected pig ileum samples.

The number of *L. intracellularis* shed in faeces was measured, as an indicator of a successful challenge infection, using a monoclonal antibody specific for *L. intracellularis* (McOrist *et al.*, 1987). Faecal swabs were taken from day 51 until the conclusion of the experiment on day 61, or until an excess of 100 bacteria per high powered field (hpf) were observed and the animal was killed prior to the development of severe clinical disease. Vaccination reduced the mean counts of *L. intracellularis* within faeces by 98.5% and 91.3% in the bacterin and pCIGH-EL groups, respectively, when compared to the challenge control group (pCIGH); the uninfected control animals did not shed any bacteria. Confirmation of PPE was based upon gross thickening of the ileum at post mortem, and histological demonstration of *L. intracellularis* in parrafin embedded ileal tissues stained with haemotoxylin-eosin. All the uninfected control pigs, the bacterin vaccinated and three out of four pCIGH-EL vaccinated pigs had no gross or histological evidence of PPE. Whereas, three of the infected control animals shed greater than 100 *L. intracellularis* bacteria/hpf in their faeces, had grossly thickened regions of the ileum and histologically severe lesions of porcine intestinal adenomatosis (PIA).

From this study, it can be concluded that vaccination with a *L. intracellularis* bacterin or a DNA vaccine construct of *L. intracellularis groEL* can prevent the development of PPE disease in pigs and may also decrease disease transmission by reducing the level of faecal shedding of bacteria within piggeries.

Supported in part by the Pig Research and Development Corporation.

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SENSITIVITY AND SPECIFICITY OF ELISAs FOR SEROVARS 1 AND 12 OF *ACTINOBACILLUS PLEUROPNEUMONIAE*

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Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia, a severe respiratory disease of pigs. In Australia, pleuropneumonia is widespread and recognised as one of the most important diseases of the pig industry (Thornton, 1995).

A recent report concerning the development and initial validation of ELISAs for serovars 1, 7 and 12 demonstrated strong cross-reactions between serovars 7 and 12 (Bowles *et al.*, 1997). The present paper provides details of the specificity and sensitivity of the serovar 1 and 12 assays based on experimental infection trials and field evaluation.

The purpose of the infection trial was to raise control sera for the serovar 1 and 12 ELISAs. It involved two groups of mixed-sex Large White pigs; one group of 14 pigs was infected with a serovar 1 strain and the other group of 12 pigs was infected with a serovar 12 strain. All pigs were bled before infection to provide negative control sera and at 6 weeks post infection to provide positive control sera. The mean ELISA titres of the trial animals (positive and negative controls) and the presently used cut-off values for each assay (determined through statistical analysis of both trial and field data) are presented in Table 1. Based on these results, the serovar 1 ELISA has a sensitivity of 92.8% and a specificity of 100%, and the serovar 12 ELISA has a sensitivity of 87.5% and a specificity of 100%.

Table 1. Mean ELISA titres, 6 week post-infection titre ranges and positive cut-off titres for *A. pleuropneumoniae* serovars 1 and 12 in experimentally infected pigs.

	Pre-infection titre		6 week titre (range)		Positive
Infection	Serovar 1	Serovar 12	Serovar 1	Serovar 12	cut-off
Serovar 1	1469	1392	18590 (11720-22659)	1794 (1248-2390)	>16568
Serovar 12	1401	1375	4073 (2150-6501)	21209 (13707-25524)	>17561

The ELISAs have been used to perform full herd profiles on 24 different herds. The ELISA results are presented in Table 2 and are compared with the known disease status of the herd (based upon data for clinical signs, culture and serotyping). If the herds of unknown status are omitted, the results indicate that, on a herd basis, the serovar 1 and serovar 12 ELISAs both have a sensitivity and specificity of 100%.

Table 2. Field evaluation of *A. pleuropneumoniae* ELISAs for serovars 1 and 12.

	Percentage of herds of known status in each category				
ELISA status	Negative (3)[1]	Serovar 1 (2)	Serovar 12 (11)	Serovar 1 &12 (3)	Unknown (5)
Negative	100	0	0	0	80
Serovar 1	0	100	0	0	0
Serovar 12	0	0	100	0	20
Serovar 1 & 12	0	0	0	100	0

[1]Number in brackets is the number of herds in each category.

These results confirm that the serovar 1 and 12 ELISAs are reliable and can be used with confidence in developing prevention and control programmes to reduce the economic losses associated with porcine pleuropneumonia.

Supported in part by the Pig Research and Development Corporation

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"SWISS" DEPOPULATION OF A NEW ZEALAND PIGGERY

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In December 1996, a 200-sow nucleus herd of purebred Large White and Landrace stock underwent a partial depopulation/medication regime adapted from Zimmerman *et al.* (1989). The objective was to enhance financial performance by improving health status while preserving genetic merit, and maintaining the rate of genetic gain and sales of breeding stock. Successful eradication of *Mycoplasma hyopneumoniae* and several other pathogens from herds of 30-340 sows by using this program has been reported by Szancer *et al.* (1988) and Baekbo *et al.* (1994).

The feed for the breeding herd was medicated for 10 d with 0.045% dimetridazole and 88g/t lincomycin-spectinomycin. All growing stock were removed, leaving only adult stock over 10 months of age. The feed for the herd was then medicated with 200 ppm tiamulin for 14 d during which time no farrowings were scheduled, and successive cleanup and disinfection of all housing and equipment began. This was followed by medication of the feed with 0.045% dimetridazole for a further 14 d. All stock were injected with doramectin at 300 mcg/kg twice, 14 d apart. Seventy young stock re-entered the herd upon reaching 10 months of age after undergoing the medication regime des-cribed above.

Table 1. Performance of breeding and growing herd 6 months before and 6 months after depopulation.

	Before	After	Difference
Sow Performance	*Mean (SD)*	*Mean (SD)*	
No. litters	194	205	--
Parity	2.5 (1.8)	2.8 (1.8)	+ 0.2
Total pigs/litter	11.9 (1.2)	12.9 (1.5)	+ 1.0*
Pigs born alive	10.7 (1.0)	11.4 (1.1)	+ 0.7**
Pigs weaned/litter	9.8 (1.5)	10.0 (1.4)	+ 0.2
Weaners			
Number of pigs	1552	1949	--
End weight (kg)	23.2 (1.4)	24.8 (1.9)	+ 1.6**
Days	36.0 (1.2)	35.5 (1.9)	- 0.5
ADG (g)	422 (42)	472 (45)	+ 50*
Grower/Finishers			
Number of pigs	1840	1125	--
End weight (kg)	80 (2.9)	80.4(1.9)	+ 0.4
Days	79 (2.5)	72 (3.9)	- 7*
ADG (g)	749 (37)	839 (64)	+ 90*
Birth to Bacon			
Days	139.6 (2.5)	129.9 (3.9)	- 10.3*
ADG (g)	563 (21)	608 (24)	+ 45*

*P< 0.05, **P< 0.01. Analysis performed on weekly average figures.

The results are shown in Table 1 and indicate that litter size, ADG and days to market have improved significantly. Total cost of the program was NZ$500 per sow, including medication, extra labour and downtime vs an estimated NZ$1000 per sow for full de-population. The method described appears to be an economical and practical alternative to depopulation and restocking with SPF stock, and is particularly relevant for herds of high genetic potential, and producers with limited capital. This is the first report of the application of "Swiss" depopulation in Australasia and it is intended as an introduction of the technique as a potential method of improving herd performance. As this is a preliminary report, the outcome of disease eradication is not yet known. However, the method appears to be cost-effective in improving the general health and productivity of the herd described in this report.

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A SYMPOSIUM - SUSTAINING SUPPLY AND IMPROVING THE UTILISATION OF FEED GRAINS BY THE PIG INDUSTRY

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Introduction

Profitability of the intensive livestock industries is determined primarily by the cost of nutrients relative to the prices received for the product. The primary source for the pig industry of the major nutrients, utilisable energy and amino acids, is from the feed grains, comprised of cereals, pulses and oilseed meals. The use of feed grains by the Australian animal industries has grown rapidly from 4.5 million tonnes in 1990 to a projected amount of almost 8 million tonnes by the year 2000. These projections depend partially on the future of the cattle feedlot industry and, if it recovers to the previously anticipated level, the total requirement for feed grains in Australia by the turn of the century could exceed 10 million tonnes. In addition to the domestic market, significant quantities of grains are exported for consumption by livestock overseas.

Although some grains such as sorghum, triticale and lupins are grown specifically for the animal industries, most of the grains available for animal feed are those that have not met the standards for manufacture of products for human consumption. Consequently, there is concern from intensive livestock producers that insufficient grain of adequate quality will be produced domestically for their expanding industries. The vulnerability of producers to insufficient grain supply will be increased substantially if large areas of the country are again subjected to droughts similar to those experienced in the early 1990's.

There are opportunities for feed grains to be produced in the higher rainfall areas of Australia typically not suitable for producing grains of milling quality and also for growers in traditional regions to produce and market grains specifically for animal production. However, for grain growers to be attracted to the production of feed grains, they must be paid prices for the grain that reflects their value in terms of animal production. Similarly, benefits will be derived by the animal industries through the more economical formulation of rations if the precise nutritional value of grains for different livestock enterprises were known.

There is considerable variation in the nutritional value of grains for pigs depending on the species, cultivar and production environment of the grain. For example, in this symposium van Barneveld (1997) estimates that the digestible energy content of wheat for pigs ranges from 13.3 to 17.0 MJ/kg dry matter (DM). Similarly, estimates of the digestibility coefficients for lysine in wheat range from 0.56 to 0.81. Differences of these magnitudes would have an enormous effect on the productivity of animals and the profitability of enterprises if mean values were assumed for all wheat. Similar variation in nutritional value exists for other grains.

Clearly, to encourage grain growers to focus production systems towards feed grains, it is important to first identify the reasons for variation in the nutritional value of grains and then to develop rapid, cheap and accurate methods of measuring these factors. The analytical methods should ideally be suitable for application either at the site of grain delivery from the farm or within the place of stockfeed manufacture so that the nutritional value of the grain is known before it is used. The rational marketing of feed grains could then be achieved with the benefits from more efficient animal production being shared between the grain grower and animal producer.

In the first paper of this symposium, Edwards (1997) examines the demand for feed grains and the reliability of supply. Van Barneveld (1997) then identifies the variation that exists in the nutritional value of grains and the likely causes of this variation. Finally, Hafi (1997) describes a model of regional feed markets and examines the effects of reduced feed availability due to drought and other factors on regional feed use within Australia.

FACTORS INFLUENCING THE SUPPLY OF FEED GRAINS TO THE AUSTRALIAN PIG INDUSTRY

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Abstract

The issue of an adequate, reliable and reasonably priced supply of feed grains has arisen in recent years as a potential threat to the viability of the Australian pig industry. Although feed grain demand has increased at only a moderate rate for the pig and poultry sectors, total feed grain demand has increased substantially over the last decade due to increased supplementary feeding of dairy cows and the emergence of large scale beef feedlotting. Grain production in Australia is erratic due to frequent changes in growing conditions and a traditional focus on the production of milling wheats, malting barley and grain legumes for export. When the erratic grain supply in Australia is compared with the increased feed grain demand, it is clear that feed grain demand will frequently exceed supply in the future. The problem is further compounded, when supply and demand are considered on a regional basis, by the geographic dislocation of feed grain production and the end users. Geographic dislocation is exacerbated by prohibitively high internal freight costs which favour the exporting of grains rather than transport within Australia. To address the issue of increased feed grain demand, ways to improve feed grain supply to the Australian pig industry must be considered. Possible avenues include increased grain production, reduced grain exports, the use of imported grain, greater utilisation of by-products and alternative feedstuffs, and improved utilisation of Australia's existing feed grain supply.

Introduction

The issue of an adequate and reliable supply of feed grains has arisen in recent years as a potential constraint to the expansion and even the basic viability of the Australian pig industry.

Supply becomes a problem when it is overtaken by demand. To put the feed grains supply in perspective, an appreciation of the nature and the magnitude of feed grain demand is needed. Since average herd feed conversion efficiency in the pig industry has remained relatively static (less than 1% improvement/annum from 1991 to 1997), feed grains demand is directly linked to total pigmeat production. The average annual increase in total pigmeat production from 1991 to 1997 has been 1.6% and feed grain demand has increased at a similar modest rate. However, the pig industry represents only one part of the total feed grains demand equation, and substantial increases in demand from other livestock sectors have brought pressure to bear on supply, thus indirectly compromising the pig industry.

Historically, the usage of feed grains for livestock production has been dominated by the pig and poultry industries. Despite these industries remaining relatively static over the last decade, the emergence of large scale beef feedlotting and substantial increases in the level of supplementary feeding of dairy cows, have induced a rapid increase in total feed grains demand (Figure 1).

An analysis of total grain production and disposal channels from 1986-1996, and projected domestic demand to the year 2000, demonstrates the basis for concerns about feed grain supply and the urgency of finding a solution (Figure 2). While domestic grain demand from the beef and dairy sectors is ever increasing, other domestic uses such as flour milling, malting, processed cereals and legumes, and seed requirements have remained relatively static. In contrast, total grain production is quite erratic from year to year as a consequence of seasonal conditions. In addition, if grain exports simply involved the disposal of the balance between total grain production and domestic demand, there would be little problem. However, the grain export industry is an

independent entity and is largely competitive with, rather than subservient to, the domestic grain trade. When preferred export volumes (involving premium grain types, contractual commitments and expedience due to geographic dislocation from the domestic markets) are added to the domestic demand, the probability that this total demand will exceed supply is becoming much higher. This pressure on supply will be more acute in specific regions. For example, in Queensland there is a strong dependence on sorghum as the prime feed grain, yet sorghum production is highly erratic and often falls far short of the increasing demand for feed grain (Figure 3). In recent years, Queensland has become a net importer of grain and may represent the forerunner of the national position.

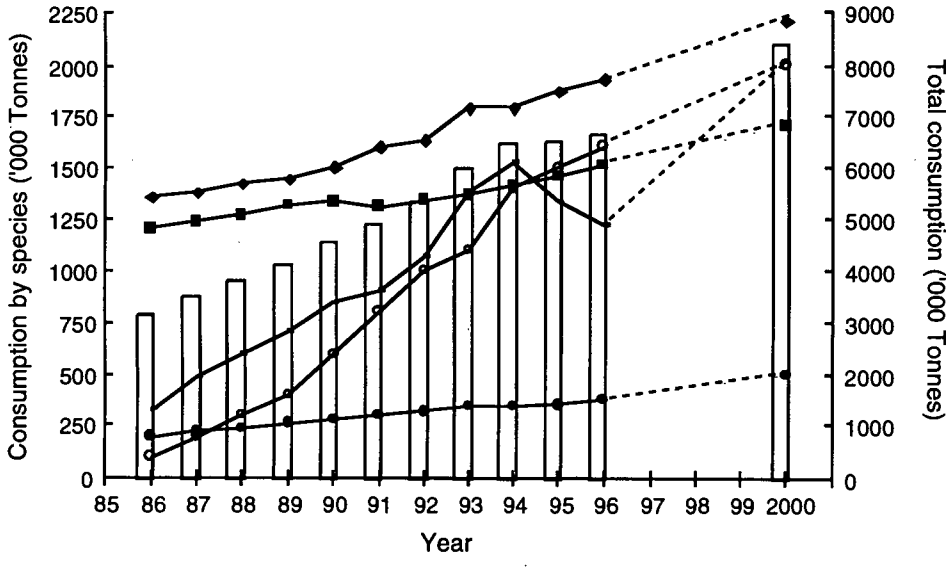

Figure 1. *Feed grain consumption (1986-1996) by poultry (◆), pigs (■), beef cattle (-), dairy cattle (○) and other livestock (●), with projections for consumption by the year 2000, compared to total feed grain consumption (bars). (Source: Australian Bureau of Statistics, 1994; Minett, 1994; Meyers Strategy Group, 1995; ABARE, 1996; Meo and Cleary, 1997).*

Options to improve feed grain supply include increased grain production, reduced exports, imported grain, greater utilisation of by-products and alternative feedstuffs, and improved utilisation of current feed grains. These will now be discussed in more detail.

Increased feed grain production.

The greatest single factor which limits grain production in Australia is rainfall, or more specifically the reliability of that rainfall, as recurring drought and reduced crop yields will attest. There is little that can be done to alter the rainfall pattern, but better use could be made of the rainfall that is received by a more appropriate choice of plant varieties in grain production or improved regional land use strategies.

Cereal grain production in Australia has been dominated by wheat and barley. The varieties of wheat and barley grown have been selected to meet the domestic and export requirements for flour milling and malting, respectively. The stockfeed industry has traditionally utilised that proportion of these grains which did not meet the required standards for export or has had to pay import parity prices to retain the grain in Australia.

With a rise in feed grain demand to the order of 30% of total grain production by the year 2000, and possibly 50% by 2005 (Meyers Strategy Group, 1995), the need for purpose specific (feed) varieties of wheat and barley has become increasingly apparent. The higher yield characteristics of these varieties should ensure improvements in both the

supply and price of feed grains, more so than utilising milling quality varieties for feed as a secondary alternative to their original purpose. Although the need for specific feed varieties for wheat and barley has long been recognised, their development has been delayed. Three contributing factors to this delay identified by Marshall (1993) are:

i) The existence of producer controlled statutory marketing authorities with compulsory acquisition rights and a strong focus on export markets
ii) The "opportunistic, even predatory" grain purchasing policies of the stockfeed industry. Through their negotiation for grains unsuitable for other markets at discounted prices, the stockfeed industry have engendered an image of "market of last resort" to many grain producers
iii) A lack of plant variety rights and the fact that crop improvement was dominated by public plant breeding programmes jointly funded by government and producers. This removed any incentive the end-users might have to invest in plant breeding and development for their specific needs.

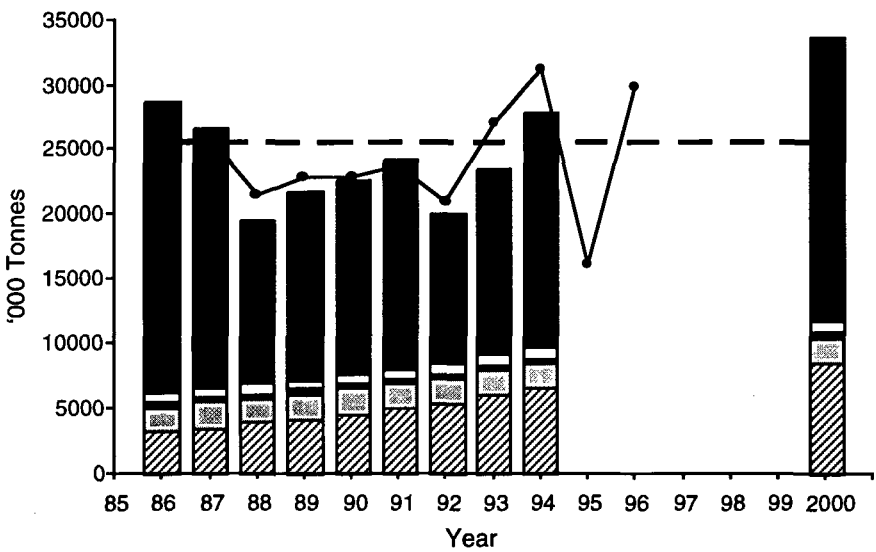

Figure 2. *Total grain production (wheat, barley, sorghum, oats, maize, triticale, peas, lupins, faba beans, chick peas, oilseed meals other than cotton, (●); ten year average (1984-1994,—) and disposal for stockfeed (▨), domestic wheat (▣), domestic coarse grains/legumes (■), seed (□) and exports (■) with projection to the year 2000. (Source: Minett, 1994; ABARE, 1996)*

With the deregulation of the domestic grain market, the ever increasing significance of domestic feed grain demand, and the introduction of plant variety rights, things have changed. Not only have specific feed varieties of wheat and barley been released, but grain producers and local end users have recognised their interdependence and their need to collaborate to consolidate their mutual profitability. This has created a more favourable environment for the contract growing of feed grains which should bring a degree of stability to both industries.

The new feed wheat varieties (e.g., Lawson) are reported to be capable of producing 6-7 tonnes/hectare under favourable conditions, after grazing (Marshall, 1993). Compared to the national average yield of 1.5-2.0 tonnes/hectare for wheat (ABARE, 1996), this represents a substantial improvement in productivity. It has also been suggested that these feed wheats could contribute to feed grain supply without necessarily displacing traditional cereal production by being introduced into higher rainfall areas in lieu of wool production.

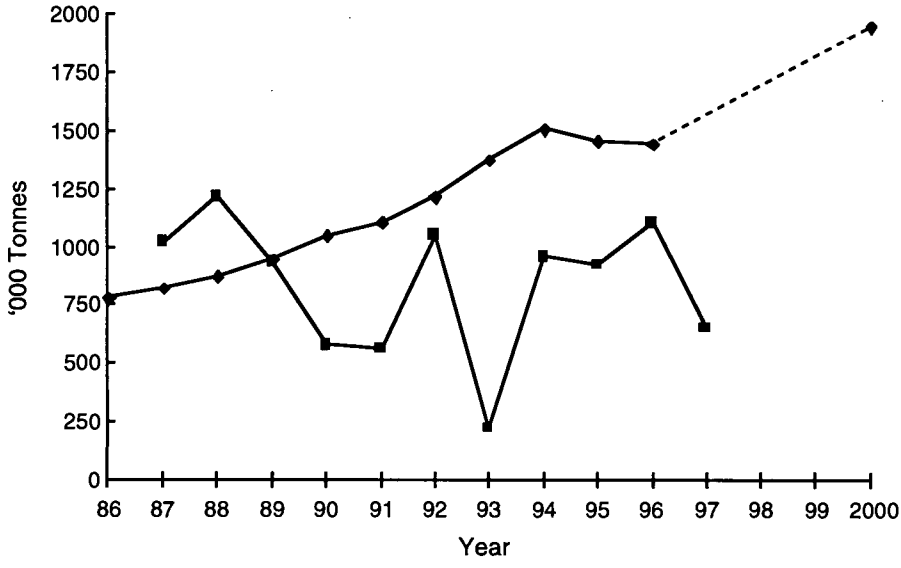

Figure 3. *Feed grain demand in Queensland (◆) compared with grain sorghum production (■).*

Grain legume production in eastern Australia has evolved with a large focus on the human food market, but the ability of grain legumes to serve as both major protein and energy sources in livestock diets has attracted a lot of interest, particularly from the pig and poultry industries. The positive role that grain legumes play in crop rotation programmes is well appreciated by the grain growers, but there is still a need for the livestock industries to encourage further production of grain legumes for use as stockfeed by contractual commitment or at least signalled interest.

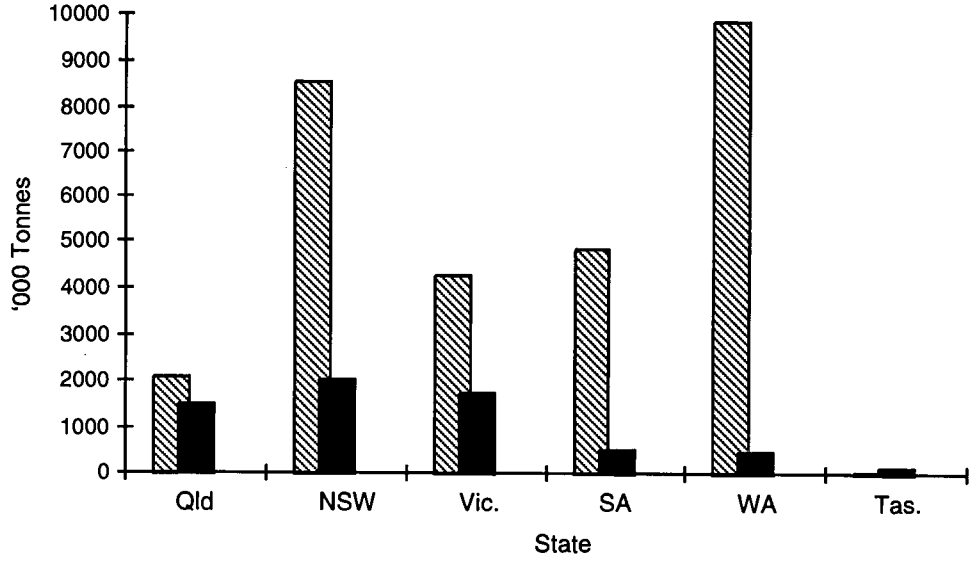

Figure 4. *Feed grain demand (■) and total grain production (▨) by State (1993-94).*

Reduced export of potential feed grains.

Part of the export component of cereal grains and pulses in Australia is material of feed quality (as distinct from milling or human food quality) which could be used domestically if it could be relocated economically. The geographic dislocation of supply and demand for feed grains and the prohibitive cost of transport in Australia to reconcile this, results in the export option being the most profitable for the grower (e.g., lupins and wheat in Western Australia and sorghum in central Queensland). The imbalance between feed grains demand and the total grain production in each state for 1993-94 is presented in Figure 4. Factors which would improve the utilisation of this potential resource are listed below:

i) Relocation of livestock production activities to the grain growing areas
ii) Development of grain growing activities that are closer to the centres of livestock production
iii) Review of transport logistics to minimise the freight penalties involved. For example, revision of domestic sea freight rates in line with international rates and the upgrading of rail services.

Extension of the feed grain supply equation to include imported produce.

Even though the widespread cultivation of specific feed wheats, barleys, and grain legumes will go a long way to meeting the requirements of the domestic livestock, the occasional occurrence of severe and widespread drought could render the local grain supply inadequate to meet the domestic demand. In these circumstances there will be enormous pressure to facilitate the importation of grain from the international market.

Largely as a result of geographic isolation and quarantine vigilance, Australia enjoys a unique freedom from many of the plant diseases and insects which trouble other countries, and this is an asset well worth protecting. However, if the livestock industries have as much right to survive as grain growers, then the question of grain imports at times when demand cannot be met by local production will need to be addressed and facilitated.

If the Australian livestock industries are to be internationally competitive, they need to be able to take advantage of international supply options. It may be in the best interests of Australia for grain growers to continue growing prime hard wheat and malting barley for export, and for the livestock industries to obtain their requirements as sorghum, maize, barley and cassava from elsewhere. By carefully selecting the sources of grains and using appropriate importation protocols, the risks of introducing harmful diseases or pests to the existing grain industries should be minimal. It would be to the net detriment of the livestock industries and the country as a whole, if Australian grain production were to be compromised in any way. In a reciprocal manner, it would be to the long term detriment of domestic grain production if the livestock industries were to be compromised by unreasonable constraints to access of feed grain. The domestic livestock production industries not only represent the most reliable and accessible market for feed grains, but also by "value adding" they deliver a greater net benefit to the national economy.

Greater utilisation of by-products and alternative feedstuffs.

There are a large number of potential feedstuffs which are currently underutilised in livestock production. If these feedstuffs were able to be incorporated to a greater extent in livestock diets they could significantly ease the demand on traditional feed grains. Examples of such potential feedstuffs include:

- Sugar industry by-products
- Oilseed industry by-products
- Grape and fruit industry wastes
- Dairy industry by-products
- Confectionary wastes

- Brewing industry by-products
- Human food wastes (bread, restaurant and institutional waste food, bakery wastes, pasta, potatoes)
- Cereal milling wastes
- Forestry wastes
- Animal wastes
- Crop residues.

Many of these potential feedstufss may require considerable research into the logistics of recovery, handling and the technology of processing, but nonetheless represent a considerable feed resource. There are numerous alternative crops which could be competitive in terms of nutritive value if the appropriate infrastructure was in place to process them. For example, cooking and/or drying facilities for potatoes, cassava, artichokes, turnips, as well as more exotic options such as aquatic plants, single cell proteins, worm or maggot meal.

With the increased interest in liquid feeding of pigs and cattle, the logistics of incorporating some of the by-products and exotic feedstuffs into commercial diets is far more feasible than in systems based on traditional milled feed. Not only is there an energy saving in not having to dry the materials, but there is also increased flexibility in formulation by being able to accommodate materials of variable moisture content simultaneously.

Improved utilisation of current feed grains

There is still considerable room to improve the efficiency of conversion of ingested feedstuffs into animal tissue. For example, energy digestibility coefficients of around 0.8 and protein retention rates of less than 50% for commercial diets fall considerably short of biological potential. Average commercial pig herd feed conversion efficiencies of 3.05 (Meo and Cleary, 1997) compared to industry leaders at 2.60 kg feed/kg live weight produced reflects the magnitude of current feed utilisation inefficiency. Areas where potential improvements could be made include:

Digestibility

Through the physical processing of feed grains (grinding, pelleting, steam flaking, expansion, extrusion, micronising, reconstituting) and the use of supplementary enzymes either as a feed additive or as a part of a preliminary hydro-thermal treatment, the digestibility of specific nutrients for each animal species can be significantly enhanced. These processes and additives need to be cost-effective at a commercial level, and with increasing supply pressure this becomes more likely. Increasing concerns about the environmental impact of animal effluent will reinforce the need to improve this aspect of production (e.g., volume of effluent solids and their biological oxygen demand, odours, and control of nitrogen and phosphorus outputs).

Improved feed formulation

By providing diets which more closely match the animal's requirements for maintenance and tissue synthesis (be this milk, meat, eggs or foetus), the efficiency of nutrient utilisation will be optimised. This not only reduces the kilograms of feed required to facilitate each unit of production but it also minimises the biological load in the effluent. The application of modelling tools (e.g., Auspig) to commercial production situations in recent years has demonstrated the potential for significant improvements in efficiency through a better appreciation of tissue requirements, more appropriate dietary specifications and identification of the physical constraints to production.

Promoting maximum productive output

In all animal species there is an obligatory requirement to meet the maintenance requirements of the animal before any nutrients can be directed to production. The nutrients consumed in the maintenance function are essentially non-productive and need to be minimised. This is achieved by promoting maximum production rates so that the proportion of ingested nutrients directed to maintenance is minimised.

Promoting lean growth

In the case of meat producing species, substantial benefits in the form of reduced feed conversion ratios (kg feed/kg live weight gain) are achieved when lean deposition is promoted in lieu of fat deposition. The energetic cost of lean deposition is approximately 20% of that of fat deposition and hence lean growth requires far less feed. This could be achieved by genetic improvement of the capacity for protein deposition or the use of tissue repartitioning agents in pigs.

v) Avoiding feed wastage

In many livestock feeding operations the proportion of feed on offer which is physically wasted is still unacceptable. If the feed is not ingested, the nutrients cannot be digested, absorbed, metabolised or utilised for production. Attention has to be directed to the design of feeders and the methods of feed administration to ensure that feed wastage is minimised. If the total feed volume employed in Australia is of the order of 10 million tonnes/year and feed wastage is of the order of 10%, it represents a very significant component in the overall feed grains supply equation. It is probably much easier to save the million tonnes wasted than generate an additional million tonnes of feed grain.

Conclusions

Australian livestock producers are rapidly approaching a situation in which demand for feed grains can be expected to exceed supply capacity on a frequent basis. There is a need to address the problem on multiple fronts to increase the production of feed grains, to improve their utilisation, to minimise waste and to explore alternative supply options, be these by-products, exotic materials or imported products.

The problem of shortfalls in the supply of feed grains is not exclusive to the pig industry and hence requires a coordinated, multi-industry, national approach to resolve it.

Symposium continued on next page

CHARACTERISTICS OF FEED GRAINS THAT INFLUENCE THEIR NUTRITIVE VALUE AND SUBSEQUENT UTILISATION BY PIGS

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Abstract

Development of rapid and objective analytical tests for the assessment of amino acid and energy availability in feed grains is a high priority of the Australian grains and livestock industries. This priority is based on the perceived variation that exists in amino acid and energy availability in feed grains and the influence this variation has on their nutritive value. There is a need to define the variation in the available energy and amino acid content of feed grains and to subsequently understand those factors that influence nutritive value. Differences of up to 3.7 MJ/kg dry matter (DM) in digestible energy (DE) content were observed following a review of data for more than 70 cultivars of wheat. Similarly, analysis of data for more than 125 cultivars of barley revealed a range in DE estimates from 11.7-16.0 MJ/kg DM. Differences of this magnitude are economically significant to pig producers. Cultivar has a minimal effect on the availability of energy and amino acids in cereals, although the variation is larger in legumes, particularly lupins. The cultural conditions and agronomic practices (e.g., fertiliser rate) have a greater influence on amino acid and energy availability than the growing region or the growing year. Many factors are shown to influence the availability of energy and amino acids in feed grains including protein source and type, starch characteristics, fat source and type, and non-starch polysaccharide components. Although all of these factors can influence the nutritive value of a feed grain for pigs in some way, the availability of energy and amino acids will ultimately depend on the particular combination of these components in a grain and how they behave in the presence of nutrients from other feed ingredients. Thus, an understanding of the factors that influence the nutritive value of feed grains is more likely to eventuate when multiple regressions of grain components are made against the availability of energy and amino acids.

Introduction

The extent of use of grains in monogastric diets is governed by their nutritive value or the cost of supplying nutrients (e.g., amino acids, energy) relative to the cost of supplying these nutrients from other ingredients. Expressing nutrient content on an "available basis" and the ability to rapidly assess the degree of availability represents the best way to assess nutritive value.

There is currently no rapid way of assessing the available amino acid or available energy content of feed grains used in pig diets. Because standard methods of analysis are time consuming and expensive, the number of grain samples that have been analysed for the above parameters is small. Consequently, there are questions pertaining to the apparent variability in nutritive value that exists amongst grains and whether this variability is due to inaccurate methods of analysis or real differences in the composition of the grains.

The objectives of this paper are to:

1. Define the variation in available energy and available amino acid content of feed grains for pigs.
2. Discuss the chemical and physical components of feed grains that influence the availability of amino acids and energy.
3. Suggest methods for the prediction of amino acid and energy availability in feed grains for pigs.

Variability in amino acid and energy availability in feed grains

Before discussing factors that may be influencing the availability of amino acids and energy in feed grains for pigs, it is important to first establish that variation does in fact exist, and that the range of values that are currently reported in the literature are not just a result of differences in experimental methodology. In this discussion, "available energy" will include measurements of DE, metabolisable energy (ME) or net energy (NE) and "available protein or amino acids" will include measurements of apparent or true ileal digestible amino acids or amino acid availability determined using growth studies subjected to slope-ratio analysis. It is recognised that net energy and available amino acids determined using metabolism or growth experiments, respectively, are the best ways to measure availability, however, the literature reporting these values is limited, and the methods used are subject to high levels of variation. For this reason, it would be difficult to assess the variation that exists amongst feed grains using these values alone.

Variation in energy availability in feed grains

Range in estimates of energy availability

Cereals: Differences of up to 3.7 MJ/kg DM in DE content were observed following a review of data for more than 70 cultivars of wheat (Eggum, 1977; Batterham *et al.*, 1980; Anderson and Bell, 1983a, 1983b; Lin *et al.*, 1987; Smith *et al.*, 1987; Bell and Keith, 1989; Fuller *et al.*, 1989; Haydon and Hobbs, 1991; Shi and Noblet, 1993; Kopinski, 1997; Wiseman, 1997). The lowest reported DE was 13.3 MJ/kg DM (Fuller *et al.*, 1989) and the highest 17.0 MJ/kg DM (Kopinski, 1997). Gross energy (GE) ranged from 16.89 MJ/kg DM (Fuller *et al.*, 1989) to 20.71 MJ/kg DM (Anderson and Bell, 1983b) while energy digestibility coefficients ranged from 0.74 (Anderson and Bell, 1983b) to 0.92 (Shi and Noblet, 1993).

Analysis of data for more than 125 cultivars of barley (Eggum, 1977; Batterham *et al.*, 1980; Hanrahan, 1981; Anderson and Bell, 1983a, 1983b; Bach Knudsen *et al.*, 1987; Lin *et al.*, 1987; Smith *et al.*, 1987; Bell and Keith, 1989; Fuller *et al.*, 1989; Yin *et al.*, 1993; A.R. Barr and R.J. van Barneveld, 1997, unpublished data, University of Adelaide, Australia; Gabert *et al.*, 1996; Kopinski, 1997; Wiseman, 1997) reveals a range in DE estimates from 11.7 MJ/kg DM (A.R. Barr and R.J. van Barneveld, 1997, unpublished data, University of Adelaide, Australia) to 16.0 MJ/kg DM (Gabert *et al.*, 1996). This corresponds with a range in GE content of 17.61 MJ/kg DM (Fuller *et al.*, 1989) to 19.63 MJ/kg DM (A.R. Barr and R.J. van Barneveld, 1997, unpublished data, University of Adelaide, Australia) and a range in energy digestibility coefficients of 0.62 (A.R. Barr and R.J. van Barneveld, 1997, unpublished data, University of Adelaide, Australia) to 0.85 (Gabert *et al.*, 1996; Kopinski, 1997). Considering that there are differences of just over 2 MJ/kg DM in the GE content, that the lowest and highest GE values do not correspond with the lowest and highest DE values, and that there are differences of more than 4 MJ/kg DM in DE, it appears that energy digestibility does not reflect GE content, and that energy digestibility rather than GE content has the greatest influence on DE.

Legumes: Wigan *et al.* (1994) summarised recent estimates of the DE content of lupins for pigs. Estimates ranged from 12.3-15.3 MJ DE/kg for lupin seed meal and 15.4-16.6 MJ DE/kg for lupin kernels. Reasons for this wide range in energy values may include:

1. The use of wheat or sugar as a base for the experimental diets. Because wheat and sugar have different digestion and fermentation characteristics, the measurement of the DE content of a test legume in these diets may be influenced when the value is calculated by difference.
2. The degree of crushing/grinding of the lupins prior to inclusion in the experimental diets.

As well as being variable, DE may not be the most appropriate measure of available energy in lupins for pigs due to the fact that a large proportion of lupin dry matter and

energy is digested in the hind-gut compared with other legumes (Taverner *et al.*, 1983). By examining the effects of graded levels of lupin kernel inclusion in a sorghum-based diet, van Barneveld *et al.* (1995a) showed there was no significant difference in the faecal digestibility of diet dry matter or energy or diet DE (Table 1). In contrast, measurement of dry matter and energy digestibility at the terminal ileum revealed that dietary inclusion of lupin kernels at 36% significantly depressed these parameters. Hence, despite all diets having the same DE, the efficiency of use of this energy would be significantly lower in pigs fed higher levels of lupins. It is likely that the differences between the ileal and faecal digestibility of nutrients in lupins would be even greater when whole seed is fed.

Table 1. Effect of graded levels of lupin kernel inclusion on the ileal and faecal digestibility coefficients of dietary dry matter and energy and diet DE (MJ/kg, air-dry) in growing pigs (van Barneveld *et al.*, 1995a).

	Ileal digestibility				Faecal digestibility			
Diet	1	2	3	4	1	2	3	4
Kernel (%)	0	12	24	36	0	12	24	36
Dry matter	0.85[a]	0.82[a]	0.77[a]	0.67[b]	0.90[a]	0.91[a]	0.90[a]	0.90[a]
Energy	0.90[a]	0.87[a]	0.83[a]	0.76[b]	0.94[a]	0.94[a]	0.93[a]	0.93[a]
Diet DE	16.85[a]	16.50[a]	15.84[a]	14.47[b]	17.71[a]	17.67[a]	17.64[a]	17.67[a]

[a,b]Values within a row (Diets 1-4) with different superscripts differ significantly ($P<0.05$).

Gatel and Grosjean (1990) reported the DE content of peas for pigs to range from 14.23 to 17.11 MJ/kg DM. Higher values were found for white flowered (15.62-16.40 MJ DE/kg DM) than coloured types (14.22 MJ DE/kg DM; Hlodversson, 1987) and round seeds have a higher DE content than wrinkled seeds. Like lupins, the relative DE content of peas may not be an appropriate comparison for peas with ingredients such as soya bean meal since Taverner and Curic (1983) reported identical net energy values for these ingredients.

Variation in energy availability between cultivars

Within a method, site and growing year, the greatest difference in DE of 2.5 MJ/kg DM between cultivars of wheat was observed by Anderson and Bell (1983b). In the majority of other studies, the observed differences were in the vicinity of 0.8-1.4 MJ DE/kg DM with the exception of Kopinski (1997) who reported differences of only 0.3-0.4 MJ DE/kg DM among eight cultivars grown in the same region at the same time. Further analysis reveals that the samples studied by Kopinski (1997) were very similar in chemical composition on a dry matter basis. Crude protein content ranged from 16.4-19.8%, β-glucan content ranged from 0.61-0.75% and total starch ranged from 57.5-61.5%.

Within a method, differences of up to 3 MJ DE/kg DM were reported for barley (A.R. Barr and R.J. van Barneveld, 1997, unpublished data, University of Adelaide, Australia), however, these values were determined using *in vitro* analytical techniques (Boisen and Eggum, 1991). Using other measurement techniques, a much smaller variation between cultivars was detected. In a study of more than 10 cultivars, differences of no more than 0.4-0.7 MJ DE/kg DM were detected (Kopinski, 1997), although these differences were significant on an air-dry basis. In similar studies by Peers and Taylor (1977) and Batterham *et al.* (1980), the differences in DE estimates for five and eight cultivars were only 0.5 and 0.7 MJ/kg DM, respectively. These differences were even less on an air-dry basis. Using total faecal collection procedures in pigs, the greatest difference in DE that was detected amongst cultivars was 2.1 MJ/kg DM (J. Patience and S. Fairbairns, 1997, unpublished data, Prairie Swine Centre, Canada).

Variation in energy availability due to growing conditions and experimental methodology

Kopinski (1997) and Wiseman (1997) respectively conducted extensive studies on the influence of site and year on the DE content of wheat. Both studies revealed minimal

effects of site and year on the DE content of individual cultivars. The greatest difference in DE content within a cultivar was 0.9 MJ/kg DM between two growing sites in a single year (Wiseman, 1997). Anderson and Bell (1983b) reported a difference of 1.9 MJ DE/kg DM over two growing seasons. Fuller *et al.* (1989) reported that the application of N fertiliser increased the DE content of wheat cultivars by up to 1.1 MJ/kg DM.

Anderson and Bell (1983b) and J. Patience and S. Fairbairns (1997, unpublished data, Prairie Swine Centre, Canada) reported significant differences in the DE content of barley cultivars grown at different sites. Similarly, Fuller *et al.* (1989) reported a difference of 2.5 MJ DE/kg DM when the same barley cultivar was grown with supplementary nitrogen fertiliser. In contrast, Bach Knudsen *et al.* (1987) and Kopinski (1997) reported only small differences in the DE content of the same cultivars grown at different sites. The latter observations may have been due to similar growing conditions between sites and years in these experiments. It appears that cultural conditions have a greater impact on DE content than site alone.

Kopinski (1997) compared the DE content of a range of sorghum cultivars at a single site and between two sites. The largest difference in DE observed in sorghum cultivars at a single site was 0.71 MJ/kg DM (17.26-17.97 MJ DE/kg DM) with a range in digestibility coefficients of 0.898 to 0.920. The greatest difference observed within a cultivar grown simultaneously at two sites was 0.5 MJ DE/kg DM (16.99-17.49 MJ DE/kg DM) with a range in digestibility coefficients of 0.901-0.906.

Variation in amino acid availability in feed grains

Range in estimates of nitrogen and amino acid availability

Cereals: Apparent nitrogen digestibility coefficients differed by up to 0.15 for wheat (Taverner *et al.*, 1981; Anderson and Bell, 1983b; Sauer and Ozimek, 1986; Lin *et al.*, 1987; Fuller *et al.*, 1989; Haydon and Hobbs, 1991). Taverner *et al.* (1981) reported the widest range of coefficients (0.71-0.83) from a single study, which is consistent with the range reported by Sauer and Ozimek (1986). Lysine digestibility coefficients ranged from 0.56 (Fuller *et al.*, 1989) to 0.81 (Taverner *et al.*, 1981) with a similar range evident for most other amino acids.

A range in nitrogen digestibility coefficients from 0.47 (Anderson and Bell, 1983b) to 0.80 (Lin *et al.*, 1987) has been reported for barley, however, a considerable proportion of this variation can be attributed to differences in the methodology used to obtain the estimates. Lysine digestibility in barley ranged from 0.43 (Anderson and Bell, 1983a) to 0.84 (Taverner *et al.*, 1981).

Legumes: Estimates for lysine availability in whole *Lupinus angustifolius* for pigs range from 0.55 (Standing Committee on Agriculture, 1987) to 0.73 (van Barneveld *et al.*, 1997a). Values of 0.70 and 0.80 for lupin-seed meal and lupin kernels, respectively, have been applied in diets used in commercial piggeries and have maintained excellent pig growth, yet these values are obviously well above the estimate of 0.55 recommended by the Standing Committee on Agriculture (1987). Further, the results of van Barneveld *et al.* (1997a) were similar to Godfrey and Payne (1987) who suggested that the availability of lysine in lupin kernel meal (*L. angustifolius*) exceeds 0.70. The reasons for the differences between estimates of lysine availability in lupins for pigs determined experimentally and those used commercially are difficult to explain. One explanation may be the inadequacies in the slope-ratio assay when defining amino acid availability in lupins due to influences from other lupin components such as non-starch polysaccharides. Alternatively, there may be a significant difference between the availability of lysine in older lupin cultivars such as Uniharvest, used in lysine availability experiments by Batterham *et al.* (1984), and new cultivars such as Gungurru that dominate current commercial use.

Gdala *et al.* (1992) determined the ileal and faecal digestibility of protein and amino acids in six varieties of white-flowered and three varieties of coloured-flowered peas. The true ileal digestibility of protein ranged from 0.66-0.83 with the greatest differences in amino acid digestibility evident for methionine, cystine and tryptophan.

Variation in amino acid availability amongst cultivars

Cultivars are rarely distinguished in studies of amino acid digestibility/availability. Taverner *et al.* (1981) compared the *in vitro* nitrogen digestibility and *in vivo* lysine availability of 47 wheat samples of different cultivars and/or growing sites. Nitrogen digestibility coefficients ranged from 0.87-0.95 while lysine digestibility coefficients ranged from 0.77-0.92. Anderson and Bell (1983a) completed a comprehensive study of differences in apparent amino acid digestibility amongst different wheat cultivars, however, faecal collection techniques were used which may have reduced the range in estimates due to the influence of hind-gut microflora. Apparent nitrogen digestibility coefficients ranged from 0.72-0.82, while apparent lysine digestibility coefficients ranged from 0.66-0.76.

Anderson and Bell (1983b) reported the apparent faecal nitrogen and amino acid digestibility of two cultivars of barley (cv Bonanza and Fergus, respectively). Within a growing season, differences in digestibility of the order of 0.06-0.16 were reported.

Variation in amino acid availability due to growing conditions and experimental methodology

Due to the higher costs associated with assessing the digestibility and availability of amino acids in feed grains for pigs, there are significantly less data available on the differences in these parameters within a cultivar grown in subsequent years.

Cereals: The experimental method can influence amino acid digestibility/availability estimates. For example, the crude protein content of the diets used in experiments to determine amino acid digestibility in wheat reported by Taverner *et al.* (1981), Anderson and Bell (1983b), Sauer and Ozimek (1986), Lin *et al.* (1987), Fuller *et al.* (1989) and Haydon and Hobbs (1991) varied from 114-162 g/kg, air-dry basis. Values determined using the lower protein diets were consistently lower than those determined using diets with a crude protein content greater than 140 g/kg due to a greater proportion of endogenous nitrogen losses in the digesta affecting the calculations. As expected, true digestibility estimates were significantly higher than apparent digestibility estimates and there appeared to be less variation in the estimates determined using faecal collection techniques. In addition, Taverner *et al.* (1981), Anderson and Bell (1983a, 1983b), Sauer and Ozimek (1986); Lin *et al.* (1987), Fuller *et al.* (1989), Yin *et al.* (1993), Jorgensen *et al.* (1997) and Nyachoti and de-Lange (1997) used either simple T-piece cannulation, re-entrant cannulation and/or faecal collection methods to determine the apparent and true amino acid digestibility of barley. Apart from surgical technique and site of collection influencing the digestibility estimates, the crude protein content of the experimental diets varied from 94 (Anderson and Bell, 1983a) to 246 (Jorgensen *et al.*, 1997) g/kg DM and the vast majority of these barley samples were tested with a dietary protein content of less than 13%. This may have resulted in a significant underestimate of amino acid digestibility (Sauer and Ozimek, 1986).

Anderson and Bell (1983b) reported the apparent faecal nitrogen and amino acid digestibility of two cultivars of barley (cv Bonanza and Fergus, respectively) grown in two consecutive seasons. Coefficient differences in the order of 0.08-0.18 were reported for Fergus and Bonanza cultivars, respectively. In a similar study, Fuller *et al.* (1989) noted that increasing application of N fertiliser to wheat and barley crops induced differences in grain protein content that were similar to the differences observed amongst varieties and yet there was no comparable effect on protein digestibility. Despite this, the digestion of lysine and a number of other amino acids up to the terminal ileum was significantly higher in the high protein varieties.

Legumes: Batterham (1992) compared the availability of lysine in three samples of *L. angustifolius* cv Uniharvest for pigs, rats and chicks using the slope-ratio assay (Table 2). The results suggest that there are large differences in the ability of different species to utilise available lysine from lupins. The results also suggest that lysine in lupins is highly available for poultry whereas in pigs the availability is much lower and very variable. Batterham *et al.* (1984) suggested the low lysine availability in lupins for pigs may be due to an unidentified growth depressant (which has a linear effect on performance with increasing inclusion level), however, the results of van Barneveld *et al.* (1997a) suggest that

the low lysine availability values reported by Batterham *et al.* (1992) are more likely to be due to the experimental methodology employed.

Table 2. Comparison of the availability of lysine (proportion of total) in three samples of *L. angustifolius* cv Uniharvest for pigs, rats and chicks (Batterham, 1992).

		Pig	Rat	Chick
Lupin seed meal	No. 1	0.37	0.81	0.98
	No. 2	0.54	0.70	0.93
	No. 3	0.54	0.80	0.84

Factors influencing amino acid and energy availability

Protein type/source

Cereals: The starchy endosperm is the major storage tissue in cereal grains, containing most of the starch and storage proteins. The storage proteins account for about half of the total nitrogen in grain and fall into two groups: prolamins and globulins/glutelins. The prolamins are the major storage proteins in all cultivated cereals except oats and rice, although smaller amounts of globulins may also be present. The converse is the case in oats and rice, where globulin-type proteins are the major components with smaller amounts of prolamins.

The prolamin storage proteins are low in certain essential amino acids, which limits the overall nutritional quality of all cereals for pigs with the exception of oats and rice (which are dominated by the globulin-type proteins). Thus, lysine is the major limiting amino acid in barley, wheat and maize, followed by threonine in barley and wheat and tryptophan in maize (Shewry, 1996). The digestibility of amino acids in prolamins and globulins does not appear to differ, however, Fuller *et al.* (1989) observed a difference in the N digestibility of high and low protein varieties of wheat and barley.

In addition to prolamins and globulins, cereal grains contain a multitude of other proteins. These include hydrolytic enzymes which hydrolyse the grain storage reserves (e.g., starch) during germination, starch granule proteins, antimicrobial and cysteine-rich proteins and inhibitors of hydrolytic enzymes. Cereal seeds are rich sources of low molecular weight proteins which inhibit the activities of proteases and/or carbohydrases. Levels of these inhibitors in cereals, such as barley and wheat, are sufficient to affect their bread and pasta making qualities, however, there is little published information about the effect these factors have on the nutritional value of cereals for pigs.

Legumes: Pea protein is composed of two main fractions - globulins (60%) and albumins (25%). The remaining 15% is in the form of insoluble proteins. The globulins are the major storage proteins, characterised by high molecular weight molecules and deficiencies in sulphur amino acids and in tryptophan. The albumins are biologically active proteins, some of which have anti-nutritional properties. LeGuen (1993) suggested that the globulins in peas are highly digestible. In contrast, it appears the albumins are partly responsible for a reduction in N apparent ileal digestibility in peas through their protease inhibiting activity and because their structure may be resistant to the action of hydrolytic enzymes.

Starch type/source

Cereals: Starch occurs in a granular form in the cells of the starchy endosperm of the grain but is not present in aleurone cells. At maturity, some residual starch may be found in the cells of the embryonic tissues and the scutellum. Starch granules in cereal endosperms occur in a variety of shapes and sizes, each characteristic of the species. Starch is an association of two polymers of glucose - amylopectin and amylose, and the proportions in which these two polysaccharides occur influences the nature of the starch.

Cereal grains differ greatly in their starch content. Wheat and maize contain the highest amounts of starch (76%), followed by sorghum (75%) and then barley and oats

(61% and 42%, respectively; Rowe and Pethick, 1994). Grain cultivars also vary in starch levels. Engstrom *et al.* (1992) and Ovenell and Nelson (1992) reported a range in the starch content of different varieties of barley from 483-659 g/kg. Some sweet maize cultivars have been found to contain as little as 200 g starch /kg (Inoue *et al.*, 1991).

Most starch containing grains contain a fraction of starch which escapes digestion in the small intestine. This undigested starch is termed resistant starch and it behaves in a similar way to dietary fibre in the large intestine. There are many factors that can affect the digestion of starch in the small intestine (Blakeney, 1993; Muir *et al.*, 1994). These include:

- Starch granules that are physically inaccessible to digestion such as when grains are whole or only partly ground. This type of resistant starch is released when the plant cell walls, which restrict the access of digestive enzymes, are ruptured by more thorough grinding.
- Ungelatinised starch granules. When feed grains are included in unpelletted diets (i.e., mash), the degree of gelatinisation is limited, increasing the potential for starch resistance. Gelatinisation of starch granules occurs during cooking and processing in the presence of excess water and heat, where starch granules swell and rupture releasing the amylose and amylopectin. Pelleting compound feeds will increase the level of starch gelatinisation within a grain.
- Particle size. The size of food particles consumed determines the surface area that is available for enzymic attack. The larger the particle size, the smaller the total surface area.
- Amylose content. High amylose cereal starches are poorly digested. The higher the amylose content, the more slowly the starch is digested. Amylopectin, with its open, branched structure appears to be more susceptible to enzymic degradation than the more compact amylose (Betschart, 1988) which has coiled, unbranched chains of glucose molecules.
- Inhibitors. Protein or glycoprotein inhibitors of α-amylase are present in many cereals and legume seeds, however, they are usually destroyed by heating. Tannins, which can also be present in the seed, can significantly inhibit the action of starch degrading enzymes.

Legumes: Jenkins *et al.* (1982) reported that the starch in lentils, with a relatively high content of amylose, was digested more slowly by humans than the starch contained in white bread. This data supports the hypothesis that starches with a higher amylopectin content are more easily digested due to greater access for digestive enzymes. Similarly, Robertson (1988) suggested that the digestion of starches from legumes is slow and incomplete due to a higher content of amylose.

Lipid type/source

Compared with the total fat in the diets of most livestock, the amount contributed by feed grains is relatively small. Despite this, the total fat content of a feed grain can have a significant influence on the DE contributions to a diet. For example, the DE content of naked oats is significantly influenced by its crude fat content (van Barneveld *et al.*, 1997d). Samples of naked oats with a difference in crude fat content of 20 g/kg differ in DE content by 0.5 MJ/kg DM. The samples with the higher crude fat content have a higher DE.

Apart from potentially increasing the DE content, the contribution of fats from feed grains to pig diets is small, and the type of fat in a grain has only a small effect on the nutritive value of the diet. Of greater importance are the interactions between fats from feed grains and supplemental fats. In pigs, saturated fatty acids alone are less efficiently digested than unsaturated fatty acids (Freeman *et al.*, 1968). However, the potential to form micelles in the gut and the absorption of fatty acids are increased in the presence of unsaturated fatty acids and monoglycerides (Stahly, 1984). Therefore, the digestibility of a particular supplemental fat source in pigs is dependent on the fatty acid composition of the total diet. The digestibility of fat in diets containing a ratio of unsaturated to

saturated fatty acids greater than 1.5 is relatively high, averaging 85-92%, whereas the digestibility of fat in diets with an unsaturated to saturated ratio of less than 1.0-1.3 is substantially lower, ranging from 35-75% (Stahly, 1984). On this basis, the digestibility of tallow when included as 5% of the diet would be expected to be 10-15% higher in pigs fed corn-based diets as compared with barley based diets in which the total dietary ratio of unsaturated to saturated fatty acids would be 1.5 and 1.0%, respectively (Stahly, 1984).

Non-starch polysaccharides

β-Glucan

Bach Knudsen *et al.* (1993a) showed that the addition of oat bran, a rich source of soluble dietary fibre in the form of β-glucan, significantly depressed the ileal digestibility of fat in pigs. Protein and energy digestibility was also negatively correlated with increased dietary fibre intake. The aleurone and subaleurone cell walls, concentrated in oat bran, act as barriers against digestive enzymes and in this way lower the ileal and faecal digestibilities of protein and fat (Bach Knudsen *et al.*, 1993b). This negative effect of oat bran on the digestibility of protein and fat, however, is mainly associated with the insoluble residue fraction, which presumably is more abundant in the aleurone than the subaleurone cell walls.

It appears that amongst soluble non-starch polysaccharides (NSP), only those with significant intrinsic viscosity can reduce blood sterol levels. This property has been assumed by the majority of authors to be directly responsible for blood sterol reduction, in that it causes the digesta to become very viscous. The unstirred layer of digesta next to the gut wall is believed to thicken so that lipid micelles diffuse more slowly, and thus, may be swept past the regions in the gut where they can be absorbed. Coles *et al.* (1996) demonstrated that an alternative binding mechanism was possible, between β-glucan and bile salts, and hence was also a feasible mechanism for the reduction in blood sterol levels. This was accomplished by showing that β-glucan reduced the activity of glycocholic acid solution over a range of intrinsic viscosities.

An increase in the dietary level of insoluble NSP from oat bran also had a significant effect on the activity of the flora in the colon as measured by the concentration of adenosine triphosphate (ATP) in the digesta, but had little effect on the density of microorganisms in the large intestine (Bach Knudsen *et al.*, 1993a). Non-starch polysaccharide-induced increases in microbial activity are thought to also decrease the ileal and faecal digestibility of other nutrients such as amino acids and vitamins (Sauer *et al.*, 1977; Just *et al.*, 1983; Graham *et al.*, 1986).

D-Xylose and L-Arabinose

Hemicellulose consists primarily of pentose sugars, joined together in a polysaccharide chain with D-xylose as the most abundant component. Schutte *et al.* (1991) investigated the ileal digestibility and urinary excretion of D-xylose and associated effects of this pentose sugar on ileal and faecal digestibility of dry matter, organic matter, GE and nitrogen by pigs. D-xylose was included in experimental diets at a rate of 100 or 200 g/kg with D-glucose, included in a diet at a level of 200 g/kg, used as a control. Ileal digestibility of D-xylose was found to be close to 100% but a large proportion of this was subsequently excreted in the urine, indicating poor utilisation. Ileal and faecal digestibility of dry matter, organic matter, GE and nitrogen decreased significantly in pigs offered the 200 g D-xylose/kg diet. Schutte *et al.* (1991) suggested that this decrease in digestibility was due to an increase in microbial activity in the small intestine of the pigs.

In a similar study, Schutte (1991) investigated the apparent ileal digestibility of L-arabinose in diets containing graded levels of the sugar. L-Arabinose is one of the most abundant components which will become free in a complete hydrolysis of NSP from feed ingredients of vegetable origin. Schutte (1991) found that only 70% of dietary L-arabinose was digested by the end of the small intestine and that L-arabinose had a lower nutritional value than D-glucose.

Oligosaccharides

The term 'oligosaccharide' is used to denote a group of carbohydrates consisting of 2-10 sugar units. Oligosaccharides may contain similar or different sugar building blocks, different linkage structures and be linear or branched. Most oligosaccharides are soluble in water and physiological fluids (Mul and Perry, 1994). Despite their solubility, oligosaccharides, such as rhamnose, stachyose and verbascose, are generally poorly digested in the small intestine of the pig, with some, such as fructo-oligosaccharides, providing a soluble substrate for specific microbial populations in the digestive tract of monogastric animals.

The nutritional role of oligosaccharides has not been clearly defined. Mul and Perry (1994) suggested that the effects of oligosaccharides are structure specific and dose dependent. The observed effects on health, feed efficiency and performance are dependent on the composition of the intestinal flora before the deliberate inclusion of oligosaccharides. Fructo-oligosaccharides, when used strategically, could be used as a preventative measure against intestinal pathogens or act as a performance enhancer (Mul and Perry, 1994).

Howard *et al.* (1993) reported that supplementation of diets with fructo-oligosaccharides increased epithelial cell proliferation in the caecum and colon of neonatal pigs. Gabert *et al.* (1995) investigated the effect of oligosaccharides on the ileal digestibilities of amino acids, monosaccharides and bacterial populations and metabolites in the small intestine of weaning pigs (9-15 kg). Commercial oligosaccharide supplementation of diets did not affect nutrient digestibilities or bacterial populations in the small intestine of young pigs. Similarly, Zuo *et al.* (1996) found that nutrients in soya bean meal low in oligosaccharides were well digested, but no better than conventional soya bean meal when fed to ileal-cannulated dogs.

van Barneveld *et al.* (1996) reported that the extraction of oligosaccharides from *L. angustifolius* and *L. albus* had a significant effect on the digestion of energy by growing pigs (Table 3). An ethanol extraction removed 73% and 67% of the oligosaccharides from *L. angustifolius* and *L. albus*, respectively, but did not change the GE content. The extraction process did not influence the ileal energy digestibility of diets containing *L. angustifolius*, but significantly improved the ileal and faecal energy digestibility of diets containing *L. albus* and the faecal energy digestibility of those containing *L. angustifolius*. Ethanol extraction improved the DE of diets containing *L. angustifolius* and *L. albus* by 0.5 and 0.7 MJ/kg, respectively, and significantly improved the digestion of all reported amino acids in both *L. angustifolius* and *L. albus* (van Barneveld *et al.*, 1997c). The average amino acid digestibility was improved by 9.6% for *L. angustifolius*, and by 7.6% for *L. albus*.

Table 3. Effect of oligosaccharide extraction on the digestion of energy from *L. angustifolius* and *L. albus* in the small intestine and in the entire digestive tract of Large White, male growing pigs (35-45 kg LW), (van Barneveld *et al.*, 1996).

	Dehulled *L. angustifolius*		Dehulled *L. albus*		Statistics	
Treatment.	Control	Extracted[1]	Control	Extracted[1]	Diet	SEM
Oligosaccharides[2] (g/kg DM[3])	40.5	11.1	63.2	20.4	-	-
Gross energy (MJ/kg)	18.73	18.75	17.33	17.34	-	-
Energy digestibility (ileum)	0.63[a]	0.67[a]	0.68[b]	0.76[c]	*	0.017
Energy digestibility (faeces)	0.85[a]	0.87[b]	0.87[b]	0.90[c]	**	0.005
Diet DE[4] (MJ/kg DM)	13.1[a]	13.6[b]	14.1[c]	14.8[d]	***	0.077

[a,b,c]Values within a row with different superscripts differ significantly ; *P<0.05; **P<0.01; ***P<0.001. [1]Modified ethanol extraction. [2]Sum of raffinose, stachyose and verbascose. [3]DM, dry matter. [4]DE, digestible energy.

The nutritional role of oligosaccharides in poultry appears to be very different to the role observed in pigs by van Barneveld *et al.* (1996, 1997a). Irish *et al.* (1995) removed the α-galactosides in soya bean meal using either ethanol extraction or α-galactosidases and then fed the extracted meal to broiler chicks. The results suggested that removal of up to 90% of the α-galactosides of sucrose in soya bean meal has no beneficial effect on the nutritional value for chickens. In fact, removal of α-galactosides reduced the apparent metabolisable energy (AME) content of soya bean meal when fed to broiler chicks. Similar results were observed when the oligosaccharides were removed from lupins fed to broilers (R J Hughes, personal communication, South Australian Research and Development Institute).

Pectins

Mosenthin *et al.* (1994) investigated the effect of dietary pectins on pancreatic secretions and on apparent ileal and faecal digestibilities of protein and amino acids in growing pigs. Dietary pectin included at a level of 7.5 g/100 g in a diet based on corn-starch significantly depressed apparent ileal and faecal protein and amino acid digestibilities. The depression in ileal digestibilities could be attributed to both an increase in endogenous protein secretions and a decrease in the efficiency of digestion. In the large intestine, pectin was used by intestinal microbes as the principle energy source to catabolise nitrogenous compounds and to stimulate bacterial nitrogen assimilation. The inclusion of pectin in the experimental diets did not affect the flow of pancreatic juice or the total secretion of protein, lipase, trypsin or chymotrypsin. The results suggest that the pectin may have a detrimental effect on the processes of protein digestion and absorption but does not affect the secretion of pancreatic proteolytic enzymes in pigs.

Soluble non-starch polysaccharides

van Barneveld *et al.* (1994) investigated the effect of viscous NSP (guar gum) on protein digestion, and the ability of the pig to adapt to high levels of viscous NSP in the diet. Diets containing graded levels of guar gum were offered to pigs fitted with simple T-piece ileal cannulae, twice daily for two consecutive 7 d periods with 2 d of continuous digesta collection at the end of each period. There was a significant increase in digestibility of all amino acids in diets containing guar gum between collection 1 and 2 (Table 4). Significant diet x collection interactions occurred for isoleucine, leucine and phenylalanine. All amino acids showed a significant linear decrease in digestibility with increasing levels of guar gum for the first collection. The reduction in the anti-nutritive activity of guar gum at collection 2 may have been due to the development of microflora in the small intestine which can cleave the guar gum and hence reduce its viscosity and anti-nutritive activity. Guar gum may also stimulate increased secretions of pancreatic juice, enzymes and endogenous N. These results suggest that diets high in soluble NSP from legumes and cereals may limit the efficiency of production of growing pigs.

Table 4. The apparent ileal digestibility (%) of amino acids in semi-purified diets containing graded levels of soluble NSP (guar gum) fed to Large White, male growing pigs (35-45 kg LW) measured at 7 d intervals (van Barneveld *et al.*, 1994).

NSP (g/kg)	0		20		40		60		Statistics[1]				
Collection	1	2	1	2	1	2	1	2	DxC	Diet	C	Linear	SEM
Threonine	79	85	71	77	64	83	62	79	NS	NS	**	*	4.1
Valine	85	89	82	84	74	87	70	85	NS	NS	***	**	2.8
Isoleucine	86	89	81	83	73	87	69	85	*	NS	***	**	2.5
Leucine	85	88	80	82	69	86	64	85	*	NS	***	**	3.3
Phenylalanine	86	89	83	84	72	87	68	86	*	NS	***	**	3.0
Lysine	87	91	85	87	81	90	78	88	NS	NS	**	*	2.5
Histidine	85	89	80	82	72	87	69	85	NS	NS	***	**	3.0
Arginine	85	89	82	85	78	90	71	86	NS	NS	**	*	3.1

[1]D, Diet; C, Collection; NS, Not significant; *P<0.05; **P<0.01; ***P<0.001.

The effects of adding guar gum to pig diets may not be entirely attributable to increases in gut viscosity. Leclere *et al.* (1994) investigated how digesta viscosity, increased through the addition of guar gum to the diet, affected starch digestion and glucose absorption in humans. By infusing meals containing glucose into the small intestine, Leclere *et al.* (1994) was able to demonstrate that bolus viscosity did not significantly affect the delay in glucose absorption through the duodenal mucosa. Similarly, by infusing meals containing starch, Leclere *et al.* (1994) showed that a decrease in the digestion of starch in the upper small intestine accounted for part of the effect of viscosity on glycaemic response, whereas the main effect of guar gum was apparently to slow gastric emptying.

Carbohydrate/protein interactions

Hansen *et al.* (1991) used digestibility coefficients of protein, energy, starch and dietary fibre in individual feedstuffs to predict the digestibility coefficients of feed mixtures. It was found that in diets containing high levels of soluble dietary fibre, such as peas plus barley and peas plus wheat, there were often significant differences between the calculated digestibility coefficient and the measured digestibility coefficient (Table 5). For all significant differences between calculated and measured values, the measured digestibility coefficient was lower than the calculated value. This suggests that combinations of ingredients with high levels of soluble NSP may result in a further reduction in the digestibility of specific nutrients, including protein and energy.

Table 5. A comparison of measured and calculated true digestibility of protein, energy, starch, soluble and insoluble dietary fibre in diets containing peas and barley, and peas and wheat, respectively (Hansen *et al.*, 1991).

	Peas + Barley	Peas + Wheat
True digestible protein		
Measured	0.879	0.908
Calculated	0.907	0.924
Difference	0.028 **	0.016 NS
Digestible energy		
Measured	0.849	0.867
Calculated	0.859	0.886
Difference	0.010 NS	0.019 **
Digestible starch		
Measured	0.995	0.996
Calculated	0.997	0.997
Difference	0.002 **	0.001 NS
Digestible soluble dietary fibre		
Measured	0.825	0.754
Calculated	0.908	0.873
Difference	0.083 ***	0.119 **
Digestible insoluble dietary fibre		
Measured	0.505	0.504
Calculated	0.475	0.545
Difference	0.030 NS	0.041 NS

NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

van Barneveld *et al.* (1997b) determined the apparent ileal amino acid digestibility and DE of wheat, barley, triticale, *L. angustifolius* (cv. Gungurru; lupins) and *Cicer arietinum* (cv. Desi; chick peas) and then formulated diets to contain 50% of each cereal, respectively, and 35% of each legume, respectively. Diets were equalised for ileal digestible amino acids with lysine limiting at 0.40 g/MJ DE and the growth rates of pigs fed these diets determined (Table 6). Pigs fed combinations of chick peas and cereals

exhibited no significant differences in their empty-body weight gains. In contrast, a highly significant difference was observed in the empty-body-weight gain of pigs fed the diet containing lupins plus barley compared to lupins plus wheat and lupins plus triticale, respectively. Based on the original diet formulations, all pigs should have grown at the same rate if the apparent ileal lysine digestibility and DE values were additive when the lupins and cereals were combined in a mixed diet. It appears that the anti-nutritive effects of soluble and insoluble NSP from lupins and barley are amplified when these feed ingredients are combined.

Table 6. Daily live weight gain (DRG), daily empty-body-weight-gain (DEBWG) and empty-body-weight feed conversion ratio (EBWFCR) of growing pigs (25-55 kg LW) fed diets formulated to equal levels of ileal digestible lysine and containing specific combinations of lupins (*L. angustifolius* cv Gungurru) and either wheat, barley or triticale or chick peas (*C. arietinum* cv Desi) and either wheat, barley or triticale (van Barneveld *et al.* 1997b).

	DRG (g)	DEBWG (g)	EBWFCR
Lupinus angustifolius			
+wheat	677	620[a]	2.71[a]
+ barley	662	590[b]	2.99[b]
+ triticale	681	630[a]	2.60[a]
Cicer arietinum			
+ wheat	692	645	2.45
+ barley	676	624	2.55
+ triticale	671	629	2.58

[a,b]Values in a column with different superscripts differ significantly ($P<0.05$).

When different sources of fibre are combined in mixed diets, it appears that the properties of the resulting combination of fibre does not necessarily resemble those of the constituents. Laplace *et al.* (1989) investigated the associative effects between wheat bran and soya bean hulls in semi-purified diets on the ileal and overall digestibility of amino acids, energy and cell-wall components (Table 7). For some amino acids, such as methionine, the combination of wheat bran and soya bean hulls resulted in a significantly higher ileal digestibility than for diets containing either wheat bran or soya bean hulls alone. Overall digestibility proved even more difficult to predict when the two sources of fibre were combined. The overall digestibility of crude protein and all amino acids was significantly less in diets containing wheat bran plus soya bean hulls compared to those that contained either wheat bran or soya bean hulls (Table 7).

If combinations of NSP have different properties to their constituents resulting in a further depression of amino acid and energy digestibility, it is likely that the cause for reduced digestibility is increased gut viscosity rather than a change in microbial activity. van Barneveld *et al.* (1995b) reported that growing pigs fitted with ileal cannulae and fed diets containing graded levels of a lupin NSP isolate experienced a significant linear decrease in lysine and energy digestibility (Table 8). This coincided with a significant linear increase in digesta viscosity, but there was no significant effect on the microbial activity in either the small intestine, the large intestine or the caecum.

Antinutritional factors

Anti-nutritional factors (ANF's) are described as non-fibrous naturally occurring substances exerting negative effects on the performance or health of animals (van Barneveld *et al.*, 1997b). Most of these substances, which include trypsin inhibitors, tannins, haemagglutinins, saponins, gossypol, glucosinolates, oxalic and phytic acids, cyanogens and lathyrogens, lectins and goitrogens, provide the plant with a natural protection against attacks from moulds, bacteria, insects and birds. A variety of antinutritional factors exist in feed grains and their nutritional role has been widely reported (Liener, 1983). Antinutritional factors are far more prominent in legumes than cereals and their presence restricts the maximum inclusion level of legumes in pig diets.

Table 7. Ileal and faecal apparent digestibility of crude protein and amino acids in diets consisting of equal quantities of total fibre derived from wheat bran or soya bean hulls or a combination of both (Laplace *et al.*, 1989).

	Wheat bran		Wheat bran+Soya bean hulls		Soya bean hulls		Probability[1]	
	Ileal	Faecal	Ileal	Faecal	Ileal	Faecal	Ileal	Faecal
Crude protein	0.808	0.872	0.807	0.835	0.800	0.864	NS	**
Sum of 17 amino acids	0.850	0.895	0.850	0.867	0.843	0.890	NS	**
Lysine	0.866	0.884	0.858	0.858	0.856	0.887	NS	*
Methionine	0.887	0.867	0.914	0.843	0.906	0.883	***	***

[1]NS, not significant; *P<0.05; **P<0.01; ***P<0.001.

Table 8. The digesta viscosity, lysine and energy digestibility and ATP activity of digesta from the small intestine, caecum and large intestine of growing pigs fed diets containing graded levels of isolated lupin non-starch polysaccharides (van Barneveld *et al.*, 1995b).

Diet	Lupin NSP inclusion (%)	Viscosity (mPa.s)	Digestibility		ATP (mg/g digesta)		
			Energy	Lysine	Small intestine	Caecum	Large intestine.
1	0	1.43	0.85	0.91	0.72	4.78	1.97
2	5	2.28	0.71	0.90	0.61	5.73	1.66
3	10	2.33	0.50	0.82	0.88	7.14	1.98
4	15	4.89	0.58	0.85	0.47	5.45	3.38
	Statistics[1]						
	Diet	***	***	**	NS	NS	NS
	SEM	0.304	0.031	0.014	1.185	1.185	1.185

[1]SEM, mean standard error; NS, not significant; **P<0.01; ***P<0.001.

Germination

Cereals: The degree of sprouting, density and chemical composition of weather-damaged wheats and the subsequent effect on DE levels was investigated by Batterham *et al.* (1980). Samples contained from 0-96% sprouted seeds with corresponding densities ranging from 770 down to 500 kg/m^3. The DE content of the samples decreased as the degree of sprouting increased, with the greatest effects noted when more than 50% of the seeds in the sample were sprouted (680 kg/m^3). Similar results were reported by Taverner *et al.* (1975) and King (1976).

Mechanical treatment

Cereals: Peer and Leeson (1985) showed a massive increase in the DE content of whole barley when it was ground (4.95 to 15.9 MJ DE/kg DM). Similar results were reported by Hlodversson (1989) who investigated the effect of processing diets based on high-moisture barley (24% moisture). Physical processing of the diets was by hammermill, whole grain pelleting through 3.1 mm and 3.9 mm dies, and by cold rolling prior to pelleting through a 4.8 mm die. Rolling the barley prior to pelleting significantly improved the DE content (15.0 MJ DE/kg DM) of the diet compared to just whole grain pelleting. Rolling possibly increased the rate of starch pre-gelatinisation during the pelleting process. Hammermilling without pelleting also had a significantly higher DE (14.7 MJ DE/kg DM) compared with diets containing whole barley prior to pelleting through a 3.1 mm die (14.4 MJ DE/kg DM). These results suggest that milling is an essential first step when feeding cereals to pigs, however, further processing in addition to coarse crushing can increase DE levels. Patterson (1984) also showed a significant improvement in the DE content of milled barley compared to whole barley, however, subsequent treatment with sodium hydroxide had no effect. Sodium hydroxide treatment did improve the energy digestibility in whole barley.

The influence of grain particle size on the DE content of wheat and barley was reported by Wiseman (1997; Table 9). Wiseman (1997) clearly demonstrated that the DE content of wheat and barley is significantly lower when the whole seed is fed to pigs. Grinding increases the DE content, however, the degree of grinding has little effect on the energy digestibility coefficient. That is, there is little benefit to fine grinding over coarse grinding or rolling, unless the grind is so fine that the aleurone and sub-aleurone layers of the seed are ruptured.

Table 9. Influence of processing method on dry matter and energy digestibility of wheat and barley for pigs (Wiseman, 1997).

	Whole	Fine ground	Coarse ground	Rolled
Dry matter				
Wheat	0.76	0.86	0.82	0.87
Barley	0.64	0.81	0.78	0.81
Gross energy				
Wheat	0.75	0.86	0.83	0.86
Barley	0.64	0.80	0.78	0.80

Predicting amino acid and energy availability

The processes of digestion in a pig are complex and it is difficult to simultaneously account for all factors that can affect the nutritive value of grains. It is also difficult to simulate the processes of digestion *in vitro*, and for this reason, there is currently no widely accepted *in vitro* analytical methods for the assessment of the nutritive value of feed grains. Methods based on three step (pepsin, pancreatin, viscozyme) multi-enzyme closed *in vitro* systems for the prediction of organic matter and GE digestibility in the pig show particular promise for practical feed evaluation, but require further validation

(Moughan, 1997). Similar *in vitro* protein digestibility assays are less promising and require further development before they can be applied with confidence.

Further research is required to assess multiple regressions of grain components against the availability of energy and amino acids in feed grains. A range of studies have been completed to date (Batterham *et al,*. 1980; Morgan and Whittemore, 1982; Yin, 1993) and all conclude that neutral-detergent fibre is the best predictor of the nutritive value of feed grains for pigs. Correlations between nutritive value and more specific fibre components have been variable, with no one study assessing the nutritional role of all non-starch polysaccharide components against amino acid or energy availability. In particular, oligosaccharide content, in conjunction with β-glucan, xylose, pectins and soluble non-starch polysaccharides, may explain a significant proportion of the variation in the availability of amino acids and energy in feed grains for pigs.

Conclusions

The variability in amino acid and energy availability in feed grains is due to differences within the grains themselves and is sufficient to be of economic concern to pig producers. Differences in the DE content of cereals and legumes is in the order of 20-30%, with even greater differences observed for amino acid availability estimates. Cultivar has a minimal effect on the availability of energy and amino acids in cereals, although this variation is larger in legumes, particularly lupins. The cultural conditions (e.g., fertiliser rate) have a greater influence on amino acid and energy availability than the growing region or the growing year.

A range of intrinsic factors influence the amino acid and energy availability in feed grains including protein type and source, starch characteristics and lipid source. Of particular note are the effects of NSP from feed grains on the availability of amino acids and energy. These components also have a significant influence on the digestion of other nutrients supplied from both the grains themselves and other components in mixed diets. If the nutritional efficiency of pigs is to be optimised, further research is required to fully define the nutritional role of specific NSP components including oligosaccharides, β-glucans, pectins and soluble non-starch polysaccharides. Starch characteristics can also influence amino acid and energy availability and to address this a better understanding of the nutritional effects of diiferences in the ratio of amylose to amylopectin is required. In addition, an understanding of the influence of grain milling (coarse crushing, pelleting) on the quantity of starch resistant to digestion in the small intestine is needed.

An understanding of those factors or combinations of factors that influence the availability of energy and amino acids in feed grains increases the potential for the development of rapid and objective tests for these parameters. Development of methods for the rapid assessment of nutritional quality will improve the efficiency of use of feed grains in pig diets and hence, will help optimise the pig production process.

Symposium continued on next page

AN ANALYSIS OF THE BALANCE OF REGIONAL SUPPLY AND DEMAND FOR FEED AND THE INFLUENCE OF THE AUSTRALIAN PIG INDUSTRY

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Abstract

The differing locations of livestock and feed industries in regional Australia make it necessary to transfer feed from surplus to deficit regions. Such feed transfers are particularly important in drought situations. A model of regional feed markets which incorporates a feed mixing and a market component was developed. The model was then used to analyse separately the impacts of drought-reduced feed availabilities and medium term developments on regional feed usage, inter-regional transfers, regional prices, exports and imports. The results highlight the role of feed transfers from Western Australia and South Australia in meeting deficits in eastern regions. These transfers reduce the need for imports of feed grains.

Introduction

Grains, oilseed meals and pulses are the main feed ingredients used in diets for pigs and poultry and beef cattle in the feedlot segment of the beef industry. Total feed use of grains grew by 7% per year over the five years to 1993-94, but fell by 14% to 5.8 million tonnes in 1994-95 (Hafi and Andrews, 1997). Almost 20% of total usage of feed grains over the three years to 1994-95 was in the pig industry (Meyers Strategy Group, 1995). Continuous advancements in animal performance are expected to contribute to increasing profitability of the pig industry in the medium term (Abdalla *et al.*, 1997). As demand for feed is a derived demand arising from the demand for pig meat products, increasing profitability is expected to result in increasing feed demand by the pig industry in the medium term.

The issue of sustaining the availability of feed in extreme situations like drought and/or through a period of sharp increases in animal numbers is common for all the livestock industries including the pig industry. The high cost of grain transport within Australia is an impediment to the transfer of feed from surplus to deficit regions within Australia. For example, the high cost of transport has resulted in limited transfers of surplus grain from Western Australia to eastern regions even when the latter regions experienced feed shortages. Feed transfers from surplus to deficit regions is particularly important in drought situations, as was the case in 1994-95, when a number of normally grain-surplus eastern regions faced feed deficits and only limited inter-regional feed transfers took place. The interstate movement of grains could also be adversely affected due to lack of coordination between statutory grain marketing organisations, making it difficult to avoid grain deficits in some states. In particular, some marketing organisations operating independently would sometimes have to decide whether to service their traditional overseas markets and contractual obligations or domestic markets which have temporary feed deficits.

Reduced feed availability during droughts and the possibility of increased feed demand over the medium term have led to debate over Australia's grain import arrangements. Even though maize and sorghum are currently imported, there are significant barriers to grain imports under current quarantine protocols. In 1994-95, Australia imported a total of 357,000 tonnes of sorghum, maize and barley under special protocols developed to redress grain shortages (Hafi and Andrews, 1997).

The principal objective of the present paper is to report some key results obtained from recent research conducted by the Australian Bureau of Agricultural and Resource Economics (ABARE) to assess the impact of reduced feed availability resulting from drought as well as the implications of projected changes in the livestock and grains

industries over the medium term. The research included the development of a model to analyse regional feed markets in Australia. The model has two components - a feed mixing component and a market component which are linked together. In the model, the Australian feed market is divided into ten regions. The model was used to analyse the potential impacts of the reduced availability of feed as a result of drought and medium term growth in demand for feed ingredients on regional prices, supply and demand balances, import requirements, exports and inter-regional transfers which are solved endogenously in the model.

Regional feed markets

The issue of sustaining feed availabilities in Australia is mainly related to the distribution of feed from surplus to deficit regions. A need to distribute feed from surplus to deficit regions has arisen because most livestock industries are located far away from the regions specialising in feed production. The location of livestock industries, the availability of feed ingredients, the cost of transporting feed and the location of markets for final products influence the formation of regional feed markets.

Regional livestock industries

The pig industry, once aligned with the dairy industry, has moved to the grain belt. The pig industry's move to the grain belt is due mainly to an increase in intensive farming, environmental concerns and health factors which have led to a change in the feeding of pigs. New South Wales accounts for around 30% of Australian production, while Queensland and Victoria each account for around 24%. In New South Wales, the pig industry is heavily concentrated in southern regions while in Queensland it is concentrated on the Darling Downs and in south east regions. Queensland and New South Wales together account for about 80% of total capacity of cattle feedlots. New South Wales and Victoria account for 38 and 32% of Australian chicken meat production, respectively. The egg industry is concentrated in Tamworth and the Riverina region of New South Wales and the Darling Downs in Queensland. The dairy industry is concentrated in high rainfall coastal areas and some inland areas such as the Murray valley.

Regional Grain Supplies

Grain growing areas of Australia are found along two inland belts. The eastern Australian grain belt stretches through central Queensland, New South Wales, Victoria and South Australia while the Western Australian grain belt is found in an area bordered by Geraldton in the north, Albany to the south and Esperance to the east. Regional crop concentrations largely reflect the diversity in temperature, rainfall and soil conditions in these areas (Table 10).

Relative availability of feed ingredients influences the feeding regimes that are adopted. For example, piggeries in southern Queensland and northern New South Wales use mostly sorghum, while field peas are popular in Victoria and South Australia and lupins in Western Australia. Similar patterns are evident for poultry diets, with the heavy use of sorghum in northern regions and pulses in southern regions. However, downgraded wheat at discounted prices is preferred by pig and poultry farmers when it is available. While lupins are not preferred, they are often included with other pulses in poultry diets. Pasture remains the primary feed source for the bulk of the dairy industry while grain is being increasingly used for supplementary feeding to improve productivity

A model of regional feed markets

A model of regional feed markets was developed as part of a research project to analyse the regional feed markets in Australia funded by the Grain Research and Development Corporation (GRDC). The model simulates simultaneous achievement of two objectives. First, the objective of meeting feed demand at a minimum cost by mixing different feed ingredients while meeting the nutrient requirements of each type of livestock

in a region is addressed. In this manner, the complex substitution and complementary relationships that exist between feed ingredients are exploited. Second, the objective of the allocation of thirteen main feed ingredients available to a region between competing demands (demand within the region and demand by other regions and countries) and, if necessary, importation of the main feed ingredients from other regions and countries in a manner consistent with the behaviour of a competitive market is simulated. The model, therefore, has two components - a feed mixing component and a market component which are linked together. The feed mixing component of the model includes fifty different feed ingredients, however, the market component of the model includes only the thirteen most important feed ingredients. These feeds include six grains (wheat, barley, maize, sorghum, oats and triticale), four pulses (lupins, peas, mung beans and faba beans) , two oilseed meals (soya bean meal and canola meal) and cottonseed.

Table 10. Crop distribution by region (Meyers Strategy Group, 1995).

Region	Crops*
Central Queensland	***sorghum*** and sunflower
South-west Queensland	***sorghum, feed barley,*** malting barley, high-protein wheat, sunflower and cotton
Northern New South Wales	***sorghum, feed barley,*** malting barley, high-protein wheat, sunflower, soya bean and cotton
Central New South Wales	***feed barley, oats and low protein wheat***
Southern New South Wales	***feed barley, oats, triticale, low-protein wheat*** and canola
Western Victoria	***feed barley, oats, triticale,*** soft wheat, canola, safflower and linseed
South-east South Australia	***feed barley,*** malting barley, ***oats, triticale,*** soft wheat, ***peas,*** canola, safflower and linseed
Western Australia	***feed barley, feed wheat,*** feed wheat, ***lupins*** and canola

*Crops produced primarily for feed are in bold italics.

In the model, the Australian feed market was divided into ten regions (Figure 5) - Southern Queensland (QLD1), Northern Queensland (QLD2), Far West New South Wales (NSW1) Northern New South Wales (NSW2), Southern New South Wales (NSW3), Northern Victoria (VIC1), Southern Victoria (VIC2), South Australia (SA), Western Australia (WA) and Tasmania (TAS). The regional demarcations chosen to divide Queensland, New South Wales and Victoria into smaller regions reflect the regional concentrations of grains and livestock production systems as briefly discussed in the previous section.

The availability of feed ingredients in each of the ten regions consists of domestic production plus net shipments from other regions and imports from other countries, while demand consists of feed demand within the region and export demand for feed.

The model is formulated as a revenue maximizing problem. First, separate models were developed for the feed mixing and market components. The models are linked through endogenous prices and usage for the main feed ingredients. The various relationships and linkages in the model are illustrated in Figure 6. A detailed documentation of the model is given in Hafi and Andrews (1997).

In the feed mixing component for each region, the least cost of 17 different types of feed mixes for pigs, poultry and ruminants were estimated (Table 11). Therefore, the feed mixing component of the model solves for 170 least-cost ration formulations. The minimum and maximum requirements of 34 nutrients for each type of livestock are also specified in the model.

In the market component, the model solution for the main feed ingredients is subject to three arbitrage conditions on regional prices and commodity balance conditions. These arbitrage conditions ensure that the difference in the price of an ingredient between two

regions is less than, or equal to, the transport cost between the regions, and the price of an ingredient in a given region is less than, or equal to, the region's import parity equivalent and greater than, or equal to, the region's export parity equivalent for that ingredient.

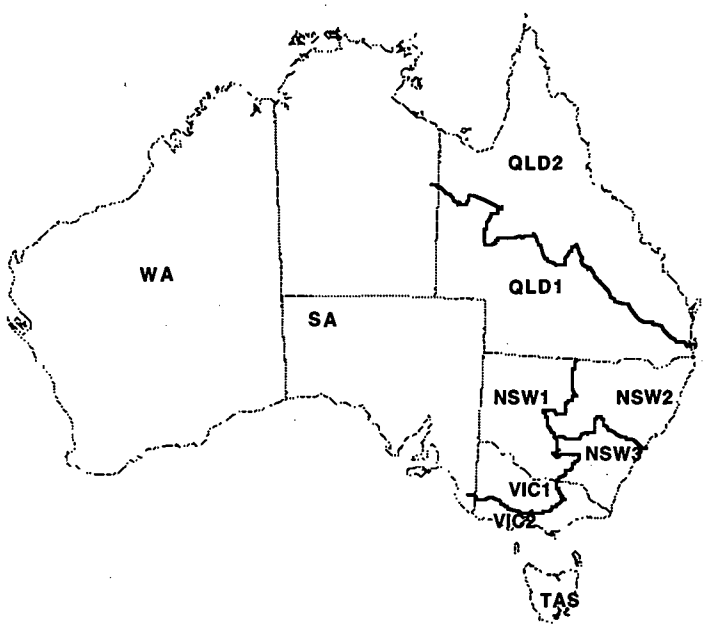

Figure 5. *Regions in Australia used in the modelling framework for regional feed markets.*

Table 11. Feed mixes for pigs, poultry and ruminants on which least-cost was estimated for use in the model.

Pigs	Poultry	Ruminants
Weaner	Broiler starter	Dairy
Grower	Broiler grower	Beef feedlot starter
Finisher	Broiler finisher	Beef feedlot finisher
Lactating sow	Pullet starter	Breeding cattle and sheep
Dry sow	Pullet grower	Live sheep for export
Breeding sow	Layer	

The market component solves the price of each main feed ingredient by observing the three arbitrage conditions on prices described in the previous section. The feed mixing component takes the market component solved price and imputes values to all the nutrients in each feed using shadow prices of nutrients. At the optimal point, the price solved for each main feed ingredient in the full model should be equal to the total value imputed to all the nutrients in one unit of that ingredient. The two components of the model were linked by specifying this relationship in the model. The linking of the two components through quantity used is achieved for each main feed ingredient by equating the regional usage in the market model to the total of optimal quantities of feed solved for the 17 categories of livestock for that region in the feed mixing component.

The model solves endogenously for feed demand for all feed ingredients and, for inter-regional transfers, regional prices, exports and imports of the 13 main feed ingredients. The model uses exogenous values of regional feed ingredient supplies, inter-regional transport costs, indicative world prices of the main feed ingredients, regional prices of other ingredients, freight rates for imports from third countries and port handling charges.

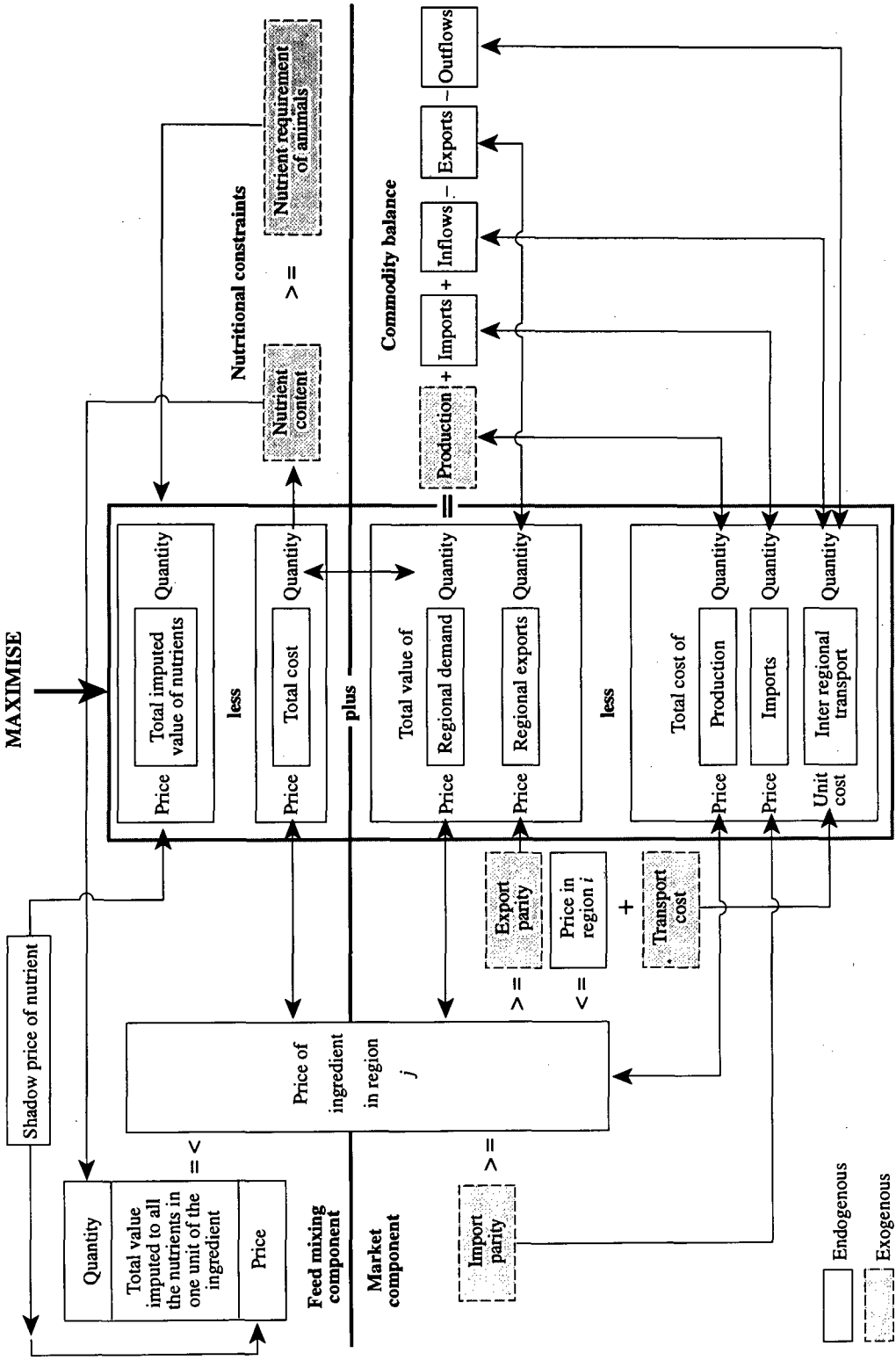

Figure 6. *Schematic diagram showing relationships that exist in the regional feed markets model.*

The model was used to develop a reference simulation based on the 1993-94 crop year and to analyse the potential impacts of the reduced availability of feed as a result of drought and medium term growth in demand for feed ingredients, on regional prices, supply and demand balances, import requirements, exports and inter-regional transfers. The reference simulation provides a base against which the results of other simulations can be assessed.

Reference Simulation

In the reference scenario, regional livestock numbers, prices for other ingredients and feed availabilities were set at their levels in the 1993-94 crop year. Australian production of the main feed ingredients totalled 12.2 million tonnes, of which 7.7 million tonnes were used by the Australian livestock industry with the remainder being exported (Table 12). Half of Australian production of the main feed ingredients is in the eastern states, with New South Wales being the major producer. Southern Queensland (QLD1) and northern New South Wales (NSW2), where most lot feeding of beef cattle is practiced, accounted for one third of total usage of the main feed ingredients in Australia.

Table 12. Estimated feed supply and disposal in the reference scenario (1993-94 crop year).

	Production (kt)[c]	Usage (kt)	Surplus (kt)	Inflow[a] (kt)	Imports (kt)	Outflow[b] (kt)	Exports (kt)
QLD1	1176	1509	-333	332	52	0	51
QLD2	348	204	144	12	3	0	159
NSW1	530	405	125	3	0	128	0
NSW2	1088	1105	-17	189	16	188	0
NSW3	1209	644	565	20	0	580	5
VIC1	1675	939	736	4	6	742	4
VIC2	177	1273	-1096	1090	8	0	3
SA	2263	693	1570	1	53	14	1610
WA	3651	693	2958	1	41	129	2872
TAS	39	205	-166	129	38	0	0
Total	12156	7669	4487	1780	218	1780	4704

[a] Quantities of feed ingredients received from other Australian regions. [b] Quantities of feed ingredients transferred to other Australian regions. [c] kilo (thousand) tonnes.

Table 13. Feed usage in the pig industry by region.

	Pig industry											
	QLD1 (kt)	QLD2 (kt)	NSW1 (kt)	NSW2 (kt)	NSW3 (kt)	VIC1 (kt)	VIC2 (kt)	SA (kt)	WA (kt)	TAS (kt)	Total (kt)	All[ab] (kt)
Grains												
Wheat	0	0	58	136	65	103	0	173	114	0	648	1264
Barley	0	0	24	0	0	0	0	0	0	0	24	2218
Others	360	23	3	26	15	43	15	39	21	12	559	2697
Sub total	360	23	85	162	80	146	15	212	135	12	1231	6179
Pulses	13	0	61	53	60	103	29	99	54	0	471	626
Oilseed meals	33	3	5	13	7	9	9	37	25	9	150	455
Total	406	25	151	229	148	258	53	348	214	21	1852	7260
By-products	0	0	0	0	0	0	35	0	0	16	51	51

[a]Excluding cottonseed which was not used in the pig industry. [b]All livestock.

Of the total quantity of feed used, 23% was obtained from inter-regional transfers (1.8 million tonnes), with imports accounting for almost 3%. Significant feed deficits occurred in southern regions of Victoria and Queensland, while significant feed surpluses were found in Western Australia, South Australia, northern Victoria and southern New South Wales. Northern Victoria (VIC1) and the three regions of New South Wales accounted for almost 92% of the total quantity of feed outflows. Southern Victoria (VIC2) received the bulk of the total quantity of feed outflows.

The Australian pig industry used one quarter of total feed usage with New South Wales accounting for the largest share (28%, Table 13). The pig industry was the single largest user of feed wheat accounting for over half of the total feed wheat used. Feed grains accounted for almost 66% of the total quantity of the main feed ingredients used in the pig industry with pulses accounting for another 25%. Sorghum was the predominant feed ingredient in pig diets in Queensland, while feed wheat and pulses were included in other states (Table 13).

Modelled impact of drought

The drought scenario incorporates the first round (short term) impacts of drought by allowing substitution between feed ingredients to take place in response to increases in feed prices resulting from reduced availability of some feeds, while holding total feed usage constant at the level in the reference simulation. Constant feed usage is achieved by holding livestock numbers, feeding rate per animal and nutritional requirements constant.

In setting up the drought scenario, the observed changes in the availability of the main feed ingredients in the 1994-95 drought year were incorporated into the model. While prices of the main feed ingredients which were used in estimating feed usage were solved endogenously within the model, prices of other feed ingredients were assumed to remain at the reference scenario level. Transport costs were also assumed to remain at the reference scenario levels.

Impact on the balance of supply and demand

Production of the main feed ingredients declined by 35% to 7.9 million tonnes in 1994-95 as a result of drought. The usage of some feeds fell in the drought scenario as increased prices encouraged substitution toward relatively cheaper feeds. In the drought scenario, total feed usage declined by 17% while the major impact was on exports, which declined by 54%. Usage of the main feed ingredients in the pig industry declined by one quarter to 1.4 million tonnes. Reduced availability of the main feed ingredients resulted in widespread deficits in a number of regions.

Table 14. Estimated feed supply and disposal in the short run drought scenario (1994-95 crop year).

Region	Production (kt)	Usage[a] (kt)		Surplus (kt)	Inflow[b] (kt)	Imports (kt)	Outflow[c] (kt)	Exports (kt)
QLD1	852	1547	(355)	-695	633	72	0	10
QLD2	308	197	(24)	111	3	7	12	110
NSW1	182	183	(45)	-1	12	37	49	0
NSW2	721	805	(142)	-84	93	102	110	1
NSW3	276	479	(52)	-203	159	63	19	0
VIC1	511	585	(155)	-74	2	77	6	0
VIC2	86	1025	(40)	-939	824	115	0	0
SA	1286	675	(339)	611	1	72	631	53
WA	3678	692	(214)	2986	0	46	1031	2000
TAS	36	205	(21)	-169	129	41	0	0
Total	7936	6394	(1386)	1542	1857	633	1857	2174

[a]Feed usage by the pig industry is given in parenthesis. [b]Quantities of feed ingredients received from other Australian regions. [c]Quantities of feed ingredients transferred to other Australian regions.

In a normal year, southern regions of Victoria and Queensland, Tasmania and northern New South Wales are likely to face feed deficits. However, in the drought scenario, southern New South Wales and northern Victoria also faced feed deficits. Deficits in southern regions of Queensland, New South Wales and Victoria, were largely met with feed inflows from other regions (Table 14). Total imports of the main feed ingredients almost tripled to 630 kt in the drought scenario. Imports comprised exclusively of soya bean meal and triticale in both the reference and drought scenarios. Total imports of soya bean meal increased to 540 kt from 200 kt in the reference scenario and total imports of triticale increased to 92 kt from 16 kt. The total quantity of feed ingredients traded between regions increased by 4% to 1.9 million tonnes and exports to other countries declined by 54% to 2.1 million tonnes (Table 14). The share of inflows in meeting regional feed requirements increased from 23% in the reference scenario to 29% in the drought scenario, while the share of imports increased from 3-10% (Table 14).

The drought scenario resulted in a marked change in the pattern of inter-regional trade. Approximately 89% of the total volume of outflows from regions originated in Western Australia (55%) and South Australia (34%), compared with just 8% in total in the reference scenario.

The three regions of New South Wales accounted for just 10% of the volume of outflows compared to 50% in the reference scenario. Northern Victoria's share of the total volume of outflows declined to less than 1% in the drought scenario from 42% in the reference scenario.

Impact on price and usage of individual feed

Reduced feed availabilities resulting from drought caused the regional prices of each of the main feed ingredients to increase except in the cases of regions which continued to export or import that feed ingredient. In the latter cases, the prices remained unchanged at the respective export or import parity equivalents. The increases in the weighted average regional prices of oats, maize, barley, triticale, sorghum, cottonseed and canola meal were in the range of 12-24% (Table 15).

Table 15. The effect of drought scenario on weighted average regional prices[a] and total usage of feed ingredients.

	Price ($/t)	Change (%)	Usage (kt)	Change (%)
Feed wheat	162	7	1264	-58
Barley	136	17	2218	7
Oats	109	24	1190	-44
Maize	174	19	134	56
Sorghum	154	12	1104	9
Triticale	180	14	269	-27
Lupins	179	7	345	-60
Peas	223	5	167	-63
Faba beans	201	9	114	-75
Cottonseed	200	17	409	-24
Canola meal	243	12	116	6
Soya bean meal	332	2	339	67

[a]An average of regional prices weighted by the quantities used

Impact on the usage of feed ingredients by the pig industry

As the main feed ingredients can substitute for each other, at regional levels, the usage of any of these ingredients responds to change in its relative price. At the aggregate level, the usage of feed wheat, oats, triticale, lupins, peas, faba beans and cottonseed decreased as expected, however, the usage of other ingredients increased. The increase in the total usage of barley, maize and sorghum was the result of the increases in the usage in

some regions as a response to falling relative prices more than offsetting the decreases in the other regions as a response to increasing relative prices. In South Australia, barley price remained unchanged at the export parity, while prices of other feed grains increased resulting in a large increase in barley usage. The increase in the price of sorghum in southern Queensland and both sorghum and maize in New South Wales was less than the increase in the prices of other grains resulting in an increase in the usage of sorghum and maize.

The prices of feed grains and pulses increased more than the increase in the prices of oilseed meals in the drought scenario. Meanwhile, grain byproduct prices were assumed to remain unchanged at the reference scenario level. As a result, grain byproducts have substituted for feed grains and oilseed meals have substituted for pulses leading to the usage of feed grains and pulses declining, while the usage of oilseed meals and grain byproducts increasing in the drought scenario (Table 16).

Table 16. Impact of price increases on feed usage by the pig industry in the drought scenario.

	Base (kt)	Change (%)
Grains		
Wheat	648	-81
Barley	24	966
Oats	274	-44
Maize	8	-100
Sorghum	269	34
Triticale	8	-100
Sub total	1231	-27
Pulses	471	-68
Oilseed meal	150	128
Total main feeds ingredients	1852	-25
Grain by-products	51	749
Total	1903	-7

While the overall usage of feed grains in the pig industry decreased, the usage of barley and sorghum increased as a result of these ingredients substituting for relatively expensive wheat, oats, maize and triticale. All of the increase in the usage of barley in the pig industry occurred in South Australia, where the price of barley remained unchanged at export parity, while all the increase in sorghum usage occurred in Queensland and New South Wales where the prices of other grains increased more than the increase in the price of sorghum.

The total usage of the main feed ingredients and grain byproducts declined by 7% in the pig industry. The flexibility in formulating pig diets by exploiting the substitution possibilities that exist between feed ingredients (the main feed ingredients and grain by-products) enabled the pig industry to meet the nutritional requirements of animals with a minimum reduction in the overall usage of these ingredients.

Impact of drought scenario on pig feed cost

The average unit cost of pig diets increased by 9% from the $195/t observed in the reference scenario as a result of increases in the prices of the main feed ingredients (Table 17). The feed cost increase was highest in all New South Wales regions reflecting large increases in the prices of the main feed ingredients in that state.

Table 17. Impact of drought scenario on pig feed costs.

	Weighted average feed cost[a] in the reference scenario ($/t)	Change in feed cost in the drought scenario (%)
QLD1	203	5
QLD2	202	1
NSW1	189	20
NSW2	195	16
NSW3	186	22
VIC1	196	12
VIC2	211	5
SA	190	3
WA	182	0
TAS	214	0
Total	195	9

[a]Weighted average of six diets for pigs (see Table 11)

It should be noted that the drought scenario does not take into account the effect of drought on pig numbers in the short run. In a drought year, pig numbers may be reduced as a result of forced selling due to unavailability of feed and/or the high cost of feed. As the length or severity of a drought increases, feed shortages and higher feed prices may force farmers to reduce pig numbers through sale for slaughter, resulting in further reductions in total usage of feed. High cost of feed during the recent years as a result of drought has accelerated the structural adjustment process resulting in a large number of small scale producers leaving the pig industry (Abdalla *et al.* 1997). However, the medium term impacts of drought cannot be readily handled in the model because dynamic relationships, such as the relationship between feed costs and livestock numbers, are not currently incorporated in the model.

Medium term growth in feed demand

In the medium term scenario, feed usage and inter-regional transfers of the main feed ingredients in the year 2000-01 were simulated using forecasts of regional livestock numbers and the availability of the main feed ingredients. The 1993-94 dollar values of the forecast world indicator prices of the main feed ingredients in year 2000-01 were used to capture the changes in the price relativities of the main feed ingredients in the medium term. The 1993-94 dollar prices of animal proteins in year 2000-01 are assumed to be 7% lower than the levels in 1993-94 based on an estimated 7% fall in the real prices of oilseed meals during the same period. The real prices of other feed ingredients are assumed to remain at 1993-94 levels as limited data are available to determine future prices. As the other feed ingredients collectively account for only a small share of the feed rations, this assumption is unlikely to be critical. The transport costs in year 2000-01 in real terms were assumed to remain at 1993-94 levels.

Total Australian production of the main feed ingredients is likely to fall by 1 million tonnes from the level in 1993-94 to 11 million tonnes by the year 2000-01. The expected fall in production of the main feed ingredient is due mainly to an expected increase in the share of malting barley over the medium term and a consequential reduction in the share of feed barley (Gordon *et al.* 1997). The decline in feed production by the year 2000-01 was introduced into the medium term scenario as an exogenous assumption.

The share of eastern states feed production is estimated to increase to 60% from 51% in the reference scenario, mainly because feed production in Queensland is assumed to increase by 38% from the actual level in 1993-94. There are no significant changes in the pattern of use compared to the reference scenario. The total usage of the main feed ingredients is estimated to increase by 11% to 8.5 million tonnes, while usage by the pig industry is projected to increase by 10% to 2 million tonnes (Table 18).

The share of inter-regional trade in total usage of the main feed ingredients is projected to decline to 19% in the medium term from the reference scenario level of 25%.

However, the share of total quantity of feed outflows from Western Australia to eastern regions is estimated to increase from 7-20%.

Total exports of the main feed ingredients are projected to decline by around 1.5 million tonnes from the reference scenario level to 3.2 million tonnes in the medium term, while the share of imports in total usage of the main feed ingredients is projected to increase to 7% from 3% in the reference scenario.

Conclusions

The analysis of drought and medium term developments in regional feed markets using the developed regional feed markets model highlights the importance of the substitution between different types of feeds, particularly at times when there are shortages of some feed grains. In particular, the role that transfers of feeds from Western Australia and South Australia to eastern Australia plays at times of reduced feed availability in the eastern states was highlighted. The inter-regional transfers of feed reduced the need for imports of feed grains, even in times of a severe reduction in the availability of feed grains, such as in the drought scenario. The estimated effects of drought and the medium term developments on feed movements highlight the importance of such feed movements in mitigating the impacts of drought on the livestock industries. A strategic plan by the livestock and feed industries could be formulated to facilitate such feed movements.

Table 18. Estimated feed supply and disposal in the medium term scenario (year 2000-01).

Region	Production (kt)	Usage[a] (kt)		Surplus (kt)	Inflow[b] (kt)	Imports (kt)	Outflow[c] (kt)	Exports (kt)
QLD1	1549	1759	(437)	-210	186	50	4	23
QLD2	553	214	(20)	339	3	1	87	257
NSW1	528	470	(161)	58	7	5	70	0
NSW2	1440	1183	(245)	257	93	2	333	21
NSW3	1217	757	(165)	460	355	9	635	189
VIC1	1203	1083	(280)	120	21	31	171	0
VIC2	169	1280	(89)	-1111	839	271	0	0
SA	1518	761	(380)	757	58	51	0	866
WA	2947	798	(227)	2149	0	20	331	1837
TAS	29	250	(42)	-221	68	154	0	0
Total	11153	8555	(2047)	2598	1631	595	1631	3193

[a]Usage by the pig industry is given within parenthesis. [b]Quantities of feed ingredients received from other Australian regions. [c]Quantities of feed ingredients transferred to other Australian regions.

The model developed in the study can also be used to estimate the impacts of other changes in market conditions and industry policy on regional feed and livestock industries. The changes might include the introduction of intensive livestock industries into new regions, the expansion of the existing regional intensive livestock industries, diversification away from feed grain crops to higher value crops, introduction of feed ingredients with higher nutrient content, reform in the transport and storage sectors and reform of quarantine protocols. Analyses of the impact of the developments would assist in strategic planning by participants in the feed and livestock industries, including industry organisations, statutory marketing authorities, transport and handling authorities, feed millers as well as individual feed and livestock producers.

SYMPOSIUM CONCLUSIONS

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The supply of feed grains for the intensive livestock industries has, on two occasions in recent years, failed to meet demand. This deficit resulted in substantial increases in the price of grain and in a loss of profitability and international competitiveness of the Australian pig industry. The projected steady increase in the demand for feed grain by the animal industries means that such a deficit is likely to occur with increasing frequency unless either grain producers focus on the production of grains specifically for the livestock industries, thereby increasing supply or alternative sources of the major nutrients required by animals are obtained. Edwards (1997) identified a wide range of potential by-products that could be used to reduce the reliance of the pig industry on grains, but considerable research into the logistics of recovery and technology for processing is required for many of these by-products before they could become profitable alternatives to grain. A modest reduction in the demand for grain can be obtained by reducing feed waste and by increasing the efficiency of grain use through the formulation of animal diets that more closely match nutrient requirements with supply than occurs currently. Nevertheless, the most effective way of guarding against a future deficit in grain supply is to increase production.

Grain growers will be attracted to produce grains specifically for the animal industries only if there is not a financial penalty relative to the production of grain for human consumption. Currently most grains fed to livestock have not met the standards for manufacture of products for human consumption and the prices received are lower than for those going to the human market. However, if the factors determining the nutritional value of grains for different forms of animal production could be identified and measured rapidly either at the site of grain delivery or within the place of stockfeed manufacture, a rational basis for the marketing of feed grains could be developed and prices paid that are appropriate to their value for animal production. van Barneveld (1997) presented information confirming the wide range in nutritional value of grains for pigs. These differences in nutritional value are economically important for the industry. The digestible energy (DE) content of cereal grains appears to be related closely to the proportion of several chemical compounds including neutral detergent fibre, xylose and amylose and the bulk density of the grain. The chemical compounds that affect the DE content of the grain and the structure of the starch granules are likely to affect relative digestion within the small and large intestine. Clearly, further research is needed to determine quantitatively the extent to which various compounds in grains alter their nutritional value for the pig, to develop methods for their rapid analysis and to derive algorithms that allow prediction of nutritional value for a specific batch of a grain.

In recognition of the need for further research to promote the growing and use of feed grains, several Rural Research and Development Corporations have funded jointly a project entitled "Improving Feed Grains Quality". The principal objectives of the project are similar to those outlined in this Symposium, namely: (i) identify the reasons and magnitude of differences between grains in their nutritional value for ruminants, pigs and poultry so that improvements in feed grain quality can be achieved through plant breeding and the processing and storage of gain, (ii) develop rapid tests to measure the nutritional value of grain so they can be priced in accordance with their suitability as an animal feed, (iii) develop and upgrade computer simulation models that predict accurately the consequences of grain characteristics and of grain processing and storage on the productivity of animals and profitability of animal enterprises.

Although more research will ultimately lead to a better understanding of the determinants of nutritional value that should lead to a more rational marketing system, it is important also to be able to predict accurately the regional supply of and demand for grain within Australia. The model described by Hafi (1997) demonstrates the importance of being able to transfer grains from one region to another and the consequences of drought, exports or other interruptions to supply on the price and substitution of grains

and on the introduction or loss of intensive livestock enterprises within specific regions of Australia. A combination of research, economic modelling and industry planning is required to assure a reliable supply of nutrients to meet the increasing demands of the future Australian livestock industries.

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β-GLUCAN AS A PREDICTOR OF PROTEIN DIGESTIBILITY AND DIGESTIBLE PROTEIN CONTENT IN BARLEY

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The β-glucans are soluble non-starch polysaccharides found in barley, and are linear polymers of glucose characterised by β-(1-3) and β-(1-4) glycosidic links. The β-glucans are known to inhibit protein digestion in pigs (Li *et al*, 1996). The present work investigates the potential of using total β-glucans and β-glucans extracted after an *in vitro* digestion as predictors of protein digestibility in barley.

In vivo apparent protein digestibility (DP, %) and apparent digestible protein content (ADP, g/kg DM) were determined in 150 g body weight Sprague-Dawley male rats given different New Zealand barleys (n=10) as the sole source of dietary protein. Chromic oxide was included in each diet as an indigestible marker, also included was a vitamin and mineral premix. Ileal digesta samples were collected at slaughter (6 rats per diet, Moughan *et al.*, 1984).

The total β-glucan content (% DM) of barley samples was measured using the method of Jørgensen and Aastrup (1988). *In vitro* extracted β-glucan (IV-β-glucan, %DM) levels were determined by suspending 4.0 g of barley in 20.0 ml HCL buffer (pH 1.5) and incubating the suspension for 2 h at 37°C. The material was neutralized by adding 0.8 ml 10% NaOH solution to the suspension which was incubated for a further 3.5 h at 37°C. The supernatant was then analysed using Flow Injection Analysis.

No relationship was found between the total β-glucans and DP (%) (r= -0.10, P>0.05). However, a strong relationship existed between DP (%) and IV-β-glucan (%DM):

$$DP = 82.15 - 3.09 \text{ IV-}\beta\text{-glucan} \qquad R^2=0.67, \text{ rsd}=1.53$$

Therefore, the ADP in barley can be accurately predicted on the basis of crude protein content (CP, g/kg DM) and IV-β-glucan content, as follows:.

$$ADP = 7.30 + 7.59 \text{ CP} - 3.68 \text{ IV-}\beta\text{ -glucan} \qquad R^2=0.97, \text{ rsd}=1.9$$

As the laboratory rat has been demonstrated to be a good model for barley protein digestion in the pig (Moughan *et al.*, 1987), this work suggests that *in vitro* extracted β-glucans have the potential to be used as a quick test to predict apparent protein digestibility in barley for pigs. Further work is required to confirm this finding in pigs.

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DIGESTIBILITY OF AMINO ACIDS AND ENERGY IN UNTREATED AND AUTOCLAVED VETCH (*VICIA SATIVA* CV. BLANCHEFLEUR) FED TO GROWING PIGS

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A perceived limitation to the use of *Vicia sativa* cv Blanchefleur (vetch) in livestock diets has been the presence of L-β-cyanoalanine and its derivatives which are known to be neurotoxic (Tate and Enneking, 1992). To investigate this limitation, information on the nutritional value of vetch is required. In addition, if L-β-cyanoalanine does not influence pig performance in subsequent growth studies, the values determined here can be used in routine diet formulations. In the present experiment the apparent ileal digestibility (AID) of amino acids and the digestible energy (DE) content of untreated and autoclaved vetch fed to growing pigs were determined.

Untreated and autoclaved vetch comprised 390 g/kg of the sugar/starch based diets and were the sole sources of the 110 g/kg of protein in each diet. Celite® was added as an acid-insoluble ash marker. The AID of amino acids and DE was determined using Large White male pigs (35-40 kg body weight) fitted with simple T-piece ileal cannulas as described by van Barneveld *et al.* (1994). Diet allocations were based on a 4 x 4 latin square design. The additional two treatments comprised soya bean meal, which acted as a control, and *Lupinus luteus* which was being assessed separately. Diets were offered at 3 x maintenance for 7 d prior to 8 h digesta collections over two consecutive days. Faeces sub-samples were collected over these 2 d for determination of DE.

Table 1. Ileal digestibility coefficients for some essential amino acids and DE (MJ/kg; air-dry basis) in untreated and autoclaved *Vicia sativa* cv Blanchefleur fed to growing pigs.

	Treatment			Significance	
Amino acid	Soya bean meal	Untreated vetch	Autoclaved vetch	SEM	Diet
Threonine	0.79	0.70	0.65	0.050	NS
Valine	0.84	0.78	0.78	0.032	NS
Isoleucine	0.86	0.82	0.82	0.025	NS
Leucine	0.86	0.83	0.84	0.027	NS
Phenylalanine	0.86	0.82	0.82	0.027	NS
Lysine	0.87^{a}	0.88^{a}	0.72^{b}	0.031	*
Histidine	0.88^{a}	0.87^{a}	0.80^{b}	0.015	*
DE	15.09	14.88	14.96	0.114	NS

^{ab}Values in the same row with different superscripts differ significantly ($P<0.05$)

The AID of lysine and histidine in autoclaved vetch was significantly lower ($P<0.05$) than in either soya bean meal or untreated vetch (Table 1). The AID of the other amino acids and the DE content of the untreated and autoclaved vetch was not significantly different to soya bean meal.

The pigs consumed all of their daily rations indicating that *Vicia sativa* cv Blanchefleur was palatable; also no neurotoxic symptoms were observed in animals fed vetch. Inactivation of the L-β-cyanoalanine through autoclaving did not improve the digestibility of any amino acid or DE. It is likely that heating of vetch during the autoclaving process reduced the AID of lysine and histidine (van Barneveld *et al.*, 1994). The high digestible amino acid content of the untreated *Vicia sativa* cv Blanchefleur suggests it has potential as an ingredient for use in pig diets.

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GROWTH RESPONSE OF PIGS FED GRADED LEVELS OF *VICIA SATIVA* CV. BLANCHEFLEUR

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The neurotoxins L-β-cyanoalanine (0.1%) and g-L-glutamyl-L-β-cyanoalanine (0.6%) are present in *Vicia sativa* cv. Blanchefleur (vetch). There is very little published information available about the effects neurotoxins and other anti-nutritional factors in vetch have on pig production. In poultry, inclusion of *Vicia sativa* cv. Blanchefleur in diets at 100 g/kg resulted in an immediate drop in feed intake and rate of lay of hens, despite selective avoidance during feeding (Glatz *et al.*, 1992). The aim of the present experiment was to assess the growth response of pigs from 25-100 kg body weight (BW) when fed diets containing graded levels of vetch.

Vicia sativa cv. Blanchefleur was included in grower diets (25-55 kg BW) at levels of 100 (diet 2), 200 (diet 3) and 300 (diet 4) g/kg, respectively and finisher diets (55-100 kg BW) at levels of 70 (diet 6), 140 (diet 7) and 210 (diet 8) g/kg, respectively. Diets were formulated using a wheat and barley base to contain equal levels of apparent ileal digestible (ID) lysine/MJ digestible energy (DE) with 0.70 and 0.55 g digestible lysine/MJ DE in the grower and finisher diets, respectively. All other amino acids were added so that they were at least 30% in excess relative to lysine. The DE content was also equalized in the grower (14.02 MJ/kg) and finisher (13.6 MJ/kg) diets. Soya bean meal was used as a control (diets 1 and 5). Diet allocations to 20 male and 20 female Large White pigs housed in individual pens (1 x 1.5 m) were based on a randomised block design. Diets and water were offered *ad libitum*.

Table 1. Growth response of pigs (25-100 kg BW) fed diets containing equal levels of ileal digestible lysine/MJ and digestible energy, and graded levels of *Vicia sativa* cv. Blanchefleur.

	Average daily gain (g)			Feed intake (g/d)		
Weight range	25-55 kg	55-100 kg	25-100 kg	25-55 kg	55-100 kg	25-100 kg
Diet	1-4	5-8	1-8	1-4	5-8	1-8
1 (5)	934^{a}	957	941^{a}	1847^{a}	3000^{a}	2396^{a}
2 (6)	878^{b}	892	883^{a}	1842^{a}	2756^{a}	2398^{a}
3 (7)	855^{b}	934	898^{a}	1640^{b}	2742^{ab}	2263^{ab}
4 (8)	768^{c}	824	800^{b}	1685^{ab}	2441^{b}	2129^{b}
Statistics						
Diet	***	NS	**	*	**	**
Linear	***	NS	**	*	**	**
SEM[1]	20.7	37.9	26.8	64.3	107.6	60.7

^{a,b,c}Values in the same column with different superscripts differ significantly (P<0.05). *P<0.05; **P<0.01; ***P<0.001; NS, not significant. [1]SEM, Standard error of mean.

Graded dietary inclusion of vetch resulted in a linear decrease (P<0.001) in average daily gain (ADG) over the 25-55 kg growth phase but had no effect over the 55-100 kg growth phase. There was a significant linear decrease (P<0.01) in feed intake over both growth phases.

No neurotoxic effects were evident and no L-β-cyanoalanine or its derivatives were detected in muscle tissue suggesting factors in vetch other than these compounds were responsible for the observed decrease in feed intake and growth over the 25-55 kg growth phase. *Vicia sativa* cv. Blanchefleur is unsuitable for use during the 25-55 kg growth phase, however some potential exists for its inclusion in pig diets up to levels of 140 g/kg when fed from 55-100 kg BW.

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INTERRELATIONSHIPS BETWEEN ENERGY INTAKE AND LIVE WEIGHT ON THE GROWTH AND TISSUE ACCRETION OF MALE PIGS BETWEEN 25-50 AND 50-70 kg LIVE WEIGHT

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Previous experimental work at Bunge Meat Industries has identified that grower pigs (63-112 days of age) initially require higher lysine:DE levels than suggested by AUSPIG v2.99 simulations, and furthermore that the lysine:DE levels required to support maximal growth performance change quite rapidly over this period. To investigate the requirements for dietary amino acids at the tissue level over this period, an experiment was conducted to determine the relationship between energy intake and tissue accretion between 25-50 and 50-70 kg live weight (LW). Sixty-five male (Large White x Landrace) pigs were allocated among an initial slaughter group of five for determination of initial carcass composition, two weight ranges (25-50 kg or 50-70 kg LW) and five intake levels, 60% *ad libitum* to *ad libitum* (n=6 per treatment group). Two protein adequate diets were prepared and offered to animals in individual pens. After slaughter half carcasses were ground, sub-sampled and proximate analysis was conducted.

The results (Table 1) demonstrate that between 25-50 kg LW protein deposition increased with increasing energy intake. Between 50-70 kg LW however, the response plateaued at an energy intake of approximately 28 MJ DE/d, indicating the presence of a maximum limit to carcass protein deposition of 143 g/d.

Table 1. The effect of energy intake on growth and carcass measurements and tissue accretion of entire male pigs between 25-50 kg and 50-70 kg LW.

	Growth performance			Tissue accretion (g/d)		
DE intake (MJ/d)	Daily gain (kg)	Feed:gain	Feed intake (kg/d)	Protein	Fat	Fat:protein
25-50kg LW						
14.45	0.608^{a}	1.70	1.033^{a}	85.2^{a}	23.9^{a}	0.286^{a}
16.30	0.712^{b}	1.64	1.164^{b}	98.6^{a}	21.0^{a}	0.216^{a}
18.73	0.855^{c}	1.57	1.339^{c}	124.7^{b}	61.6^{b}	0.499^{b}
21.49[1]	0.939^{d}	1.64	1.535^{d}	133.1^{b}	66.6^{b}	0.502^{b}
50-70 kg LW						
20.09	0.670^{a}	2.18	1.435^{a}	95.1^{a}	46.4^{a}	0.484^{a}
23.16	0.861^{b}	1.93	1.654^{b}	125.6^{ab}	47.5^{a}	0.383^{a}
27.12	0.930^{b}	2.12	1.937^{c}	122.4^{ab}	117.3^{b}	0.996^{b}
28.14	0.948^{b}	2.13	2.009^{d}	143.3^{b}	157.6^{b}	1.094^{b}
31.47	0.966^{b}	2.35	2.248^{e}	142.6^{b}	101.9^{ab}	0.719^{ab}
SED intake	0.032	0.077	0.034	7.172	10.361	0.081
SED weight	0.021	0.015	0.022	4.536	6.553	0.051
Significance[2]						
Intake	***	*	***	***	***	***
Weight	**	***	***	*	***	***

[1]Pooled data for 90% *ad libitum* and *ad libitum* groups; *ad libitum* intakes were 10% lower than expected between 25-50 kg LW. [2]*P<0.05, **P<0.01, ***P<0.001. [a,b,c,d]Within columns and within weight ranges means with different superscripts are significantly different (Tukey's HSD test; P<0.05).

The results indicate that between 50-70 kg LW maximal rates of protein deposition are being attained and thus the dietary amino acids required to support this deposition will remain constant and independent of DE intake. The data supports the concept of phase feeding as a method of increasing the accuracy of dietary amino acid supply to grower pigs.

ENZYME (BIOFEED PLUS) SUPPLEMENTATION MAY BE MORE BENEFICIAL IN BOARS AND OLDER WEANING AGE PIGS

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Pigs are commonly weaned at 23-27 days of age. By weaning earlier and providing pigs with high quality diets, it may be possible to increase growth performance up to slaughter. However, digestive capacity of early weaned pigs may be insufficient to fully digest many ingredients currently used in weaner diets. The aim of this experiment was to determine whether an exogenous enzyme preparation with broad carbohydrase activity could benefit pigs that were possibly developmentally immature at weaning.

Eighty Large White x Landrace pigs were used in a factorial design with the factors being; weaning age (14 or 24 d), weaning weight (heavy (H) or light (L)), sex (boar or gilt) and dietary Biofeed Plus (0 or 500 ppm). Biofeed Plus contains fungal xyalanases, pentosanases and ß-glucanases. Pigs were housed individually and provided a wheat-based (55%) diet containing 15.5 MJ DE and 15.9 g lysine/kg *ad libitum* for 21 d. The diet also contained 5% soya bean meal and lupin (*Lupinus angustifolias*) kernels. The mean live weight of H and L pigs, weaned at 24 or 14 d, were 7.9 and 5.3, and 5.2 and 3.9 kg, respectively.

Table 1. Effect of age (A) and weight (W) at weaning, sex (S) and dietary Biofeed Plus on rate of gain (ROG), feed intake (FI) and feed:gain (FCR) over a period of 21 days post weaning.

		Boar				Gilt					
	Biofeed	24 d		14 d		24 d		14 d			
	Plus[1]	H	L	H	L	H	L	H	L	sed	Significance[2]
ROG,	-	384	250	169	151	335	314	171	210	30.8	A***
g/d	+	419	365	149	172	360	334	102	130		
FI,	-	415	262	216	177	388	339	211	219	27.7	A***,W*
g/d	+	436	362	186	188	427	350	163	156		
FCR	-	1.08	1.05	1.31	1.42	1.18	1.16	1.27	1.05	0.17	A***,W*
	+	1.04	1.01	1.56	1.14	1.19	1.05	1.99	1.29		

[1]0 (-) or 500 (+) ppm BioFeed Plus. [2]*P<0.05; ***P<0.001.

Pigs weaned at 14 d grew more slowly (157 vs 345 g/d) than those weaned at 24 d although there was a suggestion of an interaction between age and weight at weaning (P=0.081). Thus, H and L pigs weaned at 14 d grew at 148 and 166 g/d whereas H and L pigs weaned at 24 d grew at 374 and 315 g/d, respectively. While there was no main effect of Biofeed Plus on ROG (248 vs 254 g/d, P=0.801), FI (278 vs 284 g/d, P=0.793) or FCR (1.19 vs 1.25, P=0.349) there were interactions with weaning age on ROG (P=0.050) and FI (P=0.060). Pigs weaned at 14 d grew more slowly (176 vs 138 g/d) and ate less (206 vs 174 g/d) whereas pigs weaned at 24 d grew more quickly (321 vs 369 g/d) and ate more (351 vs 394 g/d) when supplemented with enzymes. During the third week post-weaning there were interactions between dietary enzymes and sex (p=0.060) and dietary enzymes and age (P=0.023) on ROG. Thus, pigs weaned at 24 d and supplemented with Biofeed Plus grew more quickly during the third week (559 vs 460 g/d) whereas the converse was true for pigs weaned at 14 d (286 vs 334 g/d). Also, enzyme-supplemented boars grew better over this period (457 vs 371 g/d) whereas the converse was true for gilts (388 vs 423 g/d). In conclusion, dietary enzyme supplementation is most efficacious in boars weaned at an older age but benefits do not become apparent until 2 weeks post-weaning.

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GROWTH OF WEANED PIGS IS INCREASED BY FRUCTO-OLIGOSACCHARIDES AND ISOMALTO-OLIGOSACCHARIDES

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Fructo-oligosaccharides (FOS) are synthesized by the enzyme action of β-fructofuranosidase of *Aspergillus niger* on sucrose (Mitsuoka *et al.*, 1987). Isomalto-oligosaccharides (IMOS) are a mixture of isomaltose, pentose and isomaltotriose. Dietary FOS can increase bifidobacteria populations in the large intestine (Bunce *et al.*, 1995a), increase the population of faecal streptococci (Fukuyasu and Oshida, 1986) and improve nitrogen balance of weaned pigs (Bunce *et al.*, 1995b). It was hypothesized that supplementing diets with FOS and IMOS may stimulate growth of weaned pigs.

One hundred and sixty weaner pigs (Landrace x Yorkshire x Duroc or Hampshire) 39 days old, weighing 9.61 ± 0.46 kg were used in a 29 d trial. A completely randomized block design was used, the pigs were divided into 16 pens of 10 pigs each and each pen was assigned to one of the four treatments with four replications (two pens of castrated males and 2 pens of females each). Both FOS (available FOS=55.3%) and IMOS (available IMOS=44.2%) were substituted for glucose on a weight basis according to treatments in a basal diet of corn and soya bean meal. The treatments consisted of control (glucose 5%), FOS 5%, IMOS 3% and IMOS5 % for the feeding trial.

Table 1. Weight, weight gain, feed intake and feed conversion ratio (FCR) for weaner pigs fed diets with FOS and IMOS.

	Weight (kg)		Average daily	Average feed	FCR
	Initial	Final	gain (g)	intake (g/d)	
Control	9.5	20.0	465	888	1.91[b]
FOS (5%)	9.8	24.8	517	985	1.90[b]
IMOS (3%)	9.5	25.1	535	996	1.86[ab]
IMOS (5%)	9.6	24.0	498	912	1.83[a]

[a,b]Mean values within a column with different superscripts differ significantly (P<0.05).

Feed intake and body-weight gains of young pigs fed IMOS at the level of 3% and FOS at 5% tended to improve over that of the controls (Table 1). Feed conversion ratio of pigs fed IMOS at the level of 5% was significantly improved (P<0.05) but was associated with reduced growth and feed intake when compared to 3% IMOS.

Although FOS or IMOS stimulate the piglet growth the mechanisms are not yet understood, but they could include improvement of gastrointestinal health by maintaining optimum microbial balance. It is suggested that FOS and IMOS might be used as feed additives for growth promotion of weaner pigs. However, further research is needed to elucidate the mechanisms of how FOS and IMOS affect intestinal microbial metabolism and growth.

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LUPIN OLIGOSACCHARIDES DEPRESS THE APPARENT ILEAL DIGESTION OF AMINO ACIDS BY GROWING PIGS

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Extraction of oligosaccharides from lupins has been shown to improve the digestible energy (DE) content for growing pigs (van Barneveld *et al.*, 1996). An ethanol extraction process removed 73% and 67% of the oligosaccharides from *Lupinus angustifolius* and *L. albus*, respectively, but did not change the gross energy content. Ethanol extraction improved the DE of diets containing *L. angustifolius* and *L. albus* by 0.5 and 0.7 MJ/kg, respectively. The aim of the present experiment was to assess the influence of lupin oligosaccharides on the apparent ileal digestion of amino acids by growing pigs.

Dehulled *L. angustifolius* and *L. albus* were hammer-milled and subjected to an ethanol extraction to remove oligosaccharides. Four sorghum-based diets were formulated to contain 350 g/kg of either extracted or unextracted lupin meal, respectively. Celite® was added as an acid-insoluble ash marker. The apparent ileal digestibility of amino acids was determined using Large White male pigs (35-40 kg body weight) fitted with simple T-piece ileal cannulae. Diet allocations were based on a 4 x 4 Latin square design. Diets were fed for 7 d prior to continuous 8 h digesta collections over two consecutive days.

Table 1. Apparent ileal digestibility coefficients for some essential amino acids in untreated and ethanol extracted dehulled *L. angustifolius* and *L. albus* fed to growing pigs.

	Dehulled *L. angustifolius*		Dehulled *L. albus*		Statistics	
Treatment	Nil	Extracted	Nil	Extracted	Diet	SEM[1]
Threonine	0.71[a]	0.81[bc]	0.78[b]	0.86[c]	**	0.018
Valine	0.77[a]	0.84[bc]	0.81[ab]	0.88[c]	*	0.017
Isoleucine	0.81[a]	0.88[bc]	0.85[ab]	0.91[c]	*	0.014
Leucine	0.78[a]	0.88[b]	0.84[b]	0.90[b]	*	0.016
Phenylalanine	0.80[a]	0.88[bc]	0.84[ab]	0.90[c]	*	0.014
Lysine	0.80[a]	0.86[b]	0.84[ab]	0.89[b]	*	0.015
Histidine	0.80[a]	0.85[bc]	0.81[ab]	0.87[c]	*	0.012

[a,b,c]Values within the same row with different superscripts differ significantly ($P<0.05$). [1]SEM, Standard error of mean. *$P<0.05$; **$P<0.01$.

Ethanol extraction significantly improved ($P<0.05$) the digestion of all reported amino acids in both *L. angustifolius* and *L. albus*. The average amino acid digestibility was improved by 9.6% for *L. angustifolius* and by 7.6% for *L. albus*.

From the present study lupin oligosaccharides appear to hinder the digestion of amino acids in the small intestine of pigs. This is in contrast to the findings of Gabert *et al.* (1995) and Zuo *et al.* (1996) and suggests that the osmotic effects of these oligosaccharides on the digestive and absorptive capacity of the gut in pigs may change nutrient digestion in the small intestine. The results also suggest that the increase in lupin DE consistent with oligosaccharide extraction observed by van Barneveld *et al.* (1996) was due to more than a digestible energy dilution when oligosaccharides were present.

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DIGESTIBILITY OF LYSINE IN LUPINS AND CEREALS FED TO GROWING PIGS ALONE OR IN COMBINATIONS

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When ingredients are combined in a compound pig feed, it is assumed that the respective nutrient digestibilities will be proportionately the same as when the ingredients are fed separately. This assumption allows the use of linear relationships to formulate least-cost diets. In diets containing high concentrations of non-starch polysaccharides, however, the digestibility of nutrients may differ significantly from the digestibility determined in the individual feed ingredients (Hansen *et al.*, 1991). The aim of the present experiment was to compare the measured and calculated apparent ileal digestibility of lysine in mixed diets containing specific combinations of lupins (*Lupinus angustifolius* cv Gungurru) and either wheat, barley, triticale or sorghum fed to growing pigs.

Three 4 x 4 Latin square experiments were completed to determine lysine digestibility coefficients of wheat (0.85), barley (0.74), triticale (0.83) and sorghum (0.77), lupins (0.87) and the mixed diets (Table 1), respectively, using Large White male pigs (35-40 kg live weight) fitted with simple T-piece ileal cannulae (van Barneveld *et al.*, 1994). When determining lysine digestibility in the individual ingredients, experimental diets comprised 940 g/kg (air-dry basis, AD) of each cereal or 313 g/kg AD of the lupins in a sugar/starch base. Mixed diets comprised 350 g/kg AD of lupins and 500 g/kg AD of wheat, barley, triticale or sorghum, respectively. Lysine digestibility in the mixed diets was calculated using $(L_L \times D_L + L_C \times D_C)/(L_L+L_C)$ where L_L and L_C are g lysine/kg diet contributed by the lupins and cereals, respectively and D_L and D_C are their respective digestibility coefficients.

Table 1. A comparison of measured and calculated apparent ileal digestible lysine coefficients in mixed diets containing lupins (*Lupinus angustifolius* cv. Gungurru) and wheat, barley, sorghum or triticale fed to growing pigs.

Mixed diet	Measured IDL[1]	SEM[2]	Calculated IDL	Difference	Significance[3]
Lupins + wheat	0.8638	0.010	0.8657	0.0019	NS
Lupins + barley	0.7929	0.010	0.8282	0.0353	*
Lupins + triticale	0.9098	0.013	0.8787	-0.0311	NS
Lupins + sorghum	0.8354	0.010	0.8485	0.0131	NS

[1]IDL, Ileal digestible lysine. [2]SEM, Standard error of means. [3]NS, not significant; *P<0.05.

The measured apparent ileal digestibility of lysine in the mixed diet containing lupins and barley was significantly different from the calculated value (P<0.05). No significant difference was observed between the two values for mixed diets containing lupins and wheat, triticale or sorghum, respectively (Table 1). Regression analysis between measured (X) and calculated (Y) lysine digestibility (based on mean values; Y=0.5838+0.3198X, R^2=0.64, P<0.001, SEM=0.066) shows a significant correlation between the two values, despite the result for the lupin and barley diet.

As the barley contributed significantly more soluble non-statch polysaccharides than the other cereals to the mixed diets, the results support those of Hansen *et al.* (1991). Regression analysis reveals that the effects of ingredient interactions over a wide range of mixed diets have a minimal effect on lysine digestibility. The significant reduction in lysine digestibility in the lupin and barley diet, however, is likely to translate into reduced pig performance.

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EVALUATION OF A NEW AVAILABLE LYSINE ASSAY

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A new method for determining true ileal digestible reactive lysine (available lysine) in processed feedstuffs has been developed† (Moughan and Rutherfurd, 1996). It utilises the guanidination reaction to determine the reactive lysine content in a feedstuff and in the digesta of animals fed that feedstuff. The aim was to evaluate with growing pigs the accuracy of the conventional ileal amino acid digestibility assay (based on total lysine measurement) and the new assay.

A test diet containing a heated skim milk powder (HSMP) for which the true total lysine digestibility and true reactive lysine digestibility had been previously determined, and in which lysine was shown to be the first limiting amino acid, was formulated. Two enzymatically hydrolysed casein/free amino acid based control diets were also formulated to contain lysine contents equal to that of the HSMP diet based on either total lysine digestibility (EHC-A) or reactive lysine digestibility (EHC-B). It was assumed that the lysine in the control diets was fully absorbed and chemically available. All three diets contained the same ratios of other amino acids to lysine, and were formulated to contain the same content of fibre, vitamin and mineral. Net energy content was similar in all three diets. The DE to lysine ratio was calculated to be 1.8 MJ DE/g lysine for the HSMP and EHC-A diets and 2.4 MJ DE/g lysine for the EHC-B diet. The test and two control diets were fed at a level of 10% of metabolic body weight for 18 d to entire male Large White x Landrace pigs (30 kg live weight). Whole body lysine deposition was determined by body compositional analysis and compared using analysis of covariance with initial live weight as the co-variate (Table 1).

Table 1. Least squares means (±SE) for lysine deposition (g/d) for pigs fed a heated skim milk powder (HSMP) based diet and two enzymatically hydrolysed casein (EHC) diets.

	HSMP	EHC-A	EHC-B	Overall significance	
				LW0[1]	Treatment
Lysine deposition	9.1 (0.62)[a]	5.4 (0.63)[b]	9.1 (0.58)[a]	*	***

[1]Initial live weight. [a,b]Values with different superscripts are significantly different ($P<0.01$). *$P<0.05$; ***$P<0.001$.

Lysine deposition for the pigs given the HSMP diet was not significantly different from that for the pigs fed EHC-B formulated to contain the same digestible reactive lysine content as the HSMP diet (new assay), but was significantly higher than that for the pigs fed the EHC-A diet formulated to contain the same digestible total lysine content as the HSMP diet (conventional ileal amino acid digestibility assay).

The new true ileal digestible reactive lysine assay is accurate in predicting lysine digestion and absorption in the growing pig for the heated skim milk powder used here. In contrast, the results of the conventional ileal digestibility assay for this processed protein were inaccurate. The new assay appears to offer considerable promise for determining available lysine in processed foods.

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AN *IN VITRO* METHOD FOR PREDICTING DIGESTIBLE ENERGY IN CEREAL GRAINS FOR PIGS

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Information on the apparent digestible energy (ADE) content of cereal grains is needed to allow for cost effective diet formulation to maximize on-farm profit. As there may be a considerable amount of variation in ADE content among different cereal grains and also among samples of the same cereal, the use of tabulated values has limitations. Recent studies have indicated that *in vitro* techniques, which give rapid and reproducible results, may be a useful alternative to more expensive and time consuming pig digestibility trials (Boisen, 1991). In this study, the apparent digestibility of energy (DEc, %) *in vivo* and the digestibility of dry matter (DDM, %) *in vitro* were determined in 10 samples each of barley, wheat and maize. The accuracy of the *in vitro* method for predicting ADE content in cereal grains was evaluated.

For each cereal sample, six Large White x Landrace pigs (≈ 30 kg live weight) were kept in single pens for 15 d. The animals received the test diet (comprising 99.35% ground cereal, 0.4% Cr_2O_3, 0.25% vitamin and mineral premix) twice daily (0830 hours and 1600 hours) at a fixed daily rate of 10% of metabolic body weight. During the last 5 d of the trial, a fresh faecal "grab" sample was collected daily. The diets and faeces were analyzed for gross energy using an adiabatic bomb calorimeter, and chromium concentration according to the method of Costigan and Ellis (1987). The DEc was determined with reference to the marker compound in the diet (Cr_2O_3).

The DDM *in vitro* was estimated using a three-step enzymatic method (Boisen, 1991). Each sample was analysed twice using duplicate sub-samples The samples (0.5 g of finely ground material) were incubated at 40°C with pepsin at pH 2 for 75 min, then with pancreatin at pH 6.8 for 3.5 h, and finally with Viscozyme (Novo, Denmark) at pH 4.8 for 18 h. After incubation, the residue was filtered to remove the digested material and the residue was dried overnight at 80°C. A blank containing only enzyme was included. *In vitro* DDM was calculated from the dry matter (DM) in the sample and in the undigested residue, after correction for the dry matter in the blank.

The correlation between replicates within each cereal was found to be 0.92, 0.79 and 0.88 for barley, wheat and maize, respectively. The correlation between DEc *in vivo* and DDM *in vitro* was statistically significant from zero across cereals ($r=0.81$, $n=30$, $P<0.001$), but not within cereal. Multiple regression analysis of logarithm transformed data showed that the ADE could be predicted accurately across cereals on the basis of DDM and GE ($R^2=0.91$). However, within cereal, fitting DDM after GE did not improve the prediction significantly. It is concluded that DDM *in vitro* is a good predictor of DEc *in vivo* when there is a wide range of digestibility, e.g., across cereals in the present experiment, or for ingredients such as wheat milling by-products (Chen *et al.*, 1995).

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DIGESTIBLE ENERGY VALUES OF WHEAT, SORGHUM AND BARLEY

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Energy in diets is an important economic factor in pig production. More than 80% of the energy content of conventional diets used in intensive pig production comes from the grain component, with wheat, sorghum and barley being the most important grains fed in Australia. Previous Australian work with pigs has shown that the digestible energy (DE) content of these grains is quite variable with intra-species differences of 15% being typical for apparently 'sound' grain and differences for 'weather-damaged' grain being even greater (SCA, 1987).

In a series of pig metabolism studies, the DE content of 86 grain samples were assessed using a modification of the total faecal collection method of Batterham *et al.* (1980). Entire male pigs (initial body weight ~25 kg) were housed individually in metabolism crates. Faecal collection was carried out over 5 d following an initial pre-collection adaptation period of 5 d. Ferric oxide was added to the diet as a faecal dye to indicate the start and end of collection. There were four pig replicates for each grain sample. Diets were based on 90% grain and a 10% basal component consisting of casein, vitamins, minerals, lysine and oil.

Table 1. Digestible energy content (MJ/kg air-dry basis, 87-89% DM) of Queensland barley, sorghum and wheat grains from 1992 and 1993 compared with SCA (1987).

	DE (MJ/kg)			SCA (1987) DE (MJ/kg)		
Grain	Mean ± SEM	Range	n	Mean	Range	n
Barley	13.65 ± 0.014	12.87-14.29	30	12.7	11.5-13.7	16
Sorghum	14.76 ± 0.020	14.19-15.38	25	14.4	14.1-14.9	8
Wheat	14.68 ± 0.012	14.23-15.90	31	14.3	13.8-14.6	21

The results of the analyses on 1992/1993 grain samples (Table 1) from various locations in Queensland indicated a higher mean value DE for barley (0.9 MJ higher), sorghum (0.36 MJ higher) and wheat (0.30 MJ higher) than published values (SCA, 1987). In addition, the results from analyses of 1992/1993 grains indicate that the range of DE values has increased for all grains. A number of reasons for this increase in DE may exist. Firstly, in 1992/1993, Queensland was in the midst of a drought which may have influenced DE value of grain. Secondly, these results are on newer improved grain varieties in which nutritive improvements may have occurred. Finally, Owsley *et al.* (1981) have shown that smaller particle size increases faecal energy digestibility. As the diets in this study were finely ground this may have resulted in the higher DE values observed.

These results show that DE values published previously may need modification to enable pig producers to maximise profitability through diet formulation.

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EFFECT OF LIVE WEIGHT ON ENDOGENOUS ILEAL NITROGEN AND AMINO ACID EXCRETION IN THE GROWING PIG

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Endogenous ileal amino acid losses (EAAL) from the digestive tract are an important component contributing to amino acid requirements in humans and farm animals. The aim of the present study was to determine whether there is an effect of pig live weight on EAAL when expressed on a food dry matter intake (DMI) basis.

A post valve T-caecum (PVTC) cannula was surgically implanted into the caecum of each of seven 33 kg live weight Large White x (Large White x Landrace) pigs. The pigs were fed four semi-synthetic diets containing 0%, 5%, 10% or 20% enzyme hydrolysed casein (EHC, MW<5,000 Da) as the sole source of nitrogen (N). The diets were offered to the pigs at 10% of metabolic body weight ($W^{0.75}$) for 8 d experimental periods using a 4 x 4 latin square design. A 12% casein-based diet was fed for 6 d between each experimental diet to allow the pigs on the low and protein-deprived diets to regain a positive body N balance. On days 5 and 8 of each experimental period, ileal digesta were collected continuously for 24 h. Although a total collection of digesta was attempted, chromic oxide was included in each diet to allow correction for incomplete collection as necessary. The live weight of the pigs varied from 30-87 kg when digesta collections were made. The enzyme hydrolysed protein/ultrafiltration method proposed by Moughan *et al.* (1990) and applied by Butts *et al.* (1991) was used to determine endogenous flows of total N and all amino acids except for tryptophan. With this method any unabsorbed dietary amino acids are removed in the ultra-filtrate. The EAAL are somewhat underestimated as some endogenous free amino acids and small peptides are also removed in the ultrafiltrate. The data were analysed (ANOVA) using a model which included terms for diet, live weight and diet x live weight. There was no statistically significant interaction, but there were significant effects of live weight ($P<0.05$) and diet ($P<0.01$).

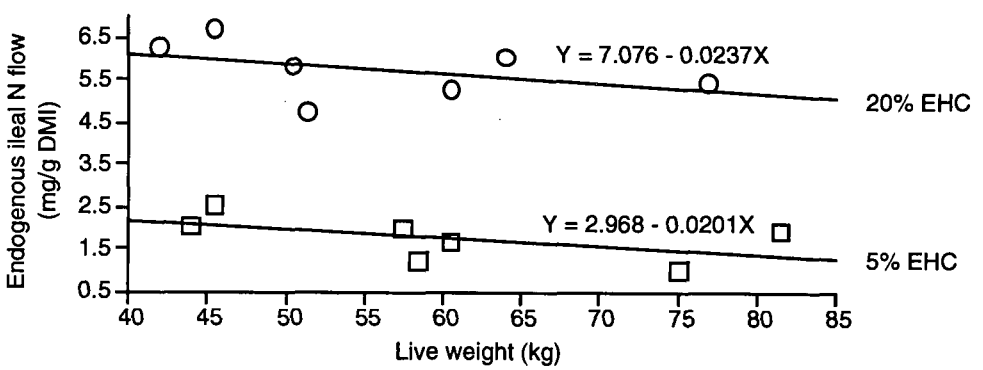

Figure 1. *Endogenous ileal N flows in growing pigs fed diets containing 5% or 20% enzyme hydrolysed casein (EHC).*

There were significant effects of live weight ($P<0.05$) on the endogenous ileal flows of total N and all the amino acids determined except for glutamic acid and proline. The EAAL increased with increasing level of inclusion of EHC in the diet. The endogenous ileal N flows for the low (5%) and high (20%) EHC diets are shown in Figure 1. It appears that an adjustment for live weight needs to be made when determining ileal endogenous amino acid losses in pigs of widely varying live weight.

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CAN NAKED BARLEY REPLACE WHEAT IN WEANER DIETS?

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Barley is avoided as the cereal base in weaner diets (Miller *et al.*, 1991) because of its variable content of largely indigestible components, β-glucans and fibre. As most of the fibre found in barley resides in the husk, the recent developments of naked barley cultivars may lead to an alternative to wheat as the cereal component in weaner diets. Naked barley has a digestible energy similar to that of wheat, 13.6 MJ/kg and 14.3 MJ/kg respectively, higher levels of important amino acids, such as lysine and threonine (Miller *et al.*, 1991) and lower levels of crude fibre than wheat, 2.1% vs 2.6% (Mullan, B.P. personal communication, 1996). These characteristics give naked barley the potential to become a useful ingredient in weaner feeds. It was hypothesized on the basis of nutritional similarity that naked barley would allow weaners to grow at a similar rate to those fed wheat, and at a faster rate to those fed conventional barley. The β-glucans also occur in the endosperm of the grain so it was expected that treatment of the naked barley diet with a corresponding enzyme might further improve the digestibility and stimulate even faster growth of weaners.

Forty-eight, newly-weaned pigs, 24 d old, were allocated at random to four treatment groups, each containing six males and six females. There were two controls, a wheat diet and a conventional barley diet, and two treatments, naked barley and naked barley + enzyme, the latter containing a β-glucanase enzyme (*PORZYME SP*, Finnfeeds International LTD). The diets were formulated to contain 14.5 MJ/kg and 0.81 g available lysine/MJ DE. All diets contained the same proportion of cereals (570 g/kg), and variable amounts of lupins, soya bean meal, skim milk powder, canola meal, fishmeal, blood meal and micro ingredients.

Table 1. Liveweight, food intake and efficiency of weaner pigs fed four different diets for a period of 21 d after weaning.

	Wheat	Barley	Naked barley	Naked barley + *PORZYME SP*	SE
Live weight (kg)					
Start	5.1	5.1	5.1	5.1	0.20
Day 7	5.5	5.6	5.6	5.5	0.27
Day 14	7.3	7.4	7.3	7.3	0.38
Day 21	10.2	10.6	10.8	11.1	0.57
Growth rate (g/d)	243	262	271	286	23.9
Voluntary intake (g/d)	481	498	506	504	18.5
Efficiency (g feed:g gain)	1.98	1.90	1.87	1.76	0.26

There were no significant differences between any of the treatments, supporting the hypothesis that naked barley can replace wheat in weaner diets. Weaners fed naked barley with or without enzyme ate 5% more food (P=0.7) and grew 15% faster (P=0.7) than the weaners fed wheat. Surprisingly the pigs fed conventional barley shared similar performances (food intake, growth rate and feed conversion ratio) to those fed wheat, perhaps because the wheat and barley diets had similar fibre contents.

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YELLOW LUPINS (*Lupinus luteus*): A NEW FEED GRAIN FOR THE PIG INDUSTRY

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Australian Sweet Lupins (ASL) (*Lupinus angustifolius*) have become an important component in the diets of pigs in many areas of Australia, and are included at up to 30% in diets for finisher pigs. However, they have not been widely accepted by the international feed industry as an alternative to soya bean meal (SBM), partly because of their lower crude protein (CP) content (310 vs 460 g/kg, respectively). The yellow lupin (YL) (*L. luteus*) is native to Portugal, Western Spain and the wetter parts of Morocco and Algeria. Recent selections have been found to have a higher CP content than that of ASL (380 vs 310 g/kg, respectively) and to yield better than ASL varieties on acid soils of low fertility (700 vs 470 kg/ha, respectively) (Cowling, unpublished). The aim of this study was to record the performance of grower pigs fed diets containing varying proportions of YL as a replacement for SBM. As a comparison, a diet containing ASL, at approximately the maximum recommended level, was included.

Twenty-five female and 35 entire male Large White x Landrace pigs were fed a wheat/SBM diet after weaning at 19 d, and were then allocated on the basis of live weight (LW) and sex to wheat-based diets in which the SBM component was progressively replaced by an increasing proportion of YL (0, 100, 180 or 260 g/kg) or with 245 g/kg of ASL. Experimental diets were formulated to contain 14.0 MJ DE/kg and 0.65 g available lysine/MJ DE. Pigs were fed the diets *ad libitum* for 35 d, and voluntary feed intake (VFI), average daily gain (ADG) and feed conversion ratio (FCR) were recorded. Treatment effects, including the linear and quadratic effects of level of YL, sex effects and treatment by sex interactions were examined using analysis of variance with initial LW (start) as a covariate.

Table 1. The effect of inclusion level (g/kg) of YL and ASL on the performance of growing pigs (covariate adjusted means).

Treatment	0	100YL	180YL	260YL	245ASL	l.s.d.
Number of pigs	12	12	12	12	12	
Live weight at start (kg)	20.7	20.3	20.7	20.6	20.8	1.15
Live weight at end (kg)	54.2	55.6	54.5	53.1	54.3	3.36
Voluntary food intake (kg/d)	2.16	2.22	2.12	2.04	2.03	0.315
Average daily gain (g)	962	1002	971	936	965	96.0
Food conversion ratio	2.25	2.22	2.19	2.20	2.11	0.294

The CP content of the YL, ASL and SBM used in this experiment were 420, 310 and 455 g/kg, respectively. There was no significant difference in either VFI, ADG or FCR when pigs were fed a diet containing up to 260 g/kg of YL (Table 1). There was a significant quadratic decline (P=0.038) in VFI as the proportion of lupins in the diet increased, which was attributed to the increase in crude fibre content of the diets (23, 32, 42, 49 and 53 g/kg for the 0, 100YL, 180YL, 260YL and 245ASL diets, respectively). Yellow lupins have the potential to be a high quality feedstuff for growing pigs, with a maximum inclusion level of 180 g/kg suggested for pigs between 20-55 kg LW. If YL are dehulled (approximately 25% of total seed weight), then they will contain higher levels of CP to that of SBM.

EFFECTS OF PORCINE COLOSTRUM ON NEWBORN INTESTINAL DEVELOPMENT IN AN ORGAN CULTURE SYSTEM

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In a previous study it has been demonstrated that porcine colostrum stimulates intestinal brush border enzyme maturation in neonatal pigs, and that the effect can be eliminated by pre-hydrolysis of porcine colostrum (Wang and Xu, 1996). It is not known whether the stimulatory effect is a direct action or is via metabolic changes following colostrum ingestion. In the present study the effects of porcine colostrum on intestinal enzyme activities in an organ culture system were examined.

Small intestinal explants were obtained from a newborn unsuckled piglet. Following euthanasia with an overdose of thiopentone sodium, the whole small intestine was removed from the piglet and washed in Hanks' solution. The jejunal part of the small intestine was opened longitudinally and cut into small explants (4 x 4 mm). The explants were placed in organ culture dishes (Falcon, Dickinson & Co. Cockeysville, MD, USA) with the mucosal side facing up. One ml of culture medium, DMEM/F12 (Sigma, St Louis, MO, USA) supplemented with 100 units/ml of penicillin and 100 mg/ml of streptomycin, was placed in each culture dish and the medium was replaced every 12 h. To examine the effect of porcine colostrum on the intestinal enzyme activities the serum of either natural porcine colostrum or pre-hydrolyzed colostrum was added to the culture medium at a concentration of 2.5 mg/ml protein equivalent. The colostrum and pre-hydrolyzed colostrum were obtained and processed following the procedure described by Wang and Xu (1996), and the serum was obtained by centrifugation at 12,000 g for 30 min at 4°C. All tissue explants were cultured at 25°C for 48 h after which the protein and DNA content and the activities of lactase, maltase, alkaline phosphatase, and amino petidase were measured as described by Wang and Xu (1996).

Table 1. Protein and DNA contents (mg/g) and brush border enzyme activities (units/g) in intestinal explants cultured for 48 h (mean ± SEM).

Treatment	n	DNA	Protein	Lactase	Maltase	Alkaline phosphatase	Amino peptidase
Control	3	6.2 ± 0.4	54 ± 3.7	5.6 ± 0.4	0.28 ± 0.03	3.2 ± 0.4	3.2 ± 0.3
Pre-hydrolyzed colostrum	3	6.1 ± 0.4	57 ± 3.1	5.4 ± 0.2	0.29 ± 0.04	3.7 ± 0.5	3.3 ± 0.1
Colostrum	3	5.1 ± 0.3	49 ± 0.7	5.7 ± 0.4	0.34 ± 0.01	9.6 ± 0.4**	3.8 ± 0.2*

***Significantly different from the control: *P<0.05; **P<0.01.

It was observed that addition of colostral serum to the medium increased the activities of alkaline phosphatase and amino peptidase, but not lactase or maltase, in the tissue explants (Table 1). In contrast, addition of the serum of pre-hydrolyzed colostrum had no effect. The observations agree, in part only, with those in a previous report that oral feeding of porcine colostrum, but not pre-hydrolyzed colostrum, stimulated intestinal lactase, maltase and alkaline phosphatase activities in newborn piglets (Wang and Xu, 1996). The results of the present preliminary study indicate that the stimulatory effect of porcine colostrum on the activities of alkaline phosphatase and amino peptidase is by direct action on the intestinal epithelial cells. It has been postulated that growth factors found in the colostrum may play a regulatory role in postnatal development of suckled animals (Xu, 1996). However, the compounds in porcine colostrum which stimulate the maturation of intestinal enzymes in neonatal pigs remain to be identified.

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SMALL INTESTINAL TRANSPORT OF GLUCOSE AND AMINO ACIDS DURING PERINATAL DEVELOPMENT IN PIGS

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Mortality is highest during the first few days after birth, particularly for pigs born slightly premature. Although problems of digestion and nutrition have been suspected as causes for failure to thrive and survive, there is little information about perinatal intestinal development. The available data suggest that the majority of intestinal growth and maturation occurs during the final trimester in anticipation of the shift from placental nutrition to external foods (Buddington and Malo, 1996). However, it is not known when fetal pigs actually acquire sufficient functional capacity to absorb the dietary loads expected at birth. In the present study the rates of intestinal uptake of glucose and some amino acids in premature pigs (obtained by Caesarian section at 90% gestation; n=11) were compared with the uptake in normal newborn unsuckled pigs (n=6) and in 1 week-old sucking pigs (n=6).

The pigs (Danish Landrace x Large White) were killed (pentobarbitone; 200 mg/kg, i.v.) and the small intestines removed and separated into three regions of equal length (proximal, mid and distal). From the middle of each region a segment was used to prepare 1 cm everted sleeves for *in vitro* measurements of the regional distribution and kinetics of transport for four nutrients, glucose, leucine, lysine and proline, using the methods of Puchal and Buddington (1992) and Zhang *et al.* (1997). The tissues were exposed for 2 min to Ringer's solution (37°C) containing tracer + increasing concentrations of unlabelled nutrient (up to a saturating concentration of 50 mmol/l). Rates of nutrient uptake per min were normalized to tissue wet weight, and total uptake capacity was calculated as the sum of the regional transport capacities (means ± SEM). To determine if a portion of nutrient uptake was mediated by saturable, carrier-mediated pathways, tracer accumulation from a solution with tracer alone was divided by the accumulation from the solution with tracer + 50 mmol/l nutrient. Accumulation ratios of more than 1.0 indicate that a saturable, carrier-mediated transport pathway is present. Finally, uptake kinetics were determined by non-linear regression analysis (Michaelis-Menten) which included a linear component to descibe the passive nutrient influx (Enzfitter software).

The characteristic pattern of a declining proximal to distal gradient of glucose transport found in sucking and adult pigs (Puchal and Buddington, 1992) was already well established in the premature pigs. The maximal rate of carrier-mediated glucose transport (V_{max}) in the proximal intestine was lower for premature pigs than for newborn pigs (3.1 ± 0.2 vs. 4.5 ± 0.3 nmol mg^{-1} min^{-1}; Zhang *et al.*, 1997). Since apparent affinity constants (K_m) for glucose transport were similar for these two groups (1.3 vs 1.5 mmol/l), differences in V_{max} probably reflect changes in the density, not the type, of glucose transporters. Total rates of leucine, lysine and proline uptake (carrier-mediated + diffusion) at the saturating concentration of 50 mmol/l did not differ among regions. The rates in premature pigs (2.4 ± 0.1, 2.0 ± 0.1 and 3.2 ± 0.1 nmol mg^{-1} min^{-1}, respectively) were similar to those in newborn pigs (2.9 ± 0.2, 2.1 ± 0.1 and 3.0 ± 0.2 nmol mg^{-1} min^{-1}) and sucking pigs (2.2 ± 0.2, 1.8 ± 0.2 and 3.8 ± 0.2 nmol mg^{-1} min^{-1}). The tracer accumulation ratios for amino acids were also similar across the three groups of pigs (5.3 ± 0.6, 10.0 ± 2.7 and 1.8 ± 0.3 for leucine, lysine and proline, respectively, n = 23).

The findings indicate that at 90% of gestation the intestines of pigs 1) are able to absorb nutrients using carrier-mediated uptake pathways, and 2) potential intestinal uptake capacities, as determined *in vitro*, are more than sufficient to absorb nutrients present in the amniotic fluid swallowed by fetuses, and in the dietary loads at the onset of suckling. Problems of digestion and nutrition in newborn pigs are therefore unlikely to arise from an inability of the intestinal mucosa to absorb dietary nutrients at birth.

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THE RESPONSE OF PIGS BETWEEN 80-120 KG LIVE WEIGHT TO ENERGY INTAKE

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Australian pig producers should increase slaughter weights to increase production efficiency by capitalising on the faster lean growth of the genetically-improved pigs that are now available. However, there is little information available on the response of these pigs to feed intake beyond 90 kg live weight (LW). Such information however, is crucial for establishing tissue amino acid requirements and for devising feeding strategies to maximize feed efficiency and prevent excessive fat deposition. The aim of this experiment was to determine the response of pigs between 80-120 kg LW to energy intake.

Eighty crossbred pigs were allocated at 80 kg LW to a 2 x 5 factorial experiment involving two sexes (male and female) and five levels of energy intake ranging from about 55% *ad libitum* up to 100% *ad libitum*. All pigs received a protein-adequate diet containing 14.5 MJ DE/kg and 9.4 g lysine/kg.

Table 1. Effects of energy intake on the growth performance (feed conversion ratio, FCR; average daily gain, ADG) and P2 backfat thickness of male (M) and female (F) pigs grown from 80-120 kg LW.

		Feeding level					SEM	Main Effect[1]		
								Sex	Feeding level	
		1	2	3	4	5			Linear	Quadratic
Intake (MJ DE/d)	M	23.5	27.3	31.8	34.5	47.9	3.4	**	***	NS
	F	22.3	26.5	30.5	34.1	41.2				
FCR	M	3.61	3.18	2.96	2.67	2.41	0.39	***	***	NS
	F	3.74	3.70	3.53	2.96	2.76				
ADG (g/d)	M	461	598	751	899	1376	120	***	***	NS
	F	423	501	598	802	1040				
P2 (mm)	M	8.9	12.9	11.3	13.1	15.0	2.2	NS	***	NS
	F	10.0	12.7	13.7	13.4	15.9				

[1]NS, not significant, **P<0.01, ***P<0.001.

Growth rate increased linearly while FCR decreased linearly, in response to increasing energy intake, with males consistently out-performing female pigs at each level of energy intake. Backfat depth at P2 increased linearly in response to energy intake, and the carcasses from males tended (P=0.07) to have lower backfat depths than those from female pigs. These responses are consistent with a linear relationship between protein deposition and energy intake, which in turn suggest that there is no intrinsic limit to protein deposition up to 120 kg LW. This linear relationship is similar to that found in younger pigs and improved genotypes (Campbell and Taverner, 1988) which have a high potential for protein growth relative to appetite. These data indicate that the pigs used in the present experiment have considerable potential for protein deposition during the period between 80-120 kg LW and they are ideally suited for production of heavy carcasses in production systems which promote high feed intake.

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THE RESPONSE OF PIGS BETWEEN 80-120 KG LIVE WEIGHT TO DIETARY LYSINE

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Australian pig producers should consider increasing pig slaughter weights so as to lower unit costs during both the production and processing stages. However, extensive examination of the nutrient requirements of pigs has been restricted to the growing period up to 90 kg live weight (LW) (Standing Committee on Agriculture, 1987). There is little information on the amino acid requirements of pigs, particularly improved genotypes, at live weights in excess of 90 kg LW. The aim of this experiment was to determine the responses of pigs between 80-120 kg LW to dietary lysine.

Sixty crossbred pigs were allocated at 80 kg LW to a 2 x 6 factorial experiment involving two sexes (male and female) and six levels of dietary lysine. All diets contained 14.5 MJ DE/kg and the lysine and available lysine concentrations ranged from 4.8-9.7 g/kg and 4.0-8.1 g/kg respectively. The ratios of other essential amino acids to lysine were in excess of the ratios suggested by the Standing Committee on Agriculture (1987) for growing pigs. The major sources of dietary protein were soya bean, fishmeal and blood meal. All diets were offered *ad libitum* between 80-120 kg LW.

Table 1. Effects of dietary lysine on the growth performance (feed conversion ratio, FCR; average daily gain, ADG) and P2 backfat thickness of male (M) and female (F) pigs grown from 80-120 kg LW.

		Dietary lysine g/kg						SEM	Main Effect[1]		
									Sex	Dietary lysine	
		4.8	5.8	6.8	7.7	8.7	9.7			Linear	Quadratic
FCR	M	2.83	2.79	2.39	2.44	2.38	2.38	0.29	*	***	*
	F	3.00	2.72	2.67	2.65	2.40	2.71				
ADG	M	913	910	925	976	1085	911	111	NS	*	NS
(g/d)	F	858	834	934	912	929	960				
P2	M	13.9	14.1	12.5	13.4	14.6	13.0	3.6	*	NS	NS
(mm)	F	18.7	14.5	14.0	16.4	14.6	16.3				

[1]NS, not significant, *P<0.05, ***P<0.001.

Based on FCR, the optimum level of dietary lysine for males was about 6.8 g lysine/kg (0.40 g available lysine/MJ DE). The corresponding level for females appeared to be lower at about 5.8 g lysine/kg (0.34 g available lysine/ MJ DE). The mean (± SE) concentrations of skatole and androstenone (Salvatore *et al.*, 1995) in samples of belly fat collected from the carcasses of male pigs were 0.11 µg/g (0.03) and 0.54 µg/g (0.08), respectively and were unaffected by dietary lysine. Fifteen male pigs had levels of androstenone in excess of the threshold level of 0.50 µg/g whereas 4/30 pigs had skatole levels above the threshold level of 0.2 µg/g (Salvatore *et al.*, 1995). Only 2/30 of the male pigs had levels of both indicators of boar taint in excess of the threshold levels of skatole and androstenone. The feed efficiency and carcass fat of males and, to a lesser extent, females, were in line with those generally accepted for pigs slaughtered at 90-100kg, suggesting that modern genotypes are capable of being grown to 120 kg LW without compromising efficiency of production or carcass quality.

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THE EFFECTS OF INCLUDING BETAINE IN THE DIET OFFERED DURING LACTATION ON THE SUBSEQUENT REPRODUCTIVE PERFORMANCE OF SOWS

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Betaine is a methyl donor which has been reported to alter nutrient partitioning in growing pigs (Cadogan *et al.*, 1993) and to alleviate heat stress in poultry. The effect betaine might have on the performance of lactating sows which are subjected to considerable physiological stress and tissue changes over a relatively short period of time have not been investigated. The present experiment was conducted to investigate the effects of parity (first litter and older sows) and betaine (zero and 2.0 kg/tonne) during a 25 d lactation on piglet performance and subsequent reproductive performance.

One hundred and four Large White x Landrace sows comprising equal numbers of gilts and older parity animals were allocated between the two betaine treatments during lactation. The basal diet contained 14 MJ DE/kg and 0.8% total lysine. The experimental diets were offered *ad libitum* from parturition to weaning at 25 d.

Of the 104 animals which started the experiment 96 completed lactation and 76 had another litter. Betaine had no effect on sow feed intake (5.56 vs 6.10 kg/d, P=0.503) or piglet growth rate during lactation (210 vs 220 g/d, P=0.235). There was however, a significant interaction between the effects of parity and betaine for subsequent litter size (Table 1). For first litter sows betaine had no effect on subsequent litter size. For older sows betaine increased the number of pigs born alive in the subsequent litter.

Table 1. Effects of parity and including betaine in the diet offered during lactation on subsequent reproductive performance of sows.

Parity	Betaine (kg/tonne)	Total born	Born alive
1	0	12.0	10.7
	2.0	12.0	10.9
2-4	0	10.2	9.5
	2.0	12.5	12.3
Significance (P=)			
Parity (P)		0.332	0.983
Betaine (B)		0.215	0.048
P x B		0.148	0.022

The results suggest that betaine, either through a nutrient sparing effect (its methyl donor function) or via a reduction in tissue and/or heat stress, improves the reproductive capacity or preparedness of sows after weaning. The results have implications with respect to the nutrient "requirements" of lactating sows and suggest current diet formulations can be improved to better match the needs of the animal.

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THE EFFECTS OF BETAINE ON PROTEIN AND ENERGY METABOLISM OF GROWING PIGS

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Betaine (tri-methyl glycine) is known to influence both the performance and body composition of growing pigs (Cadogan *et al.*, 1993). The mode of action however, remains unclear and may differ between situations in which amino supply is adequate or marginal. The present experiment was conducted to investigate the effect of betaine on the partition of energy between fat, protein and maintenance in pigs offered a protein adequate diet between 30-55 kg live weight (LW).

Sixty-four entire male pigs were allocated at 30 kg LW among eight treatments in 2 x 4 factorial array. The respective factors were dietary betaine (zero and 1.25 kg/tonne) and four levels of intake of a protein adequate diet (14.0 MJ DE and 1.06% available lysine) from 15.3 MJ/d to *ad libitum*. Protein and fat deposition rates were determined by comparative slaughter.

Table 1. Effects of betaine[1] and energy intake on the performance and protein and fat deposition rates of male pigs grown from 30-55 kg LW.

		DE intake (MJ/d)				Significance	
		15.5	17.1	19.3	*Ad lib.*[2]	DE	B
Daily gain (g)	Control.	414	479	573	929	0.000	0.069
	Betaine (B)	439	540	624	977		
Feed:gain	Control	2.65	2.56	2.39	2.06	0.000	0.073
	Betaine	2.52	2.24	2.19	2.05		
Protein deposition (g/d)	Control	71.8	84.3	97.4	140.0	0.000	0.597
	Betaine	73.7	95.9	93.0	138.0		
Fat deposition (g/d)	Control	5.4	6.2	24.6	102.3	0.000	0.107
	Betaine	12.8	25.3	40.0	124.0		

[1]Betaine was supplied by Cultor Pty Ltd, Finland. [2]*Ad libitum* energy intake was 26.4 and 27.8 MJ/d for pigs offered the control and betaine supplemented diets respectively.

The results (Table 1) showed that betaine tended to improve both growth rate and feed:gain when energy intake was restricted. Protein and fat deposition increased linearly with increasing energy intake; the former was unaffected by betaine. The latter was higher for pigs fed the diet supplemented with betaine.

Total energy retained was linearly related to DE intake (r=0.997) for pigs offered both diets. Based on these relationships the estimated maintenance energy requirement for pigs offered the control and betaine supplemented diets were 12.9 and 11.4 MJ DE/d respectively.

The results suggest that betaine, a methyl donor and osmoregulator, improves the rate and efficiency of growth under protein adequate situations by reducing the pig's energy requirement for maintenance. Under these situations betaine also increases body fat content.

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EFFECTS OF PHYTATES AND PHYTASE ON FEED CONVERSION RATIOS OF WEANER PIGS

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Phytates are salts of phytic acid which are the substrate for phytase, an enzyme of microbial origin which may be used as a feed additive. Phytic acid has a phosphorus component (phytate P) which constitutes 28.2% of its molecular weight (660.1). The amount of phytate P in the diet is commonly expressed as % phytate P. Surprisingly little attention has been given to the amount of phytate P in the diet when the effect of phytase as a dietary additive has been evaluated. Five weaner feeding studies with microbial phytase (Natuphos®) have been completed in Australia; one at Wollongbar (Barnett *et al.*, 1993) and four at Corowa.

From the five studies, eight comparisons were made where available P levels were considered adequate so that any response to phosphorus released from phytate P by the action of phytase was eliminated. In four studies substrate levels were estimated from the formulations of the diets using phytate P values determined by BRI for the relevant ingredients up to March, 1997. For the fifth study (96C49) the actual values of the relevant ingredients were established and the dietary phytate P level calculated. The eight comparisons involved a total of 1620 pigs with an average post-weaning feeding period of 34 d (range 21-40 d). Microbial phytase was included in the wheat based diets at an average of 675 FTU/kg (range 500-1000). The response in feed conversion to phytase addition was calculated and the results are presented in Table 1. Correlations between dietary substrate levels and feed conversion efficiency in the control diets plus responses to phytase in the treatment diets in relation to phytate P levels were investigated.

Table 1. Effects of phytates and microbial phytase on feed conversion ratios (FCR) of weaner pigs.

Report	Phytate P (%)	FCR		Response to phytase
		Control	Phytase	
Barnett *et al*,.1993	0.20	1.49	1.43	4.0%
BMI 93C49	0.39	1.49	1.36	8.7%
BMI 94C69	0.14	1.22	1.26	-3.3%
BMI 94C69	0.20	1.49	1.48	0.7%
BMI 95C133	0.21	1.40	1.41	-0.7%
BMI 96C49	0.12	1.29	1.28	0.8%
BMI 96C49	0.22	1.43	1.36	4.9%
BMI 96C49	0.33	1.57	1.40	10.8%
Mean	0.226	1.423	1.373	3.24%

There is a significant negative correlation ($P<0.05$; $r^2= -0.5425$) between the % phytate P in the diet and feed conversion efficiency in the control diets. There is also a significant correlation ($P<0.01$; $r^2= 0.7253$) between % phytate P in the diet and improvement in feed conversion following the addition of phytase. The regression equation for the second correlation is $Y = 44.874X - 6.90$, where X is % phytate P and Y is the % improvement in feed conversion following the addition of phytase. The two correlations indicate that increasing the % phytate P in the diet has a negative impact on feed conversion, and that the responses to the addition of phytase increase with increasing % phytate P in the diet. The rationale for the responses to phytase in diets considered adequate for available P requires elucidation; nevertheless, dietary phytate P levels should be determined whenever the addition of microbial phytase is being evaluated.

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EFFECTS OF DIETARY PHYTATE PHOSPHORUS AND MICROBIAL PHYTASE ON THE PERFORMANCE OF WEANER PIGS

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Dietary phytate P may inhibit the performance of weaner pigs because of its effects on the digestibility of protein (Rutherfurd *et al.*, 1997). The effects of differing amounts of phytate P and phytase in diets on the performance of weaner pigs were investigated in a feeding study in which the diets were formulated to marginal protein levels. The diets contained three levels of phytate P (0.12, 0.22 and 0.33%) and two levels of added phytase activity (0 and 625 FTU/kg). Phytate P contents of wheat (0.17%), peas (0.15%), rice pollard (1.49%), Canola meal, (0.56%) and soya bean meal (0.41%) were determined, and the proportions of these ingredients were manipulated to acheive the three phytate P levels. The diets contained 14.8 MJ/kg DE, adequate phosphorus (0.46% available P) and an average Ca:P ratio of 1.30. The steam pelleted diets were sprayed with microbial phytase (Natuphos®) after cooling. A total of 120 individually housed, mixed sex, commercial crossbred pigs were allocated among six diets (offered *ad libitum*) for 28 d following weaning at a live weight of 6.13 ± 0.03 kg. Performance was measured from weaning to a live weight of 16.6 ± 0.22 kg and the results are presented in Table 1.

Table 1. Effects of phytate P levels and microbial phytase on the performance of weaner pigs.

Phytate P (%)	Phytase (FTU/kg)	Daily gain (g/d)	Feed conversion	Feed intake (g/day)
0.33	0	330^{bc}	1.57^{c}	486^{b}
0.33	625	370^{b}	1.40^{b}	511^{abc}
0.22	0	351^{bc}	1.43^{bc}	491^{b}
0.22	625	406^{a}	1.36^{ab}	546^{a}
0.12	0	392^{ab}	1.29^{ab}	505^{ab}
0.12	625	388^{ab}	1.28^{a}	495^{b}
Significance (P =)				
Microbial phytase		0.046	0.109	0.199
Phytate P		0.055	0.006	0.519

^{a,b,c}Treatment means with different superscripts are significantly different (P<0.05).

The results showed that overall growth performance was negatively related to the phytate P content of the diet, and phytase significantly improved growth rates and tended to improve feed efficiency. The effects of phytase on growth performance were more evident at the two higher levels of dietary phytate P. This was further illustrated by the marked difference in the slope (b) of the regression equations relating feed conversion efficiency to phytate P. With the control diets feed efficiency declined linearly with increasing dietary phytate P (b=1.3323; r^2=0.9992). Whereas with the phytase supplemented diets there was a less pronounced linear decline (b=0.5680; r^2=0.9534).

The positive effect of phytase on growth performance may have been due to the release of phytate bound amino acids as the available lysine (AL) content of the diets (0.78g AL/MJ DE) was less than 0.90g AL/MJ DE which is the estimated requirement to support maximal growth performance in pigs of the same genotype and live weight. Biehl and Baker (1996) reported that phytase improved amino acid utilisation in young pigs fed corn-soya bean meal diets. The results demonstrate the adverse impact of phytate P on performance, and the capacity of phytase to counter this in weaner diets with adequate P.

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BASAL DIET CAN INFLUENCE APPARENT ILEAL AND FAECAL DIGESTIBILITY OF NITROGEN IN PROTEIN MEALS

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Previously, it was reported that the apparent digestibilities of nitrogen (N) and organic matter in cottonseed meal (CSM) were negligible or even negative between the terminal ileum and rectum (Prawirodigdo *et al.*, 1995). Recently, Wigan *et al.* (1995) found that the digestibility of energy was higher when lupins were fed in a wheat-based diet compared to when they were fed in a sugar based diet. The results indicated that the higher estimates of digestibility may be related to more complex carbohydrates passing to the hindgut when pigs were fed the wheat-based diets. The aim of the present study was to determine the ileal and faecal N digestibilities of CSM and soya bean meal (SBM) in simple carbohydrate and wheat-based diets.

Twenty five Large White x Landrace boars (40.9 ± 2.6 kg, live weight) were randomly allocated to either a wheat diet (W) or one of four diets containing 40% CSM or 40% SBM in a sugar:starch (1:1) or a wheat base diet (SS + CSM, SS + SBM, W + CSM or W + CSM) for 14 d. All diets contained a vitamin and mineral pre-mix, and Cr_2O_3 was included as an indigestible marker. Iron ($FeSO_4.7H_2O$) was added to the CSM diets to inactivate gossypol. Rations were offered (1800 g/pig/d) in 3 meals/d from day 1 to day 11 and in 8 meals/d from day 12 to day 13. On day 14 the pigs were fed hourly for 8 h and after the 8th meal they were anaesthetised with isoflurane. Digesta was sampled from the terminal ileum and rectum before the pigs were euthanased by injection of barbiturate.

Table 1. Effect of base diet (B) and site of digesta collection (S) on apparent nitrogen digestibility (%) of cottonseed meal (CSM) and soya bean meal (SBM).

	Sugar : starch		Wheat			
	Ileal	Faecal	Ileal	Faecal	sed[1]	Significance
CSM	55.1	52.9	67.5	58.7	2.18	D***, B*, DxB***, DxS***
SBM	74.9	81.2	70.4	80.2		DxBxS*

[1]Standard error of the difference for protein meal (P) x base diet (B) x site (S). *P<0.05; ***, P<0.001.

Apparent ileal and faecal digestibilities of N in the wheat only diet were 71.7 ± 4.0 and 74.2 ± 6.0%, respectively. Apparent ileal and faecal digestibilities of N in CSM and SBM fed in the simple carbohydrate base were similar to those previously reported (Prawirodigdo *et al.* 1995). Apparent ileal digestibility of N was greater (12.4%; P<0.05) when CSM was fed in a wheat as compared to the simple carbohydrate base. Apparent ileal N digestibility of SBM was slightly lower (-4.5%; P<0.05) in the wheat-based diet as compared to the sugar:starch-based diet. Therefore, while there was a wide difference in apparent nitrogen digestibilities of SBM and CSM when the meals were fed in the sugar:starch-based diets, these differences were less apparent in pigs fed a wheat-based diet. There was a net output of N in the hindgut in pigs fed CSM in both base diets. Conversely, there was quite substantial apparent digestion of N in the hindgut of pigs fed SBM in both base diets. In conclusion, the basal diet used can substantially influence the estimate of apparent N digestibility and the response can differ for different protein meals.

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DETERMINATION OF THE CONTRIBUTION OF AN ENZYME COMBINATION (VEGPRO) TO PERFORMANCE IN GROWER - FINISHER PIGS

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Responses to enzymes added to pig diets have been less dramatic than in poultry; however the amount of energy and protein which remains unutilized when pigs are fed diets containing soya bean meal suggests that there is potential for improvement in feed conversion efficiency. The objective of this study was to determine the relative value of an enzyme complement (Vegpro, Alltech Inc.) in high and low energy diets on the performance of grower/finisher pigs. The product (VegPro) is a mixture of protease, cellulase, pentosanase, -galactosidase, and amylase enzymes and is formulated to increase the availability of nutrients from soya bean meal.

A total of 120 pigs (Hampshire x Yorkshire; 60 barrows and 60 gilts) weaned at an average of 21 d were fed a common diet until they reached a mean live weight of 26.3 kg. A grower diet was then fed until 64 kg live weight whereupon a finisher diet was fed until each pen had a mean live weight in of 109 kg. All diets were formulated to meet or exceed National Research Council (1988) estimates for lysine, minerals and vitamins. Amino acids other than lysine were supplied in sufficient quantity to ensure that lysine was first-limiting. Treatments were arranged in a 2 x 2 factorial with factors being energy level (high energy [a corn/soya bean meal diet] vs low energy [a corn/soya bean meal diet containing 20% wheat mill by-product]) and enzyme inclusion (± Vegpro) at 1 kg/tonne. The difference in energy level between the high and low energy diets was 6.3% (14.56 vs 13.64 MJ ME/kg for growers, and 14.86 vs 13.92 MJ ME/kg for finishers). The energy:lysine ratio was kept constant. Animals were weighed and feed use was recorded at 2 week intervals.

Table 1. Effect of addition of Vegpro to diets for grower/finisher pigs on performance.

	High energy		Low energy		Factorial P values		
	Vegpro		Vegpro				
	-	+	-	+	Energy level	Vegpro	Energy x Vegpro
Grower							
Gain (g/d)	771	838.5	723	711	0.001		0.088
Intake (kg/d)	1.99	1.92	2.1	1.97			
Feed:gain	2.57	2.29	2.91	2.77	0.003	0.070	
Finisher							
Gain (g/d)	823.5	883	789	776	0.007		0.127
Intake (kg/d)	2.71	2.78	3.08	3.03	0.010		
Feed:gain	3.28	3.15	3.90	3.90	0.001		
Total period							
Gain (g/d)	801	860	771	748	0.002		0.043
Intake (kg/d)	2.38	2.36	2.68	2.56	0.026		
Feed:gain	2.97	2.75	3.48	3.42	0.001		

The addition of wheat milling by-product decreased daily gain and increased daily feed intake and feed:gain (Table 1). The inclusion of Vegpro decreased feed:gain in the grower period. An interaction between between energy level and diet observed for daily gain in the grower and total periods indicated a greater response to the enzyme supplement in the high energy diet. The data indicate that the enzyme complement tended to have a beneficial effect on pig performance.

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EFFECT OF PHYTASE ON LYSINE-RICE POLLARD COMPLEXES

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It is generally accepted that phytate can bind to protein present in a feedstuff. Furthermore, it is believed that protein binds primarily through the amino side chains of lysine residues to the phosphate moieties of phytate. It is quite possible therefore, that free amino acids added to feedstuffs as supplements, all of which possess free amino groups, may also bind to phytate either during processing, storage or in the gut post ingestion. The aim of the present study was to investigate the extent to which synthetic lysine can bind to phytate, and the effectiveness of microbial phytase (Natuphos®) to release bound lysine.

A suspension containing 2 g of Rice pollard (RP) (1721 mg phytate P/100 g RP) and 27 mg of synthetic lysine was prepared and made up to 25 ml with 0.1M sodium acetate pH 4.5 (A). The mixture was incubated at 50°C for 5 h to permit lysine-RP binding (B), then aportioned equally. The two portions were incubated at pH 4.5, 50°C for 8 h either with the addition of microbial phytase (2000 FTU/kg) (C), or without the addition of phytase (D). A control solution containing lysine but no RP was also prepared (Control). Aliquots (labelled A-D or Control) were taken after each step and the free lysine content determined using HPLC after ultrafiltration (exclusion limit 3000 Da) (Table 1).

Table 1. Free lysine content (mean ± SE)[1] after incubation with rice pollard (RP) and synthetic lysine and further incubation either in the presence or absence of added phytase.

	Free lysine (mg/25 ml)	% recovery of free lysine
Control	28.0 ± 1.04	100
RP + lysine before initial incubation (A)	21.9 ± 0.76	78
RP + lysine after initial incubation (B)	22.6 ± 0.61	81
RP + lysine after incubation with phytase (C)	25.3 ± 0.31	91
RP + lysine after incubation without phytase (D)	22.3 ± 0.29	80

[1]Means of two experiments; in each experiment analyses were carried out at a minimum in duplicate.

There was a 20% reduction in the free lysine concentration after addition of RP. Furthermore, incubation of the lysine + RP mixture at pH 4.5 at 50°C for 5 h did not increase lysine-RP binding. The free lysine concentration remained unchanged after further incubation in the absence of phytase. In contrast, after incubation with phytase 50% of the bound lysine appeared to be released.

Considerable amounts of free lysine appear to bind to compounds (perhaps phytate) present in RP. The affinity of lysine for these compounds appears to be high since most of the binding occurred under very mild conditions. Phytase was effective in releasing a quantitatively significant portion of the bound lysine suggesting that phytate was involved, at least to some degree, in the lysine binding. It is possible that a nutritionally significant portion of the lysine added to commercial pig feeds as a supplement may bind to compounds, including phytate, present in the feed and consequently will be lost to the animal. Binding of lysine may occur either during processing, storage or in the pig's intestine post feeding. Addition of phytase to diets appears to offer considerable promise in improving the availability of lysine supplements in pig diets.

A SYMPOSIUM - THE IMPACT OF PIG PRODUCTION ON THE ENVIRONMENT, AND OPPORTUNITIES FOR FUTURE CONTROL

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Introduction

Intensive animal production will come under increasing pressure to account for its effects on the environment. Consumers, already becoming increasingly wary about the practice of intensive animal farming, will have little sympathy for an industry that does not consider the environment as a high priority. Few people would realise that a 500 sow, farrow-to-finish operation producing 20 pigs per sow per year produces a similar amount of effluent as that of a town of 25,000 people (Headon, 1992). On this basis, it is hardly surprising that the environmental guidelines for piggeries are frequently under review. Changes to environmental guidelines are already affecting the existence or operation of the pig industry in many countries, such as Singapore where pig production is now banned, and others such as The Netherlands and Taiwan where quotas exist based on effluent production.

In Australia there is a community focus on effluent production and its effect on air, the land and water (Edwards, 1996). Added to this is economic pressure resulting from the introduction of load-based licensing systems. As in Europe, the environmental impact of piggeries is and will be increasingly a matter for concern.

With regard to the pig industry, Honeyman (1996) considers that sustainable swine production is a combination of production techniques that enhance profit and that improve environmental and socio-economic conditions. Opportunities that enhance sustainability include:

1. Feeding with increased use of forages and byproducts
2. Nutrient recycling through improved handling of manure
3. Low capital, high management housing systems that offer a better environment for the operator and reduced financial risk
4. Management systems suited to the pig's health and welfare and
5. Preventative approaches to pig health and a broader genetic base.

A sustainable pig production system should, according to Honeyman (1996), enhance the following areas:

1. The environment and resource base (land, water, air, human, animal)
2. The quality of life for producers, pork consumers and society
3. The profit level of producers
4. The quality of pork produced.

It is the objective of this symposium to describe some of the important issues relating to how the pig industry relates to the environment, and what possibilities exist for there to be a sustainable system developed along the guidelines of Honeyman (1996). The paper from O'Shea (1997) describes some of the procedures that are currently required to be undertaken to establish a piggery in some Australian states. To put the problem in perspective, Pluske *et al.* (1997) describe two of the major nutrients of concern in relation to effluent production, and discuss some of the methods that are available to reduce the amount of effluent produced per pig. The primary concerns with effluent are the amount and form of solids, liquid and odour that is produced. The issue of how to measure odours emanating from piggeries is discussed by Smith *et al.* (1997), while Rate (1997) describes the magnitude of the problem in relation to the amount of solids produced by a typical piggery, and describes the strategies available to process and recycle this waste.

ENVIRONMENTAL ISSUES OF CONCERN TO THE PIG INDUSTRY

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Abstract

In this paper the regulations and procedures required to develop a new piggery in Australia are described. An increasing amount of time and capital will need to be directed towards meeting these regulations in the future, especially in areas close to urban developments or in environmentally sensitive areas. The predominant issue is odour, but at this stage there is no standard method for its measurement with the result that both regulators and piggeries are aiming at an undefined and moving target. There are also problems with the models used for the calculation of nitrogen loading on to agricultural land, as the contribution of ammonia evaporation and denitrification are ill-defined at this stage.

Introduction

Sustainable development in the agricultural industries is an objective of the Australian Government and the National Farmers Federation. Pig meat producers need management systems to ensure that land, water and air environments are not adversely affected (PRDC, 1996). To this end there has been enacted environmental legislation, mainly since 1980, that has made it increasingly difficult for new producers to enter the pig industry and more restrictive and costly for existing producers to continue.

Planning approval

There are, at present, no uniform environmental regulations that cover all states within Australia. Therefore, because each state has a different set of circumstances and hence a different level of tolerance, new investors should be encouraged to examine the regulations of more than one location during the early phase of planning. In New South Wales, for example, piggeries are now required to prepare an Environmental Impact Statement (EIS) if they accommodate more than 200 pigs or 20 breeding sows and are located in one of the following situations:

1. Within 100 metres of a natural water body or wetlands
2. In an area of high water table or highly permeable soil or acid sulphate, sodic or saline soils
3. On land which slopes at more than 6 degrees to the horizontal
4. Within a drinking water catchment
5. On a flood plain or
6. Within 5 km of a residential zone and, in the opinion of the consent authority, having regard to the topography and local meteorological conditions, are likely to significantly affect the amenity of the neighbourhood by reason of noise, odour, traffic or waste.

Even if none of the above apply, an EIS is still required when the piggery is going to accommodate more than 2000 pigs or 200 breeding sows.

At a minimum, the EIS must be advertised for at least one month and any queries or complaints answered to the satisfaction of the local council and state regulatory authorities (e.g., Environmental Protection Authority, National Parks and Wildlife, Department of Agriculture). In NSW, at least, this process can take anywhere between six months and three years before final approval is given. In addition, if it is necessary for the EIS to go before the Land and Environment Court or a commission of enquiry is

convened, then substantial legal fees may be payable. If the pig industry is to expand, yet be part of a sustainable agricultural industry, then a number of issues must be considered.

Sustainable production

There are four principles of ecologically sustainable development which need to be taken into account:

1. The precautionary principle, namely that if there are threats of serious or irreversible environmental damage, lack of full scientific certainty shall not be used as a reason for postponing measures to prevent environmental degradation. Acceptance of this principle acknowledges that the environment is maintained or enhanced for the benefit of future generations.
2. Inter-generational equity, which means that the present generation should ensure that the health, diversity and productivity of the environment is maintained or enhanced for the benefit of future generations.
3. Conservation of biological diversity and ecological integrity.
4. Improved valuation and pricing of environmental resources.

With the above principles in mind, it is necessary to demonstrate, especially in an EIS, that a project proposal is applying all reasonable methods of energy conservation. The proponent must show that they are aware of the potential to generate greenhouse gases and can demonstrate that they have taken steps to minimise the effect. The development must not pollute soil, ground or surface water, disturb neighbours with noise and/or odour, and must not contribute to an increase in flies.

The precautionary principle is perhaps the most difficult to contend with as it leads to uncertainties which can draw associations between any number of possible threats of environmental damage and elements within the proposal. Another problem is the question of what constitutes environmental degradation, which might only have been reported at a molecular level but still must be considered seriously at a macro level. The reference here is not necessarily to regulatory agencies, as it is often consultants who evaluate proposals by either Councils or State Regulatory Authorities.

Piggery operation

Having received approval via the EIS procedure, producers must adopt an approach to pig production that meets with the requirements of a sustainable industry, even if in the short term it appears to influence their own economic viability. A pig production system must operate so that:

1. There is no irreversible change to soil structure as a result of effluent application.
2. There must be a balance of all nutrients applied to the soil in terms of biomass produced, runoff and what remains in the soil. There should be no nutrients from the effluent, particularly N and P, flowing below the root zone. The concern is that once the nutrients pass the root zone they will eventually find their way into the groundwater.
3. A hydraulic balance is completed. The volume of water entering (drinking, washdown, cooling, flushing) and leaving the piggery (effluent) must be measured. The volume of final effluent after treatment in the waste treatment system and going to long term storage must also be measured. It must be demonstrated that the pond system has the capacity to hold all the effluent in the event of one in 20 storm events.

A number of questions arise. Nitrogen and phosphorus both occur in organic and inorganic forms. The latter form is available for plant growth whereas the organic form must decompose before it can be converted to ammonia, nitrate or phosphate. There is an opinion that the organic N can remain in the soil for a number of years and is available for use by plants. Regulatory authorities take the view that the decomposition of organic N is

quite rapid and that any N applied, that does not either evaporate or is removed in runoff, by denitrification or by uptake as biomass, ends up below the root zone in the water table. This has implications in regard to the area of land that must be developed for irrigation.

Another issue is the removal of N, P and K from the system so that there is less to disperse and convert to biomass. In this symposium, Pluske *et al.* (1997) discuss options that are available for reducing the amount of N and P in effluent, and Rate (1997) describes a range of techniques for treating the effluent so that the levels of N and P contamination should be minimal. Attention also needs to be given to the removal of K and Na from effluent material.

Odour

Odour is the key environmental issue with existing and proposed piggeries. Existing piggeries are under threat due to urbanisation of rural zones immediately adjacent to towns. Many councils have local environmental plans which have, as an objective, the protection of the potential of agricultural lands and the prevention of ribbon developments on rural land adjacent to the roads leading into towns. Despite this, settlement is occurring near existing piggeries and there are subsequently objections to odour.

In proposals for new developments, proponents are required to produce an estimate of the odour impact of the development on the closest neighbours. Currently, this is carried out using software packages such as AUSPLUME (Lorimer, 1986). The target in terms of odour to be experienced by the receptor varies between states. There is variability among consultants who provide an odour measurement in regard to the method of collecting samples, the analysis of samples by odour panels, the level at which odour is detected (guess, certainty or identification of the odour), the calculation of ventilation rates for pig sheds and treatment plants, and the extent to which the models have been validated for area sources such as piggeries. In addition, with current technology, it is difficult to model the movement of cold air down gradients at low speeds. These are the conditions that can lead to very poor dispersal and high odour concentrations. Despite all these caveats, regulatory authorities still use odour as the basis for decision making.

There are many treatments which are claimed to control odour. Despite this there has never been a properly controlled evaluation of these technologies based on a standardised odour assessment protocol.

A clash of priorities

A major problem in environmental management is that investors are looking for opportunities for a return on capital investment, usually within a short period of time. Councils are generally keen to attract jobs to their district and there are often flow-on benefits to other nearby agricultural industries (e.g., grain supplies). However, there are those who object to any such development, and they often have the influence, financial support and education to either stop or seriously hamper such developments. Councils are therefore often in a no-win situation. If they do not support the project then the viability of their district may be affected, while if they do give it their support then they may have to argue their case in the Land and Environment Court, at Council and even at State Government Ministerial level.

New developments

When assessing a location as a possible piggery site there are many important considerations, such as proximity to markets and reliable supplies of grain and water, and an adequate supply of good labour. These are, unfortunately, the same factors that determine population density of an area. In terms of getting the proposal to be accepted by Council it is important that neighbours are as far as possible from the proposed site. However, it is difficult to find sites that have an adequate water supply and are sparsely

settled to the extent that there are only one or two neighbours within a 5 km radius. Irrespective of whether it is a new proposal or the continuation of an existing piggery, it is important to establish a network in the local community, the local council, and the relevant State Government Regulatory Authorities. A cooperative relationship should be forged rather than a confrontational one. The local council members, as well as State and Federal members of Parliament, should be aware of the benefit of the piggery to the socio-economic state of the region.

Conclusion

It is going to be increasingly difficult to establish piggeries, because of environmental and community concerns. It is essential to understand the basic regulations and to be careful about how and where a development is planned. Existing piggeries that are not licensed may also have to go through a similar process of review within the next five years to receive formal approval for their operation.

Symposium continued on next page

OPPORTUNITIES AND STRATEGIES TO REDUCE EFFLUENT PRODUCTION BY PIGS

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Abstract

There is increasing concern about the impact that agriculture is having on the environment. As far as the pig industry is concerned, a significant proportion of nutrients that are fed to pigs end up in the effluent. There are two basic options for reducing the amount of effluent produced by pigs. The first is to reduce the oversupply of nutrients being fed, and the second is to improve the efficiency with which nutrients are utilized by the animal. In this paper some of the strategies that currently exist for reducing the output of N and P in piggery waste are discussed. Particular emphasis is given to the use of computer modelling techniques to calculate the nutrient requirement of pigs at various stages of growth, and feeding strategies that make greater use of synthetic amino acids and enzymes.

Introduction

Increasing concern about the impact that agriculture is having on the environment has begun to direct attention towards the amount of nutrients produced by piggeries, and the current and potential strategies that might be available for this to be reduced. With an estimated 60% of N and 80% of P that is ingested being subsequently excreted (Lenis and Jongbloed, 1994), there would appear to be ample scope for improvements to be made. Much of the attention has rightly been focused on the growing-finishing pig, since these contribute 60-70% of the total effluent produced from a typical commercial piggery (Lenis and Jongbloed, 1994). While there are other nutrients, such as Cu, Zn and K, which also have the potential to be of concern in piggery effluent, these will not be discussed as part of this paper. However, similar principles and strategies apply to all nutrients.

There are two basic options for reducing the amount of effluent produced by pigs. The first is to reduce any oversupply of nutrients being fed, and the second is to improve the efficiency with which nutrients are utilized by the animal. The cost, and hence impact on profitability, of both options needs to be taken into account, whilst maximising the quality of the end-product. It is also important to account for the economic value of piggery effluent, and this issue is discussed in detail by Rate (1997).

Nitrogen

The pig requires the amino acids in dietary protein mainly for synthesis of body protein. A proportion of dietary protein is indigestible and this is excreted in the faeces, along with some losses from endogenous sources. However, a much higher proportion of the pigs' N excretion appears in the urine, mainly as a result of an oversupply and/or imbalance of amino acids which cannot be used for body protein deposition (Jongbloed and Lenis, 1992). Urinary N is rapidly degraded to ammonia, whereas faecal N is more resistant to degradation. Coppoolse *et al.* (1990) (cited by Jongbloed and Lenis, 1992) have calculated that of the total intake of N, the average slaughter pig excretes about 20% in the faeces and 50% in the urine. Also, protein digested in the hindgut is excreted almost entirely as urinary N (Lenis, 1989).

A major concern about N pollution is the leaching of nitrate into supplies of drinking water, and N pollution also results in emission of ammonia into the air, contributing to bad odour and to acid rain (Lenis, 1989). In this respect, Lenis (1989) considered N pollution to be of greater significance than that of P.

Calculation of N requirements

Dietary allowances for pigs are commonly set according to the requirements of those individuals within a population that have the highest genetic potential, or to the most demanding period within a particular physiological stage (Henry and Dourmad, 1993). The amino acid requirements for one herd are also often extrapolated to other herds, even though there are subtle differences in environment, genotype or management that can influence a pig's requirement for dietary amino acids. In addition, a wide safety margin is often allowed to account for fluctuations in feed quality. The combined effect of these adjustments and assumptions explain why the supply of N to pigs was, and in many cases still is, far in excess of the animal's real needs.

Computer modelling techniques have been used extensively in recent years to calculate the optimum amino acid requirement for animals in commercial piggeries. Simulation models, such as those developed by Whittemore (1983), Black *et al.* (1986) and Moughan *et al.* (1987), can be used to calculate the amino acid requirements of pigs, of different genetic potentials and live weights, under a range of environmental and physiological conditions. Others (Lenis and Jongbloed, 1994; Henry and Dourmad, 1993) have developed models to look specifically at the issue of N production in effluent.

Smits and Mullan (1996) give several examples of how diet specifications for commercial piggeries in Australia have been re-calculated using the AUSPIG computer simulation model (Black *et al.*, 1986). According to this report, it is not uncommon for diets to be over-formulated by 30% or more, and the re-calculation of those requirements has meant a major reduction in feed costs without any adverse effect on animal performance. Many producers have the potential to reduce the N content of effluent from their piggeries, and at the same time possibly increase profitability, by having their diet specifications calculated using this technology.

Feed quality

Protein digestibility is highly variable among feedstuffs, or even within samples of the same feedstuff, which leads to significant variations in faecal N excretion (Gatel, 1993). Some of this variability is related to the protein itself (quality and quantity), and some is dependent upon other components of the diet (e.g., occurrence of anti-nutritional factors, fibre content). The measurement of the quality of feed ingredients has been well reviewed by van Barneveld (1997). Developments in this area of research will help to reduce the uncertainty in preparing animal diets to specification, and will complement the above mentioned approach to calculating a pig's requirement for dietary amino acids. All of the above will help lower the N content of piggery effluent. However, if higher quality ingredients are to be used in pig diets in an attempt to reduce the nutrient content in effluent, then will there be a surplus of lower quality feedstuffs and by-products that will create an environmental problem of their own?

Feeding strategies to reduce the N in effluent

In a comparison of some of the options available for reducing the N content in effluent, Edwards (1996) concluded that the approach of improving the balance and supply of amino acids was a more cost-effective approach than was treating the waste material. A number of strategies have been investigated.

Phase feeding

An animal's requirement for amino acids is continually changing because it is a function of body weight. Therefore, as an animal grows, the concentration of dietary amino acids can be progressively lowered. Traditionally, up to three different diets have been fed to pigs from the time of weaning until they are slaughtered at about 100 kg live weight (LW). There is now considerable interest in using an approach called phase feeding, whereby a greater number of diets (e.g., five) are fed to a pig from weaning until sale. Each diet is progressively lower in its content of amino acids and, therefore, more closely matches the requirements of the animal being fed. Dourmad *et al.* (1992) have

calculated that the adoption of a phase-feeding strategy during the grower period should result in a 15-20% reduction in the N content of effluent. These findings are supported by calculations made by Lenis and Jongbloed (1994).

A similar approach has been taken with sow diets. According to Lenis (1989), until recently in The Netherlands pregnant and lactating sows were fed the same type of diet which was formulated for lactation. The requirements of the pregnant sow are much lower than those during lactation, and the adoption of a two-feed system was estimated to reduce N excretion from 21 to 16 kg per sow per year, a reduction of about 25%.

Blend feeding

A further advancement of phase feeding is called blend feeding, in which the diet could be changed on a weekly, or even daily, basis by mixing together, in various ratios, diets of different composition. In this way, a large range of diets (e.g., 12 from weaning until slaughter) can be prepared from a smaller number of base feeds. Using two diets, which were blended on a weekly basis, Mullan *et al.* (1997) found significant reductions in N intake (20%) and urinary excretion of N (25%) when pigs were blend fed in comparison to being fed a single diet during the growing period. There was no effect of reducing N intake on performance. The cost of more complex feed delivery systems, and the need to have a small weight range within the group of pigs being fed, obviously needs to be taken into account.

Use of synthetic amino acids

Another approach to reduce ultimately the excretion of N is to reduce the crude protein content of the diet by adding synthetic amino acids, while maintaining a balanced supply of essential amino acids. The increasing range of synthetic amino acids that are available at a cost-effective price, could make this an attractive proposition for piggeries located in environmentally sensitive areas. Kerr (1987) for example, formulated diets to either 16% crude protein or 12% crude protein, with the lower crude protein diet containing a number of synthetic amino acids. Total N intake was reduced from 25-19 g/d when the lower crude protein diet was fed and, although faecal N was unaltered, there was more than a 50% reduction in the output of urinary N. Similarly, Gatel and Grosjean (1992) decreased dietary crude protein from 17.0-15.5% in the growing period, and from 14.5-13.5% during the finishing period, by adding more synthetic amino acids, and reported a 15-20% reduction in N excretion.

In a laboratory study conducted by Turner *et al.* (1996), the ammonia emission from a simulated manure pit was measured. In this study, reducing the crude protein content of the diet for grower-finisher pigs from 16-12%, whilst maintaining the balance of essential amino acids by the use of synthetic amino acids, reduced the level of ammonia emissions from piggery effluent by 80%. In another study, Latimier and Dourmad (1993) concluded that improving the amino-acid balance, and hence lowering the crude protein content of the diet, was an efficient way to decrease both N output in the slurry (by 23%) and gaseous N emission from the building (by 25%). Therefore, the potential to reduce N excretion by increasing the biological value of pig diets has been well proven, but the adoption of this approach will depend on how it can be applied in commercial practice and the impact it has on profitability.

Computer modelling to reduce N excretion

It is difficult to measure the N excretion from a commercial piggery, and in this respect simulation models can be used to predict the effect on performance, profitability and N excretion before or following a change in feeding strategy. Black (personal communication) has used AUSPIG (Black *et al.*, 1986) to calculate the amino acid requirements of pigs between 50-100 kg LW. Compared to the existing commercial diet (Control), feeding a diet (AUSPIG) that was formulated to supply 103% of the requirement for amino acids of the pig at 55 kg LW reduced feed costs by $8 per tonne, reduced total N excretion by 17%, and increased profit by $0.60 per pig (Table 1). When another four diets were formulated (Phase-1) to reflect a phase-feeding system, diet costs were progressively reduced and profit per pig increased by $2.00 as compared to the

existing commercial diet. It was possible to reduce the dietary crude protein content of the diets further by the addition of synthetic amino acids (Phase-2) without any effect on animal performance. However, the further reduction in the N content of the effluent would need to be balanced against the extra cost of these particular diets.

Table 1. Predicted profitability and N excretion of male pigs grown from 50-100 kg LW when offered a range of diets differing in crude protein content.

	Control	AUSPIG	Phase-1	Phase-2
Dietary protein %				
diet 1	21.8	18.6	18.5	15.3
diet 2			18.2	15.0
diet 3			17.8	14.5
diet 4			17.2	13.5
diet 5			16.6	11.8
Feed cost ($/pig)	35.23	35.62	34.40	37.91
Change in profit ($/pig)	0	+$0.60	+$2.02	-$2.70
Total N intake (g/d)	73.7	64.1	60.4	45.2
Total N excretion (g/d)	51.4	41.7	38.1	22.8
N excretion as % of Control	100	83	75	44

Phosphorus

Phosphorus is an essential element in animal diets, being vital for the development and maintenance of skeletal tissue, and has an important role in many biochemical and metabolic functions. The amounts of P available to the pig from feedstuffs of plant origin are insufficient to satisfy the pigs' requirement for P for adequate growth and skeletal development, so it is necessary to add inorganic P to most pig diets. This is because 60-90% of the total P in plant feedstuffs is in the form of phytic acid or phytate-P.

Phytic acid (phytate) is an anti-nutritional factor present in all feedstuffs of plant origin as a variety of poorly-soluble, predominately Ca and Mg salts. Phytates are poorly digested because pigs lack the phosphatase enzyme required to cleave the phosphate groups from the phytin molecule (Cromwell *et al.*, 1993). Phytate is the name given to the phosphoric acid ester of the cyclic alcohol inositol (myo-inositol hexakisphosphate). The structure and chemistry of phytic acid is described comprehensively in a review by Reddy *et al.* (1982). In plant seeds, phytic acid is a reservoir for P, as P constitutes 28% of the molecular weight of phytic acid. Phytic acid carries up to 12 negative charges and has a tremendous chelating potential to combine with positively-charged nutrients, including Ca and trace minerals. Phytate-mineral complexes such as these are unavailable for absorption. Similarly, phytic acid can combine with amino acids such as lysine and arginine to form protein-phytate complexes which reduces the digestibility of bound protein, together with possible binding to endogenous digestive enzymes (e.g., Honig and Wolf, 1991; Caldwell, 1992).

The concentration of P in plant material in the form of phytic acid varies considerably, ranging from 0.5-1.9% in cereals (except polished rice), 0.4-2.1% in legumes, 2.0-5.2% in oilseeds (except soya bean meal), and 0.4-7.5% in protein products (e.g., wheat gluten, soya protein concentrate) (Reddy *et al.*, 1982). Although feedstuffs of vegetable origin contain adequate amounts of P, only 20-50% is digestible by pigs. To counteract the low availability of phytate-P, animal by-products (e.g., meat and bone meal) and mineral (inorganic) P compounds (e.g., dicalcium phosphate), both of which have a high concentration of P and a high availability (70-90%), are commonly added to pig diets (Jongbloed and Kemme, 1990). However, the continued supplementation of pig diets with inorganic P, together with indigestible phytate, ultimately means that there is an increase in the amount of P excreted and hence present in piggery effluent.

Calculation of P requirements

The most logical way to reduce the amount of P excreted by the pig is to supply P in better agreement with the pig's requirement. As a result of anti-pollution legislation and codes of recommended practice in various parts of Europe, feed manufacturers and pig producers have considered the mineral content of pig diets. In The Netherlands, for example, pig diets have had their P content reduced to about one-half of the previously recommended levels. In order to comply with output quotas for P, diets containing as little as 3 g/kg digestible P for growing pigs, and 2 g/kg for finishing pigs, have been proposed. In contrast, the conventional requirement in the UK is about 8 g P/kg diet dry matter, with an assumed digestibility of 80% for inorganic and 50% for organic P (Whittemore and Manson, 1995).

While reductions in dietary mineral supply are clearly beneficial from a pollution perspective, research needs to be conducted to ensure that any recommended reductions in requirements do not compromise the health, well-being and performance of the pig. This requires a better knowledge of the supply of P in the feedstuffs used routinely in pig production, especially in regard to the digestibility and availability of P to the pig rather than just using the level of "total" P in a diet. Furthermore, a better knowledge of the animal's requirement at its different stages of growth needs to be known, and the factorial method of calculating the requirements for P has been used to do this (see Jongbloed *et al.*, 1991). Similarly, split-sex feeding (i.e., where female and male pigs receive different diets to account for genetic differences in nutrient requirements) is a strategy which can also be used effectively to reduce P excretion.

Feeding strategies to reduce the P in effluent

As with N, there needs to be better agreement between supply and requirement of P to the pig, and one strategy to ensure this is phase feeding. Phase feeding is a strategy used by a number of pig producers at present, and should be encouraged as a cost-effective means of reducing P excretion from pig herds. In the simplest instance, the use of a grower diet from 45-70 kg LW and a finisher diet from 70-106 kg LW instead of a single diet over the same weight range reduced P excretion by 6% (Coppoolse *et al.*, 1990; cited by Jongbloed and Lenis, 1992). Although Mullan *et al.* (1997) did not measure P intake and excretion in their blend-feeding experiments, it can be assumed that P intake was also reduced with a concomitant reduction in P excretion. In a similar vein, a slightly bigger reduction in P excretion by growing pigs has been reported by mixing a feed rich in minerals and vitamins with a feed having a low concentration of protein and minerals in a changing ratio (multi-phase feeding). This results in the supply of P being brought closer to the pig's actual requirements (Jongbloed and Lenis, 1992).

Use of liquid feeding systems to increase P availability

The use of liquid-feeding systems in conjunction with added phytase offers a means of improving P digestibility due to an increase in contact time between the enzyme and the substrate. Most naturally occurring phytases have a pH optimum between 5.0 and 5.6 (Reddy *et al.*, 1982), and Séguier *et al.* (cited by Brooks *et al.*, 1996) found that maintaining a mixture of feed, water and exogenous phytase at pH 5.4 improved phytate hydrolysis. More recently, Geary (unpublished, cited by Brooks *et al.*, 1996) soaked wheat and soya bean meal in water alone, or water plus phytase, at a temperature of 50°C, and followed the release of inorganic P at 24 h and 48 h after steeping commenced. Geary (unpublished, cited by Brooks *et al.*, 1996) reported significant increases in the concentration of soluble P from raw materials such as soya bean meal which possess little or no endogenous phytase. Even in materials such as wheat, which possess relatively high levels of endogenous phytase, there is considerable release of inorganic P when the grain is soaked in water (Figure 1).

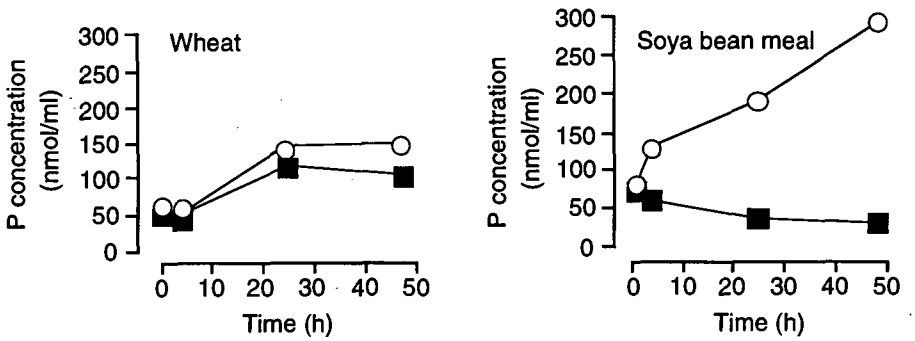

Figure 1. *The effect of steeping wheat and soya bean meal in water* (■) *or water plus phytase* (○) *on the concentration of soluble phosphorus in the liquid medium kept at 50°C (Geary unpublished; cited by Brooks et al., 1996).*

These data demonstrate that in a liquid medium where microbial fermentation is present, the heat produced by fermentation is capable of stimulating the catalytic reactions of enzymes. This may reduce the costs associated with having to heat the liquid feed to the optimum temperature for enzyme activity. A system such as this may be even more advantageous if the diet could be maintained in the liquid medium in contact with phytase over a considerable length of time, say 12-24 h before feeding.

Improving the utilisation of nutrients by pigs

Besides ensuring that the supply of nutrients to the animal closely matches their requirement, the other approach can be to improve the efficiency with which the pig can utilise those nutrients. Much attention has been given to the use of feed enzymes, but alternatively others have promoted the idea that improving the potential of the animal to deposit body protein would have beneficial effects on reducing the N content of effluent.

Enzymes

Digestive enzymes hydrolyse components of feedstuffs, however, in many circumstances the pig's natural enzyme levels are too low or the requisite enzymes are missing (Easter *et al.*, 1993). This, therefore, makes it possible that the use of exogenous dietary enzymes could improve digestibility, particularly of N-rich proteins. To date the use of feed enzymes has not given consistently positive results, but further developments are likely to give improvements in N digestibility. Most research and development has concentrated on using enzymes to increase P digestibility.

The use of phytase

Undoubtedly, the major advance in recent years to increase P digestibility and reduce P excretion is the use of microbially-derived phytase. A large number of studies conducted in a variety of countries have shown that the addition of microbial phytase to diets causes an increase in the digestibility of P and a decrease in P excretion. For example, in an experiment conducted in Australia involving 640 weaner pigs, Campbell *et al.* (1995) investigated the interrelationship between the concentration of available P (0.15, 0.25, 0.35 and 0.45%) and phytase supplementation (0 or 100 g/tonne) on pig performance from 19 d (when pigs weighed 11.1 kg) to 40 d after weaning (Table 2). The diets contained wheat, lupin kernels, rice pollard, canola meal and soya bean meal, and had an estimated phytate-P content of 0.35% or 1.25% phytic acid. Pigs offered the diets supplemented with phytase showed superior performance to pigs offered the corresponding diets without phytase. Pigs offered diets with phytase also had equal or better performance when offered the diets containing 0.25% available P than their counterparts offered the unsupplemented diets containing higher levels of available P. These data suggest that phytase improves pig performance independent of its effect on

the availability of P in the diet, which may in part be explained by improvements in the availability of other minerals such as Zn and Mg (Adeola, 1995; Adeola *et al.*, 1995).

Table 2. Effects of dietary available P and phytase supplementation on the performance of pigs from 19-40 d after weaning (from Campbell *et al.*, 1995).

Phytase (g/tonne)	Available P (%)	Daily gain (g)	Feed intake (g/day)	Feed:gain (g feed:g gain)
0	0.15	403	640	1.58
	0.25	481	720	1.48
	0.35	530	800	1.51
	0.45	540	820	1.55
100	0.15	472	740	1.56
	0.25	540	770	1.42
	0.35	629	850	1.40
	0.45	595	820	1.38
Significance (*P*):				
Phytase		0.004	0.175	0.093
Available P		0.001	0.133	0.307
Phytase x Av. P		0.939	0.790	0.739

In another experiment conducted in Western Australia to measure apparent P retention, Mullan *et al.* (1994) fed female pigs (45 kg LW) either a standard grower diet containing 0.40% total P (Control) or a diet formulated to contain the same energy and amino acids level but only 0.32% total P (Low-P). A third group (Low-P + Phytase) received the low-P diet plus the phytase enzyme Natuphos® added at 200 g/tonne (3850 U/g). Pigs were fed at 3 x maintenance and digestibility of phosphorus was determined over a 7-day collection period following 12 d of acclimatization. The digestibility of P was increased when pigs were fed the low-P diets and this was further enhanced by the use of phytase (Table 3). Similar improvements in the digestibility of P using low-P diets were reported in an earlier study conducted in Western Australia by Godfrey *et al.* (1993), although these authors reported higher rates of P retention. It appears that differences between the two studies may be accounted for by differences in the Ca:P ratio used, as there is some suggestion that the Ca:P ratio is important in determining the response to added phytase (Qian *et al.*, 1996). Based on the data of Mullan *et al.* (1994), the addition of phytase to a diet containing a low concentration of P would cause a reduction in total P excretion of 56% from the grower/finisher herd. For the piggery where this study was conducted (approximately 41,000 pigs sold/year), this represented a reduction in P excretion from 21.8 t/year to 14.0 t/year.

Table 3. Effects of feeding diets differing in P concentration and addition of phytase on P digestibility (from Mullan *et al.*, 1994).

	Treatment			
Item	Control	Low-P	Low-P + Phytase	SED
No. of pigs	6	7	6	
P intake (g/d)	7.9^{a}	6.3^{b}	6.7^{b}	0.48
P output (g/d)	5.0^{x}	3.2^{y}	3.1^{y}	0.33
P retention (g/d)	2.9	3.2	3.6	0.40
P digestibility (%)	38.1^{a}	48.1^{b}	53.3^{c}	3.81

Values in the same row with different superscripts differ significantly ($^{a,b,c}P<0.01$; $^{x,y}P<0.001$).

Improved animal efficiency

A number of technologies (e.g. β-agonists, porcine somatotrophin (pST)) are available that will increase the potential of the animal to deposit body protein. Some researchers have suggested this as an approach to reduce the nutrient load in piggery effluent. For example, Quiniou *et al.* (1993) reported that when pigs between 51-101 kg LW were treated with pST, there was a significant improvement in growth rate, muscle growth and N retention without apparently increasing the daily protein requirements. As a consequence, N output in urine was reduced by 25% while faecal N remained unchanged. Other research would indicate that the daily protein requirements are increased when pigs receive pST (Dunshea and Walton, 1995), and it would seem likely that there would be a concomitant decrease in N excretion. Anything that reduces the difference between supply and demand for dietary amino acids, has the potential to reduce N output.

Apart from their efficacy as a growth promotant, β-adrenergic agonists may contribute to an important reduction in N excretion in piggery effluent. In reviewing the results of several experiments, Easter *et al.* (1993) reported that their use with pigs from 60-100 kg LW resulted in a 19% increase in N deposition and a 9% increase in N retention. This was estimated to be equivalent to a reduction in annual waste N production of about 760 kg for a commercial unit producing 5,000 pigs (100 kg LW) per year. The same author has made calculations on the potential benefits to reducing N excretion by the use of probiotics or antibiotics. For example, as a result of improvements in feed conversion efficiency, it may be possible to reduce protein consumption without influencing performance. Similarly, it has been suggested that an effect of dietary antibiotics is to increase the digestibility of N and amino acids in the small intestine, but it is unlikely for this to be accepted as an environmentally friendly approach to reduce N output.

There are no accurate figures available on the extent of feed wastage in commercial piggeries, although estimates range between 5-30%. This could therefore be a major contributor to total effluent production, and one that in many instances could be improved through better management. Increasing the number of pigs reared per sow per year will also help lower effluent production from a piggery, although the magnitude of change is likely to be small. Finally, anything that will help a pig grow more efficiently, such as improvements in the animal's environment and/or its health status, will reduce N output to some extent.

Conclusion

A high proportion of nutrients that are fed to pigs end up in effluent. The development of computer models has played a major role in improving our understanding of the animal's requirement for nutrients, especially N. As a consequence, the N content in the diet, and subsequently in effluent, is being reduced without any change in animal performance. With P, the use of the phytase enzyme to improve the availability of P in ingredients has meant that the P content of diets can be reduced, again without a decrease in growth rate. Improving efficiency, especially by reducing feed wastage, will also help reduce effluent production. Such strategies will become commonplace as the pressure to reduce effluent production from piggeries increases.

Symposium continued on next page

MANAGEMENT AND RE-USE OF PIGGERY EFFLUENT

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Abstract

Intensive piggeries produce substantial quantities of wastes which can be re-cycled to supply nutrients for crops and pastures. There is, however, the potential for adverse effects on the environment if this practice is not managed correctly. In this paper the management of wastes from piggeries is discussed, with a focus on the fate of N and P following land application of piggery wastes. The origins and properties of wastes are considered briefly with implications both for the use of piggery wastes as fertilizer substitutes, and for their potential environmental impact. The fates of N and P following application of piggery wastes to land are considered in detail. The nutrient cycles for N and P from piggery waste into the plant-soil system are presented, with reference to transformation and loss for each element from the system. The transformations of the components in each cycle and the factors that affect them are shown to be strongly interactive and interdependent. The fate and behaviour, in the plant-soil system, of potentially toxic elements (Cu and Zn) derived from piggery wastes are discussed briefly.

Introduction

The appropriate use of wastes is a significant issue for the pig industry in Australia and world wide due to increasing pressure from regulatory authorities to protect the environment. Piggeries are readily identifiable point sources of wastewater and/or solid waste, and as such are possibly more vulnerable to community pressures than more extensive agricultural operations.

The major concerns with the re-use or disposal of piggery waste are related to the nutrient element content of the materials produced, coupled with the potential for waste-derived fluxes of nutrients (particularly N and P) to enter the environment at a rate exceeding an ecosystem's assimilatory capacity. Considerable attention has been directed towards the potential for pollution and eutrophication of surface and ground waters as a result of N and P inputs derived from organic waste materials, including those from piggeries. Beneficial re-use of piggery wastes, however, is a viable option for productive agriculture and horticulture, given that nutrient input fluxes can be matched closely with plant demand to avoid the risk of nutrient export through processes such as leaching, runoff and atmospheric emissions.

In this paper an attempt is made to review the properties of wastes, the options for waste management, and the nutrient transformations and fluxes which occur following land application of piggery wastes with a particular focus on the fate of N and P.

Definitions and properties of piggery wastes

Origins

Piggery wastes are defined as pig excreta (faeces and urine), plus feed wastage, and residual detergents and disinfectants which are generated from intensively housed pigs. This waste is commonly diluted with wash-down water, leading to a liquid waste. Alternatively, the waste may include bedding material, for example in deep-litter or tunnel-house management systems where pigs excrete directly on to relatively large amounts of bedding material (e.g., sawdust, straw) which provides a substrate for composting.

Amounts of waste produced by piggeries

Housed pigs may produce between 3-10 l of urine and 0.2-0.3 kg dry solids per d per 40 kg animal. For the same 40 kg animals, urine + faeces contains approximately 20-25 g N and approximately 7.5 g P per pig per day (Vanderholm, 1985; ASAE, 1993). Estimates of the amounts of piggery wastes produced annually in Australia appear in Table 4.

Table 4. Annual waste production from intensively-housed animals in Australia (ABS, 1996).

	Animal numbers	Dry matter per animal (kg/y)	Total dry matter (t/y)	N (t/y)	P (t/y)
Pigs	2,646,000	250	662,000	29,000	17,000
Poultry	65,586,000	15	984,000	52,000	34,000

Piggery waste composition

The chemical and physical composition of raw piggery wastes is very dependent on the characteristics of the waste collection system used (e.g., amount of wash down water; use of recycled water for flushing; pigs on deep litter). It is also strongly influenced by the composition of feed (Kruger *et al.*, 1995), such as the presence of phytase which increases the digestibility of P and decreases P levels in effluent (Pluske *et al.*, 1997). Some examples of chemical and physical properties of fresh liquid piggery wastes are presented in Table 5; it should be noted that most published studies do not describe waste collection or storage systems in any detail. In terms of the fate of N, the most relevant properties of these wastes are the significant N content, the high proportion of ammonium N, and the low C:N ratio, all factors expected to lead to rapid release of available N.

Table 5. Some examples of chemical and physical compositions of liquid piggery effluents from different sources.

	Source of data		
Property	New Zealand[1,2]	Spain[3]	Australia[4]
Type of waste	Shed flushings, unscreened, fresh	Fresh slurry	Liquid manure from farm dam
Total C (mg/kg)	820	2000-24500	-
Total N (mg/kg)	1352-1628	1310-5210	2500
Total P (mg/kg)	150-440	30-730	73
Total K (mg/kg)	-	1100-3300	812
C:N ratio	0.58	1.53-6.96	-
NH_4^+-N (mg/kg)	746-1200	1100-3810	-
NO_3^--N (mg/kg)	0	-	-
NH_4^+/total N (%)	55-85	45-84	-
Cu (mg/kg)	-	43	1.4
Zn (mg/kg)	-	29	1.1
pH	7.4-7.5	7.7-8.3	-
EC[5] (µS/cm)	-	7510-21100	-
Dry matter (%)	1.4	0.76-6.88	-

[1]Cameron *et al.* (1995). [2]Carey *et al.* (1997). [3]Bernal and Roig (1993). [4]Brechin and McDonald (1994). [5]Electrical conductivity.

Fertilizer value

The significant concentrations of N, P and K in piggery wastes contributes to their ability to supply these nutrients to plants (Van Faassen and Van Dijk, 1987; Smith and Van Dijk, 1987). Fertilizer value can be calculated from the net savings in conventional fertilizer achieved by applying wastes to land. Efficiency is limited from a plant nutrition standpoint in that a particular waste will contain nutrients in proportions which differ from those required by plants. Management options vary between applying sufficient waste to completely supply the most limiting nutrient (inefficient in terms of the higher amount of waste required and potential for oversupply and subsequent losses of nutrients) or applying waste at a rate which supplies sufficient of the least limiting nutrient and supplementing with conventional fertilizers. The second option reduces the net value of the effluent as a fertilizer but is a more environmentally sound practice.

Options for management and re-use of piggery effluents

The most rudimentary method for managing wastes from intensive livestock operations such as piggeries is direct, uncontrolled discharge to land or to a water body. The environmental consequences of direct discharge of organic wastes are well-documented with respect to sewage outfalls, and include pollution with pathogens, eutrophication from excessive nutrient inputs, induced anoxia from high biochemical oxygen demand (BOD), excessive turbidity, salinity, and odour (Cameron *et al.*, 1996a).

Pond treatment

Pond treatment of nutrient-rich wastewaters such as piggery effluents utilizes one or more ponds in sequence which rely on different (anaerobic or aerobic) microbially mediated or physicochemical processes to remove nutrient elements (Dakers *et al.*, 1985). Anaerobiosis is maintained by high BOD in waste streams and sufficient depth of water to allow stratification of the water-sediment column. Anaerobic pond treatment removes N by breakdown of nitrogenous organic material and volatilization of ammonia (Shilton, 1996); C is lost by organic matter decomposition as carbon dioxide and/or methane (Dakers *et al.*, 1985). Phosphorus and K removal is effected by precipitation and sedimentation processes and both these elements, therefore, accumulate in pond sludges.

Aerobic, or oxidation, ponds are shallow to allow convection- and wind-induced aeration; N removal is by nitrification - denitrification, since the systems are unlikely to maintain completely aerobic conditions. A variety of methods have been proposed for increasing aeration efficiency including mechanical aeration (Vanderholm and Warburton, 1985), and the use of biological trickle filters (Boiran *et al.*, 1996). Phosphorus and K depletion occurs by similar precipitation and sedimentation processes to those which occur in anaerobic ponds, and aerobic ponds are commonly used in sequence following anaerobic pond treatment. Although both types of treatment pond are often considered to be self-sealing (Dakers *et al.*, 1985), this may not be true for ponds constructed on sandy soils (Hills, 1976). Leakage from soil beneath ponds may be a significant loss mechanism for N (Dakers *et al.*, 1985); Huffman and Westerman (1995) reported that pond construction materials had the greatest influence on the severity of N losses as a result of pond leakage.

Pond systems have the advantage of allowing discharge to water bodies (given that they perform correctly) or the re-use of waste water for shed flushing, although treated waste-water may also lead to contamination (Cameron *et al.*, 1996a) if the treated waste-water stream has higher contaminant concentrations than any water it enters. Incorrect pond construction or management, however, may lead to substantial pollution of surface and ground waters, and the intention of the design of pond systems to remove nutrients means that the fertilizer value of the wastes is underutilized or ignored. Waste water from anaerobic or aerobic ponds can be applied to land, as an alternative to secondary or tertiary treatment or discharge to waterways, and this is a common practice for Australian piggeries.

Anaerobic digestion

Also known as "biogas production", anaerobic digestion involves the decomposition of organic waste materials in the absence of oxygen by methane-forming bacteria (Dakers *et al.*, 1985). Gas evolved during the decomposition process (mainly methane and carbon dioxide) is a potentially useful energy source and is commonly termed "biogas". Anaerobic digestion is not generally considered to remove nutrients, and therefore the disposal of digested waste is subject to similar problems to untreated material, and it may be treated further (Yang and Chen, 1994) or applied to land (Pain *et al.*, 1990). Anaerobic digestion of pig slurry may require addition of a carbon source (e.g., straw) to optimize methane production (Masciandaro *et al.*, 1994).

Land application

Land application is taken to mean application of raw or partially treated piggery wastes to land in a manner that accounts for the capability of the land to assimilate water and nutrients applied in the waste. Application of liquid wastes by spray, flood irrigation or tanker, or solid wastes (e.g., separated solids, pond sludges) fall under this description. Land application has advantages in that a high proportion of waste nutrients are potentially available to replace conventional fertilizers. The potential drawbacks of land application are the risk of pollution and/or stock health problems due to excessive or poorly timed applications (Cameron *et al.*, 1996a), the high initial infrastructure cost for some (e.g., sprinkler) systems, and the possibility that disposal may be limited to certain times of the year (Giffney, 1985).

Beneficial removal of nutrients by land application is by plant uptake and various retention processes in soils including microbial immobilization, cation exchange, adsorption and precipitation. Nutrient losses following land application of wastes include ammonia volatilization, denitrification, leaching and runoff; the environmental impact of losses to water has probably received greater emphasis than atmospheric losses, which are intentional in some cases. The fate of N and P following land application of piggery wastes will be considered in greater detail in later sections of this paper.

Composting

Composting is the process by which waste materials are stabilized by digestion at low moisture contents through the action of thermophilic aerobic bacteria (Vanderholm and Warburton, 1985). The bacteria present in the waste and/or carbon source have been found sufficient to facilitate composting without inoculation (Tam, 1995).

There are two broad objectives of composting; firstly to convert raw waste into a product with better handling properties and without potential health hazards. Secondly, to produce soil amendments that release nutrients at rates which more closely match plant requirements and have no adverse effects on plant growth or soil quality (Vanderholm and Warburton, 1985; Harada *et al.*, 1993). Composting may utilize either liquid piggery effluents (Bernal *et al.*, 1996) or separated solids (Bhamidimarri and Pandey, 1996), but the C to N ratios of both waste fractions are low, meaning that an additional carbon source (e.g., straw, sawdust or green waste) is required for successful composting. Addition of material as a carbon source also fulfils a function of compost aeration, especially for static-pile systems (Bhamidimarri and Pandey, 1996; Forshell, 1993), and may also provide thermal insulation (Georgocakis *et al.*, 1996). Alternatively, piggery wastes and a carbon source may be combined in deep litter systems (Tam, 1995; Chan *et al.*, 1994) and composting of deep litter/waste mixtures may proceed *in-situ* or following removal from the piggery. Maintenance of water content of composts at approximately 50-60% is also critical (Vanderholm and Warburton, 1985; Chan *et al.*, 1994) with higher water contents being found less than optimal for composting due to development of anaerobic conditions (Tiquia *et al.*, 1996b).

The environmental impact of composts used as soil amendments is related to their ability to release nutrients at rates more closely matched to or lower than plant demand.

This is particularly relevant for N; composting of piggery wastes has been found to decrease water-soluble N species such as nitrate (Chan *et al.*, 1994). Gethin *et al.* (1996) showed that application of composted piggery waste to land did not increase nitrate and phosphate leaching losses. The decrease in N availability is caused by an increase in the proportion of organic N relative to other forms (Bernal *et al.*, 1996; Chan *et al.*, 1994), and is possibly related to a shift towards N being present in organic compounds which do not decompose readily (Govi *et al.*, 1995). Nitrogen losses can occur during composting of piggery wastes (Bhamidimarri and Pandey, 1996) mainly by ammonia volatilisation (Chan *et al.*, 1994); denitrification may also lead to significant losses of N (Burton *et al.*, 1993; Groenstein and Van Faassen, 1996). The final N content may actually increase relative to the uncomposted mixture due to losses of organic carbon following microbial respiration (Bernal *et al.*, 1996). Phosphorus and K are conserved in the composting process (Chan *et al.*, 1994; Bhamidimarri and Pandey, 1996).

Composting of piggery wastes can also decrease the amounts and/or availability of phytotoxic waste components such as ammonia or soluble Cu which inhibit plant germination (Tiquia *et al.*, 1996a).

Constructed wetlands

Chemical, physical and biological processes in natural wetlands (e.g., precipitation, adsorption, volatilisation, sedimentation, filtering, immobilisation and denitrification) are important in nutrient removal from wetland systems (Mitsch and Gosselink, 1993). Similar processes may be exploited for treatment of piggery and other wastewaters (Hunt *et al.*, 1994), especially those which have undergone primary or more advanced treatment by other methods.

The simultaneous existence of both anaerobic and aerobic zones in wetland environments is considered to provide conditions necessary for N removal. Aerobic conditions facilitate nitrification of the predominantly ammonium-N content of wastewaters, and denitrification occurs in zones of reducing conditions. In some cases, however (e.g., Reaves *et al.*, 1995) N removal is low (approximately 30%) due to the predominance of anaerobic conditions. Phosphorus removal is generally considered to be by adsorption on wetland soil or precipitation. Szogi *et al.* (1995) observed a decrease in P removal from piggery effluent by a constructed wetland as the wetland soil became saturated with phosphate. Phosphorus removal may be more sustainable if other components entering the system, such as Fe, react to yield insoluble forms of P as observed by Cooke *et al.* (1992) in a natural wetland used for disposal of treated sewage wastewater.

Nutrient uptake by wetland plants is also an important removal mechanism for N and P; plants (e.g., rice) may be harvested for human consumption (Hunt *et al.*, 1994) or for subsequent composting.

Environmental issues associated with land application of piggery effluents

Water and soil quality

The leaching of nitrate into groundwater is of concern because of the frequent use of groundwater for human consumption (more than 60% in Australia; LWRRDC, 1996) and the known adverse effects of excessive nitrate consumption such as methaemoglobinaemia or gastric disorders (Mirvish, 1991). The entry, by leaching or runoff, of N into surface waters is also of concern from the point of view of eutrophication (Keeney, 1982), although eutrophication is more commonly associated with excessive P inputs (Cullen, 1996). Loss of N and P into water bodies also represents an agronomic loss, providing further incentive for its minimization. Application of animal wastes to land can also result in leaching of bacteria (e.g., faecal coliforms) to groundwater, especially during intense rainfall events (Rate *et al.*, 1994). The physical properties of soil may be adversely affected by liquid animal effluent applications; for example, plugging of soil pores and subsequent development of anaerobic conditions in temperate climates (Cornforth, 1973).

Atmospheric emissions

Volatilization of ammonia can occur at most stages during the collection, treatment and re-use or disposal of effluents (Jarvis *et al.*, 1987). Atmospheric ammonia is linked to acid precipitation (Haynes and Sherlock, 1986) and odour emissions (Pain *et al.*, 1990). Denitrification may be enhanced following land application of piggery wastes (Cameron *et al.*, 1995) and a proportion of N lost by denitrification is as nitrous oxide, which is known to be both an ozone-depleting and a greenhouse gas (Haynes and Sherlock, 1986). Gaseous emissions have, in some cases, been considered favourably in terms of reducing final N loadings, but the potential for atmospheric pollution may alter this attitude.

Potentially toxic elements

Copper and Zn are commonly added to pig feeds as growth promoters (Cooke, 1981; Kruger *et al.*, 1995), and these elements are known to be present in piggery wastes (see Table 5). While both these elements are essential micronutrients for plants, microorganisms and higher animals (Clark, 1979), both Cu and Zn can be toxic to organisms when present at higher concentrations (Alloway, 1995). In general, the more toxic trace elements such as As, Cd, Cr or Pb are not found in significant concentrations in piggery wastes (Wong, 1985; Kruger *et al.*, 1995).

Nitrogen cycling following land application of piggery effluents

Relevant chemistry and biology and transformations of N in soils

The most abundant forms of N in piggery effluents are organic N (comprising a range of organic compounds such as urea and proteins) and ammonium-N. Nitrate is often observed to be absent from pig manures or liquid effluents (Cameron *et al.*, 1995; Bernal and Kirchmann, 1992) and urea has been observed in trace amounts (Bernal and Kirchmann, 1992).

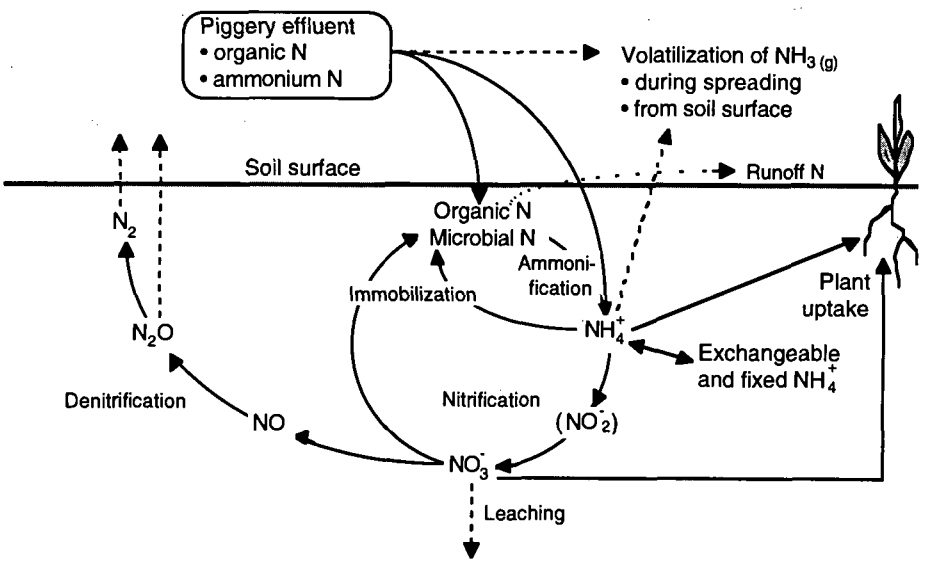

Figure 2. *Components of the N cycle having particular relevance to land application of piggery wastes. Undesirable losses of N are shown with dashed lines* (---).

The environmental N cycle incorporates a number of pools of N which are interconnected by chemical or biochemical transformations. Additions of piggery effluent to soils contributes N to the organic and ammonium-N pools. The transformations of N

relevant to application of pig manure or effluent to land are outlined below, and these are summarized graphically in the N cycling diagram presented in Figure 2.

Previous reviews (Haynes, 1986a & b; Stevenson, 1986) have covered N cycling and transformations in great detail. A similarly comprehensive review will not be attempted here but the important transformations and losses of nitrogen following additions of piggery effluent will be summarized, and some of the factors which influence these fluxes in the plant-soil system will be described.

Loss of ammonia gas (volatilization)

Ammonia volatilization often represents the first loss of N from the soil-plant system. Gaseous loss of ammonia is a purely chemical reaction controlled by the following: the pH of the soil (Black *et al.*, 1985), ammonium + ammonia concentration in solution, the partial pressure of ammonia in the soil atmosphere and above the soil surface, soil temperature and the amount of water infiltration into soil (Haynes and Sherlock, 1986). For wastes varying in N content, the amount of ammonia volatilized is closely related to the ammonium content.

In field experiments, ammonia has been shown to be volatilized from surface-applied pig slurries over a period of 7-14 d following slurry application (Cameron *et al.*, 1995; Carey *et al.*, 1997) with most ammonia loss occurring during the first 1-2 d. Similar patterns of ammonia volatilization have been observed in laboratory studies (Bernal and Kirchmann, 1992), and further ammonia emissions above background levels do not generally occur following the initial 7-14 day period. The presence of significant amounts of urea may cause transient pH rises in soil to which effluent is applied due to production of ammonium carbonate from hydrolysis of urea. This causes a delay in maximum ammonium volatilization flux, and increases the total amount of ammonia volatilized (Black *et al.*, 1985). Ammonia volatilization in piggery sheds, from treatment ponds (Shilton, 1996), during effluent irrigation (Safley *et al.*, 1992) and from storage (Sommer and Husted, 1995) decreases the amount of N applied to land prior to spreading of effluent.

The magnitude of losses due to ammonia volatilization range from 10% (Cameron *et al.*, 1995) to greater than 80% (Bernal and Kirchmann, 1992) of ammonium-N applied in effluent. The amount of ammonia volatilized decreases with an increasing amount of water infiltration following effluent application, whether the water is derived from rainfall following application (Fraser *et al.*, 1994) or from the effluent itself (Sommer and Ersbøll, 1994; Carey *et al.*, 1997). Ammonia volatilization may also be suppressed by acidification of effluent (Stevens *et al.*, 1989) or by subsurface injection, rather than surface spreading of effluent (Cameron *et al.*, 1996b).

Ammonification and nitrification of organic N

Conversion of organic N to inorganic ammonium (ammonification) is a process mediated by many groups of heterotrophic organisms in soils (Haynes, 1986a). The factors which control the amount and rate of ammonification are the same as those influencing decomposition of any organic material in soils and include: C:N ratio of the organic substrate, microbial population, soil water content, degree of soil aeration, temperature, soil pH and availability of other nutrients.

Nitrification in soils (oxidation of ammonium to nitrate) is a two stage process mediated by specialized groups of bacteria. The first stage is ammonia (ammonium) oxidation:

$$NH_4^+ + {}^3/_2O_2 \rightarrow NO_2^- + H_2O + 2H^+$$

The transformation is most commonly performed in soils by bacteria from the genus *Nitrosolobus* (with *Nitrospira* species predominating in acid soils, and some contribution from *Nitrosomonas* species) (Prosser, 1989). Ammonium oxidation is the rate limiting step for nitrification (Haynes, 1986b). The second step, nitrite oxidation, normally mediated in soils by bacteria from the genus *Nitrobacter*, is:

$$NO_2^- + \tfrac{1}{2}O_2 \rightarrow NO_3^-$$

Conversion of nitrite to nitrate is relatively rapid (meaning that nitrite does not accumulate in soils, except when free ammonia is present).

The amount and rate of nitrification is primarily controlled by the availability of ammonium (NH_4^+), nitrifier populations and the external factors influencing microbial activity (such as temperature, soil water content and soil pH). Since fewer bacterial genera can facilitate nitrification, this process is more sensitive to environmental variables than is ammonification which is mediated by a diverse microbial population.

Rate and Cameron (1992a) found that most N released from pig slurry-soil mixtures in aerobic incubations was in the form of nitrate. A 1-2 week delay preceded the maximum rate of N release. This was probably because the population of ammonifying and/or nitrifying bacteria needed to increase before significant ammonification and nitrification could occur. An alternative explanation is that the delay in N release was due to limiting S release, since the release of sulphate in their systems did not involve a similar lag phase. No lag phase, in terms of carbon mineralization, was observed by Bernal and Kirchmann (1992), suggesting that delayed production of nitrate may have been due to changes in the population of nitrifiers.

Most studies of N mineralization from mixtures of pig slurry and soil (Flowers and O'Callaghan, 1983; Bernal and Kirchmann, 1992; Rate and Cameron, 1992a) show that nitrate becomes the dominant inorganic N species shortly (15-20 d) following addition of pig slurry to soils. Nitrogen dynamics observed in closed systems shows net immobilization of N (Flowers and Arnold, 1983; Bernal and Kirchmann, 1992). Open-system incubations (Rate and Cameron, 1992a), which may represent field conditions more accurately, show net mineralization of N, and indicate that piggery effluent addition to soil may even stimulate enhanced mineralization of native soil N, an observation also made by Bernal and Kirchmann (1992).

Nitrification is known to be a process causing soil acidification when followed by nitrate leaching (Helyar and Porter, 1989). Transient decreases in soil pH have been observed in the field following waste application (Cameron *et al.*, 1996b), and long-term application of pig slurry has been shown to cause permanent soil acidification (Straczynska and Filipek, 1994).

Immobilization

Incorporation of N into microbial cells (Haynes, 1986a) and/or refractory organic compounds such as humic substances (Stevenson, 1994) are processes categorized together as N immobilization. Evidence that N from piggery effluent can be immobilized is found in the results from stable-isotope-labelling (^{15}N), mass-balance studies (Cameron *et al.*, 1995; Carey *et al.* 1997) which show that approximately 12% of pig slurry applied remained in soil one year following slurry application at 200 kg N per ha. The highest levels of N immobilization were observed in the top 5 cm of soil (>50% of the total N retained by soil).

Nitrogen in inorganic forms is subject to loss from the soil as ammonia (discussed previously), by plant uptake of ammonia and nitrate, by leaching (predominantly of nitrate) and denitrification. Immobilization, therefore, is a process which maintains soil fertility for a longer time and mitigates the environmental impact of inorganic N additions.

Plant uptake

Plant uptake of N derived from piggery effluent is frequently a major, and desirable, mechanism of N removal (Brechin and McDonald, 1994; Spallacci, 1989; Thompson *et al.*, 1987; Cameron *et al.*, 1995; Sutton *et al.*, 1978). Increased N uptake by plants following piggery effluent addition to crops and pastures is due both to higher plant productivity (which may be influenced by effluent supplying additional nutrients) and to increased plant N content (Cameron *et al.*, 1995; Sutton *et al.*, 1978; Brechin and McDonald, 1994). Nitrogen uptake increases with increasing amounts of effluent application but the efficiency of N use by plants decreases; this is observed as a lower proportion of N from

effluent being taken up by plants at higher application rates (Sutton *et al.*, 1978; Cameron *et al.*, 1995).

The timing and method of application of piggery wastes has been shown to affect the amount of N uptake by plants. Wolt *et al.* (1984) showed that crop yield and N uptake were strongly dependent on the timing of application of liquid piggery effluent. This was attributed to fluctuations in the N content of the waste, environmental conditions such as soil temperature and seasonal differences in the amount of ammonia volatilization or nitrate leaching (Wolt *et al.*, 1984). Fischer *et al.* (1984) compared corn yield and N uptake after applying pig waste to the soil surface and by subsoil injection. Yields varied with application method in two out of three years, but no application method consistently gave higher plant yields than the other due to interactions with climatic conditions.

An important consideration when assessing the fertiliser value of piggery wastes is how long the improvements in soil fertility are sustained. The residual effects of liquid effluent application have been observed to be erratic or effectively zero one year after application (Wolt *et al.*, 1984; Smith *et al.*, 1985; Cameron *et al.*, 1995). Some authors, however, have observed increases in plant growth and N uptake more than one year following pig slurry application, but this was not observed for all soil types (Bernal and Roig, 1993). Most N applied in pig slurry has been observed to be taken up early after applications (Cameron *et al.*, 1995), and this reflects the high proportion of soluble N (as NH_4^+) in the waste, with the relatively small proportion of organic N resulting in small amounts of sustained N release.

Application of N-rich effluents to pastures can alter the proportion of leguminous species (Benckiser and Simarata, 1994), meaning that the sustainability of perennial legume-based pastures may be decreased if effluent or N-fertilizer application is not continued.

Nitrate leaching

Leaching of nitrate is potentially a very serious loss of N from the soil-plant system in terms of its impact on the quality of groundwater, and in some cases surface water. Application of piggery and other organic wastes in excess of the assimilatory capacity of an ecosystem (immobilization and plant uptake) can result in undesirable losses of N (Figure 2), including leaching of nitrate (Burden, 1984; Cameron and Haynes, 1986; Sherwood, 1986; Spallacci, 1989; Cameron *et al.*, 1995, 1996) or denitrification (Cameron *et al.*, 1995). Nitrate leaching in itself, however, does not always result in contamination of groundwater (Joseph, 1983); the amount of contamination will depend on a number of factors including the concentration of nitrate in drainage water or the amount of drainage in relation to groundwater flow. The amount of nitrate leached depends on a number of factors, including: the amount and form of N applied in effluent (Cameron *et al.*, 1995; Vetter and Steffens, 1981); season of application (Vetter and Steffens, 1981); the presence of conditions conducive to nitrification (Cameron and Haynes, 1986); the contributions of other N loss mechanisms (e.g., volatilization, denitrification, and plant uptake); the potential for preferential (macropore) flow (White, 1985; Cameron *et al.*, 1995); timing and intensity of rainfall events (Jarvis *et al.*, 1987); and soil texture and structure (Sherwood, 1986).

The most reliable measurements of nitrate leaching following land application of piggery effluent are those made using large undisturbed soil monolith lysimeters with edge-flow suppression, a technique developed by Cameron *et al.* (1992). Such a technique allows the effects of soil structure on water and solute transport to be retained, and allows element mass balances to be calculated due to the constrained soil volume. In this regard, the studies by Cameron *et al.* (1995) and Carey *et al.* (1997) are particularly useful.

Loss of N by nitrate leaching has been measured at 5-19% of total slurry N applied (Cameron *et al.*, 1995) or 1.5-15.5% of N applied (Sherwood, 1986). The proportion of N leached in these and other studies is observed to increase with increasing application of N in the effluent, and this has generally been attributed to lower N use efficiency by plants (Jarvis *et al.*, 1987; Cameron *et al.*, 1995) rather than to increased hydraulic loadings.

The amount of nitrate lost by leaching can also be affected by preferential flow of water and solutes through soil macropores (White, 1985), a mechanism which decreases the residence time of nitrate in soil and reduces the opportunity for plant uptake. Such behaviour has been observed for nitrate leaching following pig slurry application to stony soils where many preferential flow pathways would be expected to exist (Cameron *et al.*, 1995) and in structured sandy soils (Carey *et al.*, 1997).

Grazed systems would be expected to incur higher leaching of nitrate due to animal urine returns which, although not necessarily contributing a high average N loading, constitute localized high N inputs with uneven distribution (Fraser *et al.*, 1994).

The method of effluent application will also have an influence on nitrate leaching losses. Different methods of surface application differ in the amount of ammonia volatilized (Giffney, 1985) and therefore effective N loading. For example, surface application of piggery effluent by spray irrigation may result in up to 26% of the N applied being lost by volatilization (Carey *et al.*, 1997), not including ammonia losses during the irrigation process itself. In contrast, injection of organic waste materials below the soil surface can decrease ammonia volatilization losses 20-fold (Cameron *et al.*, 1996b). Subsoil injection of effluent may increase (Cameron *et al.*, 1996b) or decrease (Jarvis *et al.*, 1987) the amount of nitrate leaching, and this is probably dependent upon whether or not the subsurface injection system (including factors such as the season of application and the degree of root pruning by the injection equipment) favours plant uptake of N.

Denitrification

Denitrification is the loss of N to the atmosphere under anaerobic conditions via the microbial reduction of nitrate to produce gaseous nitrous oxide (N_2O) or molecular N (N_2) (Haynes and Sherlock, 1986).

Denitrifying organisms are able to metabolize carbon anaerobically using nitrate and nitrite as terminal electron acceptors (Haynes and Sherlock, 1986). As a result, denitrification is observed to occur at high soil moisture contents where oxygen availability is low, and also depends on the availability of nitrate in soils and the presence of readily mineralizable carbon (Comfort *et al.*, 1990). Soil temperature also has a significant effect, with denitrification occurring at lower temperatures following slurry addition than following inorganic fertilizer application, an observation attributed to the increased supply of mineralizable carbon (Thompson *et al.*, 1987).

The magnitude of denitrification losses observed following land application of piggery wastes are variable; Van den Abbeel *et al.* (1990) measured a denitrification loss of 7% of applied N using an acetylene inhibition technique. Studies in New Zealand, using pig slurry labelled with $^{15}NH_4Cl$ (Cameron *et al.*, 1995; Carey *et al.*, 1997), have estimated denitrification losses at between 29-39% of N applied, depending on soil type. These relatively high denitrification losses were attributed to the development of transient anaerobic conditions following autumn application of slurry, caused by saturation of soil in the surface layers as a result of relatively impermeable underlying soil.

Phosphorus cycling following land application of piggery effluents

Phosphorus in piggery effluent is present in predominantly inorganic forms (van Riemsdijk *et al.*, 1987) with only small amounts of soluble phosphate species (Gerritse, 1976). The reactions of P added to soils are dominated by strong adsorption to soil minerals, and formation of sparingly soluble compounds, for example with Fe or Ca (Wild, 1988). A P cycle showing transformations of, and sinks for, P following addition of piggery effluent to soils is presented in Figure 3.

Phosphorus in piggery effluents

The P content of pig slurries is related to their solids content (O'Dell *et al.*, 1995), and shows considerable variability. This variability poses problems for the management of P loading for effluent applied to land; if P is the most limiting element from either a

plant nutrition or environmental standpoint, uncertainties about its concentration in effluent make estimation of application rates less precise than would be desirable. Phosphorus in piggery effluents is predominantly present in insoluble inorganic forms (Van Riemsdijk *et al.*, 1987; Gerritse, 1976; Van Faassen and Van Dijk, 1987) and is typically present in phosphate minerals such as struvite ($MgNH_4PO_4.6H_2O$), octocalcium phosphate ($Ca_4H(PO_4)_3.3H_2O$) or dicalcium phosphate ($CaHPO_4.2H_2O$) (Fordham and Schwertmann, 1977). Organic forms of P comprise approximately 15-20% of total slurry P (Van Faassen and Van Dijk, 1987; Van Riemsdijk *et al.*, 1987). Only 5-10% of P in pig slurries is present as dissolved inorganic P (which would be directly available to plants or mobile in soil systems) (Van Faassen and Van Dijk, 1987; Gerritse, 1976).

Transformations of P in soils

Phosphorus applied to soils in piggery effluent undergoes transformations from its original chemical forms which alter its mobility in soils and its availability to plants. Both inorganic P (Van Riemsdijk *et al.*, 1987) and organic P (Zhang *et al.*, 1994) are initially in forms which are relatively plant-available, but these become less available with increasing time following application of piggery effluent to soils. Phosphorus from pig slurry tends to accumulate in soils as inorganic forms of P with little or no accumulation of organic forms of P (Sharpley and Smith, 1995; Papini *et al.*, 1991). The inorganic forms of P which accumulate in soils may be calcium-bound P, or insoluble calcium phosphates (Sharpley and Smith, 1995). Phosphorus applied in piggery wastes may become more available with successive applications if phosphate derived from the waste tends to progressively saturate adsorption sites, as observed by Holford *et al.* (1997) whose experiments revealed decreases in soil P sorption capacity following pig effluent addition. The addition of organic matter in pig effluent may increase soil P retention by providing additional adsorption sites (Holford *et al.*, 1997) or decrease soil P retention by blocking adsorption sites on soil minerals (Fardeau and Martinez, 1996).

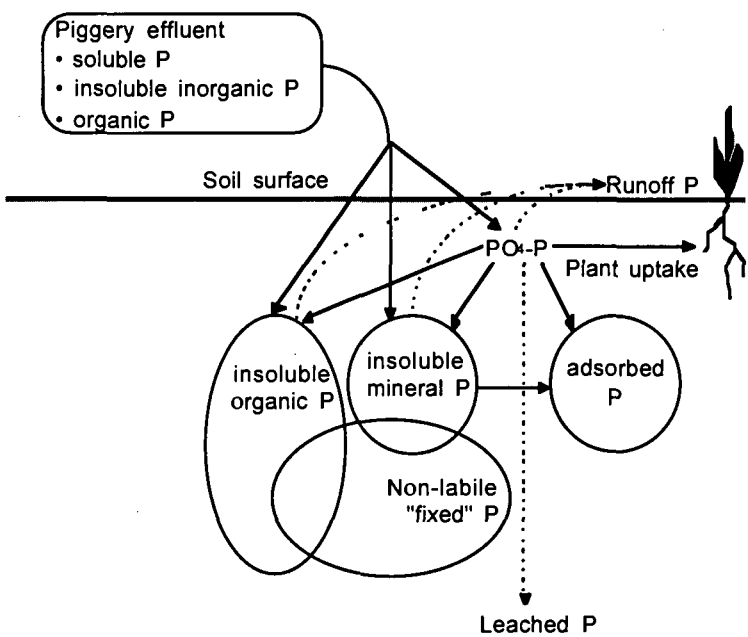

Figure 3. *Components of the P cycle having particular relevance to land application of piggery wastes. Undesirable losses of P are shown with dashed lines* (---).

Runoff losses of P

Phosphorus from organic wastes may enter water bodies via runoff (Cameron *et al.*, 1996a) or leaching (Weaver and Ritchie, 1994), and P from piggery wastes may contribute to eutrophication of surface waters (Humphries and Bott, 1987). Phosphorus from piggery waste applications almost exclusively accumulates at or near the soil surface (Furrer and Gupta, 1985; King *et al.*, 1990; Papini *et al.*, 1991; Weaver and Ritchie, 1994) and this increases the potential for transport of P via runoff (King *et al.*, 1990). Losses of P via runoff, however, may be lower from organic wastes than from fertilizers (Cameron *et al.*, 1996a).

Phosphorus leaching

Phosphorus retention processes in soils are generally considered to reduce the risk of P leaching (Cameron *et al.*, 1996a), but retention mechanisms may be less important for sandy soils (Papini *et al.*, 1991; Weaver and Ritchie, 1994). In addition, downward movement of P has been observed for soils with high P retention characteristics (Fardeau and Martinez, 1996). Leaching of P may be enhanced if long-term pig slurry applications have saturated soil P adsorption capacity (Holford *et al.*, 1997). Chardon *et al.* (1997) emphasize the substantial contribution of organic P species to P leaching, and point out that P leaching is also enhanced in intense rainfall events, an observation consistent with particulate P transport. Leaching of P is strongly dependent on soil type (Van Riemsdijk *et al.*, 1987; Weaver and Ritchie, 1994). Some authors have found no effect of addition of pig slurry on leaching or mobilization of P (Furrer and Stauffer, 1986; Rate and Cameron, 1992b). Weaver and Ritchie (1994) found, however, that 39-100% of dissolved P in piggery effluent could be leached from sandy soils with low P sorption capacities. In the same study, it was shown that 40% of P in solid phases applied to soils in pig effluent could be leached. For soils in The Netherlands, Van Riemsdijk *et al.* (1987) calculated average P movement downwards through the soil at 0.5-4 cm per year, depending on waste application rate. This rate of P movement is consistent with data for sewage effluent at Wagga Wagga, Australia (Cameron *et al.*, 1996a).

Plant uptake of P

Plant availability and uptake of P from animal wastes is variable (Smith and Van Dijk, 1987). Brechin and McDonald (1994) found no effect of P on barley growth, but Cameron *et al.* (1996a) found a significant increase in P nutrition of pasture following organic waste applications. Bernal and Roig (1993) also observed a plant response to P in pig slurry, with availability of soil P to plants increasing with successive slurry application. Animal waste may be less (Smith and Van Dijk, 1987) or similarly (Fardeau and Martinez, 1996) effective at supplying plants with P than mineral fertilizer, but the long-term P-supplying ability of organic wastes and fertilizers would be expected to be similar (Smith and Van Dijk, 1987). Christie and Kilpatrick (1992) found a decrease in mycorrhizal infection of pasture grasses following long-term pig slurry application which was related to soil P status.

The fate of potentially toxic elements (Cu and Zn) following land application of piggery wastes

Copper and Zn are added to pig feeds as growth enhancers and the concentration of Cu added in feeds may be up to 200 mg/kg (Cooke, 1981). These elements are generally not retained by pigs, and therefore appear in piggery wastes at concentrations in the range 100-800 mg/kg on a dry weight basis (Huysman *et al.*, 1994).

Copper and Zn are relatively immobile in soils (Alloway, 1995), and therefore tend to accumulate in soils (Coppenet, 1981), especially at or near the soil surface. This accumulation is more pronounced when high rates of waste are applied (Christie and Beattie, 1989). As is the case for P, the bioavailability of Cu changes with time following piggery waste addition to soils. Unwin (1981) reported that Cu applied to soils in pig

slurries became less extractable with EDTA solution (a relatively mild extractant) in the first few years following slurry application. This was likely to be due to slow formation of insoluble Cu compounds and ongoing reactions between soil particles and Cu. Copper is well known to be strongly adsorbed in soils, especially by organic matter (Beckwith, 1955). Pre-treatment of piggery wastes before application to land may further decrease the bioavailability of Cu and Zn. Both Tiquia *et al.* (1996a) and Tam and Wong (1995) found that composting reduced the amounts of potentially toxic elements in bioavailable forms.

Copper added to soils in piggery wastes may also have an effect on plants and animals in agricultural ecosystems. Christie and Beattie (1989) did not observe any reduction in total microbial biomass or populations of nitrifying bacteria following application of pig slurry at typical farm application rates. Huysman *et al.* (1994), however, found that relatively small concentrations of Cu in soils (5-10 fold lower than European Community recommendations) caused changes in microbial populations, favouring metal-tolerant bacteria. Increases in the Cu concentration of plant tissues have been observed following piggery waste application to soil (Coppenet, 1981; Unwin, 1981). Following excessive pig slurry applications, plant Cu concentrations have been found to increase to levels which are potentially toxic to stock (McGrath, 1981; Christie and Beattie, 1989). Bremner (1981), however, suggested that the health risks to stock fed on crops or pasture to which piggery wastes containing copper have been applied are minimal.

Conclusions

Piggery wastes contain substantial amounts of useful plant nutrients, especially N and P. There is some potential for adverse environmental impacts if excessive levels of these nutrients enter the environment, especially water bodies, and for this reason many treatment systems have focussed on nutrient removal rather than beneficial re-use. A number of waste re-use options, for example land application, composting and use of constructed wetlands, offer the possibility of efficiently and safely using N and P from piggery wastes. Potentially toxic elements (Cu and Zn) derived from piggery wastes do not appear to have an adverse impact unless excessive applications of waste are made.

The forms, transformations and losses of N and P following piggery waste application to land are strongly interdependent. In order to make a useful assessment of both the beneficial and adverse the effects of piggery wastes on the plant-soil ecosystem, it is necessary to consider these interacting factors.

Symposium continued on next page

ENVIRONMENTAL ODOURS FROM PIGGERIES

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Abstract

A piggery is a complex and large areal source of odour. The complexity stems from: (i) the many individual odour sources within the piggery, all of which will be emitting odours of different strength and different character, and (ii) the variation of these emission rates with time, due to the effects of piggery management and atmospheric factors such as temperature and wind speed. In this paper the directions and outcomes to date of two inter-related projects funded by the Australian Pig Research and Development Corporation (PRDC) aimed at quantifying emissions from piggeries are discussed. Rates of odour emissions from the various odour sources within piggeries are reviewed. The rates presented show considerable variation and uncertainty and are of a magnitude that warrants some concern on the part of the industry. The preliminary results from the current research suggest that the shed emissions from a modern, well-designed and managed piggery are relatively low and should be of little concern. This work has also demonstrated the considerable difficulty in estimating shed emission rates in a typical piggery, where the individual sheds are surrounded by other odour sources which influence the odour concentration of the incoming ventilation air. Pond emissions are clearly a significant contributor to the gross emissions. Their contribution ranges between 30 and 50% of the gross emissions and they appear to offer the most profitable target for the application of strategies for reduction of emissions and hence reduction of potential odour nuisance.

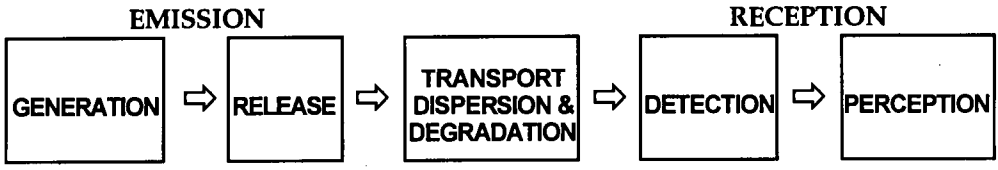
Introduction

With the expansion in Australia of the intensive animal industries (such as beef cattle feedlotting and large scale piggery developments) comes concerns about their possible impact on the environment and in particular about the nuisance to neighbouring communities caused by the odour emitted from them.

The nuisance caused by an odour is a function of the concentration, frequency and duration of odour events. For a particular receptor (neighbour) these are determined principally by: the magnitude or rate of odour emissions; the wind direction; and the degree of dispersion in the atmosphere. The latter two factors are either readily measured or are relatively well understood. The magnitude of and the processes involved in the emission of odours are less well understood but must be estimated if such facilities are to be managed to reduce their potential odour nuisance.

The rate at which odours are produced in organic manures and wastes, emitted to the atmosphere and subsequently cause a response at some downwind location is a complex function of many variables. A conceptual model of the processes and the pertinent variables is included as Figure 4.

A piggery can be viewed as a complex large areal source of odour. The complexity stems from a number of factors. Firstly many individual odour sources comprise the entity called a piggery (in the form of the piggery buildings, manure stockpiles, areas of spread manure or slurry, effluent ponds and associated land disposal areas) all of which will be emitting odours of different strength and different character. This is a feature common to the other intensive animal industries and also the sewage treatment industry. Secondly, most of the emitting surfaces are fully or partially open to the atmosphere. As a result, the emission rate will vary considerably over short times, due largely to the effects of temperature and wind speed. Finally, the odour generation rate will vary over longer times due to the growth or decline of the emission generation factors listed in Figure 4.



Dominant factor:

Nature of the source	State of the atmosphere		Characteristics of the odour	Characteristics of the receptor
Issues:				
Manure type	Windspeed	Windspeed and direction	Concentration	Individual threshold
Manure depth	Surface roughness	Atmospheric stability	Odorant mixture	Lifestyle expectations
Moisture content	Atmospheric stability	Surface roughness	Averaging period	Social background
Volatile solids	Surface moisture content	Topography	Fluctuations	State of health
Temperature and pH	Concentration gradients	Mixing height	Background odours	Non-odour concerns
Management				

Figure 4. *Conceptual representation of odour processes.*

The fate of the odour emissions as they move downwind to any potential receptor is an equally important question. Dispersion of the odours is currently predicted using the Gaussian dispersion model AUSPLUME (Lorimer, 1986). AUSPLUME is accepted as the Australian industry standard dispersion model and has been proven for a range of gaseous and particulate pollutants. However it has not been proven for odour. As a result of the combined effects of time, varying emission rates, thermal buoyancy (particularly important in piggery ventilation), odour masking (lack of superposition of individual emissions), degradation in the atmosphere and adsorption on surfaces, the odour concentrations at receptors may be less than would be predicted by the traditional application of the dispersion model. These and other matters concerned with the dispersion of odours are discussed in more detail in Smith (1995b).

Even less is known about the processes associated with the reception of odours and exactly what constitutes a nuisance odour. Unknowns include:

1. The time constant of human response to odour, which is particularly relevant given the very short time fluctuations in odour concentration that occur
2. The relative importance of concentration, frequency and duration in causing nuisance
3. The relationship between the odour detection, recognition and nuisance thresholds of the population at large and those of the select panels used in determining odour concentrations and
4. The role of geographic and socio-economic factors.

Odour nuisance surveys carried out in The Netherlands (Miedema and Ham, 1988) suggest that the best indicator of annoyance is the predicted (by dispersion modelling) 1-hour 99.5 percentile concentration, i.e., the 1-hour time averaged concentration that would be equalled or exceeded 0.5% of the time. The situation in rural Australia is substantially different from that in The Netherlands particularly in relation to the level of background odours. Hence the results from the Dutch work cannot be applied here without confirmation by similar rigorous nuisance surveys.

Current Research

The potential for the reduction of odour nuisance from piggeries is based on two hypotheses:

1. That the magnitude or rate of odour emissions may be reduced by properly understanding the relationship between emissions and piggery management practices, and by modifying management accordingly and
2. That the impact of the emissions may be reduced through appropriate siting, design and management of piggeries; based on an understanding of how emissions vary in time and space and how they interact with the environment as they disperse downwind.

In this paper the directions and outcomes to date of two inter-related projects funded by the Pig Research and Development Corporation (PRDC) are discussed. The aim of both projects is, in part, to address the above hypotheses.

The major activities/technical objectives of the first project fall into two groups. Firstly it aims to develop techniques for measuring the odour emission rates from piggery buildings, quantify these rates, and propose management practices which might result in the reduction of odour emissions. Secondly it aims to quantify the gross rate and the fate of odour emissions from piggeries. Included in this second objective is the development of techniques for estimating the gross rate of odour emissions from piggery complexes, and investigation of the relationship between the emissions from individual sources and the gross emission rate.

The second project addresses the notion that the effluent ponds are the dominant source of the odours emitted from piggeries. This view is supported by the limited evidence from studies in Europe and Australia which suggest that pond emissions might be between 3-10 times greater than the building emissions. The work proposed in this project will measure the odour emissions from piggery effluent ponds, how they vary spatially and with time, and identify the key factors involved in the emissions.

Odour emissions from piggery buildings

Previous measurements

In Australia, Schulz and Lim (1993) measured odour concentrations within a large number of piggery buildings and attempted to relate them to some of the controlling variables. However, no conclusions can be drawn from that work regarding emission rates because of the failure to measure or estimate the concentration of the incoming air (background odour) and the ventilation rates of the buildings. As part of the Environmental Impact Statement (EIS) for the DanPork piggery proposed (but later abandoned) at Scone in NSW (CMPS&F, 1993), average emission rates from the existing piggery buildings equivalent to about 13 OUm^3/s per pig[1] were presented. These emission rates were based on measurements within the individual buildings at the site.

Table 6. Dutch standard emission rates.

Animal Type	Standard emission rate (OUm^3/s)	Measured emission rate (OUm^3/s)
1 fattening pig	5.0	3.3
1 replacement gilt	7.5	3.6
1 nursing sow	7.5	14.0
1000 laying hens	83.0	11.3

[1] In this paper, odour concentration (which is the concentration of the odorous gas relative to the threshold of detection of a select panel, as determined by olfactometry) will be treated as dimensionless and will be expressed as odour units (OU). The rate of emissions from an odour source is the product of the concentration and the volumetric flow rate (m^3/s) of the emissions. Consequently the units for the emission rate are OUm^3/s.

In The Netherlands, standard emission rates have been calculated for the purpose of modelling odour dispersion (Baan *et al.*, 1991) and are reproduced in Table 6. The only other known instance in which the rate of odour emissions from piggery buildings have been published is from France (Texier, 1994). The study by Texier was performed in a forced-ventilated shed in which two different ventilation rates were set. Emission rates were very high and ranged from 17-33 OUm^3/s. The study confirmed that lower ventilation rates do increase the concentrations within the building and in the extracted air. The ventilation rate did not markedly influence the gross emission rate.

Estimation of emission rates

There is no way of directly measuring the rate of odour emissions from any source including piggery buildings. In all cases the emission rate must be inferred from measurements of the odour concentration in an air stream after the emissions have mixed with that air stream.

Assuming that odour is a mass conservative quantity and assuming complete mixing within a piggery building then the odour emissions can be described by a continuity equation:

$$C_i Q + G - C_o Q = V\frac{dC}{dt} \qquad (1)$$

where C is the average odour concentration in the building (OU); C_i is the odour concentration in the incoming ambient air (OU); C_o is the odour concentration in the outgoing or ventilation air (OU); G is the odour generation rate (OUm^3/s) which is a function of the concentration C and the design and management of the shed; Q is the ventilation rate (m^3/s); and V is the volume of the shed (m^3).

If the unsteady term on the RHS of equation 1 is negligible and if the internal concentration represents the concentration of the outgoing air, then the odour emission rate E (OUm^3/s) will be given by:

$$E = (C - C_i)Q = G \qquad (2)$$

In the event of a zero background odour concentration, the emission rate reduces to a simple product of the internal concentration and the ventilation rate.

In either case, the emission rate will equal the generation rate irrespective of the ventilation rate. While it is obvious that emission rates are the product of a concentration and the ventilation rate, the converse, that the internal concentration is a function largely of the ventilation rate, may be less obvious. An unknown factor is the magnitude of the effect of the internal concentration on the rate of odour generation or release, with the relatively high concentrations in European piggeries perhaps tending to suppress the release of odours.

Ventilation rate

Clearly the ability to estimate ventilation rates is essential to the estimation of odour emission rates. A survey of the Australian pig industry was conducted in 1992 by NSW Agriculture (Taylor *et al.*, 1994). From this survey a 'typical' building could be described as a naturally ventilated, insulated shed with roof ridge vents, automatic control of side wall shutters or blinds and spray or drip cooling.

The two driving forces that influence air exchange in naturally ventilated sheds are wind pressure on the building and thermal buoyancy. As a result of air flow around a building the pressures on the windward side of a building exceed that of the leeward side of the building, causing air to flow through the building. Factors influencing the degree of ventilation due to wind pressure are wind velocity and direction, location of building openings and surface roughness of the surrounding terrain.

Thermal buoyancy of the inside air causes ventilation to occur due to temperature difference between inside and outside temperatures. Typical cold conditions result in

warm air flowing from high in the building being replaced by cold air entering nearer the base of the building. Management objectives would be to control this effect with wall shutters or other devices in order to achieve a balance between temperature loss and increasing humidity in the building.

Foster and Down (1987) reviewed the techniques for estimating the ventilation of animal buildings by natural convection. From this and other work it is clear that for naturally ventilated sheds the ventilation rate cannot be obtained from a single direct measurement, but must be inferred from measurements of the controlling meteorological variables.

A preferred method, which deals simultaneously with the effects of thermal buoyancy and wind induced ventilation, is that of Brockett and Albright (1987). At any height they defined a pressure difference Δp, which results from superposition of the wind-induced and thermally-induced pressure differences, as:

$$\Delta p = g(\rho_o - \rho_i)(\bar{h}_g - h_g) + \frac{\rho_o V_w^2}{2}(C_{pe} - C_{pi}) \quad (3)$$

where ρ_o is the density of the outside air; ρ_i is the air density in the building; h_g is the height above the ground; $\bar{h}_g$ is the height of the neutral axis above the ground; V_w is the external wind speed; C_{pe} is the external wind pressure coefficient; and C_{pi} is the internal wind pressure coefficient.

The velocity through an incremental opening at height h_g is given by:

$$V_j = \sqrt{\frac{2\Delta p}{\rho}} \quad (4)$$

and the volumetric flow rate through the entire opening by:

$$Q_i = C_d \int V_j \, dA \quad (5)$$

Summation of the flows (inflows and outflows) through every opening and equating to zero gives an expression in which the only unknown is the height $\bar{h}_g$ of the neutral axis. The ventilation rate can then be calculated by summing the flows through all inlets.

Down and McMahon (1990) and Down (1990) used this method in an internal climate model for Australian piggery sheds (the PHICS model). The external pressure coefficients were determined using Australian Standard AS1170.2 (SAA, 1989), although they acknowledged that these coefficients were designed for calculating wind loads for structural design and were likely to over-predict ventilation rates. The internal pressure coefficients were determined iteratively as in Bruce (1975) for wind occurring alone.

The implementation of this method requires measurement the following variables: air density inside and outside the building; building dimensions including the height and area of openings; wind pressure coefficients for the building; and wind speed.

Preliminary results from PRDC project

Methodology

The work to date has concentrated on developing a reliable technique for determining building emissions and has all been undertaken at a single piggery. The piggery, located on the Darling Downs, is constructed and managed as two 1000 sow units, each consisting of a breeder building, a farrowing building, a weaner building and three grower buildings. Measurements were taken in and adjacent to one of the grower sheds in the eastern section of the complex. This building is naturally ventilated, insulated, with roof ridge vents, automatically controlled side-wall blinds and spray cooling. According to Taylor *et al.* (1994) it represents a "typical" Australian style building. The measurements taken during odour sampling included the building dimensions and blind positions, wet and dry bulb temperatures (inside and outside the

shed), ambient windspeed and direction, time since flushing and atmospheric stability parameters. The number of pigs in the building varied between 700-1000.

All odour samples were drawn into 120 l MYLAR bags contained within a plastic drum, using the procedure described in Jones *et al.* (1994). All samples were taken over a 10 min period and therefore correspond to 10 min time-averaged concentrations. The odour concentration measurements were made using the forced-choice, dynamic-dilution olfactometer owned by the Queensland Department of Primary Industries (Jones *et al.*, 1994). A minimum of five dilutions were presented for each sample with three replicates of each dilution. The dilution step was a factor of two. Odour concentrations were calculated by the individual thresholds method (Jones *et al.*, 1994).

Trial 1 - Odour distribution in a building

The main aim of this first experiment was to quantify the spatial variations in odour concentration throughout a typical piggery building with a view to developing a rapid means of determining a representative concentration. The methodology and results are described in detail in Dalton *et al.* (1996) and are summarised in the following paragraphs. Samples of odorous air were collected simultaneously in vertical transects and in horizontal transects along and across the building under a variety of meteorological conditions. The corresponding ventilation rates were calculated from the measured meteorological data.

The odour concentrations varied considerably both spatially across the shed and with time (or ventilation rate). As expected, at the higher ventilation rates the odour concentrations were lower and more uniform. In the vertical transects the concentrations were highest near the floor and decreased with height, suggesting incomplete mixing of the emissions with the ventilation air.

When the wind direction was other than at right angles to the shed, the longitudinal transects showed a consistent variation in odour concentration along the length of the shed. Concentrations were lowest at the windward end and increased along the length to a maximum at the leeward end.

Clearly then it is difficult to determine a representative concentration for a piggery building, the pattern of concentration in the building being at least a function of the wind direction and the ventilation rate. As a first approximation it is suggested that samples for concentration measurements be taken at about 1.5 m height at multiple locations equally spaced along the centreline of the building.

Trial 2 - Effect of ventilation rate on emission rates

The aim of this trial was to gauge the effect on odour emission rates of varying the ventilation rate of the building. Variations in the ventilation rates were obtained in an uncontrolled manner from variations in the meteorological conditions and in a controlled way by raising and lowering the side blinds of the building. The premise was that the emission rate should remain relatively constant and that the odour concentration should vary inversely as the ventilation rate. The results from this trial (odour concentrations and ventilation rates) are given in Table 7, along with emission rates calculated assuming no background concentration (of the incoming air). As can be seen from these results the simple premise referred to in the previous paragraph proved false. Concentrations varied considerably and showed no consistent relationship with ventilation rate. The variation in estimated emission rates was excessive, ranging from 4.9-40.0 OUm^3/s per pig with an average of 15.2 OUm^3/s per pig. The cause for this variation was assumed to be the presence of significant and varying background odours. Wind directions varied throughout the trial so that at various times background odours would have been present from the nearby ponds or from the other sheds. Notable is measurement number 7 taken on 27 September. Here the wind was blowing directly off the nearby pond and although the ventilation rate was high the concentration was also very high, resulting in the highest estimated emission rate. Inclusion of the background odour in the estimation of the emission rate (through equation 2) would have resulted in a very much reduced estimate of the emission rate. Unfortunately the concentrations of these background odours were not measured.

Table 7. Pig building emission rates (E) from trial 2 calculated solely from measured shed odour concentration (C) and ventilation rate (Q).

Measurement		C (OU)	Q (m^3/s)	E (OUm^3/s)
No.	Date			
1	23 Sept 96	52	123.4	8.7
2	23 Sept 96	64	88.1	7.6
3	24 Sept 96	192	38.9	10.5
4	24 Sept 96	197	85.0	23.8
5	25 Sept 96	113	168.2	19.3
6	25 Sept 96	142	45.3	6.5
7	27 Sept 96	242	162.8	40.0
8	27 Sept 96	243	29.9	7.4
9	1 Oct 96	91	180.2	22.9
10	1 Oct 96	128	117.8	21.1
11	1 Oct 96	173	39.4	9.5
12	1 Oct 96	100	34.8	4.9

The emission rates were recalculated using a mathematical optimisation process which returns estimates of the emission rate and background odour concentrations to minimise the variation in emission rate. While this approach must be seen as a little approximate, it does indicate the importance of the background odour and its contribution to the internal odour concentration. The result is a substantial reduction in the estimates of the emission rates, to values more consistent with the previous Dutch measurements (Baan *et al.*, 1991). The results from the optimisation (mean emission rate of 5.8 OUm^3/s per pig) are shown in Table 8.

Table 8. Pig building emission rates (E) from trial 2 taking account of (estimated) background odour concentrations (C_{in}).

Measurement	Wind direction	C_{in} (OU)	E (OUm^3/s)
1	227	15	6.2
2	213	15	5.8
3	335	135	3.1
4	330	135	7.5
5	316	72	7.0
6	316	72	3.2
7	0	206	6.0
8	315	72	5.2
9	238	74	4.3
10	236	74	8.9
11	254	22	8.3
12	254	22	3.8

Pond emissions

Previous measurements

The best available estimates of pond emissions come from the work of the University of New South Wales (Schulz and Lim, 1993). Emission rates varied considerably depending on the type and condition of the pond and range from 15-38 OUm/s (units here are those of a specific emission rate, that is, OUm^3/s per unit area).

Typically, emission rates from anaerobic ponds ranged from 19-38 OUm/s, from facultative ponds 19-29 OUm/s, and from aerobic ponds 15 to 22 OUm/s.

Presented in this manner the rates are difficult to compare to the emissions from other sources. Values equivalent to the upper end of the range were used in the EIS for the DanPork piggery (CMPS&F, 1993) and when converted on the basis of area and number of pigs served gave an emission rate of about 22 OUm^3/s per pig. For the existing Scone piggery the emissions from the effluent pond were estimated to be 27% of the total emissions, compared to the sheds which contributed only 16% of the total. No data is available on the spatial or temporal variability of pond emissions or on the effect on emissions of pond loading rate or condition. Pond emission rates are also a function of the state of the atmosphere (wind speed, stability and temperature) and the aerodynamic roughness of the region surrounding the pond.

Sampling methodology

The emission rates reported above were based on point estimates obtained with portable floating wind tunnels identical to those used for sample emissions from land based sources such as feedlot pens and areas of spread manure (Smith and Watts, 1994a & 1994b; Schulz *et al.*, 1994). Alternatively, estimates of the average emission rate from the entire pond can be obtained from odour concentration and wind speed measurements in the ambient air stream immediately downwind of the pond. Emission rates can be obtained from these measurements by inverse calculation using an appropriate dispersion model (Smith, 1995). For a comparison of the two different approaches see Smith and Kelly (1996). The PRDC-funded pond odour project will use both of these approaches as appropriate.

Emissions from other sources

Other odour sources within piggeries include: manure stockpiles, slurry tanks, irrigation dams, and areas irrigated with effluent or spread with manure. Data on emissions from these sources are relatively sparse, with the best available estimates coming from Schulz and Lim (1993) and the EIS for the proposed expansion of the DanPork piggery at Scone. Emission rates drawn from these references are listed in Table 9.

Table 9. Miscellaneous source emission rates (Schulz and Lim, 1993; CMPS&F, 1993).

	Emission rate		
Source	OUm/s	OUm^3/s/pig	% of total
Slurry pits	70 - 125	0.3	0.4
Manure (screened)	41 - 58	12	15
Irrigated areas	2	0.15	0.2
Solids application (dry)	4	5	6

As a generalisation it can be said that the miscellaneous emissions listed in Table 9 are usually a minor contributor to the total emissions, are very dependant upon manure and effluent management practices and some are highly variable temporally.

In the case of the land application of solid and liquid wastes the emissions are short lived, being at a maximum immediately after application and declining to a fraction of their initial value within a very few hours. No local data are available but this trend is well illustrated by the many European studies on the emissions from pig and other animal slurries spread on pasture. For pig slurries emission rates as high as 200-500 OUm/s at 1 h after spreading declined to 3-14 OUm/s after 48 h (Pain *et al.*, 1991; Pain and Missellbrook, 1991). It would be dangerous to suggest that these values are indicative of the emissions that might occur under the very different manure management practised in Australian piggeries. However it does serve to illustrate how nuisance can be avoided by

timing land applications to coincide with favourable atmospheric conditions and wind directions.

Gross emission rates from piggery complexes

The DanPork EIS (CMPS&F, 1993) again provides the best estimate of the gross odour emissions from an entire piggery. In this case the emission rate from the existing piggery was 1,706,170 OUm^3/s which is equivalent to about 83 OUm^3/s per pig. This is an extremely high emission rate, caused by an excessive emission rate of about 600,000 OUm^3/s (or 38 OUm/s) from the irrigation dams, a value far in excess of what would normally be expected. Reducing the dam emissions to a more realistic value would reduce the gross emission rate to about 63 OUm^3/s per pig.

These gross emission rates were determined by the simple addition of the emission rates estimated for each of the individual odour sources within the piggery. It assumes that superposition of the different odours is valid and ignores the possible masking of one odour by another. It also takes no account of the effect of odour concentration (from one source) on the emission rate from another source. Combined with the fact that the subject piggery was old, and from an environmental perspective was poorly managed, and that the individual source emissions were all at the high end of the possible range, the likely impact is that the above emission rates overestimate the likely gross emission rate for a piggery, particularly a modern, well designed and managed piggery.

The methodology being used in the present PRDC project involves the estimation of gross emission rates from measurements of ambient odour concentration taken downwind of the site. This should give better estimates of the gross emission rates. To assist in the process of estimating emission rates for new and proposed piggeries, these estimates will be compared to the summation of individual source emission rates.

Conclusions

Odour emissions from the various sources of odour within piggeries have been reviewed. The emission rates presented show considerable variation and there is substantial uncertainty with respect to the magnitude of the rates from some sources. Nevertheless the rates are of a magnitude that warrants some concern on the part of the piggery managers and the industry as a whole.

The preliminary results from the current PRDC funded research suggest that the shed emissions from a modern, well designed and managed piggery are relatively low and should be of little concern. This work has also demonstrated the considerable difficulty in estimating shed emission rates in a typical piggery, where the individual sheds are surrounded by other odour sources which influence the odour concentration of the incoming ventilation air.

Pond emissions are clearly a significant contributor to the gross emissions. Their contribution ranges between 30 and 50% of the gross emissions and they appear to offer the most profitable target for the application of strategies for the reduction of emissions and hence reduction of potential odour nuisance.

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Symposium continued on next page

SYMPOSIUM CONCLUSIONS

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Piggeries are readily identifiable point sources of wastewater, solid waste and odour. As such the pig industry is perhaps under more pressure than more extensive agricultural operations from a community that is becoming increasingly concerned about the environment. Therefore the industry needs to develop strategies to reduce the quantity of effluent produced, and to consider how best to dispose of, or re-use, piggery waste.

Pluske *et al.* (1997) described how the quantity of effluent produced is a function of both an oversupply of nutrients to the pig and an inefficiency in the way that those nutrients are utilized. The development of computer models to calculate more accurately the nutrient requirements of the pig, together with the greater use of synthetic amino acids in pig diets is a sound approach to reducing the quantity of N in effluent. The addition of the phytase enzyme to pig diets has been shown in a large number of studies to increase the digestibility of P and to reduce P excretion. Similar approaches could be used with other nutrients in the future.

Piggery effluent contains substantial amounts of useful plant nutrients, especially N and P. Rate (1997) considers that the re-use of piggery effluent for plant growth is a viable option provided that the input of nutrients is closely matched with plant demand to avoid the risk of nutrient export through processes such as leaching, runoff and atmospheric emissions. Potentially toxic elements (e.g., Cu and Zn) in piggery wastes do not appear to result in adverse effects unless excessive applications of waste are made. Importantly, effluent is beginning to be regarded as a resource rather than as a potential cause of pollution

O'Shea (1997) notes that odour is the major environmental issue of concern to the pig industry, especially with the rate of urban encroachment into rural areas. Effluent ponds are the dominant source of odours emitted from piggeries, whereas emissions from a well-designed and well-managed piggery are relatively low and should be of little concern (Smith *et al.*, 1997). Therefore, high priority needs to be given to developing and validating management strategies that will reduce the emission of odours from effluent ponds. There is a lack of objective data as to what constitutes a nuisance odour. While the industry is right to develop techniques to measure and subsequently reduce odour emission from piggeries, it is possible that the target emission levels may be lowered to unrealistic levels.

Unfortunately there is a myriad of regulations controlling the development of new piggeries and, in some cases, the operation of existing piggeries. There is also a lack of uniformity among states within Australia in these regulations, and there can be considerable time and expense involved in meeting their requirements. All of the issues discussed in this Symposium will have a significant impact on the structure, operation and location of the pig industry in the future.

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A METHOD FOR A FLEXIBLE GREENHOUSE GAS INVENTORY

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Usually, greenhouse gas (GHG) inventories calculate the emitted gases using overall pig numbers and averaged emission data. They are therefore not able to provide information about opportunities for the mitigation of emission. The method developed relates emissions to production techniques used, enables scenarios to be described and aids in determining best practices to reduce GHG emissions while maintaining a high level of productivity.

The relevant GHG pollutants are carbon dioxide (CO_2), methane (CH_4) and dinitrogen oxide (N_2O). There are three different sources of GHG in the production process: the metabolism of the animal, the animal housing or, more generally, the husbandry process, and the waste management system. These distinctions in the process and the underlying causes for release are necessary to determine an appropriate method for mitigation of emission.

The inputs and outputs of carbon, nitrogen and phosphorus were balanced and subsequently the enteric losses of GHG computed using feed consumption and meat production data. The losses through the husbandry process were computed based on which husbandry system was used. The losses from the effluent management were calculated using temperature curves of representative locations in the different states, data about effluent management practices, storage times and manure quantities and contents, which are in turn determined by feed consumption, reproduction data, growth rate and final weight (Farran *et al.*, 1997). However, the feasibility of the method depends very much on the availability of detailed information about industry practices and the related emissions. Calculated average values for Australian GHG emissions are shown in Table 1. The CO_2 equivalents for CH_4 and N_2O were taken from IPCC (1996).

Table 1. Average emissions in Australia (g/kg meat produced) from the entire pig production process using different waste management procedures.

Waste management system	CO_2	CH_4	CO_2 equivalent[1]	N_2O	CO_2 equivalent[2]	Total CO_2 equivalent
Direct land application	14	10.5	221	2.4	744	978
Anaerobic lagoons	32	132	2999	2.0	620	3424
Aerobic lagoons	1543	23.5	494	4.3	1333	3369
Covered lagoons	(269)	17	358	2.0	620	709
Digester	(412)	10.5	221	2.0	620	429
Straw-based	613	18.1	381	12.8	3968	4962

[1]CO_2 equivalent for CH_4; [2]CO_2 equivalent for N_2O (IPCC, 1996).

The initial study shows that the waste management system produces most emissions. However, all values vary over a wide range. For instance, the methane released per kg meat produced from an anaerobic lagoon varies from 6 g in winter in Tasmania to 302 g in summer in Queensland, South Australia or Western Australia. While local weather conditions do influence emissions drastically, the efficiency of the industry, daily weight gain, reproduction or feed conversion ratio are also of major importance and must be considered when developing mitigation strategies for a particular enterprise.

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DAILY PATTERNS OF AMMONIA AND BIOAEROSOL CONCENTRATIONS IN PIG SHEDS

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Elevated concentrations of ammonia gas and bioaerosols in the air-space of naturally-ventilated pig sheds have been shown to have a negative effect on the health and growth rate of growing pigs (Cargill and Skirrow, 1997; Donham, 1991). Bioaerosols consisting of dust, dried dung, bacteria, skin flakes and other particles are measured using gravimetric methods which determine the mean dust concentrations over a set period of time. Ammonia gas is measured using gas tubes that give a snapshot of gas concentrations at the time of measurement (Cargill and Skirrow, 1997). None of these methods quantify exposure levels over a 24 h period.

In order to investigate the autumn diurnal variations in ammonia and bioaerosol concentrations in pig sheds, concentrations of both pollutants were monitored continuously for a minimum of 3 d at two locations within four naturally-ventilated grower sheds with automated blinds, and four mechanically-ventilated weaner rooms, on three farms. Ammonia and carbon dioxide were measured using a Masterman Gas Machine, which analyses the concentration of gas in air samples collected every 20 min at two locations within the shed. The concentration of bioaerosols was measured using a real time analyser (Osiris), which continuously monitors the concentration of particles at one location.

The daily patterns for ammonia, carbon dioxide and bioaerosol concentrations were similar for all locations, although the maximum and minimum values recorded varied among locations. There was also a day-to-day variation in maximum and minimum values at each location. The patterns of bioaerosol, ammonia and carbon dioxide concentrations, recorded at three-hourly intervals, during a 24 h period in one weaner room, are recorded in Table 1.

Table 1. Concentrations of bioaerosols, ammonia and carbon dioxide recorded at three-hourly intervals, over one 24 h period in a weaner room.

Time	0300	0600	0900	1200	1500	1800	2100	2400
Bioaerosols (mg/m^3)	0.42	0.77	1.80	2.67	2.58	0.45	0.32	0.45
Ammonia (ppm)	13	8	7	2	5	5	11	19
Carbon dioxide (ppm)	1,200	950	550	600	650	750	1,000	1,500

The concentration of bioaerosols was associated with pig activity, increasing from early morning and peaking in the mid-afternoon. Mid-afternoon concentrations exceeded the recommended maximum concentration of 2.4 mg/m^3 (Cargill and Skirrow, 1997) at seven of the eight locations. Concentrations of ammonia and carbon dioxide peaked at all locations between 2300 and 0400 hours, suggesting that ammonia concentrations were influenced by ventilation. Overnight maximum concentrations of ammonia were above the target value of 10 ppm in three mechanically-ventilated rooms and two naturally-ventilated sheds. Daytime minimum ammonia concentrations were from 25% to 91% lower than maximum overnight values at the same location.

The results demonstrate the value of developing continuous monitoring techniques for both bioaerosols and ammonia. They also indicate that separate approaches need to be considered in pig sheds when seeking to develop strategies for reducing the concentrations of these airborne pollutants.

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USING ALL-IN/ALL-OUT HOUSING TO IMPROVE AIR QUALITY

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All-in, all-out (AIAO) management has been used successfully in pig production to reduce the impact of respiratory disease and to increase growth rates (Tuovinen *et al.*, 1990). It also provides the opportunity to house pigs in a clean environment, which may reduce the level of immunological stress on the pig and improve productivity (Holtkamp, 1995).

The effects of AIAO management on air quality in naturally ventilated grower/finisher sheds was investigated by using partitions to create AIAO sections at the end of existing continuous flow (CF) sheds on two farms. Ridge vents in all the sheds selected for renovation were at least 1 m wide. Pigs were housed in grower sheds from 7-14 weeks and finisher sheds from 14-22 weeks. Stocking densities were similar in all AIAO and CF sections, and AIAO sections were cleaned between batches of pigs. Air quality in both CF and AIAO sections was monitored over nine batches of pigs on each farm, 2-4 weeks after each new batch entered the section. Respirable particles were measured using a cyclone attachment connected to an air pump operated at 1.9 l/min. Total particles were measured using an Institute of Occupational Medicine (IOM) attachment connected to an air pump operated at 2.0 l/min. Airborne viable bacteria were measured as colony forming units (cfu) using an Anderson sampler loaded with horse blood agar plates and connected to a pump operated at 2.0 l/min. The data collected was pooled for analysis.

The range in concentrations of total and respirable particles and airborne bacteria were similar on both farms, but the values were lower in AIAO sections (Table 1).

Table 1. The concentrations of airborne pollutants in AIAO sections compared to CF sections in the same naturally ventilated sheds.

Air quality parameter	AIAO Section (mean ± SE)	CF Section (mean ± SE)
Total particles (mg/m^3)	0.937 ± 0.074^a	1.549 ± 0.155^b
Respirable particles (mg/m^3)	0.114 ± 0.012^a	0.187 ± 0.033^a
Airborne bacteria (10^3 cfu/m^3)	94.17 ± 9.388^a	124.6 ± 9.206^b

^{a,b}Values in the same row with different superscripts differ significantly ($P<0.05$).

Although the mean concentrations of total particles in both CF and AIAO sections were below the recommended target figure of 2.4 mg/m^3 (Cargill and Skirrow, 1997), 26% of all values in CF sections were above this figure. By comparison, the maximum concentration of total particles recorded in AIAO sections was 1.5 mg/m^3. Similarly, the maximum value for respirable particles in AIAO sections was only 0.19 mg/m^3 compared with 0.65 mg/m^3 in CF sections, and 21% of values in CF sections were above the target figure of 0.23 mg/m^3. Although the mean concentration of bacteria in AIAO sections was 20% below the target figure of 120,000 cfu/m^3, 21% of values were above this figure. However, the mean concentration of bacteria in CF sections was 5% above the target figure and 55% of values were above 120,000 cfu/m^3.

The results support claims that changing continuous flow management systems to all-in, all-out systems will improve air quality in well designed, naturally ventilated pig sheds.

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PREVENTION OF BACTERIURIA AND AMMONIA EMISSION BY ADDING SODIUM BENZOATE TO DIETS FOR PREGNANT SOWS

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Bacteriuria of vesical or vaginal origin in intensive sow herds causes farrowing and fertility disorders (Madec, 1984). The occurrence of bacteriuria can be prevented by increasing urinary acidity. In addition, acidic urine inhibits a rapid indoor volatilization of ammonia (NH_3) from anaerobically degraded urea and protein. As a pungent gas, NH_3 irritates the eyes and respiratory system of stockpersons, and it is harmful to the environment (Mroz *et al.*, 1996). The objective of this trial was to investigate the effect of adding acidogenic sodium benzoate (excreted mainly as hippuric acid in urine) to the diet on the incidence of bacteriuria in pregnant sows and on ammonia volatilization in slurry.

Twenty-four pregnant, multiparous, Large White x Polish Landrace sows of 180 kg initial body weight at mating were allotted to four treatments differing in dietary Na-benzoate content (0, 2, 4 and 8 g/kg) over the whole period of pregnancy, according to a completely randomized block design. A basal diet consisting of barley (55%), oats (20%), wheat (10%), rapeseed meal "00" (8%) as major ingredients was formulated to contain 15.2% of CP, 0.62% lysine and 13.5 MJ of ME/kg. The animals were offered the diet *ad libitum* and given free access to water. During weeks 1, 8, 12 and 16 of pregnancy urine samples were collected quantitatively via balloon catheters and analysed for N, Ca, P and urea concentrations, pH and numbers of bacteria. Furthermore, measurements were made of NH_3 formation from slurry (faeces + urine) incubated for 7 d at 22°C.

All sows remained healthy during the course of the study, and their voluntary feed intake and growth performance (feed efficiency and growth rate) were not affected significantly by inclusion of Na-benzoate in the diet. Data were statistically analysed by analysis of variance (ANOVA) and are presented in Table 1.

Table 1. The effects of sodium benzoate, included in the diets of pregnant sows, on water consumption, the output and composition of urine, and the formation of ammonia from slurry.

	Sodium benzoate (g/kg)				Overall	$F_{probability}$
	0	2	4	8	SED	
Water consumption (L/d)	11.2	10.5	9.0	7.5	2.73	0.08
Urine (L/d)	8.9	8.2	7.1	5.60	1.86	0.07
Urine pH	7.7	7.2	6.4	5.5	0.31	0.01
Urinary N (g/L)	3.2	3.3	4.0	5.1	0.56	0.10
Urinary Ca (mg/L)	28.8	35.4	36.6	46.0	12.04	0.21
Urinary urea (mmol/L)	110.0	120.4	143.1	170.2	25.42	0.06
Urinary P (mg/L)	68.3	70.0	86.5	109.6	28.30	0.13
Relative NH_3 formation (%)	100.0	85.3	72.4	56.8	5.25	≤0.001

Sodium-benzoate (particularly at the highest dose) reduced the bacterial population in urine from 10^6/ml at mating to $\leq 10^3$/ml at farrowing. Also, the reduction in urine pH of up to 2.2 units was associated with a 43.2% reduction in the indoor emission of NH_3 (Table 1). The benefits of including Na-benzoate in the diets of pregnant sows under the conditions used in commercial practice (e.g., restricted feeding) have yet to be tested.

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THE DISTRIBUTION OF AIRBORNE PARTICLES IN PIG SHEDS

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Airborne bioaerosol particles and dust are important environmental pollutants in pig sheds. High concentrations of these particles have been associated with increased severity of respiratory disease and reduced growth rate in pigs (Robertson *et al.*, 1990; Cargill and Skirrow, 1997).

Most sampling routines which are used to measure concentrations of airborne dust and bioaerosols depend on the analysis of air samples at one selected location approximately one metre above the ground within the airspace (Cargill, unpublished). It is important to verify the accuracy of sampling from a single location as the results are used to predict the air quality for the whole shed.

Four sampling locations, one metre above the floor, were selected within each of two mechanically-ventilated weaner rooms of similar design, with floor level pens and a central aisle. Pen walls were open and the stocking density for each room was 1.8 m^3/pig. Sampling sites were above the aisle half way between the centre of the room and the air inlet wall (aisle A), in a similar position towards the air exhaust end of the room (aisle B), and over the solid floor (pen C) and slatted area (pen D) of the middle pen. The monitoring equipment used at each location included humidity and temperature sensors attached to data loggers, a cyclone attachment to measure particles of less than 5 microns, and an Institute of Occupational Medicine (IOM) attachment to measure total airborne particles. The last two were attached to air pumps operated at 1.9 and 2.0 l/min respectively. All sites were sampled simultaneously over an 8 h period on five occasions and the data were pooled for analysis.

The concentration of total particulate matter varied among locations and was 48% higher above the aisle than over the pens (Table 1). The lowest concentrations were recorded over the slatted area. However, the concentrations of respirable particles (those less than 5 microns) were similar at all locations, and varied less than 15% between pens and aisle. Similar values were recorded for both temperature and humidity at all locations, including the patterns of daily fluctuation.

Table 1. Concentrations (mean ± SE) of total airborne and respirable particles and temperature and humidity values at four locations within each of two mechanically-ventilated rooms.

	Aisle A	Aisle B	Pen C	Pen D
Total particles (mg/m^3)	3.00 ± 0.62^{ac}	3.38 ± 0.73^{a}	1.93 ± 0.51^{bc}	1.35 ± 0.83^{b}
Respirable particles (mg/m^3)	0.28 ± 0.03^{a}	0.24 ± 0.07^{a}	0.31 ± 0.05^{a}	$0.29 \pm 0.0.6^{a}$
Mean daily temperature (°C)	23.0	23.0	23.1	23.5
Mean daily humidity (%)	52.7	45.5	56.6	53.4

[a,b,c]Values in the same row with different superscripts differ significantly ($P<0.05$).

The results obtained in the present experiments support the validity of protocols which select a single location over the aisles for monitoring dust and bioaerosols in pig sheds with a central walkway. If only one of the central locations had been used, the results would have identified that the concentrations of both respirable and total particles were above the industry targets of 0.23 mg/m^3 and 2.4 mg/m^3 respectively (Cargill and Skirrow, 1997). However, either of the locations over the pen would have failed to detect the high concentration of total particles over the aisle, and failed to alert management to a significant occupational health and safety issue.

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THE EFFECTS OF GENERAL HYGIENE ON AIR QUALITY IN MECHANICALLY-VENTILATED WEANER ROOMS

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Poor air quality has been associated with increased severity of respiratory diseases and reduced growth rate in pigs (Cargill and Skirrow, 1997). Inappropriate environmental conditions will affect the efficiency and profitability of pig production, even if they do not produce clinical disease. The identification of risk factors associated with airborne pollution is essential to reduce the impact of poor air quality on commercial farms.

The aim of this study was to assess the effects of a dirty environment on selected air quality parameters in the presence of adequate ventilation. The dirty and clean environments were created by varying stocking rates within pens in two separate rooms with independent ventilation.

The dirty room housed 97 weaner pigs in six partially-slatted pens, and more than 60% of the floor area (excluding slats) in four of the six pens was covered with faeces. The clean room housed 78 pigs, and the solid floors in all pens were free of faeces. A Masterman Gas Machine was used to monitor ammonia and carbon dioxide levels over 4 d periods. Institute of Occupational Medicine (IOM) attachments connected to airpumps running at 2.0 l/min, and cyclone attachments connected to air pumps running at 1.9 l/min, were used to monitor total and respirable particles, daily for 4 d. The pumps were operated continuously between 0800 and 1600 hours. An Anderson sampler, loaded with horse blood agar plates and connected to an air pump operated at 2.0 l/min for 5 min, was used to measure total viable bacteria once daily at 1000 hours for 4 d. Continuous temperature and humidity sensors were installed in both rooms.

Table 1. Comparison of air quality in dirty and clean weaner rooms

	Clean Room	Dirty room	Target values[c]
Stocking density (m^3/pig)	1.47	1.18	>1.20
Stocking rate (m^2/pig)	0.37	0.30	>0.30
Total particles (mg/m^3)	4.289	4.118	<2.40
Respirable particles (mg/m^3)	0.398	0.706	<0.23
Viable bacteria (cfu/m^3)[a]	87,000	194,000	<120,000
Ammonia[b] (ppm)	3.37	37.08	<7.00
Carbon dioxide[b] (ppm)	756	956	<1,500

[a]cfu/m^3 - colony forming units/cubic metre. [b]Mean concentration over 4 d periods. [c]Cargill and Skirrow, (1997).

Pen hygiene had little effect on the concentration of total airborne particles but the concentration of respirable particles, bacteria and ammonia were 1.77, 2.23 and 11 times higher in the dirty room, than in the clean room. While the concentration of total and respirable particles was above target levels in both the clean and dirty rooms, concentrations of ammonia and bacteria were only above target levels in the dirty room.

The results of the air quality audit indicate that, in the presence of adequate ventilation, pen hygiene has a greater influence on ammonia and airborne bacterial concentrations, than on airborne particles. While maintaining clean pens reduced the concentration of respirable particles, values were still above acceptable levels, suggesting that other factors need to be considered.

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MEASUREMENT OF AIR QUALITY AND WEANER PIG PERFORMANCE IN TWO DIFFERENT ENVIRONMENTS

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Previous studies in commercial pig units in Australia have shown that weaner pigs with increased air space per animal had reduced respiratory disease at market weight and increased live weight gain from birth to slaughter (Skirrow, 1993). Although these studies suggest that adverse air quality reduces pig performance particularly during the weaner growth phase, there are few studies where measurements have been made of both air quality and the growth rate of weaner pigs. The objective of this study was to create a 'clean' and a 'dirty' environment for weaner pigs and measure air quality and pig performance.

Male pigs were weaned at 3 weeks of age (7.6 ± 0.13 kg live weight; mean ± SE) and housed in the same air space in either individual pens (n=13) or groups of 10 pigs/pen (n=12 pens). The feeding regime is described by Lee *et al.* (1997). In each environment air space was maintained at 1.0 m^3/pig by stocking additional pens at 20 pigs/pen. Floor space (0.54 m^2/pig) and floor type (metal slats) were similar for individual and group pens. The experimental design was replicated in two temperature-controlled, negatively-ventilated rooms, maintained as either 'clean' or 'dirty' environments. In the clean environment the pens were hosed, effluent was flushed with clean water, and the air space was fogged with disinfectant (Virkon S) on a daily basis. The dirty environment was created by passing re-cycled effluent beneath the floor slats and not cleaning the room prior to and throughout the experiment. Data loggers were used to record room temperature and relative humidity in the clean (27.0 ± 0.42°C; 59.5 ± 1.64%) and dirty rooms (27.3 ± 0.34°C; 63.6 ± 3.10%) respectively. Ammonia (NH_3) and carbon dioxide (CO_2) concentrations in each room were measured at pig level at 0700 h each day using gas detection tubes. Total dust (<100 µm) and respirable dust (<5 µm) were collected at 50 cm above pig level at intervals of one week using a 0.8 µm filter for 20 min each hour over 24 h. Endotoxin was determined from dust filters using the limulus amebocyte lysate test, and bacteria were collected using an Anderson sampler. The experiment continued for 5 weeks and live weight of each pig was recorded at the start and finish of the experiment.

Table 1. Ammonia, CO_2 and dust concentrations, and numbers of bacteria in the air of 'clean' and 'dirty' environments for weaner pigs from 3-8 weeks of age (mean ±SE).

Environment	Clean	Dirty	Probability
Ammonia (ppm)	6.0 (± 0.49)	12.7 (±0.70)	0.001
Carbon dioxide (ppm)	1773 (± 96.3)	2268 (± 115.0)	0.002
Total dust (mg/m^3)	1.46 (±0.209)	2.28 (±0.253)	0.037
Respirable dust (mg/m^3)	0.24 (±0.032)	0.19 (±0.063)	0.500
Endotoxin (ng/m^3)	167 (± 54.4)	312 (± 115.5)	0.300
Bacteria (cfu/m^3 x 10^3)	138 (± 36.3)	197 (± 32.9)	0.245
Daily gain (g)	593 (± 14.6)	539 (± 17.3)	0.024

Significantly lower NH_3 and CO_2 concentrations and lower total dust content in the clean vs dirty environments were associated with a 10% improvement in daily gain. This study indicates that frequent cleaning of weaner pig accommodation and effluent flushing with fresh water improves air quality and pig performance.

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A NEW UNIT FOR DEFINING THE SIZE OF A PIG ENTERPRISE FOR ENVIRONMENTAL MANAGEMENT PURPOSES

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The traditional unit for defining the size of a piggery in Australia was by the number of sow equivalents. The method evolved for the traditional farrow-to-finish piggery, where the pigs were bred and grown out to sale age on one site. A major problem with this measure of size is the differences in herd performance, including pigs being sold at different ages and weights. The 'sow equivalent' measure has often been incorrectly used by local authorities and regulatory agencies in determining waste treatment facilities and buffer distances. The 'sow equivalent' method is increasingly less relevant and inappropriate for modern production systems, including multi-site production, and does not accurately reflect the waste production of a piggery enterprise. Defining the size of a pig production enterprise by its relevant waste production would overcome many of these anomalies.

The Standard Pig Unit (SPU) has been developed as a method of defining the size of a pig production unit in terms of its volatile solids (VS) production. The Digestibility Approximation of Manure Production, DAMP (Barth, 1985) model is used to predict VS production and it requires as inputs the mass, percentage dry matter, percentage ash and percent total digestible nutrients of each feed component. The level of feed wastage can be taken into account and varied to suit the class of pig and method of feeding. A SPU multiplier is calculated for each class of pig depending on their relative VS production. Table 1 shows SPU equivalents for each class of pig.

Table 1. Standard pig unit multiplier for each class of pig.

Pig classification	Age range (weeks)		VS production (kg/year)	Equivalent SPU multiplier
Gilts	1	24 to 30	258	1.8
Boars	2	24 to 128	233	1.6
Gestating sows	3	-	233	1.6
Lactating sows	4	-	373	2.5
Suckers	5	0 to 4	14	0.1
Weaners	6	4 to 10	69	0.5
Growers	7	10 to 16	147	1.0
Finishers	8	16 to 24	237	1.6

The VS unit was chosen as the best measure of potential environmental impact of a piggery because it is possible to predict the output of VS using the DAMP model, whereas no methods of prediction exist for other indicators, such as Biological Oxygen Demand or Chemical Oxygen Demand. In addition, measuring VS is cheap and easy and the waste output of the major nutrients (nitrogen and phosphorus) for any given pig production scenario is in a relatively fixed proportion to it. That is, if nitrogen or phosphorus were used as the comparison instead of VS, SPU multipliers similar to the values in Table 1 would be obtained for each class of pig. The of output VS is also used in the design standards for agricultural anaerobic lagoons.

The DAMP model is currently being enhanced to use dry matter digestibility values for each feed ingredient, rather than total digestible nutrient values. This modification, along with a mass balance validation experiment performed on a commercial grow-out piggery is expected to further improve the accuracy of VS estimation. The SPU method offers a far superior method of defining the size of a pig production unit in terms of its potential environmental impact.

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IMMUNOLOGICAL OUTPUT AND CYTOKINE PROFILE IN PIGS EXPOSED TO HIGH AMBIENT TEMPERATURES

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In recent years, immunological and endocrine stimulation have emerged as major factors in the poor growth performance of a variety of species including chickens, pigs and humans. The failure of animals to achieve their genetic growth potential can be related to the interaction of the endocrine-immune gradient and its effect on nutrient partitioning. With increased output of endocrine and immune systems, described as a high endocrine-immune gradient, nutrient partitioning is affected and is manifest as an increase in fat deposition accompanied by a decrease in muscle accretion. The pro-inflammatory cytokines investigated in this study are known to be the major cytokines influencing systemic metabolism of disease states (Murtaugh, 1994; Myers and Murtaugh, 1995). However, little work has been done to investigate the specific cytokine response to stressors such as high ambient temperatures. This paper reports the effect of high ambient temperatures on immunological parameters in pigs.

Nine female pigs, 12 weeks of age, were housed in metabolism cages. Venous blood was collected via ear vein canulae at sampling times of 0900, 1000, 1300 and 2100 hours during the non-heat period; this sampling regimen was repeated during acute heat exposure at 30°C for 24 h. Single pigs were euthanased either prior to or after exposure to heat for the removal of tissues. Cellular and humoral immune function was assayed for each time point. Neutrophil function was determined as the % phagocytosing neutrophils, and lymphocyte proliferation was determined as a stimulation index (i.e., the ratio of 3HT [tritiated thymidine] incorporated in stimulated cells compared to unstimulated control cells). Using reverse transcriptase polymerase chain reaction (RT-PCR) and specific porcine oligonucleotide primers, expression of messenger RNA (mRNA) was examined for pro-inflammatory cytokines (IL-1 , IL-6, IL-8 and TNF) in tissue samples.

Table 1. Effects of high ambient temperatures on cellular immune parameters and cytokine levels.

Measure	Control period (n=6)	Heat period (n=6)	P (paired t-test)
Lymphocyte proliferation	389.94	141.28	0.05
% phagocytosing neutrophils	26.76	15.94	0.001
Cytokine IL-6 mRNA levels	-	++++	-
Cytokine IL-8 mRNA levels	-/+	+++	-

Acute heat exposure was found to significantly decrease both the phagocytic capacity of neutrophils and the proliferative abilities of T-cells (Table 1). The RT-PCR analysis of IL-6 and IL-8 mRNA expression showed that IL-6 production was increased in all tissues and IL-8 in four tissue samples in response to heat exposure. The results obtained for cellular immune function indicate that heat exposure has a deleterious effect on cellular responses, exhibited as a reduction in the capacity of lymphocytes to proliferate when stimulated, and a decrease in the ability of neutrophils to ingest foreign particles. Exposure to heat may therefore cause immunosuppression, rendering pigs more susceptible to infection. Conversely, the expression of IL-6 and IL-8 were increased in response to 24 h heat exposure; this increase in cytokine production is consistent with a high immune gradient. The combined effect of a high immune gradient and cellular immunosuppression during heat exposure would ultimately impair the ability of pigs to reach their genetic growth potential. Further research is warranted to elucidate the interaction of endocrine and immune factors and their subsequent effects on productivity.

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DUST AND ENDOTOXIN CONCENTRATIONS IN FOUR VICTORIAN PIGGERIES

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Endotoxin is a lipopolysaccaride found in the outer membrane of gram negative bacteria. It may be a major inflammatory component of swine dust, increase the risk of respiratory disease and reduce production (Donham, 1989). Endotoxin has also been associated with a decrease in pulmonary function of workers (Donham, 1989). Changing the management of sheds from continuous flow (CF) to all-in, all out (AIAO) with thorough cleaning between batches, has been shown to increase the growth rate, reduce respiratory disease and improve air quality (Cargill and Skirrow, 1997).

In this study the concentrations of endotoxin in dust samples, collected from pig sheds where either CF or AIAO management was practiced, were measured. Respirable dust was collected using cyclone filters, and total dust was collected on 0.8 µm filters, at 1.9 l/min and 2 l/min respectively. Samples were collected intermittently over a 24 h period, for a total of 480 min. Dust samples were collected from four farms over a 12 month period and stored at -20°C. From the 360 respirable and total dust samples, 135 total dust samples were tested for endotoxin concentrations using the limulus amebocyte lysate test (Biowhittaker QCL 1000®). The data were analysed by analysis of variance (Genstat5 3.1, Lawes Agricultural Trust) and the results are presented in Table 1.

Table 1. Mean daily concentrations of airborne endotoxin and dust, from continuous flow (CF) or all-in, all-out (AIAO) weaner, grower and finisher sheds, on four Victorian farms.

Type of shed	Weaner		Grower		Finisher		$LSD_{5\%}$
Management	AIAO	CF	AIAO	CF	AIAO	CF	
Total endotoxin (ng/m^3)	153^{bc}	176^{bc}	190^{bd}	171^{bc}	126^{ac}	141^{bc}	58
Total dust (mg/m^3)	3.33^{c}	3.50^{a}	2.07^{b}	2.35^{b}	1.90^{b}	2.01^{b}	0.70

^{a,b,c}Means in the same row with different superscripts are significantly different ($P<0.05$).

Large variations in endotoxin concentrations were observed on all farms both among farms and between collections within farms. There were no significant differences between AIAO and continuous flow within age groups, but some significant differences were apparent among age groups (Table 1). Overall, 24 of the 135 total dust samples exceeding 300 ng/m^3, nine of these exceeded 500 ng/m^3. There was no association between dust and endotoxin concentrations. There were no correlations between respirable and total endotoxin concentrations, nor between respirable dust and total dust.

It has been documented that respiratory health of workers is affected when endotoxin activity exceeds 80 ng/m^3 (Donham, 1989). Depression of growth in livestock is believed to occur when concentrations exceed 150 ng/m^3 (Donham, 1989). The recommended maximum concentration for total dust is 2.4 mg/m^3 (Cargill and Skirrow, 1997). During daylight hours pigs are more active and as a result airborne dust concentrations are higher. Weaner pigs are more active than finisher pigs and this is reflected in the significantly higher total dust concentrations in weaner sheds (Table 1). During the day workers are exposed to endotoxin and dust concentrations far in excess of those recommended.

Endotoxin and dust concentrations on some farms and sheds appear to be excessive. Prolonged exposure at these concentrations could play a role in pig health and the wellbeing of workers.

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EFFECT OF GROUP SIZE AND ENVIRONMENT ON WEANER PIG PERFORMANCE AND PLASMA CORTISOL CONCENTRATION

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Pigs housed individually in research or boar test environments grow 15-25% faster than animals raised in group pens under commercial conditions (Black and Carr, 1993). An interaction of the endocrine and immune systems in response to psycho-social stress and adverse environment may be responsible for the poor performance in commercial units. The objectives were to measure in weaner pigs the effects of group size and environment on performance and plasma cortisol concentrations (this study), and also immune function (Knowles *et al.*, 1997).

Male pigs were weaned at 3 weeks of age (7.6 ± 0.13 kg live weight; mean ± SE) and housed at 27 ± 0.4°C in either a clean or dirty environment as described by Currie *et al.* (1997). In each environment pigs were housed in single pens (n=13) or in groups of 10 pigs per pen (n=12 pens). Feed intake was recorded for the 5 week experimental period and live weight was measured at the start and finish. Pigs were offered a commercial, pelleted diet *ad libitum* and water was provided by nipple drinkers. Two blood samples were collected by venipuncture. The initial blood sample was taken at weaning after pigs were moved to the weaner environments. A second blood sample was taken at the finish of the experiment. Plasma cortisol concentration was measured by radioimmunoassay. Data was analysed by ANOVA (Minitab) and the results are presented in Table 1.

Table 1. Mean performance and plasma cortisol concentrations of weaner pigs (3-8 weeks age) housed in individual (n=13) or group pens (10 pigs/pen) in a clean or dirty environment.

Environment (E)	Clean		Dirty		SEM*	Probability		
Group size (G)	1	10	1	10		E	G	E x G
Daily intake (g)	804	767	703	760	28.8	0.075	0.740	0.117
Daily gain (g)	611	573	534	544	22.3	0.024	0.540	0.283
Feed:gain	1.32	1.34	1.33	1.39	0.028	0.266	0.182	0.389
Cortisol (ng/ml):								
Start	67	72	61	75	6.3	0.547	0.513	0.044
Finish	43	51	36	59	3.7	0.842	0.002	0.073

*SEM, Standard error of the mean for environment x group size.

Pigs tended to consume more feed per day in the clean compared to the dirty environment (786 vs 730 g; P=0.075) and grew 10% faster (593 vs 539 g/d; P=0.024). Feed:gain was not significantly affected by group size or environment. The secretory activity of the adrenal cortex (plasma cortisol) was enhanced in group housed pigs in both environments at the final bleed (P=0.002). However plasma cortisol was higher at the start of the experiment which presumably reflected the additional stress associated with weaning.

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ODOURS FROM A STRAW-BASED SHELTER FOR PIGS

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This study was conducted to measure odour emission at a range of temperatures from a straw-based shelter at the Medina Research Centre. The shelter housed 174 commercial hybrid female pigs, of 85.3 kg average live weight, on a manure pack 350-450 mm deep, built up over 12-13 weeks.

The 22 m x 9 m shelter, orientated with the long axis east-west, consists of a semi-circular tunnel of woven polyethylene, supported by steel hoops mounted on 1.5 m high vertical timber sides. The odour samples were taken at the leeward end of the shelter over two 4-5 hour periods, 8 d apart in early summer, and collected into disposable Nalophan bags in a rigid plastic sampling vessel, using the procedure described by Schulz (1996). Temperature (T) and average air speed through the end of shelter, measured with a hot-wire anemometer, were recorded for each sample. Prevailing winds forced ambient air through the shelter from west to east. Smoke tests showed that under these conditions, there was negligible leakage through the sides of the shelter, enabling the ventilation rate (V) to be calculated from the air speed through the end of the shelter and the cross-sectional area of the shelter. Odour concentration (C) was measured using a computer-controlled, forced-choice, dynamic-olfactometer in accordance with the Dutch Standard (1995) NVN 2820. Odour emission rate per pig (E) was calculated from E=CV/N, where N=number of pigs. No background odours were present during sampling.

The mean and standard deviation for T, V, C and E were 24.9 ± 4.5°C, 9.9 ± 7.5 m^3/s, 568 ± 278 odour units (OU) and 28 ± 13 OUm^3/s respectively. When two readings with an outlying T or V value were excluded from the data prior to analyses, there was a significant effect of T and V on E (P=0.012 in both cases), with T and V accounting for 75.8% and 76.2% of the variance respectively.

Dalton *et al.* (1996) measured odour concentrations and calculated emission rates ranging from about 25-450 OU and 2-5O OUm^3/s per pig respectively, in a naturally ventilated building with roof ridge vents, and automatic control of side wall blinds and spray cooling. However, no temperature data were reported, and their results were strongly influenced by background odours from nearby treatment ponds. Although it appears that C and E from the shelter in our study were higher than those measured for a conventional building by Dalton *et al.* (1996), this cannot be concluded with certainty, given the effect of T and V on E. Furthermore, it is not valid to compare the two housing systems on this basis, since excrement is commonly removed from conventional buildings at frequent intervals, using flushing systems that discharge into treatment ponds which contribute to gross odour emissions from a piggery, in contrast to shelters where it accumulates in the manure pack. Further work is required to quantify the odour emissions from all sources associated with the operation of straw-based shelters, such as stockpiled manure and subsequent land applications, before proper comparisons can be made with total odour emissions from conventional production systems.

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LEPTOSPIRAL INFECTION OF PIG KIDNEYS WITH VISIBLE LESIONS, STUDIED USING A POLYMERASE CHAIN REACTION TECHNIQUE

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Visible nephritic lesions ("white spots") on pig kidneys often indicate leptospirosis due to *Leptospira interrogans* serovar pomona. The prevalence of nephritic lesions detected in Victorian abattoirs by the Pig Health Monitoring Service increased between May 1995 and May 1997 from 1.5% to 4.1%, and herds affected increased from 24% to 59%. However, human infection with serovar pomona is decreasing. Ten percent of 99 diagnostic titres of human sera submitted to Monash University for leptospirosis testing in 1988 were due to pomona, compared with 0% of 58 in 1996.

A polymerase chain reaction (PCR) technique specific for the DNA of pathogenic leptospires was used to detect leptospiral DNA extracted from pig kidneys. The PCR, which could detect 10^4 leptospires/ml or less, was compared with culture and with immunogold silver staining (IGSS) using 70 pig kidneys collected from 36 herds in 1988-89 (Chappel *et al.*, 1992). Matched sera were tested by the microscopic agglutination test (MAT). Serovar pomona was isolated from 13/70 kidneys, 10/70 were positive by IGSS, and 18 were PCR-positive. Five of the 36 herds (represented by 39 pigs) gave sera with MAT titres to pomona of ≥512. All culture-positive and IGSS-positive kidneys came from these five herds, as did 16/18 PCR-positive kidneys. Two PCR-positive kidneys came from other herds, which had MAT titres to serovar bratislava. Nephritic lesions were observed on 7/18 PCR-positive kidneys.

Table 1. Leptospiral PCR results for 94 kidneys with visible lesions, collected at Victorian abattoirs in 1996.

PCR (number of herds)	+ve Samples (samples tested)	MAT pomona ≥512 (number of herds)
+ve (30)	40 (55)	5 (1)
-ve (28)	0 (39)	0
Total (58)	40 (94)	5 (1)

The PCR was applied to 94 kidneys with lesions, collected from 58 herds in 1996 along with matched sera (Table 1). Forty kidneys were PCR-positive. However only 5 pigs (all from one herd) had pomona titres of ≥512, and these were PCR-positive. There were MAT titres of >32 to serovar hurstbridge in 14 of the 94 sera, to serovar bratislava in 13 sera, to pomona in 6 sera and to serovar tarassovi in none. Titres to bratislava and hurstbridge bore no relationship to the kidney PCR results. These results suggest that leptospires remain the major cause of white spots in pig kidneys, but that serovar pomona is of reduced significance. It is possible that many nephritic lesions are now being caused by an unidentified leptospiral serovar.

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VASCULAR-ACCESS-PORTS FOR THE REPEATED BLOOD SAMPLING OF INDIVIDUALLY OR GROUP-PENNED CONSCIOUS SWINE

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Studies to identify the stressors which limit production in group-housed pigs require a procedure that allows for repeated blood sampling with minimal disturbance to the animal. In this study, vascular-access-ports (VAP) were used in an attempt to circumvent the problems associated with exterorized catheters. The VAP's (Access Technologies, Skokie, Illinois, USA) consisted of a titanium reservoir attached to a silicone rubber catheter.

One week before surgery, two male and two female pigs (45-50 kg live weight, LW) were moved to individual metabolism cages in a temperature controlled room (23°C) and offered a grower ration. On day 1 of the study the VAP's were implanted subcutaneously with the catheter inserted into the right jugular vein while the pigs were under general anaesthesia (Halothane: nitrous oxide). Patency was maintained by filling the VAP's with heparinized/saline (1000 units/ml) after each blood sampling.

Blood samples (10 ml) were collected three times weekly between 12-53 d after surgery. On day 40, pigs were transferred from the individual pens to a group pen. To test the efficacy of the ports two functional endocrine tests were carried out, the first on day 17, when each pig was given a bolus injection of insulin (0.2 units/kg LW), and blood samples (4 ml) were taken at 30, 15 and 2 min before the injection and every 15-30 min after for 240 min. In the second test, on day 24, each pig was given a bolus injection of growth hormone-releasing hormone (GHRH: 0.5 nmol/kg LW) and blood samples (4 ml) were taken at 20, 15, 10 and 2 min before the injection and every 5-15 min after for 90 min. All samples were assayed for cortisol by radioimmunoassay (RIA) and those collected in the second test were also assayed for growth hormone (GH) by RIA.

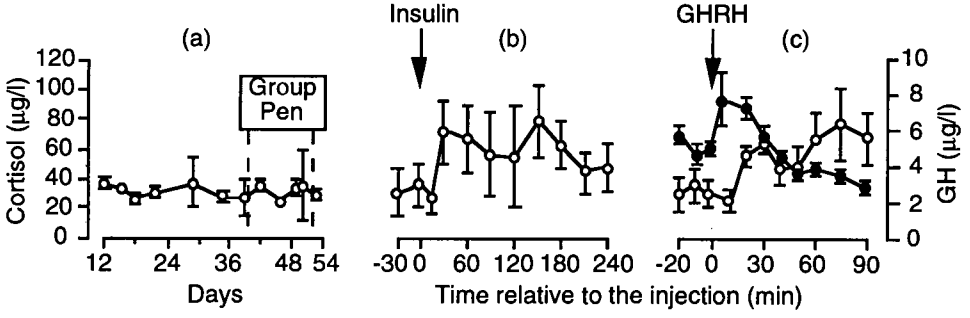

Figure 1. *Plasma cortisol (O) and GH (●) concentrations (mean ± SEM) in pigs: (a) housed individually from 12-39 d and then as a group from 40-53 d after VAP implantation (n=2); (b) after an injection of insulin (0.2 units/kg LW; n=4); (c) after an injection of GHRH (0.5 nmol/kg LW; n=4).*

Following transfer of the pigs to the group pen on day 40 the cortisol concentrations were similar to those when they were housed individually (Figure 1a). The cortisol concentrations were elevated following both insulin (Figure 1b) and GHRH injections (Figure 1c). The GH concentrations were elevated following GHRH injection (Figure 1c).

Vascular-access-ports provide a technique for the chronic catheterization of pigs without the trauma associated with maintaining exteriorized catheters and provide a suitable means of blood sampling pigs in either single or group pens without stress.

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EFFECTS OF COLOSTRUM FEEDING ON GASTRIC DEVELOPMENT IN NEONATAL PIGS

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It has been shown that colostrum feeding stimulates the maturation of digestive enzymes in the small intestine of neonatal pigs and that the effect can be eliminated by hydrolysing the colostrum with proteolytic enzymes prior to feeding (Wang and Xu, 1996). The effects of colostrum feeding on gastric development has not been demonstrated in newborn pigs, although it has been reported that natural suckling leads to rapid postnatal gastric tissue growth and morphological development (Xu and Cranwell, 1990; Xu et al., 1992). In the present study the effects of colostrum feeding on the development of gastric tissues and enzyme activity in newborn pigs were examined.

Twelve newborn unsuckled Landrace piglets from four litters were used. Piglets were bottle-fed for 3 d at 3 hourly intervals with one of three diets at the rate of 35 ml/kg body weight. One piglet from each litter received porcine colostrum, one received pre-hydrolyzed colostrum, and one received 5% lactose solution. At the end of the experiment all animals were killed with an overdose of thiopentone sodium, and the stomach from each animal was immediately removed and processed for biochemical analyses and histological examination following procedures described by Xu *et al.* (1992).

Table 1. Body weight, stomach weight, stomach weight to body weight ratio, protein and DNA content of gastric tissue, gastric enzyme activities and cell mitotic indices in piglets fed lactose solution, porcine colostrum or pre-hydrolyzed porcine colostrum (mean ± SEM).

	Lactose (n=4)	Colostrum (n=4)	Hydrolysed colostrum (n=4)
Birthweight (kg)	1.43 ± 0.10	1.31 ± 0.11	1.25 ± 0.11
Body weight (kg) at 3 d	1.28 ± 0.12	1.54 ± 0.13	1.48 ± 0.16
Stomach weight (g)	6.0 ± 0.4	8.2 ± 0.9*	8.8 ± 0.9*
Stomach weight/body weight (g/kg)	4.8 ± 0.4	5.3 ± 0.2*	5.7 ± 0.2*
Total protein content (mg)	274 ± 23	450 ± 67	436 ± 47
Total DNA content (mg)	11.4 ± 1.2	12.6 ± 2.2	14.3 ± 1.6
Milk-clotting activity[a]	4.8 ± 2.6	13.1 ± 5.1*	5.3 ± 0.4
Proteolytic activity[b]	59 ± 3	77 ± 9	67 ± 5
Muscle mitotic index (cell/mm^2)	5 ± 1.6	74 ± 19*	47 ± 5.6
Mucosal mitotic index (cell/mm^2)	76 ± 26	182 ± 38	472 ± 197*

*Significantly different from the lactose group ($P<0.05$). [a]Expressed in equivalents of pepsin activity (mg/g tissue). [b]Expressed in equivalents of pepsin activity (μg/g tissue).

Compared with piglets fed lactose solution, those fed colostrum or pre-hydrolyzed colostrum had a greater gastric tissue mass and tissue protein content (Table 1). The cell mitotic indices in both muscle and mucosal layers, as measured by bromodeoxyuridine labelling, were also greater in piglets fed colostrum or pre-hydrolyzed colostrum than in piglets fed lactose solution. Furthermore, the gastric milk-clotting activity was greater in colostrum-fed piglets than in piglets fed lactose solution or pre-hydrolyzed colostrum. It is concluded that oral ingestion of both colostrum and pre-hydrolyzed colostrum stimulated gastric tissue growth. Also, colostrum, but not pre-hydrolysed colostrum, stimulated milk-clotting activity in the gastric tissue of neonatal pigs.

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TREATMENT OF GILTS WITH PORCINE SOMATOTROPIN DURING PREGNANCY: EFFECTS ON PROGENY GROWTH

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Treatment of pregnant sows with porcine somatotropin (pST) has been reported to increase fetal growth, muscle fibre numbers of progeny and improve early postnatal growth of pigs (Kelley *et al.*, 1995; Sterle *et al.*, 1995). Since increased muscle fibre number is associated with higher growth rates and lower feed:gain in growing pigs, pST treatment during pregnancy has the potential to increase growth rates and decrease feed:gain of progeny. If effective, this treatment offers a commercial advantage to the Australian pig industry, since pST use is licensed in Australia, but not elsewhere. This study was designed to assess the effects of pST treatment of pregnant gilts on the performance of their progeny.

Pregnant crossbred gilts were fed a commercial diet (13.5 MJ DE/kg, 15.05% protein) at 1.8 kg/d and, from day 25 to day 50 of pregnancy, received 0, 2 or 4 mg pST daily by intramuscular injection. Five gilts per treatment were slaughtered at day 51 of pregnancy. The remaining gilts (n=20 sows/treatment) farrowed and progeny were weighed and tagged at birth. Two male and two female median birth weight progeny per litter were weighed regularly throughout production, including the finisher period (approximately 70-100 kg live weight).

Maternal pST treatment increased fetal weight at day 51 of gestation ($P<0.01$). Birth weight was higher in the progeny of gilts treated with 2 mg pST/d than in the progeny of gilts treated with 4 mg pST/d, but did not differ between progeny of treated and control gilts. Growth rate during the finisher period was higher in males than females ($P<0.001$), and was positively related to birth weight ($P=0.006$), but was not affected by maternal pST treatment ($P=0.482$).

Table 1. Effects of maternal pST treatment from day 25 to day 50 of gestation on fetal weight at day 51 of pregnancy, and on progeny birth weight, and finisher growth rate (Mean ± SE).

	Maternal pST treatment (mg pST/d)		
	0	2	4
Fetal weight, day 51 (g)	46.2 ± 1.4^{a}	52.7 ± 1.4^{b}	54.6 ± 1.5^{b}
Birth weight (kg)	1.44 ± 0.05^{ab}	1.54 ± 0.04^{a}	1.40 ± 0.04^{b}
Finisher growth rate (g/d)*	1003 ± 24	982 ± 25	1025 ± 24

^{a,b}Values in the same row with different superscripts differ significantly ($P<0.05$). *Adjusted to a birth weight of 1.5 kg.

Despite the positive effects on fetal growth during the period of maternal pST treatment, administration of pST to gilts during early- to mid- pregnancy did not affect progeny birth weight, nor postnatal growth rate of progeny during the finisher period in this experiment. This outcome differs from that of previous reports and is most likely due to differences in maternal nutrition between studies. Further examination of interactions between pST treatment (dose and period) and nutrition is in progress.

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PASSIVE AND/OR ACTIVE IMMUNIZATION AGAINST SOMATOSTATIN IN PIGS GROWN TO MARKET WEIGHT

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Somatostatin (SS) is a universal inhibitor of endocrine function known to suppress the secretion of growth hormone, insulin and gut hormones. Previous studies have shown that passive immunization against SS by the provision of antibodies in colostrum from SS immune sows improves weaning weight (Westbrook, 1996). In this study, the efficacy of a novel SS antigen to improve the efficiency of growth in pigs grown to market weight was assessed. The influence of subsequent active SS immunizations on growing pigs with or without passive immunization was also evaluated.

Primiparous sows (Large White x Landrace) were either actively immunized with a SS antigen (n=20), or a placebo of adjuvant alone (n=20), at 3 weeks and 1 week prior to farrowing. Litter sizes were standardized to 10 piglets per litter avoiding cross fostering within and between treatment groups. Piglets were suckled naturally. Piglets, selected on the basis of liveweight, from both control and SS immune sows were actively immunized with the same antigen at 28 and 42 days (CT, n=68, TT, n=69 respectively) and some of these animals received a further boost on day 112 (CTT, n=42, TTT, n=32 respectively). This last boost coincided with the transfer of animals to finisher accommodation where they were grown out to market weight (24 weeks). Differences in average daily live weight gain (DG) emerged during the finisher phase (Figure 1). Data were analysed in Minitab as an incomplete factorial ANOVA, and in Genstat for individual treatment comparisons.

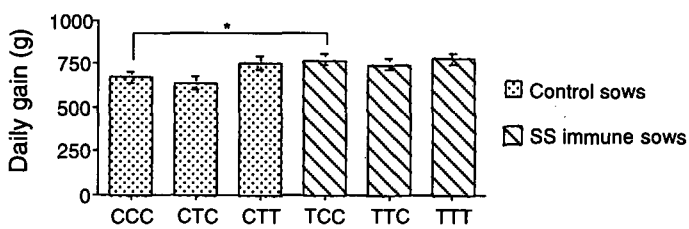

Figure 1. *Daily live weight gain (DG) (mean ± SEM) of pigs from 13-20 weeks of age (C=Control, T=SS Immunization). The first letter on each bar corresponds to the treatment of the sows, the second to the treatment of piglets at 28 and 42 d, and the third to the treatment of the pigs at 112 d. Statistical significance; *P<0.05.*

Both pigs which received two active immunizations (CTT, 749 ± 34g) and pigs which received passive immunization alone (TCC, 768 ± 32g) had significantly better growth rates over the whole study than the controls (CCC, 668 ± 28g, P=0.056 and P=0.017 respectively). Administration of booster immunizations to piglets with passive immunity had no additional effect (TTT, 772 ± 34, P=0.77). There was an overall improvement in growth performance in pigs from dams immunized against somatostatin (TCC, TTC, TTT) compared to pigs from control dams (CCC, CTC, CTT; P=0.004).

The fact that passive immunization of the piglets alone (TCC) was equally as effective as active immunization (CTT) suggests that an improvement in metabolic efficiency through an endocrine imprinting mechanism or an acceleration in gut development may have been achieved in the neonatal piglets. Thus the growth rate of finisher pigs may be enhanced simply by the provision of the antibodies from the sow at farrowing, which would be a simple technology for the producer to adopt for commercial production.

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PROVISION OF IMMUNOGLOBULINS TO SUCKING PIGS CAN ENHANCE POST-WEANING GROWTH PERFORMANCE

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Enterotoxigenic pathogens are present in most pig herds and, in the case of the young pig, may cause intestinal atrophy or loss of function in the developing gastrointestinal tract (GIT) (Martineau *et al.*, 1995). An effective response to pathogenic bacteria in the GIT relies, in part, on an increase in the local concentration of immunoglobulins (Ig). Sufficient amounts of oral bovine IgG resist digestion in the upper GIT to remain active at this site (Morel *et al.*, 1995). Minimising GIT disease during early life may provide long-term advantages in growth performance by reducing damage to the structure of the gut and subsequently improving gut function. In the present experiment, the hypothesis that providing a supplementary source of bovine IgG to sucking piglets increases post-weaning growth performance was tested.

The litters from eight multiparous Large White x Landrace sows received oral supplements by syringe. Three pigs in each litter received oral doses of whey globulin concentrate (WGC), which contained 6% IgG. A second group of three pigs per litter received oral doses of whey protein isolate (WPI) to approximate the amino acids supplied in WGC but without the IgG's. A third group of three piglets per litter received oral doses of water (CONT) to simulate the oral dosing procedure. The daily supplement of WGC and WPI provided 0.7 g/d of ideal balanced protein during the first week and 1.4 g/d thereafter. The oral doses were provided twice daily at 0900 h and 1600 h from day 3 to day 24 of lactation. A linear model including sex, sow and treatment as fixed effects, and live weight at birth as a covariate, was fitted to the data.

Table 1. Least square means for average daily gains (g/d) during growth.

Period	CONT	WPI	WGC	Pooled SE	Treatment P-value
Birth-weaning[1]	249	264	259	9	0.52
Weaning-transfer[1]	425	422	405	14	0.60
Transfer- slaughter[1]	692	638	722	20	0.02
Birth-slaughter	545	521	565	11	0.03

[1]Weaning = 24 days of age; Transfer = 62 days of age; Slaughter = 85 kg live weight.

The provision of either WGC or WPI did not increase the average daily gain up to weaning (Table 1), possibly because the piglets reduced their intake of sow's milk. To determine the effect of supplemental IgG, the most valid comparison is WPI vs WGC because the supply of ideal protein, and the time taken to provide each oral dose, were similar for these two groups. The WGC pigs grew 13% faster from transfer to slaughter ($P<0.05$), and 8% faster from birth to slaughter ($P<0.05$), suggesting that the provision of IgG during early life can lead to long-term advantages in growth rate.

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RESPONSE OF VACCINATED PIGS TO CHALLENGE WITH *ACTINOBACILLUS PLEUROPNEUMONIAE* SEROVAR 1

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Chronic respiratory disease caused by *Actinobacillus pleuropneumoniae* (AP) is a major contributor to poor herd performance. In herds where AP is endemic, vaccines combined with segregated early weaning (SEW) could become an important part of disease management. The ability of four AP vaccines (A, B, C and D), to protect pigs from experimental challenge was examined in a preliminary trial. These vaccines were obtained from four different sources.

Forty-two specific-pathogen-free Large White x Landrace male pigs, seven weeks of age were randomly allocated into six groups, i.e., two groups of five pigs (positive and negative controls), and four vaccine groups of eight pigs (week 1). All vaccines contained AP serovar 1 antigens and exotoxins. All pigs in each vaccine group received two doses of vaccine. The controls were injected with sterile saline. Pigs were weighed prior to vaccination and challenge, and at the completion of the experiment (week 10). Blood samples were collected before vaccination and challenge and analysed using an ELISA for antibodies to ApxI haemolysin, a major virulence factor of AP. Two weeks after the final vaccination, the positive controls and all vaccine groups received an intranasal challenge of AP serovar 1. Animals severely affected by challenge were euthanased then necropsied. Survivors were necropsied two weeks post challenge. Lungs were examined and sectioned, and samples for culture were taken from the turbinates, trachea and lungs.

Each vaccine provided a level of protection. Vaccines A and C substantially reduced infection in comparison to the effects seen in the positive control pigs (Table 1). Isolation of AP commonly occurred from the lungs, though in groups A, C and D, the organism was not recovered from all lung lesions. Vaccination had no effect on pre-challenge growth rates. The variation in growth rates among groups occurred after challenge (Table 1). Post-vaccination levels of ApxI antibodies for three vaccine groups (A, C, and D) increased substantially, with a strong association between vaccine induced levels and protection, apparent in these groups (Table 1).

Table 1. Effects of serovar 1 challenge in four groups of vaccinated pigs.

Treatment	Mortalities	AP isolation No. pigs	% Lung affected	Av. Growth rates post-chall.(g/d)	ApxI titres* (mean ± SE)
+ve control	5/5	5/5	69	0	0.099 ± 0.034
-ve control	0/5	0/5	0	1053	0.093 ± 0.031
A	1/8	2/8	5	988	1.447 ± 0.042
B	6/8	8/8	55	277	0.454 ± 0.108
C	0/8	1/8	5	961	1.694 ± 0.065
D	1/8	4/8	19	776	1.033 ± 0.042

*Increases above titres determined prior to vaccination.

The levels of protection provided by different vaccines were variable, though each vaccine provided some level of protection compared to the effects observed in the positive controls. From information gathered here a vaccine will be chosen for trials to determine whether hyperimmunisation of sows can confer passive protection to early weaned pigs. If ApxI antibody levels in piglets can be correlated with improved disease resistance, this should enable weaning age (to prevent pleuropneumonia) to be increased in SEW systems. Increasing weaning age should have positive ramifications on sow productivity, and reduce the costs associated with weaning very young piglets.

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NOTE: Due to confidentiality agreements the authors are not able to provide the sources of the vaccines or the vaccination regime.

EFFECT OF ENVIRONMENT AND GROUP SIZE ON IMMUNOLOGICAL PARAMETERS IN WEANER PIGS

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Pigs raised in commercial production units grow 15-25% more slowly and are often fatter than their individually housed counterparts; the combined effects of these factors have been estimated to reduce the profitablilty of a 250 sow piggery by more than $50,000 per annum (Black *et al.*, 1994). Interaction between the endocrine and immune systems in response to stress has emerged as a factor controlling the growth performance of intensively farmed livestock. With a high endocrine-immune gradient, nutrient partitioning is altered to cause increased fat deposition and decreased muscle accretion (Elsasser, 1993). The objectives of this study were to examine the effects of environment and group size on immunological parameters and plasma cortisol concentration (Lee *et al.*, 1997) and the subsequent effects on growth rate in weaner pigs.

Male pigs weaned at 3 weeks of age were housed at 27°C in either single pens (n=13) or groups of 10 pigs per pen (12 pens). The individual versus group experimental design was replicated in two rooms maintained as either clean or dirty environments as described by Currie *et al.* (1997). Blood samples were collected by venipuncture upon entry into experimental rooms (day 1) and upon exit (day 35). Feed intake and growth rate for the 5 week period were reported by Lee *et al.* (1997). Immunological parameters measured included neutrophil phagocytic function, lymphocyte proliferation and natural killer (NK) cell activity (Table 1). Data were analysed by ANOVA (Minitab).

Table 1. Mean daily rate of gain (DRG) and cellular immune function values of weaner pigs in individual (n=13) or group pens (10 pigs/pen, n=12), housed in clean or dirty environments.

Environment (E)	Clean		Dirty		Probability		
Group size (G)	1	10	1	10	E	G	E x G
DRG (g)	611	573	534	544	0.024	0.540	0.283
WBC[1] ($x10^6$/ml)	25.96	21.93	24.86	20.64	0.534	0.008	0.953
Neutrophil function (%)	38.11	34.95	39.46	42.59	0.003	0.917	0.049
Lymphocyte proliferation (SI[2])	96.16	157.16	65.84	81.92	0.003	0.033	0.173
NK[3] activity (%)	62.12	46.57	42.61	49.76	0.172	0.314	0.021

[1]WBC, white blood cell count. [2]SI, stimulation index. [3]NK, natural killer cell.

Pigs in the clean environment grew 10% faster (593 vs 539 g/d) compared with those in the dirty environment. Pigs in the dirty environment had significantly greater neutrophil phagocytic activity (P=0.003) while the proliferative responses of lymphocytes were significantly impaired (P=0.003). There was an interaction between the dirty environment and groups size to alter NK activity (P=0.021). The combination of group size and a dirty environment may increase endocrine-immune output resulting in poor growth performance of weaner pigs under conditions of increased environmental stress.

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NUTRIENT OXIDATION AND LIPOGENESIS IN GROWING PIGS

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Energy metabolism has been widely estimated by indirect calorimetry in combination with balance experiments. However, little attention has been paid to the possibility of using the data from indirect calorimetry to estimate substrate oxidation and lipogenesis. In this paper, oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF), and lipogenesis in growing pigs have been quantified by indirect calorimetry and carbon and nitrogen balances.

Energy and protein metabolism were measured in 25 Danish Landrace pigs over a live weight (LW) range of 30-90 kg. The pigs were fed at a high feeding level with metabolizable energy (ME) >1.2 $MJ/kg^{0.75}$ and digested protein (DP) between 11-15 $g/kg^{0.75}$. Results were presented in 4 groups (A-D) in relation to digested fat (DF). The pigs in groups A and D were offered a semipurified diet with or without addition of soya oil respectively. In groups B and C pigs were fed with barley and maize respectively supplied with identical protein sources. Consecutive balance experiments were carried out with a 7 d preliminary period followed by a 7 d collection of faeces and urine. A 24 h measurement of gas exchange by means of an open-air-circuit respiration unit was performed in the middle of each collection period. Nutrient oxidation and lipogenesis were calculated according to Chwalibog *et al.* (1992) and Chwalibog and Thorbek (1995).

Table 1. Digested fat (DF) and oxidized protein (OXP), carbohydrate (OXCHO) and fat (OXF) in relation to total heat production (HE). Lipogenesis in relation to total fat retention (RF) at the start and end of the growth period from 30-90 kg LW.

Group		A		B		C		D	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Balances (n)		24		82		42		18	
DF ($g/kg^{0.75}$)		0.5	0.1	1.2	0.4	2.4	0.4	7.7	0.1
OXP/HE (%)		14.2	3.4	14.3	2.4	14.8	2.4	15.2	3.8
OXCHO/HE (%)		85.8	3.4	85.7	2.4	85.2	2.4	84.8	3.1
OXF/HE (%)		0		0		0		0	
Lipoge-	Initial	70	6.4	34	5.9	17	3.5	25	1.3
nesis (g/d)	Final	369	18.8	362	11.7	322	17.8	275	6.7
Lipog.	Initial	92	0.9	64	7.1	44	4.7	20	1.1
(% of RF)	Final	97	0.2	91	0.5	82	0.7	55	0.4

In the present study no OXF was observed in spite of the variation in DF between 0.5-7.7 $g/kg^{0.75}$. The results are in agreement with Flatt *et al.* (1985), who demonstrated from short-term energy balances in humans that the presence of fat in the meal did not promote fat oxidation. With no OXF the heat production was caused mainly by OXCHO (85%) while the remaining 15% originated from protein oxidation. This indicates that digested carbohydrate is a major energy substrate which together with DP covers the oxidative energy requirements without any contribution from DF.

Lipogenesis was the major source of fat retention when DF was low, while it decreased with increasing DF level. This may indicate that the supply of DF is a dominating factor in fat accretion, while the level of lipogenesis is adjusted according to the level of DF.

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IMPACT OF FEEDING SURFACE DESIGN ON FEEDING BEHAVIOUR AND FEED WASTAGE BY GROWER PIGS

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There are two main sources of feed wastage in single-space feeders - direct feed loss as a result of rooting and pawing behaviour, and indirect loss as a result of unnecessary visits to the feeder. Feed wastage could be minimized by physically preventing direct loss and by maximizing time spent within the feeding space, i.e., meal duration.

Meal duration can be increased by increasing the cost of obtaining feed. A new prototype pig feeder, the RT (Rootin' Tootin') feeder (University of Melbourne and PRDC, 1997), which is based on this premise, has been developed. One of the key features of this feeder is that the pig must perform an operant feeding response, rooting on the floor of the feeder, in order to obtain feed. This response appears to be sensitive to the texture of the rooting substrate and therefore the influence of different feeding surfaces on feeding behaviour and feed wastage has been investigated.

Male and female crossbred pigs (20-25 kg) were individually housed in 1.1 x 2.4 m pens on raised wire mesh floors with access to a single-space tunnel feeder. Six different feeding surface treatments, 285 mm wide x 250 mm deep, were evaluated. Flat - mild steel plate; Check - mild steel checkerplate; TranSS - rippled stainless steel laid across the feeder floor, transverse to the body axis of the feeding pig; LongSS - rippled stainless steel laid along the feeder floor; TranRod - 5 mm mild steel rods welded at 30 mm centres to mild steel plate, laid across the feeder floor; LongRod - steel rods on plate laid along the feeder floor. Six pigs were observed on each of the six treatments for a 3 d period in a Latin square design, replicated twice (n=12 pigs). Daily *ad lib* feed intake was recorded, and spilled feed was collected from trays underneath each pen. Feeding behaviour was sampled for one 24 h period per treatment using time lapse video, and rooting behaviour was sampled for one 3 h period per treatment in the morning or afternoon feeding periods using real time video.

Table 1. Effect of feeding surface texture on mean number of feeding and rooting events, daily intake and wastage.

	Flat	Check	TranSS	LongSS	TranRod	LongRod	SED	P
Feeding (events/24 h)	95	80	85	87	91	84	8.9	0.596
Intake (g/d)	1871	1835	1938	1924	1856	1856	47.6	0.204
Wastage (g/d)	278	126	258	258	44	212	71.5	0.012
Rooting (events/3h)	190	99	219	176	205	89	65.7	0.230

There were no significant differences in the number of feeding events between the six feeder types (Table 1). There was no significant difference between treatments in feed intake, but planned orthogonal contrasts showed significantly (P=0.03) higher intakes on stainless steel compared to rod feeding surfaces. There were significant differences in spilled feed, with least wastage from the Check and TranRod feeders. Differences in rooting behaviour were pronounced but not significant. The least amount of rooting was observed on checkerplate and longitudinal rod surfaces.

The results presented here illustrate the dilemma facing feeder designers. The performance of rooting behaviour, which encourages longer meal durations, may be stimulated by smooth textured feeding surfaces, and contribute to enhanced intake. However, the same behaviour is responsible for direct loss of feed from feeders.

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METHOD OF FEEDING CAN AFFECT WELFARE OF PIGS

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In conventional pig production systems, dry sows are housed either in stalls or group pens. These pigs are generally restrictively fed and are either fed concurrently with drop-feeders or manually; in the latter case there is a delay between the first and last stall/group receiving food. This experiment compared sequential versus simultaneous feeding on the stress response of pigs (based on total cortisol concentrations, measured over a 3.5 h period around feeding on days 1, 2, 3, 8, 10, 17, and 24, and free cortisol concentrations, similarly measured, on days 17 and 24). After pigs were trained to expect feed once a day at a specific time (period 1), treatments were imposed so that pigs were fed simultaneously (Treatment 1 - Concurrent), or in a fixed order (Treatment 2 - Fixed), or in a random order (Treatment 3 - Random). The three treatments were imposed on a total of 24 individually housed boars (Large White x Landrace) that were housed in one shed; the pigs were approximately 60 kg starting weight and were selected from floor-fed, group-housed pigs. The treatments were: (1) Concurrent - pigs were fed simultaneously at an "announced" (the cue was a buzzer) daily presentation of feed (six pigs fed at period 1); (2) Fixed - two pigs were permanently assigned to be fed at one of six fixed consecutive 15 min intervals after period 1 (12 pigs with two pigs each fed at periods 2-7); and (3) Random - each of six pigs was randomly assigned each day to any one of the seven possible feeding times. All pigs were automatically fed once daily to approximately 90% of expected voluntary feed intake and there was an initial 2 week period to condition them to expect feed in conjunction with a unique auditory cue (a buzzer).

Changing the feeding schedule from being associated with a cue (a buzzer sounding immediately before the first feed drop of the day) to a random event occurring at some time over the following 90 min had adverse effects on pigs. Pigs in the Random treatment showed increased overall total and free cortisol concentrations ($P<0.05$; Table 1) and higher cortisol concentrations 15 min after the time of the first feed drop of the day than in both Concurrent and Fixed treatments ($P<0.001$). Free cortisol concentrations (mean values for days 17 and 24 combined) were higher in the Random treatment ($P<0.05$). There was a significant interaction between treatment and time for mean total cortisol concentrations. On day 1 of treatment cortisol concentrations were lower in the Concurrent than the Fixed and Random treatments ($P<0.05$; mean values were 47.5, 65.3 and 76.0 nmol/l, respectively; $LSD_{(P=0.05)}=17.35$), while on day 2 the mean values were only different in the Random treatment ($P<0.05$; mean values were 56.2, 54.8 and 81.1 nmol/l for the Concurrent, Fixed and Random treatments, respectively).

Table 1. Effects of feeding treatment and day on overall cortisol concentrations (nmol/L).

		Treatment			Day		
Parameter	Control	Fixed	Random	1	8	24	SEM
Mean total cortisol	48.6^{p}	56.8^{a}	66.2^{qb}	67.0^{y}	50.8^{px}	63.2^{q}	2.13
Total cortisol 15 min after the 'buzzer'	46.7^{x}	52.7^{x}	74.1^{y}	78.2^{qb}	57.4^{q}	54.0^{p}	6.46
Free cortisol	6.3^{a}	7.1^{a}	10.0^{b}	-	-	-	1.31

$^{ab, pq \text{ and } xy}$ denote significant differences at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

The data suggest that the way in which pigs are fed can have implications for their level of stress and possibly their welfare. There was evidence that the Random treatment resulted in a chronic stress response, based on both the cortisol data presented and a reduced immunological responsiveness, and in the Fixed treatment there was some evidence for an initial short term cortisol response to changing the feeding schedule.

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AUTHOR INDEX