# MANIPULATING PIG PRODUCTION III

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#### PREFACE

This is the Proceedings of the Third Biennial Conference of the Australasian Pig Science Association. The Association was formed in 1987 with the objectives of encouraging and promoting scientific discussion and collaboration amongst scientists interested in pig research and pig production. The strength of APSA is that all the relevant scientific disciplines and all the relevant areas of pig production are represented. As such the previous scientific conferences in 1987 and 1989 have created a highly interactive and effective forum for comprehensive discussion of current pig research and the problems and issues facing pig production.

In Albury in November, 1991, in accordance with the general aims of the Association, over 200 scientists with a common interest in pig research and production met to interact both scientifically and socially. This is the Proceedings of that Conference. The Conference covered the main disciplines of reproduction, welfare, nutrition, health and genetics. Several review and symposium papers on critical and topical subject areas were presented by internationally renowned scientists. These major papers were supported by 71 abstracts presented by scientists of the Society. These proceedings will be extremely valuable to people interested in pig research and pig production.

The Executive of APSA is confident that the scientific and social program organized for the Third Biennial APSA Conference, as with previous APSA Conferences, will result in improvements in the understanding of pig research and pig production. Furthermore, it is anticipated that the Conference will stimulate and encourage high standard research in the critical areas of pig production. Improvements in these aspects will undoubtably result in gains in pig productivity and improvements in animal welfare.

The Organising Committee for this Conference consisted of Drs D.P. Hennessy (Secretary), G.M. Cronin (Treasurer), R.S. Cutler, M.R. Taverner, R.H. King, E.S. Batterham, B.P. Mullan, R.G. Campbell and Messrs P.D. Cranwell and C. Hansen. I would like to thank them for their splendid support during the past 18 months.

P.H. HEMSWORTH President APSA

# PAST ACHIEVEMENTS AND FUTURE ROLE OF RESEARCH IN THE PIG INDUSTRY

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Pig research in Australia has developed rapidly in strength and size. The Australasian Pig Science Association has approximately 150 members including many established and internationally respected scientists and importantly, there are also young scientists of considerable talent.

For an industry containing approximately 1600 specialist pig producers, the Australian pig industry is well served for research expertise. This has been reflected in the rate of improvement in productivity in Australian pig farms.

From a biological viewpoint, the opportunities for research continue to increase with our greater understanding of the pig and the rapidly improving research technology. There are other existing and emerging factors, however, that may increasingly limit these research opportunities in the future.

This paper discusses the changing industry and research environment and their likely impact on the future role of research in the Australian pig industry.

## The changing pig industry

Rapid expansion in the capability, activity and output from pig research in Australia has been matched both by increase in the productivity of the pig industry and dramatic change in industry structure.

In a recent detailed study of the performance of Australia's first specialist intensive pig unit, Cleary (1991) found that between 1970 and 1990, herd liveweight feed conversion efficiency improved 25% (from approximately 4.0 to less than 3.0, Figure 1). The trend has been industry wide - an analysis of the Victorian pig management recording scheme (Mr D. Treacy, personal communication) indicated a similar improvement in herd feed conversion from 4.83 in 1971 to 3.24 in 1990.



Figure 1. Herd liveweight feed conversion at Huntly piggery during the past 25 years (Cleary, 1991).

Increased productivity has been accompanied by a change to large and increasingly specialised pig farms. As the total size of the Australian pig industry is quite stable, there is a quickly decreasing number of producers - in 1989 there were 61% fewer holdings with pigs than in 1980. However, these farms had an average of 2.7 times more pigs than those in 1980. ABS (1989) statistics indicate that the majority (57%) of pig production in Australia was derived from the approximately 430 herds (5.6% of pig farmers) whose herd size exceeded 100 sows. It is expected (CRESAP Report, 1990) that within the next decade this sector of the industry will control 90% of the market.

It is likely that much of the improved productivity results directly from the development and implementation of research information.

#### The changing research environment

During the past 20 years, research in the pig industry has primarily been undertaken and funded by public sector institutions. Table 1 shows the level of supporting industry funds which have been provided to State Departments of Agriculture, Universities and Colleges and other bodies. Industry funds have been used to "seed" research in these institutions and have generally provided far less than half of the total costs of research.

Table	1.	The	proportional	distribution	(%)	of	industry	research	funds	among
researc	ch c	organis	ations							

	1974/75	1979/80	1984/85	1989/90
State Departments	55	45	46	40
Universities & Colleges	41	49	37	41
Commonwealth Departments	4	6	10	16
Other bodies	-	-	6	3

Through the Pig Research and Development Corporation (PRDC) the pig industry has developed an efficient mechanism to support, and to an increasing extent control the direction and nature of research.

In 1971 the industry took advantage of a Commonwealth Government initiative to match industry funds for research. A levy of 5 cents on each pig slaughtered resulted in a pool of \$121,500 available to support research. A broadly-based committee of industry, Government and research representatives was established to determine the most cost effective use of these research funds.

For the first five years the priority for this funding was to establish a national carcass classification scheme - this work is one of the significant achievements of research for the Australian pig industry. During the 1970's, the industry funds were largely responsible for developing our current physical resources for pig research.

One of the greatest achievements of the industry funds has been to establish our current human resources for research - in 1991 there are more than 50 pig industry funded scholars trained or in training.

The industry support for research and development (R&D) has continued and currently the pig industry is directly contributing 55 cents per pig slaughtered (0.4% of GVP). With an annual budget of \$5.4 million to invest in R&D, the pig industry, through the PRDC, is a dominant influence in pig research in Australia.

Despite the large increase in industry funding of R&D, the competition for these funds is increasingly fierce - within and between specialties, and between individuals, research groups and institutions. Whereas in the past there has been relatively little competition for funding good science, there is now an increasing selectivity on research funding and scientific merit will not by itself ensure support.

In 1991 the PRDC fully or partly supported 43% (35/81) of new applications for research. Increasingly PRDC funds are strategically allocated and in its recent R&D

Plan (PRDC, 1991), the PRDC have identified six major objectives, 26 key strategies and 17 specific priorities which guide its activities and investments in R&D. Research into pig health has been traditionally an area of high activity and it remains a high priority. But of the 21 new applications for health research, most were good science but strategic investment limits in this area allowed only four new applications.

Some of the major features of the changing research climate for pig scientists are:

- 1. Agriculture has shared in the rapid reduction in public sector expenditure and an associated increase in accountability. Unfortunately, agricultural research (and probably science in general) is probably not now recognised to the extent it deserves for its contribution to the economy.
- 2. The industry funds are becoming more business oriented and more concerned with the likely returns to research investments.
- 3. The industry is also becoming more sophisticated in business and is requiring greater accountability and return for its investment in research. As the industry continues its trend to larger production units, the producers will generally have a clearer understanding of research needs and be better able to direct funding and resources for their perceived needs. This will increase the priority for shorter term, applied research.
- 4. As scientific knowledge advances and the technology necessary to develop its potential becomes increasingly complex, both the costs of research and the potential benefits are expanding.

Table 2. The increasing costs of R&D - the average size and cost of the research programmes of the PRC and PRDC since 1985/6

	N° of projects	Total expenditure (\$m)	Average expenditure (\$'000/project)
1985/86	52	1.01	19.4
1986/87	60	1.22	20.3
1987/88	74	1.64	22.1
1988/89	76	1.84	24.2
1989/90	87	2.82	32.5
1990/91	79	2.94	37.2
1991/92	72	3.31	46.0

As shown in Table 2, the average costs of research projects to the PRDC has doubled during the past four years. This is partly a reflection of the increasing overall costs of research, but also a reflection of the diminishing public sector support for research and greater reliance on industry funds.

There are two major consequences of this change: firstly, it is unlikely that industry funds will be able to completely compensate for this loss of public sector funds and thus total resources for pig science will diminish in the longer term. Secondly, industry funds will increasingly direct research in the pig industry.

#### Past achievements of pig research

There are many measures of achievement in research. For researchers, success is often measured in scientific publications, peer and industry recognition and continued funding. For the industry, successful research is that which has led to simple, quickly adopted and widely applicable technology resulting in large cost reductions. The most successful of our research programmes have combined all of these achievements - they have been characterised by good basic science (which has provided the success required by the scientist) from which clear and important implications and practical applications have arisen.

An example is in the area of pig nutrition, growth and development: research during the 70's and 80's provided an understanding of the pig's basic tissue responses to nutrient intake and how this is affected by gender, age, liveweight and genotype. The work enabled the development for Australia of a manual of nutrient requirements (Standing Committee on Agriculture, 1988) which has had considerable national and international impact on pig feeding. Furthermore, this basic research information enabled the development of Auspig, a simulation model of pig performance (Black *et al.*, 1986), now used as a decision support system for pig farm management in Australia.

#### Areas for future research in the pig industry

Through an analysis of current industry trends and predicted future industry developments, and through discussion with farmers, researchers, Government and industry leaders, the following areas for future research were determined:

- Production efficiency. There will be the continued demand for new technology to maintain past increases in farm productivity. Generally the terms of trade for pig producers have been in decline in recent years - the price of feed (the major cost component of pig production) has increased more than pig meat prices (G.V. Cleary, personal communication). Thus the sustained viability of the industry demands that efficiency indicators such as herd feed conversion (Figure 1) continue to improve. Specific areas identified for future research activity include:
  - i) Dietary protein quality a better understanding of the fate of dietary amino acids in the pig is needed to improve the low utilisation of dietary protein in commercial pig diets and to develop techniques to measure dietary amino acid availability.
  - ii) Constraints to lean growth developments in molecular biology have enabled new and considerably higher limits to the pig's lean growth potential. Research is required to provide a practical and acceptable implementation of this technology i.e. an adequate delivery system for exogenous growth hormone (PST) or an alternative means of manipulating the somatotropin axis of the pig. In the shorter term, research is required to establish the major factors under commercial conditions which are limiting the expression of the pig's potential for lean growth.
  - iii) Superior genetic stock methods are required for the better identification of superior genetic stock. Biotechnology will enable the use of physiological indices of growth and reproduction. These will complement electronic developments in automated measurement and recording of animal performance. Advanced breeding tools such as PIGBLUP, should be used to model economically important but difficult traits such as mothering ability and carcass and meat quality. Ultimately, individual genes controlling traits of economic importance will be identified and incorporated into transgenic programmes. Further development of artificial breeding is necessary to enhance the dissemination of improved genetic stock.
  - iv) Controlling endemic disease although continued major health problems are

expected with respiratory and enteric disease complexes, increasingly the industry will be forced to control these diseases with little or no antibacterial substances. Research effort will be required therefore to better understand the relationship of the disease organisms and the host. This should then lead to control mechanisms based on vaccines and on the manipulation of the pig's immune system and resistance capacity through a better understanding and control of the macro/micro environment and the development of systems that will boost the pig's immunity to disease entities.

- v) Sow milk production evidence suggests that growth performance of the baby piglet is considerably below its potential and is limited largely by the milk production of the sow. Research is required to establish factors affecting milk production and mechanisms controlling its production and release to the piglet. There may well be applications to pigs from research developments in molecular biology in dairy species.
- vi) Piglet mortality in the Australian pig industry there is an average mortality of 25% of the total pigs born and 20% of those born alive (Australian Pig Industry Reference Manual, APIRM 1989/90). Less than 8% die after weaning. It will be necessary to develop strategies to increase piglet survival and performance through a better understanding of the processes of parturition, lactogenesis, piglet activation and immunoglobulin transfer.
- vii) Reproductive performance - better understanding is needed of the factors limiting the pig's reproductive performance under commercial conditions. A greater understanding of reproductive endocrinology may provide solutions to the continuing problems of seasonal infertility. This work may also provide leads for the use of hormones and immunisation against hormones to enhance reproductive performance. There would be advantages for fertility and in other areas, in identifying the timing of ovulation and hence the optimum time of mating of the female. More accurate quantitative information is required on the relationships between nutrient intake and reproductive performance. Research into factors controlling feed intake of sows will be an important component of future research. Substantial improvements in reproductive performance may be possible using transgenesis with prolific Chinese breeds. Further knowledge of the sexual and physical environment at mating may allow improved reproductive performance under commercial conditions.
- viii) *Feedstuff and feed additive evaluation* new feed sources for pigs need to be identified. The emphasis will be to better utilise lower quality feedstuffs through improved processing techniques and the development of specific feed additives such as enzymes to increase nutrient availability or destroy antinutritional factors. New synthetic ingredients such as amino acids would reduce the demand for costly native proteins.
- 2. Processing and marketing efficiency. General improvements in pig breeds and in standards of pig management have resulted in the continued decrease in fatness of Australian pigs. While problems of high variability in total fat content and in fat distribution remain, there is the increased realisation of variable and poor meat quality.
  - i) *Pig meat quality* research is required to better define the carcass and meat quality requirements of domestic and export markets. Parameters

reflecting these quality requirements (including meat colour, water holding capacity, boar taint), need then to be developed and used to provide the procedures (involving pig husbandry, transport and lairage) and incentives for producers to improve pig carcass and meat quality.

- ii) New slaughter technology automation may provide research opportunities for the efficient and more hygienic conversion of the live pig into pig meat products.
- iii) *New pork products* technology is required to develop new products suited to market demands such as low fat and low salt pork products.
- iv) *Meat safety* new technology in handling, processing, packaging and storage will be required to improve the microbiological status and increase shelf life of pork.
- 3. Housing. There is considerable potential to develop new and alternate technology into pig production facilities. A greater knowledge of environmental parameters critical to pig performance will encourage research based on computer and electronic technology for environmental control in pig houses. There is research opportunity to develop new and alternate materials and designs for equipment, facilities and buildings. A greater understanding of the needs and behaviours of both animals and humans will ensure that equipment and housing systems are better suited to the needs of the animal and of the stockpeople.
- 4. Animal welfare. Techniques to assess welfare need to be developed and used to identify the level of change (both behavioural and physiological) at which welfare is at risk. Strategies are required to minimise the stresses of husbandry techniques; this will involve an evaluation of current and alternative housing systems for all age classes of pig.
- 5. Waste management. Research is required to develop improved systems for the treatment, utilisation and disposal of piggery effluent. New technology is required to reduce, remove and recycle nitrogen and phosphorus before entering groundwaters and waterways. More information and new technology is required to monitor and control the generation and release of odours from piggeries.
- 6. Communication. More efficient transfer of new technology will require a better understanding of the needs, behaviours and perceptions of the appropriate industry audience. Increasingly, this research will need to be extended to the community at large to better communicate the needs for development and new technology in pig production.

#### Setting research priorities in the pig industry

The underlying rationale for investment in pig industry R&D is the understanding that investments will be to the net benefit of the industry and of the general community. There is little evidence that specific analysis of the returns to investment in R&D has been used in research planning in Australia.

Evidence exists (Marsden *et al.*, 1980) that R&D for the primary industries can yield high rates of return and that in many cases, significant benefits are passed on to other sectors of the economy. Recent analysis of returns from six research projects in the red meat industry (Martin, 1991) revealed benefit/cost ratios from 6:1 to 74:1, with internal rates of return to investment ranging from 25% to more than 600%.

There are now tools to assess the return to investment in R&D in the pig industry. At the farm level, the implications of new technology for herd performance and profitability can be predicted using Auspig. At industry level, Morris *et al.* (1991) have developed an economic model to assess the impact of new technology on the Australian pig industry. These new techniques allow a better analysis of the distribution of the benefits between producers, processing firms and final consumers from investment in R&D at different points in the marketing chain.

For example, Griffith and Morris (1991) used the model recently to demonstrate that of a total \$100m annual net return from the implementation in Australia of new technology resulting from research with recombinant growth hormone (PST), the large majority (\$90m annually) would be captured by the consumer of the product with the pig producer benefiting by only \$9m annually.

The application of these new tools will provide better information in setting priorities to guide R&D investment. But in addition to considerations of potential size and distribution of benefits, criteria such as the potential and capacity for R&D must be assessed. In the final analysis, research priority setting is a decision making process where optimal solutions regarding the allocation of resources are sought within a political, social and institutional framework.

In the pig industry, the PRDC has become the dominant influence on national research priorities. The broad base of technical, scientific, policy, farming and marketing experience and skill on the Board of the PRDC, provides a good instrument for selecting research priorities and projects among the various disciplines. This model is overcoming many of the recognised problems of programme and project appraisal by panels of research "experts" or "peers".

As described by Turney (1990), peer review is a poor instrument for selecting among disciplines. Peer review reacts slowly to fresh opportunities in science - well established lobby groups generally succeed in promoting their interests at the expense of newer rivals and it is rare to find peer reviews suggesting that funding for some areas should be discontinued.

#### Emerging issues for research in the pig industry

The opportunities for research to continue contributing to the pig industry are not diminishing. Simulation models, such as Auspig, enable better identification of critical areas of research for the industry, and developments in fields such as electronics and molecular biology, provide tools enabling more effective and sophisticated research.

With our existing research base, we will soon have the ability to approach the pig's known physiological limits for growth and reproduction and indeed, through genetic manipulation, it will inevitably be possible to extend known limits. However, will the limits to our ability to manipulate pig production be imposed by the physiology of the pig, or will factors such as government policies and public and community attitudes mould the nature of research and indeed, the pig industry into the 21st century?

1. Government policies. The viability of the Australian pig industry can be affected rapidly by government policy associated with issues such as quarantine, international trading conditions and urban regulations and legislation. Changes in State and Federal Government policy for R&D may also have marked but longer term affects on the industry.

The Commonwealth Government directly supports pig industry R&D through matching industry contributions on a dollar for dollar basis. This support is channelled through the PRDC and is assured to a maximum of 0.5% of GVP. However, while PRDC funding generally provides the majority of "operating" funds, the basic physical and human resources for pig industry R&D are provided directly by governments through State Departments of Agriculture,

Universities and CSIRO and these funds are not assured.

There is evidence of a decline in the general opportunities for research in Australian universities and State Government organisations. In a recent survey of Australian academics, Professor B. Rolfe (personal communication) found 81% believed that opportunities to conduct research in Universities had reduced since 1987. The survey also indicated similar deterioration in teaching conditions. A study by Evans *et al.* (1990) indicated a marked (24%) decrease in manpower for agricultural research in State Government organisations during the 1980's.

The pig industry has as one of its major challenges, to maintain its ability - skills and resources, to provide effective R&D.

2. Public and community attitudes. Community attitudes will increasingly impact on the pig industry. In other countries, public attitudes have been largely responsible for legislation forcing the industry to change husbandry practices such as sow tethers and stalls. The Australian public and pig industry require objective research information for the formulation and evaluation of any similar regulations and legislation.

Public attitudes will impact not only on the subjects for future R&D in the pig industry, but also on the nature of this research. The public has a poor understanding of science and biotechnology (Couchman and Fink-Jensen, 1990) on which to base their perceptions of R&D and new technologies. Taverner (1990) described how poor communication in the development of the transgenic pig adversely influenced public perceptions of new biotechnologies in meat production. As suggested by Couchman and Fink-Jensen (1990), a dialogue between scientists and the public is needed to:

- i) Inform the public of the social and economic benefits of biotechnology, the possible hazards, and the controls in place to minimise these risks.
- Ensure that research organisations are aware of public concerns and that they take account of them in their research practice (especially ethical and safety considerations).

It would seem however, that because of a low credibility of scientists with the public, research is required to determine how to implement an effective and credible dialogue (Taverner, 1990).

As described by Taverner (1989), socio-economic acceptability has become an important feature of new product development. If negative public attitudes to biotechnology reduce the chances of success and/or increase the time to implementation/pay-off for product development, this could curtail the investment and R&D necessary for the development of new technology.

#### Conclusions

Largely through the support of State and Commonwealth Governments in providing staff and resources, the pig industry has developed a very strong and effective base for R&D. Furthermore, industry and Commonwealth Government support an effective mechanism involving a R&D Corporation (PRDC) which provides funding, direction and coordination for a national research programme.

The extent of likely reduction in Government support for R&D will not be matched by increased industry support and it is likely that although the opportunities for R&D are increasing, the total resources for pig R&D will diminish.

The diminished resources will be more specifically targeted into maintaining and developing skills and facilities. Increasingly, the combination of specialist skills in technology (such as molecular biology or electronics) will need to be closely aligned with husbandry, commercial and communication skills. It is likely that research will be increasingly concentrated at national centres for R&D in the pig industry.

There is an essential role for research in the Australian pig industry. Continued industry viability is dependent on R&D to provide technology enabling high productivity within the constraints of community standards.

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## ASPECTS OF OVARIAN FUNCTION IN THE GILT AND SOW

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#### Introduction

This review of ovarian function will consider different stages of the reproductive life-cycle of the gilt and sow, starting with ovarian maturation in the prepubertal gilt and ending with ovarian function in the lactating and weaned sow. The key regulators of ovarian function will be discussed in each reproductive stage and the need for further research identified. The role of ovarian function in providing for oocyte maturation and steroidogenic function will be discussed as an integral part of the female reproductive process.

Many aspects of ovarian function in pigs have been the subject of detailed reviews in recent years under the aegis of the International Conferences on Pig Reproduction and other meetings; this review will therefore focus on those areas in which the author has particular research interests, and will draw heavily on the results of work carried out in my own laboratory and in collaboration with other groups. Many of the ideas presented have therefore arisen from work and discussion with graduate students, researchers and technical staff and I acknowledge their contributions and continuing enthusiasm for work in the area of pig reproductive physiology.

#### Ovarian function in the prepubertal gilt

#### The origin of the block to ovarian development

Much of the evidence reviewed previously (Elsaesser, 1982; Christenson *et al.*, 1985; Foxcroft *et al.*, 1989) suggests that the primary limitation to ovarian development in the immature gilt is a lack of gonadotrophin, and particularly luteinizing hormone (LH), secretion. By inference this suggests that the luteinizing hormone releasing hormone (LHRH) pulse generator in the hypothalamus is either inherently inactive or is under inhibitory control during the prepubertal period. In either case, tonic LH secretion is controlled by a process of hypothalamic maturation that probably has a number of integrated components. Hypothalamic maturation also involves development of the positive feedback mechanisms through which rising plasma oestradiol-17 $\beta$  concentrations are able to trigger the pre-ovulatory surge of LH.

Well before hypothalamic maturation is complete, the ovary will respond to gonadotrophic stimulation and exogenous gonadotrophins have been used to induce oestrus and ovulation in the prepubertal gilt (see Paterson, 1982; Ainsworth et al., 1990). However, such treatments have been associated with a failure to establish a continuing pattern of cyclic ovarian function (Paterson, 1982; Paterson and Lindsay, 1981). This may be due to inadequate endogenous gonadotrophins and a lack of support for follicular development at the end of the induced luteal phase; deficiencies in the complex feedback signals from the ovary that are known to be crucial for the regulation of cyclic changes in LH and follicle stimulating hormone (FSH) secretion may be involved. It is also possible that the ovaries of the late prepubertal gilt are relatively insensitive to gonadotrophic stimulation, possibly reflecting metabolic immaturity. Although high doses of exogenous gonadotrophins like pregnant mare's serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG) may overcome this insensitivity, the pattern of follicular growth and steroidogenic activity may not be typical of that seen in naturally cyclic gilts (Wiesak et al., 1990) and this may contribute to abnormal cyclic ovarian activity after treatment.

#### Maturational changes in the reproductive axis

#### Central mechanisms controlling gonadotrophin secretion

Evidence for innate maturational changes in the hypothalamic control of LH secretion was recently reviewed by Foxcroft *et al.* (1989). The secretion of gonadotrophins shows characteristic changes in the mid-prepubertal period in both intact and ovariectiomized gilts as shown for LH in Figures 1 and 2. Evidence for a clearly defined change in LH secretion at pubertal onset is still lacking, but Danesbury and Rawlings (personal communication) have observed that mean plasma LH concentrations in immature gilts were inversely related to the age at which gilts could be induced to reach puberty in response to boar stimulation. In the absence of exposure to boar stimulation, it is possible that changes in ovarian sensitivity to gonadotrophins, related to changes in metabolic state or in autocrine/paracrine regulators within the ovary, may trigger the recruitment of the first wave of preovulatory follicles and pubertal oestrus, in the presence of relatively constant levels of gonadotrophins.



Figure 1. Mean plasma LH (ng/ml) in intact prepubertal gilts of different ages, derived from analysis of repeated frequent sampling (modified from Foxcroft et al., 1989).

Evidence for central mechanisms controlling developmental changes in gonadotrophin secretion in the prepubertal gilt, in the absence of ovarian feedback, is shown in Figure 2, and is similar to data from other species.

The nature of the mechanisms that regulate LHRH secretion even in ovariectomized prepubertal females is uncertain. The data in Figure 2 also indicate, however, that negative feedback of ovarian origin exists from as early as eight weeks of age in the prepubertal gilt. Studies with opiate antagonists (Barb *et al.*, 1988) suggest that the inhibitory effects of gonadal steroids and peptides are not mediated through opioid-dependent mechanisms in the prepubertal gilt, as treatment with naloxone did not increase LH secretion. However, treatment with morphine (Cosgrove and Foxcroft, unpublished observations) results in complete suppression of LH release in the prepubertal gilt, suggesting that the lack of a response to treatment with opioid antagonists such as naloxone, is most likely due to a lack of endogenous opiate activity and not to a lack of available opiate receptors.



Figure 2. Mean plasma LH (ng/ml) in intact and ovariectomized prepubertal gilts sampled throughout development by acute venepuncture in two different studies (modified from Foxcroft et al., 1989).

It is still questionable whether the patterns of gonadotrophin and steroid secretion described in existing literature on pigs (see review of Foxcroft *et al.*, 1989) are consistent with the gonadostat theory of pubertal development (Ramirez, 1963), which • postulates that decreasing sensitivity to oestrogen negative feedback results in a concurrent increase in both plasma oestrogen and LH at puberty.

Direct stimulatory inputs to the hypothalamus in response to pheromonal or photoperiodic signals may also be important. Melatonin treatment during development has produced variable effects on puberty attainment in the gilt (Evans et al., 1991; Diekman et al., 1991), possibly due to differences in the experimental design used and particularly the photoperiod under which the gilts were raised. Unequivocal evidence for a distinct nocturnal rise in endogenous melatonin in swine has also been hard to establish (Clapper and Diekman, personal communication) and appears to be dependent on a high level of light intensity. Observed diurnal rythms in reproductive hormones in pigs may not therefore be mediated through changes in melatonin secretion. Nevertheless, preliminary evidence for the existence of a diurnal rhythm in LH secretion, with increased LH release at night (Elsaesser and Foxcroft, 1978) was substantiated in a study of restrictively-fed prepubertal gilts (see Booth, 1990a; Foxcroft et al., 1989), although not in similar work conducted by Cosgrove, Booth and Foxcroft (unpublished observations). This inconsistency in observing a clear diurnal rhythm in LH secretion may again relate to a number of factors, such as previous photoperiodic changes and the season at which gilts were studied, or to genetically determined changes in the inherent maturational state of the animals. In our most recent study of nocturnal and nutritional effects on LH secretion in the gilt (Cosgrove and Foxcroft, unpublished data) a diurnal pattern of LH secretion in feed restricted gilts was again evident. These data also confirm our view that if the frequency of episodic LH is already high in prepubertal gilts, it is technically difficult to establish diurnal changes, whereas this is possible in feed-restricted gilts in which episodic LH frequency is depressed. The principal objective of these continuing studies on diurnal changes in

LH secretion is to establish whether this phenomenon is restricted to a particular period of maturation, as seen in adolescent human males (see recent reviews of Delemarre-van de Waal *et al.*, 1989 and Kelch *et al.*, 1989). An inherent diurnal rhythm, in which LH secretion increases at night, would provide the basis for both a seasonal suppression in fertility in the summer months under a long-day photoperiod (see Claus and Weiler, 1985) and for a multi-phasic surge release of LH in response to an oestrogen challenge in the prepubertal period (Elaesser and Foxcroft, 1978; Dial *et al.*, 1984; Foxcroft *et al.*, 1984).

#### Steroid-dependent maturational changes

Developmental changes in steroid metabolism may also be important in contributing to a gradual increase in circulating oestrogens in the late prepubertal period. Age-dependent differences in plasma oestradiol after the administration of body weight-related doses of oestradiol benzoate (Elsaesser and Foxcroft, 1978) led to a study which established a maturational change in the metabolic clearance rate of oestrogen (Elsaesser *et al.*, 1982). Although changes in the proportion of body fat and changes in mixed function oxidase activity in the liver have been suggested as factors which may contribute to such changes in metabolic clearance rate, direct experimental evidence to support these suggestions is lacking. Interestingly, preliminary studies to understand the physiological basis for precocious puberty in the Chinese Meishan, as compared to European Large White breeds, indicate possible differences in oestrogen metabolism and in negative and positive feedback responses to exogenous oestrogen treatment (Tilton *et al.*, 1991).

From a study of ovariectomized gilts, with or without chronic treatment with oestrogen implants in prepubertal gilts of different ages, we concluded that chronic exposure of the hypothalamus to oestrogens of ovarian origin was necessary for maturation of the oestrogen positive feedback mechanism (Foxcroft *et al.*, 1984); continued exposure to oestrogens is also necessary in the mature female for the maintenance of this LH surge mechanism. Again, a failure to ensure maturity of these mechanisms before inducing ovulation with exogenous gonadotrophins may result in a failure to establish continued cyclic ovarian function.

#### Factors interacting to change the rate of sexual maturation

#### Growth and nutrition

Classic theories of pubertal development link attainment of a critical "Body State" to the attainment of puberty (see l'Anson *et al.*, 1991). However, studies with modern hybrid gilts, selected for high growth rates and fed *ad libitum*, suggest that neither growth rate nor body composition limit age at puberty (Young *et al.*, 1990). However, both realimentation and intravenous glucose administration induced rapid increases in LH secretion in short-term restrictively-fed prepubertal gilts (Booth, 1990a; Cosgrove *et al.*, 1991) which could only have been mediated by dynamic changes in the metabolic state of the animal (see Figure 3).

The mechanism(s) mediating these central effects of nutrient intake are uncertain. A prime role for insulin is consistent with the data of Booth (1990b) in the prepubertal gilt and Armstrong and Britt (1987), Cox *et al.* (1987) and Beltranena *et al.* (1991) in the cyclic gilt. However, direct administration of insulin, whilst enhancing ovarian follicular development, produced inconsistent responses in LH secretion in cyclic gilts (Cox *et al.*, 1987). Similarly, contradictory data on insulin-induced increases in LH secretion have been reported in sheep (see Suttie *et al.*, 1991).

The data of Cox *et al.* (1987) and Matamoros *et al.* (1990) clearly indicate the potential for metabolic changes to affect ovarian function directly; indeed the effects of insulin may themselves be mediated by changes in insulin-like growth factors (IGF's) within the ovary, as reviewed by Hammond *et al.* (1991). IGF-1 enhances the responsiveness of granulosa cells to gonadotrophin stimulation in vitro and IGF-1 is secreted by granulosa cells in culture. IGF-1 mRNA has also been isolated from



Time (hours)

Figure 3. Episodic LH secretion in littermate prepubertal gilts fed at maintenance levels for 7 days and then to maintenance (Gilt 8) or to appetite (Gilt 2) at 0800 and 1600h on the day of sampling shown (data from Booth, 1989).

porcine follicular tissue (Cameron *et al.*, 1990: Charlton and Foxcroft, unpublished observations). Thus the significant increase in peripheral IGF-1 concentrations after realimentation of the restrictively-fed prepubertal gilt (see Booth, 1990b; l'Anson *et al.*, 1991), presumably of hepatic origin, may be associated with an increase in ovarian IGF-1 synthesis. As in the case of hepatic IGF-1 synthesis, this may be an indirect response to the hyperinsulinaemic state induced by increased energy intake. Further evidence that changes in metabolic state may act directly at the ovarian level is shown in Figure 4 (Cosgrove *et al.*, 1991). In this experiment ovarian responses to realimentation were studied in short-term restrictively-fed prepubertal gilts in which LH secretion was uniformly suppressed by administration of the oral progestagen allyl trenbolone.

Available evidence suggests therefore that with unrestricted feeding of modern hybrid gilts, growth rate and body composition are unlikely to affect age at puberty. However, short-term changes in nutrient intake can produce rapid responses in the reproductive axis. Inadvertent changes in metabolic state can still therefore affect critical aspects of reproductive function in gilts, such as sensitivity to boar stimuli and ovulation rate at breeding.

#### Social status

In feral pigs and probably also in well managed domestic herds, stimulation of



Figure 4. Mean ovarian follicular volume and follicular diameter in prepubertal gilts after being fed to maintain body weight at 75kg for 7 days and then either continuing on maintenance feeding (R12) or being fed to appetite (A12) from days 8 -12. Treatment-induced changes in LH secretion were suppressed by feeding the oral progestagen, allyl trenbolone, throughout the study (Cosgrove, Tilton and Foxcroft, unpublished observations).

gilts by mature boars provides the ultimate stimulus for puberty attainment. As discussed by Hughes *et al.* (1990), the physiological mechanisms that mediate boarinduced puberty are still uncertain. A pheromonal and non-pheromonal ("stress" induced by physical presence of the boar ?) component appears to be necessary for maximal responses. Recently, Kingsbury and Rawlings (personal communication) have obtained direct evidence for a boar-induced increase in LH secretion in prepubertal gilts. Further research into the neuro-endocrine pathways mediating the effects of boar-stimulation would be of great interest.

#### Genetic effects

The increased fecundity of the Chinese Meishan pig is associated with an even more dramatic effect on sexual precocity. For example, in recent collaborative studies with Meishan and European domestic gilts (Hunter *et al.*, 1991a), age at puberty occured at  $115 \pm 20$  and  $235 \pm 22$  days of age in Meishan and hybrid large-white gilts, respectively. The endocrine basis for this precocity is uncertain but differences in the pattern of gonadotrophin secretion, possibly related to differences in steroid feedback activity, would not be unexpected.

#### Practical implications

A number of factors suggest that selection for the rate of sexual maturation in pigs could lead to an improvement in lifetime breeding performance. Firstly, this has already been partly accomplished in sheep; selection of ram lambs on the basis of testis size produced some enhancement of fecundity and fertility in their ewe-lamb offspring, as reviewed in the paper of Haley *et al.* (1990). Preliminary evidence for responses to similar selection in pigs has been reported by Toelle and Robison (1985) and Schinckel *et al.* (1983). There are considerable differences between and within breeds in age at puberty, associated with differences in LH secretory activity in at least one study. Within breed differences between littermate groups of gilts; for this

reason gilts are routinely allocated to treatments on a littermate basis to reduce variability and on occasion to allow exclusion of whole litters retrospectively on the basis of abnormal endocrine status.

The social and physical environment of the replacement gilt clearly affects the time of puberty. The data of Paterson and Pearch (1990) and Paterson *et al.* (1991b) suggest that ideally, gilts should be reared in a short-day photoperiod to avoid seasonally related delays in sexual maturation. Alternatively, oral melatonin treatment may be a practical means of blocking seasonal delays in sexual maturation (Paterson *et al.*, 1991a). Management should avoid situations in which even short-term limitations in feed intake occur at critical times in the reproductive cycle; for instance mixing of strange gilts in the period immediately preceeding exposure to boars or to mating. As the induction of an early pubertal oestrus has several advantages, gilts should be routinely exposed to boars at an early age to provide a pool of cyclic gilts from which replacements can be selected on the basis of sexual precocity, sexual behaviour and established cyclic ovarian function. This approach also optimizes the use of synchronizing agents such as oral progestagens for programmed entry of gilts into the breeding herd.

## Cyclic gilts

The endocrine control of the recruitment and selection of preovulatory follicles on a cyclic basis has still to be completely elucidated, but a number of hypotheses can be advanced on the basis of existing data.

In the luteal phase of the cycle, growth and atresia of follicles provides a pool of approximately 2 to 6 mm follicles that can apparently be recruited into a preovulatory population at any time. As in other species, and particularly in cattle in which successive growth of dominant follicles has been described in detail in the luteal phase (see Ko et al., 1991), this early phase of growth and subsequent atresia occurs in the presence of unchanging patterns of LH and FSH secretion. Thus although this phase of follicular growth is gonadotrophin dependent, the dynamic changes in follicular populations appear to be regulated by intra-ovarian mechanisms. In the pig, which is polytocous, it is not clear whether the growth of these intermediate sized follicles is a highly coordinated event involving successive waves of dominant follicles, or whether asynchronous follicular development occurs which continuously provides adequate numbers of follicles for recruitment. Considerable heterogenity in follicular development has been reported at the time of recruitment in cyclic gilts (Grant et al., 1989). In herds with good fertility, the onset of oestrus soon after weaning of sows, or exposure of gilts to boars, suggests that continuous follicular development occurs in the pre-recruitment period. Because the size of the recruited pool of follicles is an important determinant of litter size, a complete understanding of the mechanisms controlling the growth of intermediate sized follicles is of great practical significance. Nutritional state and genotype are both important (see Foxcroft and Hunter, 1985). The data of Hasegawa et al. (1988) showing an inverse relationship between plasma inhibin and FSH in the pig oestrous cycle, suggests that as in other species, immunization against inhibin could affect ovulation rate by affecting the size of the recruited pool of follicles and increased ovulation rates have been reported by Brown et al. (1990) using this approach. Treatment with epostane (an inhibitor of progesterone synthesis) in the late follicular phase also increased ovulation rate (Fu et al., 1990), however the stage of follicular growth affected by this treatment was not established. Nutritionally-induced increases in ovulation rate in cyclic gilts potentially involve both increases in LH secretion (Armstrong and Britt, 1987; Cox et al., 1987; Flowers et al., 1988; Booth, 1990a,b) and direct affects of insulin acting to reduce follicular atresia as descibed earlier (see Figure 5). However, as confirmed in the recent studies of Beltranena et al. (1990), responses to flush feeding are only seen in previously restrictively-fed gilts and act to restore ovulation rate to that of unrestricted females. Furthermore, flush feeding was associated with significant differences in

plasma IGF-1, as well as insulin, in the late follicular phase (Beltranena *et al.*, 1991), again suggesting that IGF-1 could be an important mediator of nutritional effects on ovarian function.

Recruitment of preovulatory follicles is thought to occur between days 14 to 16 of the oestrous cycle (Foxcroft and Hunter, 1985). The first potential endocrine signal triggering recruitment and continued follicular growth will be a change in ovarian progesterone concentrations, associated with changes in ovarian prostaglandins. Characteristic increases in episodic LH secretion; dependent on a decline in peripheral plasma concentrations (Foxcroft and Van de Wiel, 1982), may not therefore be the primary signal for follicular recruitment in the cyclic gilt, but act to accelerate the rate of follicular development and steroidogenesis once the follicular phase is established. At other stages of the reproductive cycle (puberty induction and after weaning), however, an increase in episodic LH secretion may be the primary stimulus to follicular recruitment.



Figure 5. Follicular atresia, expressed as a percentage of all medium to large follicles present, estimated on days 17 and 19 of the oestrous cycle in control gilts and in gilts treated with insulin twice daily from day 15. Insulin treatment significantly reduced follicular atresia (after Matamoros et al., 1990).

Selection occurs between day 16 and 20 of the oestrous cycle and a defined cohort of preovulatory follicles exists at the time of the LH surge. The emergence of the preovulatory population results from continued growth of selected (dominant) follicles and atresia and arrested development of smaller follicles in the ovary (see Foxcroft and Hunter, 1985). Heterogeneity in follicular development at the time of recruitment appears to be perpetuated to the preovulatory stage, as evidenced by differences in morphological and biochemical characteristics and in oocyte maturity at time of ovulation (Grant *et al.*, 1989; Hunter *et al.*, 1989; Hunter and Wiesak, 1990). Pope *et al.* (1990) and Hunter and Wiesak (1990) discussed the possible relationship between follicular heterogeneity and subsequent embryonic development; such effects may be mediated by inherent differences in the developmental potential of the oocyte at ovulation, differences in the timing of ovulation among follicles or by effects on early luteal function.

During the follicular phase *in vivo*, follicles are exposed to an initial increase in episodic LH release, followed by suppression but not total inhibition of LH release as plasma oestradiol concentrations rise (see Foxcroft and Van de Wiel, 1982). More latent changes in FSH secretion occur after luteolysis. Plasma FSH concentrations gradually decline to reach a nadir immediately before the preovulatory LH surge,

presumably in response to the feedback effects of both oestradiol and ovarian inhibin, which are secreted in increasing amounts by developing follicles (see Ainsworth *et al.*, 1990). There are therefore probably critical changes in the LH:FSH ratio that may be important in controlling the selection process. Evidence that the pattern of follicular growth and the degree of follicular heterogeneity differ between naturally cyclic gilts and those treated with PMSG and hCG (Babalola and Schapiro, 1988; Wiesak *et al.*, 1990) supports this suggestion.

The complex intra-ovarian changes occuring during the follicular phase have been reviewed by Ainsworth et al. (1990) and will not be considered in detail. Of the many potential regulators of follicle selection and oocyte maturation, follicle regulatory protein (FRP) and oocyte maturation inhibitor (OMI) have been shown to have specific physiological effects (see Tonetta and diZerega, 1990). A critical role for factors promoting oocyte maturation has also been described in experiments with porcine oocytes co-cultured with follicular fluid extracts, granulosa cells or with entire follicle shells (see Moor et al., 1990; Yoshida et al., 1990). It has also been possible to demonstrate that the largest and smallest follicles within the presumed preovulatory population in PMSG-treated gilts have different capacities to promote oocyte maturation in vitro (J. Ding, unpublished observations; Figure 6). Unless the factors mediating such maturational effects can pass freely between follicles in a paracrine or endocrine manner, these data provide further preliminary evidence for a functional relationship between morphological heterogeneity of follicles and oocyte maturation. It was therefore of considerable interest to test the hypothesis that the greater uniformity reported in embryonic development in early pregnancy in the Meishan gilt (Bazer et al., 1988) could be associated with greater uniformity in follicular development. Preliminary data (Hunter et al., 1991a) suggest that this is not the case, but preovulatory follicles of Meishan females are smaller and more steroidogenic; the secretion of important maturational factors and the relative maturity of oocytes may therefore also differ and these possibilities are currently under investigation. Do these preliminary data also raise the possibility that the mechanisms controlling increased fecundity in Boroola merino sheep and Meishan pigs represent an example of convergent evolution?



Figure 6. Incidence of male pronuclear development in pools of pig oocytes recovered from follicles of prepubertal gilts 36 h after PMSG treatment. Oocytes were matured in vitro in cocultures with follicle shells recovered 36 or 72 h after PMSG and representing the largest (L) and smallest (S) follicles within the presumed ovulatory population. (Ding and Foxcroft, unpublished observations).

## Practical implications

Although our understanding of the process of follicle recruitment and selection is therefore rudimentary, there is great potential to apply information from this area of study to improving systems for the *in vitro* maturation and fertilization of oocytes. As in sheep, it may also be possible to identify breeding populations with characteristics which will favour the development of increased numbers of quality oocytes and follicles. Previous selection on ovulation rate alone has yielded little benefit in litter size due to adverse tends in embryonic survival. Techniques that allow these two effects to be segregated are therefore needed; further studies of the Meishan pig are therefore important as this breed may already have achieved this objective.

#### Ovarian function in early pregnancy

A number of studies have demonstrated important associations between the pattern of progesterone secretion, the uterine environment and embryonic survival in sheep (Ashworth *et al.*, 1989). The review of Pope *et al.* (1990) suggests that asynchrony between embryonic and uterine development is also critical in pigs and we postulate that this is in part progesterone dependent. Earlier data suggesting that differences in established luteal progesterone secretion may mediate effects of nutrition on embryo survival in the gilt (Dyck *et al.*, 1980) have been hard to substantiate and the overall reproductive status of gilts used in these studies has been questioned (Pharazyn *et al.*, 1991a). However, although no effect of feed intake in early pregnancy on embryonic survival was established in the data of Pharazyn *et al.* (1991a), there was an inverse relationship between plasma progesterone concentrations on day 3 of pregnancy and the variability in embryo survival (see Figure 7).

Krzymowski *et al.* (1990) reviewed evidence for the effective countercurrent exchange of steroids in the sub-ovarian plexus in the pig, resulting in local concentration of steroids in the utero-ovarian vasculature. Because of the important effects this may have on the oviductal environment (see Hunter, 1990), we elected to extend the preliminary data of Hunter *et al.* (1983) by comparing progesterone concentrations in peripheral plasma with those in the ovarian, oviductal and uterine vasculature in early pregnancy. As reported by Weems *et al.* (1989) in sheep, the countercurrent exchange of steroids resulted in a significant difference in progesterone concentrations in the sub-ovarian and oviductal vasculature compared to peripheral



Figure 7. Variability in embryo survival in primiparous gilts in relation to peripheral plasma progesterone concentrations on day 3 of pregnancy (from Pharazyn et al., 1991a).
plasma; however, this differential accumulation of steroid did not extend to the uterine drainage in pregnant and cyclic gilts (Pharazyn *et al.*, 1991b; see Figure. 8). Differences in the luteal production of progesterone therefore have the potential to selectively affect the oviductal environment at the time of fertilization and early embryonic development, before measureable changes in peripheral plasma progesterone occur.



Figure 8. Progesterone concentrations in plasma obtained from a primiparous gilt at different locations in the utero-ovarian and peripheral vasculature on day 2 of gestation. By chance, ovulations in this gilt were restricted to the right ovary. J = jugular vein; Uc, Um and Ucr = veins draining the caudal, mid and cranial sections of each uterine horn, respectively; Od = oviductal vein; Ov = arterio-venous blood from the ovarian pedicle (from Pharazyn et al., 1991b).

In their study of morphological differences in preovulatory follicles, Grant et al. (1989) observed apparent luteinization of a proportion of follicles before ovulation, which might have consequences for the pattern of progesterone secretion. Variability in the time interval between the preovulatory LH surge or oestrus and the initial rise in peripheral plasma progesterone has been reported earlier (Guthrie et al., 1972; Van de Wiel et al., 1981) and in recent experiments ranged from 31 to 53 and from 30 to 59 hours, respectively (Pharazyn, Beltranena, Aherne and Foxcroft, unpublished data). Together with data on the temporal relationship between increases in progesterone in the peripheral and oviductal vasculature shown in Figure 8, this suggests that the initiation of progesterone-dependent changes in the oviductal micro-environment may occur over a similar range of time after the LH surge. Direct evidence is needed for functional relationships between differences in follicular development, luteinization, progesterone secretion and early embryonic development. In a polytocous species like the pig, such temporal differences in progesterone production may have important effects on embryonic development, whereas differences in the absolute concentrations of progesterone in the established luteal phase may be of little consequence.

#### Ovarian function in the periparturient period

Two aspects of ovarian function will be considered. Firstly, the role of ovarian secretory products in lactogenesis and secondly, the ontogeny of the inhibitory effects of suckling on gonadotrophin secretion and hence ovarian activity.

#### Milk secretion and milk ejection

The regressing corpora lutea of pregnancy are a potential source of both progesterone and relaxin. There is general concensus that the concentration of both hormones in the regressing corpora lutea decreases rapidly, as measured by immunochemistry, immunoassay and in vitro production (see Hunter et al., 1991b). In this recent collaborative study, the rapid decline in immunostaining for relaxin by day 4 and a further decline to essentially undetectable levels by day 14 of lactation, was paralleled by a decline in relaxin in luteal extracts and in relaxin production by luteal tissue in vitro as measured by radioimmunoassay. The rate of decline in relaxin was also significantly greater in suckled than in weaned sows. Moreover, there was no effect of oxytocin in vitro on the release of relaxin from luteal tissue in short-term culture. Evidence for a non-luteal source of relaxin that would provide the basis for the suckling and oxytocin-induced release of relaxin reported by Afele et al. (1979) and Whitely et al. (1985) in mid-lactation is lacking and the pattern of relaxin secretion reported by these authors is not consistent with the known circulating half-life of relaxin nor with the reports of other authors (Sherwood et al., 1981; Kendall et al., 1983).

The suggestion that elevated levels of progesterone in the periparturient period may be detrimental to lactogenesis (Hartmann and Holmes, 1989) led us to evaluate the plasma progesterone changes before and after farrowing, and associated changes in metabolic hormones, milk composition and piglet growth and survival. Progesterone declined at a variable rate and there was a positive correlation between litter size born and plasma progesterone levels before farrowing (de Passillé, Rushen, Schaefer, Aherne and Foxcroft, unpublished observations), suggesting that progesterone of fetoplacental origin may make an important contribution to circulating progesterone concentrations in late pregnancy. A significant inverse relationship (Figure 9) between plasma progesterone in the immediate post-partum period and litter-growth rate is consistent with a functional link between progesterone and lactogenesis. Although direct evidence for such a relationship is needed, these preliminary data suggest that further studies relating periparturient endocrine status to lactational efficiency would be of interest.



Figure 9. Relationship between plasma progesterone concentrations averaged over the 48h period after farrowing of first and second parity sows and the average three day weight gain of their piglets. (de Passille, Rushen, Foxcroft, Schaefer and Aherne, unpublished observations).

#### Inhibitory effects of suckling on ovarian function

The endocrinology of the lactating sow has been extensively reviewed (Edwards, 1982; Britt *et al.*, 1985; Varley and Foxcroft, 1990). In established lactation there is clearly an inhibitory effect of suckling on hypothalamic LHRH release and the resulting decline in episodic LH secretion is probably the principal cause of lactational

anoestrus. Support for this hypothesis comes from studies in which exogenous LHRH treatment induced LH release, behavioural oestrus and ovulation during lactation. An increase in FSH but not LH after ovariectomy of the lactating sow indicates that whereas suckling exerts direct inhibitory effects on LHRH and LH secretion, ovarian regulators are also important for the suppression of FSH. Studies involving the combined treatment of lactating sows with exogenous LHRH and oestrogen established inhibitory effects of oestrogen on both endogenous and LHRH-stimulated release of LH (De Rensis *et al.*, 1991). In contrast, stimulatory effects of oestrogen were observed by Sesti and Britt (1991), suggesting that the order in which LHRH and steroid treatment are administered may be critical to the response obtained. These data clearly indicate, however, that the absence of inhibitory feedback control by oestrogen on LH secretion in lactation is due to a lack of ovarian oestrogen production and not to an insensitivity of the hypothalamus to negative feedback.

A considerable body of evidence from studies with sows weaned immediately after farrowing (zero weaned sows) indicate that active gonadotrophin secretion is possible in the absence of suckling and stimulates ovarian follicular development in at least a proportion of animals (see Varley and Foxcroft, 1990, for review). Furthermore, a detailed study of gonadotrophin and prolactin secretion by De Rensis (1989) established that active secretion of LH occurred over the first 24-36 h after farrowing, irrespective of whether the sow was suckled or weaned. Furthermore LH secretory activity was highly variable between sows and in zero-weaned animals was significantly correlated to the degree of follicular development. Between 36 and 72 h after farrowing, LH secretion was significantly suppressed in suckled compared to zero-weaned sows, indicating that a considerable period elapses before the inhibitory neuro-endocrine mechanisms mediating the effects of suckling on LH secretion become operative. Therefore, unlike domestic ruminants, in pigs there is no latent suppression of LHRH and LH secretion due to the long term effects of progesterone secretion in pregnancy and LH secretion is only suppressed when the inhibitory effects of suckling are established. Support for these conclusions comes from the data of Sesti and Britt (1991). The suggestion of several authors that the development of polycystic ovaries in zero-weaned sows is due to a failure of the oestrogen positive feedback mechanism needs confirmation. Evidence for a gradual recovery of the positive feedback response in suckled sows during lactation (Elsaesser and Parvizi, 1980; Cox et al., 1988) is, however, consistent with this proposal.

In more recent studies of suckled sows, the pattern of active LH secretion after farrowing followed by a gradual inhibition of LH secretion between 36 and 90 h of lactation was confirmed (De Rensis and Foxcroft, unpublished data). As shown in Figure 10, repetitive treatment with the opiate antagonist naloxone every three hours from 36 hours after farrowing did not block the inhibition of LH secretion, whereas a clear inhibitory response to naloxone treatment was observed on days 8-10 of lactation in all animals. These data may be interpreted as evidence for a non-opioidergic mechanism mediating the initial inhibitory effects of suckling. However, concerns that repeated treatment with naloxone as used in this experiment might in itself lead to a lack of responsiveness to opiate antagonists, led us to conduct further work to investigate the precise role of endogenous opiates in suckling-induced inhibition of LH secretion.

In practice, an understanding of the relative importance of the metabolic demands of lactation, and activation of neuro-endocrine reflexes by suckling *per se*, on the inhibition of LH secretion in lactation is essential for the development of optimal management strategies for lactating sows. The work of Grant (1989) established that stepwise reductions in the neural input associated with suckling resulted in increased LH secretion and ovarian development, whilst the total weight of piglets weaned in the different treatment groups (taken as an estimate of milk production) showed little or no change. Furthermore, the data of Mullan and Close (1991) and Mullan *et al.* (1991) provide evidence for more profound effects of litter-size on LH secretion and post-weaning fertility, than those due to differences in food intake or body condition

These data confirm, however, that the endocrine status of sows in a of the sows. serious catabolic state is likely to exert a negative influence on reproductive performance. In particular, the increase in growth hormone, in association with a decrease in insulin secretion reported by Baidoo (1989) in sows losing excessive body condition during lactation, would produce inhibitory effects at both the hypothalamohypophysial and ovarian level, as discussed previously. The data of Mullan et al. (1991) and Mullan and Close (1991) also demonstrate that a decline in plasma insulin occurred between days 17 and 21 of lactation even in sows with high feed intakes, low litter-size and showing no change in body weight or backfat thickness. As in the gilt therefore, attention must be paid to short-term dynamic changes in metabolic state at critical times in lacation, if maximal fertility of weaned sows is to be achieved. Assuming that sows commence lactation in reasonable body condition and that their nutrient intake is sufficient to prevent serious loss in body condition, management practices that reduce the neural inputs resulting from suckling will probably provide the greatest benefits in terms of increased reproductive performance.



Hours post-partum

Figure 10. LH secretion measured at 15 min intervals at different times after farrowing in a control sow and in a sow reciving repeated i.v. naloxone injections from 36 - 78h post-partum. A single naloxone injection was given to both sows on day 10 of lactation. (De Rensis and Foxcroft, unpublished observations).

#### Summary and conclusions

This discussion of ovarian function in the gilt and sow and of ongoing studies into the endocrine control of reproduction in pigs, has emphasized the complex interaction between stimulatory and inhibitory mechanisms in the regulation of the reproductive cycle. The relative importance of each mechanism changes at each stage of the reproductive cycle and generalizations as to the cause of infertility are dangerous. Adequate stimulation by the gondadotrophins, particularly LH, is essential for ovarian stimulation at every stage. The key factors that limit LH secretion must therefore be identified and management practices modified as far as possible to alleviate this primary block to ovarian function. However, important effects at the ovarian level may change gonadal responsiveness to gonadotrophic stimulation, particularly if animals enter a catabolic state. Even in well managed herds, short-term changes in metabolic state may still have an important impact on fertility and economic performance and such effects should be considered in developing management practices for the breeding herd.

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## SEXUAL STIMULATION DOES NOT AFFECT OESTROGENS IN BOAR SEMEN

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Boar semen contains high amounts of oestrogens and the main oestrogens in semen are oestradiol-17 $\beta$  and oestrone sulphate (Claus, 1985). The function of these oestrogens is not well understood, but seminal oestrogens may influence uterine motility and the timing and duration of ovulation by inducing a release of PGF2 $\alpha$  immediately following insemination (Claus, 1990).

Sexual stimulation of boars before mating has been shown to increase sperm numbers in the ejaculate (Hemsworth and Galloway, 1979). The objective of the present study was to examine the effects of sexual stimulation on the total amount of oestrogens in the seminal plasma of the ejaculate.

Four mature boars were each subjected to two treatments over a four-week period, in which semen was collected twice weekly. The treatments were semen collection with or without observation of a mating immediately prior to collection (sexual stimulation or control). Pairs of boars were alternated between treatments on a weekly basis. Semen was collected over a dummy sow with the gloved-hand technique. Means were calculated for each boar (based on 4 ejaculates per treatment) and the data were analysed by Analysis of Variance.

<b>X</b> 7. • (-	Tre	atment	s			Boars	6	
Variate	Control	SS1	SED	1	2	3	4	SED
Oestradiol-17 <b>6</b> Oestrone suplhate	14.4 20.7	3.3 23.3	7.7 9.6	32.2 16.7	2.6 26.7	0.8 3.1	5.3 33.3	10.92 10.34

Table	1.	Oestradiol-176	and	oestrone	sulphate	content	(nmol)	per	ejaculate	in
semina	ıl pl	asma								

<sup>1</sup>Sexual stimulation.

Sexual stimulation did not affect the amount of oestrogens in seminal plasma of the ejaculate (Table 1). There were significant (P<0.05) differences between the boars in amounts of oestrogens. The implication of differences between boars in the amount of oestrogens on the variation between boars in fertility and fecundity is not known and clearly warrants examination.

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## SPERM DISTRIBUTION AND THE EFFECTS OF SEXUAL STIMULATION ON SPERM NUMBER IN THE BOAR

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Little is known about the effects of sexual stimulation on the temporal distribution of spermatozoa in the ejaculate of the boar, although an increase in sperm numbers following sexual stimulation of the boar has been reported (Hemsworth and Galloway, 1979).

Experiment 1 measured the number of sperm ejaculated every 30 s during ejaculation. Five boars were trained for semen collection over a dummy sow and semen was collected from each boar by the gloved-hand technique twice weekly for four weeks. During semen collection the behaviour of the boar towards the dummy sow was recorded. The results showed that about 80% of the sperm were ejaculated in the first 90 s  $(1.82 \times 10^{10} \text{ v } 2.35 \times 10^{10})$  and support the statement of Hemsworth *et al.* (1991) that the minimum duration for a successful copulation is 90 s.

Experiment 2 investigated the effects of sexual stimulation prior to semen collection on the number and distribution of sperm in the ejaculate. Four mature boars were each subjected to two treatments over a 4-week period, in which semen was collected twice weekly. The treatments were semen collection with or without observation of a mating prior to semen collection (Sexual stimulation (SS) or Control (C), respectively). Pairs of boars were alternated between treatments on a weekly basis. Means were calculated for each boar (based on 4 ejaculates per treatment) and the data were analyzed by Analysis of Variance.

Variable	Mean for SS	treatment C	Sign. <sup>1</sup>
Time to first mount (s)	28.8	72.7	NS
Time to commencement of ejaculation (s)	120.4	198.6	NS
Duration of ejaculation (s)	353.6	318.6	NS
Total volume of ejaculate (ml)	260.4	231.9	NS
Total sperm number (x10 <sup>10</sup> )	4.57	2.94	*
Sperm number (x10°), 0-90 s	34.74	25.03	NS

Table 1.	Summary o	f the sexual	behaviour	and	semen	characteristics	in i	the	SS	and
C treatmo	ents (Experin	nent 2)								

<sup>1</sup>NS, non significant, P>0.05; \* P<0.05.

The results indicate that sexual stimulation increased the number of sperm in the whole ejaculate by 56% (P<0.05). This study provides evidence that sexual stimulation is effective in enhancing the short term sperm output of boars.

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# INDUCTION OF OESTRUS AND OVULATION IN PREPUBERTAL GILTS FOR EMBRYO COLLECTION

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Prepubertal gilts treated with gonadotrophins exhibit oestrus and ovulation. Combinations of pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG) show variable oestrus, ovulation rate and number of embryos collected.

We tested responses to different levels of PMSG and hCG in prepubertal gilts (74-90 kg; 116-196 days of age). PMSG was given (IM) at Day 0 and hCG 72 h later (Table 1). A preparation of PMSG and hCG (PG600; Intervet, Sydney) replaced PMSG in one test. Embryos were collected surgically 4-6 days after natural mating. The incidence of oestrus, ovarian cysts and endometritis were noted and the number of corpora lutea (CL) and embryos counted (Table 1).

Oestrous incidence did not differ among treatments ( $X^2 = 5.68$ ; df = 4, P>0.05). The incidence of metritis was much greater with PG600 and 1,000IU PMSG than with 750 or 500 IU PMSG ( $X^2 = 16.76$ ; df = 1, P<0.01), but the occurrence of ovarian cysts did not differ between these groups ( $X^2 = 3.61$ ; df = 1, P>0.05).

Parameters		H	ormonal regi	imen	
PMSG hCG	500 500	750 500 ,	1,000 500	1,000 800	PG600 <sup>1</sup> 500
N° animals	14	28	45	31	24
% oestrus	79	89	89	74	71
% endometritis	0	4	40	39	41
% ovarian cysts	0	8	25	14	20
N° CL <sup>2</sup>	9.9 <sup>b</sup>	15.9ª	18.2ª	16.7ª	22.0ª
(se)	(1.0)	(1.1)	(2.6)	(1.5)	(2.8)
N° embryos	6.5°	11.7 <sup>ab</sup>	16.0ª	12.3 <sup>ab</sup>	11.8 <sup>ab</sup>
(se)	(0.9)	(1.4)	(2.7)	(2.0)	(2.0)

	Tabl	e 1.	Responses (	of prep	ubertal	pigs to	o exogenous	gonadotro	phins
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<sup>1</sup>400IU PMSG & 200IU hCG. <sup>2</sup>Values in each row with the same superscript do not differ (P=0.05; Anova of log counts).

The mean number of CL differed significantly only with 500IU PMSG. Although there was a trend to the number of embryos collected to be greater with 1,000IU PMSG and 500 IU hCG, the high incidence of metritis at 1,000IU and also with PG600 indicated that 750IU of PMSG+500 hCG was the choice for oestrous induction and ovulation in prepubertal gilts.

### METABOLIC STATE OF THE LACTATING SOW INFLUENCES PLASMA LH AND FSH BEFORE AND AFTER WEANING

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When food intake is low during lactation sows will catabolize maternal tissues (fat and lean) to provide substrates for milk production and this may result in an increase in the interval between weaning and re-mating (Mullan and Close, 1989). The aim of this experiment was to examine how the nutritional, metabolic and physiological state of the sow affects concentrations of plasma LH and FSH, and hence reproductive function.

In a 2x2 factorial experiment, 28 Landrace x Large White gilts were fed the same diet either to appetite (H) or 3.0 kg/day (L) for a 21-day lactation with litter size adjusted to either 6 (6) or 12 (12) piglets at each feeding level. Blood samples were drawn via indwelling jugular cannulae at 15 min intervals for a 12-hour period immediately before and after weaning.

The treatments had a marked effect on the loss of body reserves by sows during lactation and on reproductive performance (Table 1). Suckling intensity appeared to influence LH secretion prior to weaning, whereas plasma LH after weaning was related to loss of body reserves during lactation. The concentration of plasma FSH was highest when plasma LH was low before and after weaning (L-12).

		H-6	H-12	L-6	L-12	SEM <sup>1</sup>
Intake						
Energy (MJ ME/day)		62.0ª	66.3ª	33.1 <sup>b</sup>	35.2 <sup>⊳</sup>	2.28
Nitrogen (g/day)		146.9×	147.3×	75.2 <sup>y</sup>	78.2 <sup>y</sup>	5.02
Change during lactation						
Sow liveweight (kg)		-0.1ª	-8.1 <sup>b</sup>	-17.2°	-34.0 <sup>d</sup>	3.03
Sow backfat (mm)		-0.7ª	-3.0 <sup>ь</sup>	-3.8 <sup>b</sup>	-7.0°	0.82
Litter weight (kg/day)		1.52 <sup>⊾</sup>	2.06ª	1.36°	1.94ª	0.114
Weaning to oestrus interval (d)		11.2ª	8.7ª	8.5ª	19.2 <sup>b</sup>	4.28
Mean plasma LH (ng/ml)	-pre	0.30ª	0.21 <sup>b</sup>	0.38ª	0.20 <sup>b</sup>	0.047
	-post	0.57 <sup>ab</sup>	0.49 <sup>bc</sup>	0.81ª	0.28°	0.133
LH pulsatility (no./12h)	-pre	3.2ª	2.7ª	3.7ª	1.7ª	0.84
	-post	7.2ª	6.1ª	7.7ª	3.2ª	1.34
Mean plasma FSH (ng/ml)	-pre	34.1°	37.1°	<b>43</b> .5⁵	60.4ª	1.04
	-post	44.7°	52.6 <sup>ь</sup>	46.0°	59.1ª	1.12

Table 1. Plasma concentrations of LH and FSH for sows pre- and post-weaning

<sup>1a,b,c,d</sup>Differ at P<0.05; <sup>x,y</sup>differ at P<0.01.

The secretion of LH plays an important role in regulating oestrus activity after weaning. The concentration of FSH is a consequence of a low secretion of LH and ovarian inactivity, and the level of FSH appears to have no major role in regulating the level of ovarian function during lactation.

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# METABOLIC AND ENDOCRINE CHANGES DURING THE REPRODUCTIVE CYCLE OF THE SOW

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The excessive mobilization of body reserves during lactation has a major effect on subsequent reproductive performance by reducing plasma LH concentrations and delaying the return to oestrus (Mullan *et al.*, 1991). The aim of the present experiment was therefore to record plasma concentrations of several metabolites and hormones during the reproductive cycle in an attempt to identify the primary signals which link the nutritional and reproductive states of the sow.

The experiment involved 12 gilts, one half of the animals were fed 5.5 kg/day (67 MJ ME and 956 g crude protein/day) and litter size adjusted to 6 piglets (H-6), whereas the remaining 6 animals were fed only 3 kg/day (33 MJ ME and 475 g crude protein/day) and suckled 12 piglets (L-12). Blood samples were drawn via indwelling jugular cannulae at 15 min intervals over a 12-hour period (0800-2000 h) on day 110 of gestation and days 10 and 17 of lactation, and immediately pre- and post-weaning, for the measurement of plasma glucose, insulin, non-esterified fatty acids (NEFA) and growth hormone (GH).

During lactation H-6 sows maintained bodyweight whereas L-12 sows lost 34.0 kg; the corresponding changes in backfat (P2) were -0.7 and -7.0 mm, respectively. The mean weaning to oestrus interval for H-6 and L-12 sows was 11.2 and 19.2 days (P<0.01), respectively. There was a significant increase in plasma insulin following farrowing for the H-6 but not for L-12 sows, but only in early lactation were there differences in glucose concentrations (Table 1). Plasma NEFA concentrations decreased between gestation and lactation for the H-6, but increased for the L-12 animals. NEFA concentrations were linearly and inversely related to the animal's energy balance (r=0.75). Growth hormone was secreted in an episodic manner and was extremely variable between animals.

	Pregnancy	Lact	ation	Wea	ning
	Day 110	Day 10	Day 17	Pre-	Post-
High - 6					
Glucose (mg/100ml)	72±7	69±3	60±8	53±9	68±8
Insulin (mŬ/l)	13.8±5.9	15.3±3.4	12.8±4.9	11.1±9.7	15.9±5.1
NEFA (mmol/100ml)	0.23±0.08	0.45±0.15	0.41±0.07	0.45±0.13	$0.15 \pm 0.06$
GH (ng/ml)	1.70±0.53	3.31±1.58	3.11±1.22	2.95±1.12	2.08±0.52
Low - 12					
Glucose (mg/100ml)	63±8	85±5	74±7	68±8	54±14
Insulin (mŬ/l)	15.0±3.5	34.0±11.3	28.0±0.91	15.7±5.1	15.4±4.9
NEFA (mmol/100ml)	0.23±0.11	0.12±0.02	0.13±0.03	0.14±0.06	0.45±0.18
GH (ng/ml)	2.12±0.50	2.72±0.95	2.47±0.76	2.27±0.76	1.90±0.48

Table 1. Plasma concentrations of glucose, insulin, NEFA and GH in sows at various stages of the reproductive cycle (Mean  $\pm$  SE)

In association with other studies (Mullan *et al.*, 1991), it is concluded that insulin may play a key role in linking the nutritional, endocrine and reproductive states of the sow.

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# MAMMOGENESIS IS INFLUENCED BY PREGNANCY NUTRITION

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In experiments at the University of Western Australia we have manipulated the body composition of gilts by changing their protein and energy intakes during pregnancy and have found that fat gilts produce less milk than lean gilts (7.0 versus 9.0 l/d) at the same body weight. Is it possible that these nutritional regimes imposed during pregnancy may affect mammogenesis and be responsible for differences in milk output?

Mammary tissue grows during pregnancy in proportion to bodyweight (Tucker, 1987) and, in the gilt, the potential number of secretory cells measured by DNA does not change after parturition (Hacker and Hill, 1972). Weldon *et al.* (1991) attempted to stimulate mammogenesis by altering nutrition in the last third of pregnancy. An increase in protein intake had no effect on cell number while an increase in energy decreased mammary weight. Based on the work of Tucker and Weldon we expected that nutrition during pregnancy would not affect the number of milk secretory cells of gilts with the same bodyweight.

Seven fat and seven lean gilts were slaughtered at 112 days of gestation and their mammary glands dissected free of fat, muscle and blood vessels. Each teat was weighed and a segment from each taken and frozen in liquid nitrogen, freeze dried, defatted and then analysed for total DNA content using a fluorometric method adapted from Hopkins and Tulloh (1976).

The fat and lean gilts had the same weight of mammary glands and teats and there was no difference in chemical composition of the glands (51% water, 14% fat and 34% protein). There was an extraordinary difference in the concentration of DNA in the mammary tissue (Figure 1). Given that there is  $6 \times 10^{-12}$  g DNA per mammalian diploid cell we have calculated that the number of cells in the mammary gland of the lean and fat gilts were 313 (±21) million and 75 (±8) million.





This unexpected result is very important. If this difference in DNA reflects a difference in alveolar cell numbers then it suggests that mammogenesis can be altered by nutrition during pregnancy and is not as well protected as is currently thought.

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### A SYMPOSIUM - IMPLICATIONS OF THE SENATE INQUIRY ON ANIMAL WELFARE FOR THE PIG INDUSTRY

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#### Symposium introduction

The modern animal welfare movement really began in 1964 with the publication of Ruth Harrison's book "Animal Machines". This book raised many controversial issues in intensive farming in England, Europe and USA. The British Government responded by setting up a parliamentary inquiry in 1964 conducted by Professor F.W.R Brambell. The inquiry looked at the husbandry methods under which intensive animals were farmed. The result of this inquiry was the publication of the Brambell Report in 1965 - "The Welfare of Animals Kept Under Intensive Husbandry Systems". This report generated many changes in the manner in which animals were managed and veterinarians were given the power to enter farms and check the condition of the animals and their facilities.

In 1975 the publication of Peter Singer's book "Animal Liberation - Towards an End to Man's Inhumanity to Animals" expounded the philosophy of what was to become in 1977 the basis of the Animal Liberation Movement. This book questioned the right of humans to exploit animals. A term Singer uses - speciesism - is useful to define. It is 'a prejudice or attitude of bias toward the interests of members of one's own species against those of members of other species'. Singer goes on to say that any belief that mankind has any inherently greater right to exist or any right to subjugate another species is 'speciesism' and is considered as great an evil as racism or sexism.

A more extreme view of the animal rights position is expressed by Tom Regan (1986/87). It has become the basis of the animal rights activists who are very influential in America (Cleveland, 1990). Regan states that the fundamental wrong is that which allows us to view animals as our resources, here for us to eat, surgically manipulate, or exploited for sport or money. He goes on to argue that this attitude allows us to farm animals without really worrying about it, and claims that if we made the rearing methods of farm animals 'more humane' it would require the total dissolution of commercial animal agriculture. Similarly the animal rights view of using animals in science is categorically abolitionist.

In response to animal welfare issues, Codes for the Welfare of pigs, poultry, animals transported by road, rail and air, intensive husbandry of rabbits and animals at slaughtering establishments have been produced by the Standing Committee on Agriculture, Animal Health Committee (subcommittee on Animal Welfare). Some State Agriculture Departments have also published some codes of practice for the welfare of livestock.

The Senate Inquiry into Animal Welfare has become a significant event in the history of animal welfare issues in Australia. It began in November 1983 and has not yet been concluded. Recommendations from this inquiry include those for export of live sheep, kangaroos, dolphins and whales in captivity, sheep husbandry, animal experimentation, intensive husbandry of pigs and poultry and an interim report on the racing industry.

The report by the Senate Select Committee on Animal Welfare relevant to the pig industry is "Intensive Livestock Production", June 1990. It covers several areas including recommendations for pig housing for growers and sows, tax incentives to encourage producers to upgrade their systems to incorporate improved design features, husbandry practices and off farm handling of pigs. Other important recommendations were for the development of training courses for stockpersons and the enforcement of Codes and legislation for the prevention of cruelty to animals. The views of a scientist, a pig producer and a politician on the implications of this report and its recommendations are presented in this symposium.

## THE FINDINGS OF THE SENATE SELECT COMMITTEE ON ANIMAL WELFARE: IMPLICATIONS FOR THE PIG AND POULTRY INDUSTRIES

#### Senator D. Brownhill

Deputy Chairman, Senate Select Committee on Animal Welfare, Canberra, ACT 2600.

The Senate Select Committee on Animal Welfare (SSCAW) Report on Intensive Livestock Production is the sixth issued by the Committee since its inception in 1983. The Report was tabled in the Senate on 28 June, 1990, and followed public hearings, inspections and submissions spread over almost the entire history of the Committee, which came into operation in 1983. The Government Response to the Report was tabled in the Senate on May 30, 1991.

It is true to say that community concerns about intensive animal husbandry, and most particularly the intensive production of poultry, was a key factor in the establishment of the Committee. The fact that the topic was relegated to sixth in the series of reports is less a reflection of its importance *viz-a-viz* other industries than an indication of the extent of the evidence received and the considerations to be made.

It is probably also true to say that it may be a reason why the Government took so long to table a response to the enquiry. The only other report to which a government response is still outstanding is our report on kangaroo management.

I would say at this point that the membership of the Committee changed several times over the course of this enquiry, as did the Secretariat. It is likely the emphasis of the enquiry and the inherent biases of the Committee members moved in line with these changes. I cannot be certain that the final result was merely the views of the latest members or a combination of all those who had gone before. Certainly, because of the changing membership, considerable evidence was taken and inspections were made again and again to introduce new members of the Committee to the issues at hand.

As already stated, the inquiry was undertaken because of developing concern in Australia that welfare of our domestic livestock was being jeopardised in many of the housing systems currently operating; in particular, the pig, chicken meat and egg industries.

The Report says in its opening paragraphs: "Australians face a challenge over the next decade balancing the forces which impact on their standard of living. In order to prosper as a nation we must lift our production performance while at the same time lower the exploitive nature of progress and development to date" (SSCAW, 1990).

Of course, it is not just in Australia that intensive animal production has come under the microscope, it is on the political agenda of most developed Western countries, particularly Europe. Animal welfare, and especially farm animal welfare has come under attack from a number of quarters, and in my opinion, will continue to do so. How this issue has been handled in other countries I am obviously not as familiar with, but in the opinion of others more qualified than I, Australia's approach to welfare issues, particularly at the farming and research levels, is very good and I would venture to suggest, better than most.

The Committee's Report on the poultry and pig industries has not been greeted with wholesale support, but given that the criticism has come from both animal activists and the industry itself suggests that perhaps we have trodden a middle path and brought down an objective report. Certainly that was our intention and the Committee had to weigh the considerable community concerns and criticisms of intensive animal practices against economic realities.

While the charter of the Committee is to inquire and report upon the question of animal welfare in Australia, I do not believe that we ought, nor be expected to do that

in isolation from any other consideration. Indeed the earlier report on the live sheep trade expressed the view that if the future of the trade were to be made purely on animal welfare grounds, then there was enough evidence to stop it. However, the report went on to say that "the Committee agreed that the animal welfare aspects ... cannot be divorced from economic and other considerations" (SSCAW, 1985), and that same criteria can be applied to the industries covered in this report.

With this report the Committee had to consider at some length and in some detail the views of those who opposed intensive systems. They advanced the argument that close confinement is ill-treatment, because it deprives livestock of the opportunity to express physiological and ethological needs and behaviour. The Committee believed that the recommendations should reflect realistic objectives and criteria for the industry. We needed to be mindful of the economic considerations of the reforms we were proposing, but we also had to address the concerns and objections of those in opposition.

In general, the Committee took into consideration the present situation of the respective industries, the community concerns about those industries, the relevant research into alternatives and then tried to arrive at recommendations that were realistic.

There are a total of 41 recommendations, and they are directed at both the industry and at government. As I have been asked to assess the implications of those recommendations for the industry, it ought to be said that those that the Federal Government have chosen not to support clearly will have little impact on the industry. Others fall within the auspices and legislative control of the states. As you would be aware, legislation varies from state to state, so I am uncertain as to what states will take up what recommendations.

I do not think it necessary to comment on every recommendation, but clearly there are some that concern the relevant industries more than others and I will address my remarks to those. In passing I note that to date the response from both pig and poultry industries has been subdued, and perhaps one reason for this is that over recent years those involved in intensive animal production have come to realise that welfare considerations have to be addressed.

Poultry, and to a lesser extent pig producers, have been forced to acknowledge the animal welfare debate much earlier and more vigorously than others. The respective industry organisations have had to develop a strategy to demonstrate that their growers are concerned about the welfare of their animals and that every effort has been made to reduce stress, discomfort and pain. In keeping with the Committee's previous preference for self-regulation, this Report falls very much into that category.

The Committee has taken the view that legislation can only be as good as the enforcement procedures and resources available to ensure it is adhered to. Additionally, what we were recommending was within the jurisdiction of state legislatures and therefore we had little power to direct.

Moving to the substance of the recommendations, I would consider the most significant recommendations are those which deal with housing systems and stocking densities.

In all industries that involve animals, and most particularly intensive industries, it is the quality and level of stockmanship that is the linchpin to the entire success of the enterprise. The most modern intensive facility will fail if the stockmanship does not meet the needs of the animal, and the Committee has grappled with an acceptable definition of stockmanship on almost every enquiry.

What constitutes a "good stockman"? How does an employer, an industry, a legislator, ensure that good stockmanship is in place? Is stockmanship judged by formal qualification, years of experience in the industry? Clearly neither of those are sufficient or the total answer. Good stockmanship, or husbandry, is so much an innate sense of knowing what is right for the animal at the time, and poor stockmanship will lead to reduced profits, although in some operations, profit is not a major criterion.

In dealing with this issue, the committee recommended that the subject of animal behaviour be recognised as an integral component of the curriculum in agricultural and veterinary colleges, especially as a component of animal welfare. It further suggested the development of certificate training courses for stockhands at TAFE and agricultural colleges. We suggested funding initiatives be developed on a less formal level to encourage skills training for those in the industry not able or willing to undertake formal training, and that the relevant research bodies in the Pig Industry Research Council give greater priority to this.

I am pleased that the Federal Government response has also supported that initiative and recognised that it is pivotal to increased welfare consideration. It is appropriate that I acknowledge the efforts of the University of Queensland in this regard. Their desire to establish a Chair of Animal Welfare is commendable and appropriate at a time when Australia is at the forefront of animal welfare reforms, particularly in the rural sector.

Recommendations were made in respect of housing, stockmanship, transport and slaughter in the pig industry. The Australian Pig Industry Policy Council, in its submission to the committee stated that "while community concern about animal welfare has been a more recent phenomenon, farmers have for generations generally treated their livestock in a humane and considerate manner" (SSCAW, 1990). While not disagreeing with that statement, it is patently obvious that not all farmers subscribe to that view, and the industry would do well to accept that improvements need to be made in welfare aspects.

The report notes that parties to the enquiry demonstrated a real concern for the welfare of food animals and agreed that the least stressful effective methods of production should be used. At issue is the extent to which welfare is affected by intensified production, the importance of components of the production system most likely to impose suffering and the ethological needs of the livestock involved.

The pig industry, in my view, has addressed those concerns very well. Much research has already been undertaken into behavioural problems of intensively housed pigs. Evaluation of intensive systems is essential in order to ensure the removal of undue suffering. We accept that one of the problems associated with this evaluation is determining what is normal and natural behaviour.

The Committee carried out numerous inspections of pork producers, ranging from several thousand sow corporate establishments to owner-operated farms with a few hundred sows. We saw a variety of accommodation options and came to the conclusion that an intensive system for pigs was proper if the health of the animals was not adversely affected, if their behaviour was not disturbed and if their adaptability was not overcharged.

The committee expressed disappointment that while controversy has stimulated welfare specific research on the impact of different intensive systems, there has been virtually no study of the economic implications of the different approaches. We recommended that the Pig Research Council actively encourage research to address the cost equation associated with capital costs of pig housing and loss of production with a view to clarifying some welfare stress issues. Also, a review of stocking densities for growing and adult pigs in groups to take account of the advances in understanding of physiology and behaviour, was recommended.

Issues such as housing of dry sows, examination of farrowing crate design, both in size and layout were the subject of numerous recommendations.

We have suggested that future trends in housing the dry sow should be away from individually confined stall systems and that this be reflected in the Codes of Practice. The government has supported in principle the recommendation to review housing systems, but makes the point that group systems involve greater demands in terms of stockmanship. It accepts, however, that the tethering of sows be banned. This comes after the NSW Government legislated to ban the tethering of sows, in line with representations it received from the NSW RSPCA on this subject. The Committee would also like to see alternative approaches adopted to accommodating sows through their various stages to reduce piglet mortality. The constant confinement, with lack of mobility for the animals was of concern to the Committee, although it accepted research findings that even when provided with more space and increased opportunities for activity, the pigs often did not utilise the facilities provided.

This lack of space, and the factory farming appearance of intensive pig production, sits heavily on the conscience of animal activists. A sow with piglets lying in sterile but barren and confined conditions is offensive to many and opposite to the childhood farmyard picture of animals and their young, lazing in the sun on the perennially green pastured farm.

The same can be said for the poultry industry. It is often no more than early childhood notions of how animals ought to be raised that intensive producers have to fight against. Picture books don't show the disease, the mud, the flies, the foxes and the manure and arguments that suggest that extensive farming has all those aspects is met with the response that it is "natural" and therefore alright, whereas intensive housing, with all its hygiene and sterility, is not.

It is perception, as much as anything else, that intensive producers have to fight against. Facts can always be found to support the notion and those charged with the responsibility of 'selling' the industry to the general public need to be as aware of that as they are of those producers in the respective industries who do not "do the right thing".

Finally, in the area of research, we have urged the government to encourage and financially support more study, both economic and welfare related, in both the pig and poultry industries. This has the support of the Federal Government also, and like many of our recommendations, been submitted for inclusion in the revised Codes of Practice for the respective industries. Those that involve state action and legislation have been sent, through the auspices of the Australian Agricultural Council, to state governments for consideration.

Some of the recommendations may in time appear in a legislative form; however, many will remain as a recommendation or a Code of Practice at a State or Federal level.

Both Pig and Poultry industries would do well to accept that community objections remain and there will be continuing pressure on governments and on animal welfare agencies, to ensure that improvements continue in both industries.

Consumers will continue to demand improvements in food (nutrition, convenience and price) but it is most probable that they will not be prepared to accept any lessening in welfare standards to achieve those goals.

It is up to industry to ensure the happy balance. You can be assured that if you get it wrong, you will be told.

Few will thank you if you get it right.

### IMPLICATIONS OF THE SENATE INQUIRY ON ANIMAL WELFARE FOR THE PIG INDUSTRY

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#### Introduction

Intensive livestock production is an issue that is now a political agenda item in most western countries. There is an emerging undercurrent that "intensification" has been to the detriment of animal welfare. The report acknowledges that there has been, within developed more affluent societies, a general awakening and growth of consumerism, environmentalism, naturalism, activism and the general questioning of materialism, raising concerns on animal production methods, the ethics of those methods and the perceived animal welfare negatives.

The Brambell Committee, established by the British Government in 1964, enquired into animal welfare. This was the notable first of a number of similar committees established worldwide by various governments. An outcome has been the legislation of animal welfare related laws and regulations in a number of countries, such as banning the battery hen cages in Switzerland, and the phasing out of stalls and tethers in the United Kingdom by 31 December 1998.

#### Senate Select Committee

The Australian Government established a Senate Select Committee in November 1983 to enquire into and report on "The question of animal welfare in Australia, with particular reference to:

- 1. Interstate and overseas commerce in animals
- 2. Wildlife protection and harvesting
- 3. Animal experimentation
- 4. Codes of practice of animal husbandry for all species"

The Committee to date has delivered six reports to the Senate, the last of which was "Intensive Livestock Production". This report covered the three areas of most concern within intensive livestock production: the pig, chicken meat and the egg industries. In conducting its enquiry the Committee was presented with written and verbal submissions from industry bodies, animal welfare organisations, researchers, Government Departments and veterinary bodies. The report was presented to the Senate and then published for distribution during June 1990 (SSCAW, 1990).

#### **Producer involvement**

The pig industry, having received the report, was asked to respond in full or in part. The Government has now considered the report and responded.

The average producer, while exposed to the six to seven year history of the enquiry was not directly exposed to the presentation of submissions. Those submissions were developed and presented by representatives of industry bodies. Specialists were contracted as expert witnesses to support specific areas of the submissions. The Senate Committee of Enquiry was also exposed to a cross section of industry production systems and husbandry practices.

The public release of the report and its contents were well covered by industry journals, exposing most interested producers to the summary and key recommendations. It would be somewhat surprising though if the majority of producers have read the full report. That responsibility has fallen on the industry representatives, advisers and those key producers with a vested interest in the long term status of the pig industry.

#### Code of practice

The industry has become increasingly responsive to its recognised position in the intensive animal production scene over the last two decades - the coming of age. Being one of the recognised intensive industries has necessitated an industry approach to self regulation and self responsibility regarding its management of welfare issues.

The "Model Code of Practice for the Welfare of Animals - 1. The Pig" was released in 1983 (Australian Bureau of Animal Health, 1983). This document was the culmination of two years discussion and consultation by industry bodies with the Sub-Committee on Animal Welfare (SCAW) of the Animal Health Committee (AHC) within the Australian Agricultural Council (AAC). This model code was then adapted and adopted by all states, again after further consultation with industry. One of the

overriders when both discussing and accepting the final draft code was a procedure to regularly (five yearly) update the voluntary code, taking into account:

- 1. Advances in technology and research findings relevant to the existing code
- 2. Developing controversial issues
- 3. Legislation that impinges on the code

Of recent years producers have increasingly relied on the belief that the welfare of their livestock directly impinges on the productivity of those livestock and ultimately the economic viability of that production system/unit. Therefore any emerging production system, management practice or research that suggests welfare benefits, must be seriously assessed and evaluated. Likewise, if research indicates that a current practice or system is detrimental to the welfare of the pig, then it behoves the industry to investigate potential alternatives and/or modifications to that existing practice. In essence producers are desirous of the code being regularly updated, but strongly advocate that any additions, alterations or deletions be in the light of advanced technology and validated research.

Australia is in a unique position as regards the responsiveness of the mechanism for reacting to and initiating research into specific factors of pig production. The Pig Research and Development Corporation (PRDC) is so placed to be both reactive and proactive to issues of immediate concern, be they welfare or otherwise. Comparing the PRDC "Five Year Plan" (PRDC, 1991) with the Senate report most of the recommendations are encompassed in part or total. The current program will inevitably clarify some of the Senate Committee's recommendations, open up others to further research and provide additional validation to relevant areas in the code of practice.

#### The report

The welfare of the pig stops not at the farm gate but at the conclusion of slaughter. Indeed the ramifications of the pigs' welfare post-farm gate are just as critical as pre-farm gate. Post-farm gate stress directly reflects on carcase yield and quality. The welfare issue extends to a multi-sectorial level rather than resting solely with the production sector.

The report, while being directed primarily at the production sector includes two recommendations specific to the post-farm gate.

The Senate Select Committee listed a total of 21 recommendations in the report, under the following headings:

- 1. Research and evaluation
- 2. Housing the sow
- 3. Production systems
- Stocking rates
- 5. Tax incentives
- 6. Husbandry practices
- Post-farm gate
- 8. Education and training
- 9. Legislation and regulation
- 10. Standards

While some recommendations place aspersions or questions on current systems or husbandry practices, the need is recognised for further research into the stated areas. This research will ascertain and validate the merits of the current systems and if necessary develop alternatives that adequately enhance the welfare aspect. Any intensive industry with the ability and willingness to address those genuine welfare concerns of the community, as cited within the recommendations, must be more soundly positioned for the future. Failure in this regard will only compound the misunderstandings and over-reactions, allowing the more extreme community elements greater credibility and influence.

The critical point though is for the industry, not just to be positively reacting to those community concerns, but to be actively and vigorously seen to be pursuing the issues. This necessitates a well planned and executed public relations program.

#### Recommendations - Pigs, Recommendations 20-41 and implications

The following are the Committee's recommendations and my discussion points on each. It must be noted that the discussion points are not representing a deliberated position adopted by any industry body in response to the report, but the summations of a single producer whose primary income is sourced from pig production. However, the comments do endeavour to perceive the industry's approach and stance to recommendations embodied in the Report as well as personal views and summations.

#### 1. Research and evaluations

#### i) Recommendation 20

"The Committee recommends that the Pig Research Council (PRDC) actively encourage research to address the cost equation associated with capital costs of pig housing and loss of production with a view to clarifying some welfare stress issues".

The critical issue is the balance or perceived trade-offs between acceptable welfare levels and the range of capital costs to achieve the various degrees of welfare. This contentious point is the very heart of the welfare debate. Up to recent years it has been solely based on perceptions. Under rational circumstances the industry and responsible welfare groups must accept a multi-faceted description of welfare and an objective means of measuring such, under a range of situations. Once accepted, research can be conducted and the results accepted by industry and the concerned community. Not an easy task!

Research into the so-called cost equation considering capital costs, loss of production and the welfare status is of high importance. Such research should provide producers with a clear indication of the implications of their capital options and decisions. The findings would be beneficial not only to those producers embarking on "green field" production facilities but more critically the cost benefits/negatives of alterations, repairs and upgrading of existing facilities.

It is a recognised fact that production facilities are "aging" at an alarming rate. Information that could assist with modernisation of existing facilities (taking account of welfare) would be of considerable benefit. It is imperative that the results of the research would be packaged in such a manner that they were readily and easily understood, practical to use and had a shelf life extended by regular review.

#### i) Recommendation 21

"The Committee recommends that the maximum recommended stocking densities for growing and adult pigs in groups be reviewed to take account of the advances in understanding of physiology and behaviour and the welfare consequences of pen space, stocking rules and group sizes".

Producers have always recognised that stocking density is closely related to average daily gain (ADG). Numerous stocking density trials have indicated that ADG peaks at approximately 130kg per square metre. Conversely where environmental control is inadequate, ADG may also suffer at the lower stocking density levels. Reduced ADG when linked with stocking density is considered a clear indicator of a stress welfare problem.

A review of the current recommended maximum stocking densities would be welcomed, providing that review took into account non-welfare aspects as well, that is, improved environmental control systems, high energy diets, feed dispensing systems and the physical pen design. Validated review findings could then be compared with the existing Code of Practice recommendations. It is again an area where an open mind is necessary for, as a consequence of improved technology, some maximum rates may be increased or conversely decreased.

#### 2. Housing the sow

i) Recommendation 22

"The Committee recommends that future trends in housing the dry sow should be away from individually confined stall systems and that this be reflected in the Codes of Practice for the welfare of the pig".

The intensive production sector have grave concerns regarding this recommendation. A significant proportion of the intensive systems utilise dry sow stalls. Stalls have played a critical and essential role in the development of the current Australian pig industry. Yet the very connotation of 'confinement' especially the 'confinement stalls and tether system' attracts the bulk of the welfare debate.

In January 1991, Ministry of Agriculture for Food and Fisheries (MAFF) of the United Kingdom (UK) announced that a ban would be introduced this year on construction of new tethered and narrow breeding stalls. Existing installations will be phased out by December 1998. It has been quoted (Animal Pharm, 1991) that in 1988, 60% of UK producers operated a stall or tether system. The current percentage is quoted at 50% and is anticipated to drop to 40% by 1992. Enactment of the legislation to ban tethers and stalls in the UK could be less disruptive than in some fellow European countries. The Netherlands and Denmark have both produced a number of draft "Animal Welfare Laws", but have yet to enact them. Each country also has a high percentage of stalls and tethers.

In Switzerland and Sweden, where animal welfare acts have been legislated, both are experiencing problems with the implementation of and compliance with the "Acts". In many instances the emotive sentiments were imposed by legislation without having in place viable and competitive alternative options. Availability of an option or range of options, that are capital and productively competitive, allows for a more cohesive and acceptable method of enforcing change. While the Committee recommends that future dry sow housing be away from individual stalls, clear benefits need to be identified to encourage producers to voluntarily trend towards alternatives.

Background material leading to Recommendation 22 infers the stalling of dry sows for the total gestation period. Stalling of sows post weaning or post mating until pregnancy confirmation, and then group housing was not addressed. In summary, there would be a great deal of industry disquiet if the Code of Practice reflected this recommendation without firstly proving that stalls are detrimental to the sows when compared to other alternatives.

#### ii) Recommendation 23

"The Committee recommends that tethering of sows be banned".

When negotiating a stance or position most negotiators have a sacrifice or trade item. The tether has been described as the industry

pawn by some industry personnel. To put the use of tethers in Australia in perspective, when the Code of Practice was being drafted, sources indicated that approximately five percent of intensive producers may have the tether system.

Poorly managed tether systems can have serious welfare implications, an area upon which the welfare activists have capitalised and gained community sympathy.

Under good management the tether system has yet to be proven inferior, regarding the level of sow welfare. Taking account of the above points the industry may well concede the banning of new tethers and consider a phasing out period for existing systems, following further reviews.

iii) Recommendations 24 and 25

"The Committee, noting that sow size has increased over the years, recommends that immediate attention be given to ensure that stalls and farrowing crates currently in use do not cause suffering due to cramping", and,

"The Committee recommends that the Codes of Practice for the pig be revised to ensure stalls and crates reflect the body dimensions of large sows".

The industry endorses these recommendations as good housekeeping measures. Well managed units often have either a range of crate sizes or adjustable crates to accommodate a range of sow sizes.

#### iv) Recommendations 26 and 27

"On the issue of farrowing crates, noting that piglet mortality due to sow overlay is a major welfare consideration, the Committee recommends the encouragement of some producer pilot systems to test the viability of designs which will allow sows more freedom of movement and access to a separate exercise area at least some time each day", and

"The Committee recommends that governments and the industry encourage the adoption of alternative approaches to accommodating sows through their various stages and the improvement in husbandry skills needed to avoid welfare problems".

A developing industry must constantly assess alternative housing systems and management practices. Accommodating the sow has been an evolving area, an area that is constantly under the pressure of innovation. Each subtle change to a system also requires an adjustment to the management skills. However, alternative systems demand very high standards of stockmanship. The UK Animal Welfare Advisory Body strongly supports the need for further urgent research into alternative systems and requests the government to allocate funds for both training and research and development. This request was noted after MAFF advised the banning of tethers and stalls.

Both recommendations must be endorsed by industry for they support the evolving of new systems and the refinement of existing systems, together with the necessary skills training. Placing a greater focus on these areas should identify more readily the critical points and management skills.

A considerable amount of research, evaluation and developmental work is being conducted in Europe. Because of the size of the Australian industry only a limited amount of systems research can be conducted within the country, though there is certainly the ability to review overseas work, and adapt it to current knowledge and conduct evaluation trials.

v) Recommendation 28

"The Committee questions the management practice of birth induction and

recommends that the welfare implications of prostaglandin use be investigated".

Many producers would have difficulty understanding the reasoning behind this recommendation. Birth intervention in humans is an accepted practice for many and varied valid reasons. The use of prostaglandins within piggeries is an effective management tool to maximise the ability of piglets to survive and enhance the husbandry of the sow. Any removal of this practice would be viewed by the industry as a negative welfare issue.

The industry would not be adverse to research of this issue, though it would fail to see the justification. Research to improve the use of interventionary administrations would be of merit.

#### 3. Production systems evaluation

#### Recommendation 29

"The Committee recommends that the Commonwealth Government fund a research project in Australia to examine and evaluate housing systems that may be suitable to Australian conditions and that this review:

- i) Examine overseas research findings into alternative housing systems
- ii) Assess the welfare benefits and any welfare disadvantages of such systems
- iii) Evaluate the economic viability of alternative systems
- iv) Take account of the views of producers, industry service providers, design engineers and specialist ethologists"

There is general agreement to this recommendation. It is assumed that if the Government accepted the recommendation that the resultant research project would be either submitted to or requested of the PRDC.

#### 4. Tax incentives

#### **Recommendation 30**

"The Committee recommends that the Commonwealth Government provide tax incentives to encourage producers to upgrade their systems to incorporate improved design features to improve pig welfare".

One of the industry's primary concerns is the "aging" of its production facilities. Many of the earlier intensive units are nearing the end of their economic and productive life. Major upgrading or total replacement will be necessary for many units to take advantage of improved systems, technology and husbandry skills required to maximise production. The economic challenges are closely entwined with the welfare of the stock. Government tax incentives and favourable credit facilities would greatly enhance the industry's ability to upgrade existing facilities, to meet the enhanced welfare expectations. Green field sites would have greater scope in choosing the most appropriate system and supporting technology.

The industry highly endorses this recommendation, for it is this approach that would greatly assist the industry to maximise the achievable productivity gains resultant from improved animal welfare.

#### 5. Overstocking

#### **Recommendation 31**

"The Committee recommends that the appropriate authorities ensure that regular inspections of intensive pig production units be undertaken to monitor husbandry practices generally and to ensure that stocking densities do not exceed those specified in the Codes of Practice for welfare of the pig". The appropriate authorities are not specified, though it could be assumed that Departments of Agriculture would be involved. Many departmental officers would be uneasy in the role of both adviser and policeman. Stock inspectors would be a more appropriate avenue, providing the health aspects were adequately addressed to prevent disease transfer from unit to unit.

In essence the industry is striving toward self regulation. The industry had considerable involvement with the recommended maximum stocking densities in the Code of Practice. In this light the recommendation as it stands could not be supported.

- 6. Pig husbandry practices
  - i) Tailbiting

Recommendations 32 and 33

"The Committee, noting that taildocking involves some pain and stress, recommends that stockpersons are properly trained in the procedure, so that the task is undertaken with dexterity and with as little trauma to the pig as possible", and

"The Committee recommends that further research into the causal factors of tailbiting be undertaken as the issue is so closely linked to overall aspects of pig welfare in close confinement production".

The capability of stock attendants is a critical point for the welfare of the stock during husbandry procedures. Ongoing research, training and education are vital components for the industry's development and recognition of responsibilities.

ii) Teeth clipping

Recommendation 34

"The Committee is surprised at the high susceptibility to infection which apparently occurs in intensive systems and noting the emphasis placed on the health benefits of intensive production recommends that further research be conducted into the underlying reasons for infection that necessitates teeth clipping".

Teeth clipping is a universal practice, with a general assumption of its value. However, incorrect clipping may cause more damage than unclipped piglets. Grinding the needle teeth may be a more effective method. The Danish industry has been trialing the grinding of needle teeth for over a year. The results indicate a considerable reduction in infections and described the method as a safer operation for the piglet and the worker. The operation is cited as being as quick as conventional clipping.

7. Pigs - Off farm handling

#### i) Recommendation 35

"The Committee, noting the importance of a multi-sector approach to strategies to minimise stress, deaths, and decrease yield and quality losses, during post-farm handling of pigs, recommends a State and Territory-wide multi-sectoral review of off-farm handling of pigs with a view to upgrading existing Codes of Practice and disseminating information to service providers, producers, transporters, abattoirs and interested parties. The review process should take account of the views of animal welfare organisations and specialist ethologists".

The post farm gate handling of pigs is another area of concern to the industry. It is, as stated, multi-sectoral and yet only involves a short time frame of the animal's life. The facilities and the management of the livestock during this time period are critical not only on a welfare level but also on the quality of the carcase.

The industry has made a recommendation that this multi-sectoral area be addressed by a new senate inquiry covering the transport and lairage of pigs. The brief of that enquiry should include:

- a) Logistical, management and welfare problems arising from strikes and breakdowns
- b) Issues of ownership and responsibilities: a many faceted topic
- c) Insurance of stock in transit and lairage facilities
- d) Possible accreditation of transport and lairage facilities
- e) Training of stock persons in transport and lairage
- f) Producer training/education in on farm loading facilities and stock handling

The Committee's report would be discussed with those involved sections of industry and then incorporated into a Code of Practice covering transport and lairage.

#### ii) Recommendation 36

"The Committee recommends that in addition to ensuring that information is widely disseminated on the proper handling of pigs from farm loading to slaughter, adequate monitoring should also be undertaken to ensure compliance with the provisions of the Code of Practice associated with the transport and slaughter of livestock".

There is general agreement with this recommendation. Assuming Aus-Meat accreditation becomes universal through the pig slaughtering facilities, that body would be best placed to provide the monitoring service as part of a plant's accreditation requirements. Any alterations to existing plants necessary to meet standards would require sufficient time to allow the upgrading of facilities and training of staff.

#### 8. Stockmanship, Education and Training

#### i) Recommendation 37

"The Committee recommends:

- a) That the subject of animal behaviour be recognised as an integral component of the curriculum in agricultural and veterinary colleges in Australia, especially as a component of welfare
- b) The development of certificate training courses for stockpersons in the pig and poultry industries by Technical and Further Education and agricultural courses
- c) Funding initiatives be developed to support skills training of stockpersons unable to gain access to formal training courses
- d) The PRDC, the Chicken Meat Research Council, and the Egg Industry Research Council give greater priority to welfare-related stockmanship research"

The four sections are important areas for coverage. The industry has a committed involvement in the award restructuring and career paths for employees. A part of this involvement necessitates the development of a recognised training program. This is being established on a National basis through the Rural Training Council of Victoria, acting as agents for the Rural Training Council of Australia.

a) and b) will be achieved by industry's needs and pressure. Item d) can be flagged by including welfare/stockmanship into the 'Five Year

Plans' of these industries' councils/corporations, signalling to researchers and industries, the level of priority given to this area.

#### ii) Recommendation 38

"The Committee also recommends that the Codes of Practice be revised to take account of advances in the understanding of the importance of stockmanship in the welfare of animals in intensive systems".

As previously stated the "Codes of Practice" need revising regularly to encompass new practices, new technology and welfare research findings. The existing "Code" was accepted by industry prior to the conducting of the research investigating the interactions between stockpeople and pigs. Those research findings could now be embodied within the "Code".

#### 9. Legislation and regulation

#### i) Recommendation 39

"The Committee recommends that to ensure that the Codes of Practice remain relevant there should be continuing revisions as appropriate and major reviews every five years to take account of technological changes in husbandry practices, include advances in the understanding of pig physiology and behaviour, and to reflect prevailing community attitudes. Codes should include statements on the importance of suitable education and training in maximum welfare in intensive systems. The review process should take account of the views of the industry, industry service providers, consumers and animal welfare organisations and specialist ethologists".

The pig industry is self regulated under a voluntary "Code of Practice". When reviewing voluntary codes, prevailing sectional or community attitudes, while being considered, must not govern the revised contents. However, if community attitudes are validated by sound research then those supported attitudes should be reflected in the revised code.

The industry would concur on the need to formally and regularly revise and update the current code.

#### ii) Recommendation 40

"Noting that each State and Territory Government has the responsibility to implement policies and enact and upgrade existing legislation which it thinks will best enhance animal welfare with its jurisdiction the Committee recommends:

- a) Legislation for the prevention of cruelty to animals and other relevant Acts specify that Codes of Practice for the welfare of animals must be followed
- b) That State and Territory Governments around Australia develop a complementary legislative and regulatory approach to animal welfare"

The critical issue here is that any legislation or regulation be based on validated research data and a soundly reasoned approach before adoption. Failure to apply these principles could place the industry in a tenuous position.

The industry stance is one of self regulation. Failure to achieve this would necessitate legislation.

10. Standard for Husbandry Systems

#### **Recommendation 41**

"Noting that standards are set for a range of commodities which are released onto

the market, the Committee recommends that governments with responsibility in this area develop standards for new and modified animal husbandry systems".

New and modified systems must be trialled and tested before standards can be applied. Failure to allow natural progress would stifle the industry's ability to develop to its fullest capacity.

#### Conclusion

Many of the European countries are either at the point of drafting animal welfare legislation or have enacted legislation. Numerous aspects of that legislation have been proposed and/or enacted, not as a result of industry wishes but through the pressure of welfare lobby groups. One of the more notable cases was the referendum held in Switzerland resulting in the banning of battery cages. The Swiss egg producers have experienced a significant reduction in their share of the domestic egg market since the ban. This is despite Government imposition of protective import restrictions. The messages are clear. Change is inevitable. The critical issue is who or which sector of the community is initiating the changes. It is essential for the pig industry to retain the initiative. Retention of that initiative will provide an extension of time in which to refine and formalise the following key points:

- 1. Establish a well planned and co-ordinated public relations program to extend a clear and concise image of the pig industry to the community; its capability of self-regulation, specifically welfare aspects; and its involved concern with emerging welfare issues.
- 2. A respected and effective political lobby arm.
- 3. A strengthened self-regulation capability that includes animal welfare.
- 4. Expansion and promotion of industry education and training that includes animal welfare as a critical component.
- 5. A formal review process that regularly reappraises the Pig Code of Practice and initiates necessary changes.
- 6. Co-ordination of the necessary cross linkages of the covered points, giving industry a focused direction. A component of the co-ordination group would be the identification of areas requiring research.
- 7. Research and trial of those identified issues.

The industry gained a great deal of credit and respect from the Senate Select Committee's Enquiry and resultant report. It has provided an excellent opportunity of grasping the initiative and running with it.

### IMPLICATIONS OF THE SENATE INQUIRY ON ANIMAL WELFARE FOR THE PIG INDUSTRY - A SCIENTIST'S PERSPECTIVE

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#### Introduction

The inquiry into intensive livestock production (which examined the laying hen,

the broiler chicken and the pig) was undertaken because of the developing concern in Australia (at the time of establishment in 1983 of the Senate Select Committee on Animal Welfare) that the welfare of domestic livestock was being jeopardised in many of the housing systems in current use. The basic question asked in this paper is "Are the recommendations for pigs in the report scientifically valid?" In short, the answer has to be in the negative. While some aspects of the report e.g. recognition of the importance of the stockperson and training, have considerable merit, specific areas of the report dealing with housing systems for pigs are disappointing and provide little advice to producers.

The report has attempted to incorporate into one document the perceptions/attitudes of a number of minority groups (farmer, industry and government organisations, scientists and welfare groups) and on the basis of these comments/submissions and some scientific literature makes recommendations on the future direction of the industry (SSCAW, 1990). As such it is a source document of views held by a number of different groups in the community. However, as the committee acknowledged its inability to assess the scientific content of the literature, it is unacceptable that it subsequently selectively quotes papers/reports to support specific recommendations.

From a scientist's perspective it is this latter aspect which is most disappointing. The community and animal welfare may have been better served by more appropriate recognition in the report that science was a minor player and acknowledging that animal welfare could be jeopardised by the report due to i) simplistic (and possibly incorrect) recommendations and ii) a redirection of research effort and funding away from complex areas of concern.

This paper discusses a number of issues raised both directly and indirectly in the report: The role of science in the Committee's report; a comparison of the Committee's assessment of welfare in the pig and laying hen industries; the concept of suffering; housing systems for pigs; and future welfare-related research in the pig industry.

#### The role of science in the animal-welfare debate

I believe the committee found itself in a dilemma that it was unable to resolve. On the one hand the committee acknowledged its limitations to assess research data:

"To attempt to summarise, let alone critically analyse and compose the findings of such research, is beyond the Committee's brief and area of expertise" (page 37).

Yet, at the same time, research data were selectively used to substantiate the Committee's viewpoint (see below).

There is also an attempt to play down the role of science, perhaps as a consequence of the Committee's acknowledged limitations to use science in assessing welfare. For example, in the report's conclusions there is the suggestion that science has a limited role in the subject of animal welfare. The concepts that should be considered are:

".... economic, scientific, ethical, aesthetic and practical concepts which together make up the complex subject of animal welfare, ...." (page 261).

In addition, there are a number of other statements that tend to play down the role of science by highlighting the acknowledged difficulties in the assessment of welfare:

"The difficulties involved in defining stress and evaluating its effects on animals cannot be overstated. 'Objective' measures of the impact of stress should be treated with caution" (page 39);

".... a preoccupation with the scientific approach to solving problems has come to assume an overly inflated position ...." (page 42); and

".... undue weight in their assessment of animal welfare on this scientific approach to the resolution of animal welfare problems. Evidence .... seemed to reflect too heavy a reliance on the scientific method at the expense of more experimental, intuitive approaches to data

gathering and formulating recommendations" (page 42).

This latter statement clearly indicates the committee's misunderstanding of the scientific method. The scientific method involves such abstract concepts as creativity, flair, intuition, guesswork, etc. in the development of ideas. However, scientists realise that these ideas are not an end in themselves and would not (and could not) base recommendations on their preliminary ideas. The ideas form the bases of hypotheses (guesses) which can be tested experimentally and subsequently accepted, rejected, modified, re-tested etc. to perhaps eventually end up in the form of recommendations. As stated by Medawar (1986) "Most of the day-to-day business of science consists in making observations or experiments designed to find out whether this imagined world of our hypotheses corresponds to the real one. An act of imagination, a speculative adventure, thus underlies every improvement of natural knowledge".

The committee further attempts to play down the contribution of science to the resolution of animal welfare issues by bringing the concept of suffering into the discussion:

"The scientific assessment of animal suffering is a central element in animal welfare research" (page 39);

"The Committee agrees .... that more emphasis must be given to obtaining knowledge, through experimentation, of animals' subjective feelings and to determine whether, or not, they are suffering mentally" (page 43); and

"For progress to be made in animal welfare assessment and research would entail ......... behavioural science continuing to provide evidence on aspects such as fear, frustration, conflict, pain and discomfort; and a fresh approach .... to arrive at a deeper knowledge of animals' overall well-being or suffering" (page 44).

The concept of suffering will be discussed later in this paper. Suffice to say that this area of concern was not addressed by relevant scientists either at the time of their submissions or subsequently. Considerable help could have been given to the Committee to indicate the extent to which suffering can be measured and is incorporated into research programs. Science is a tool to measure the definable. While it may not be practicable to define suffering, some of the elements that contribute to this concept are known and can be measured. It is a misunderstanding of science to expect it to be able to measure that which cannot be defined.

An example of the committee's misuse of science is as follows. When discussing current research, a summary argument of Hughes (1983) is presented to provide a statement that:

" .... there is now convincing evidence .... space available in a typical battery cage is too small" (page 74).

Some recent work of Lagadic (1989), cited in the previous paragraph of the report, indicates a more complex space requirement; this is based on operant experiments in which some birds will increase and others decrease their space allowance, indicating no clear preference for cage size. However, this work is substantially discounted by the final sentence of the same paragraph:

"However, several other studies have consistently shown that battery kept hens have shown a preference for larger rather than smaller cages" (page 74).

This latter statement is based on papers of Dawkins published in 1978 and 1981, some 10 years before Lagadic's (1989) paper. At the very least this (mis-) use of the scientific literature is inappropriate.

### Assessment of housing systems for the welfare of laying hens and pigs: A comparative approach

Comparing the way in which the report presents background material and makes recommendations on housing for the welfare of the laying hen and the pig provides a clear example of the inconsistencies in the report. Some of this material is presented in Table 1. Housing systems for the laying hen are discussed in two chapters of the report. Background information from various interest groups is presented and current research into space allowance for caged hens is documented. Overseas recommendations on space allowance and some welfare advantages of modifying cages by the addition of perches and solid sides are discussed. The committee's recommendations are reported. Even if one disagrees with specific recommendations, the procedure appears to be reasonable as the processes involved in the committee reaching its recommendations are documented. There is a systematic discussion of a number of housing systems along with details of overseas research and recommendations, prior to the committee's recommendations.

In contrast, the information on which the committee has based its recommendations for pigs is not well documented. The material presented is nowhere near as systematic as it is for the laying hen and a considerable amount of the information on advantages/disadvantages of different housing systems is presented in a Table from Muirhead (1983) without any discussion of its relevance to Australian conditions or housing systems. Concepts such as 'suffering', 'disturbed behaviour' and 'adaptability' are introduced for the pig and these concepts (irrespective of their relevance) should equally apply to poultry in formulating recommendations. Research into housing systems, particularly extensive Australian research, was not discussed and consequently any welfare advantages from modifying existing systems was ignored. Instead, a list of titles and monies spent on welfare research in Australia by the Pig Research Council (now the Pig Research and Development Corporation) were presented with no discussion. The report acknowledges that there are advantages/disadvantages in all systems:

"All pig production systems have advantages and disadvantages from the welfare p o in t of view" (page 216), and lists some of the advantages of individual housing:

" .... (protection from bullying, close monitoring and control of food intake), ......" (page 217).

The rationale for the recommendations to ban tethers and phase out stalls is unclear.

Other examples of a disparate approach to material for pigs and poultry appears in the sections on husbandry practices for the laying hen (chapter 7) and management practices for the pig (chapter 12). While there is some apparent disquiet over the way in which beak-trimming is conducted and it is acknowledged that long term pain can result, the procedure is found to be acceptable. Similarly, a moulting procedure (barley *ad libitum*) that induces considerable weight loss is also found to be acceptable and is in fact recommended as the procedure of choice.<sup>1</sup> In contrast, while taildocking of pigs is acceptable, because of the adverse consequences of tail-biting, the problem is clearly identified as a consequence of intensive pig production and the acceptability of the procedure appears to be marginal.

While the report can only draw on material that was presented to the inquiry, the committee selected the material to appear in the report. If material was required in certain formats, it could have been subsequently obtained and feedback sought on concepts that the committee, on reflection, decided were relevant to their deliberations.

My interpretation of the material presented to the inquiry on housing is that the design of the housing system, whether for pigs or the laying hen, is more important to welfare than the system *per se*. Thus, welfare research is more likely to be cost effective and the welfare of pigs is more likely to be improved by research directed towards understanding the interactions between design and welfare and subsequent application of improved principles of design to existing systems or their incorporation into new systems, rather than the simplistic approach of banning or phasing out specific systems. Presumably it is harder for politicians to accept, defend and package a more complex message for public consumption.

It has not been recognised in the report that the 'science of animal welfare' is a new science and as such its predictive capability is limited. I believe that until animal

<sup>1</sup> A loss in live weight of 17-31 % appears to be necessary for optimum postmoulting laying performance (Baker *et al.*, 1983; Karunajeewa *et al.*, 1989).

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Table 1 continued:		
Laying hen	Pig	Comment
Committee recommendations	Committee recommendations	
<li>i) cages acceptable and possibly to be phased out when efficient alternatives available.</li>	i) acknowledges advantages of individual housing.	Acknowledges advantages of individual accommodation for pigs but recommends, without
<ul> <li>ii) specified space allowance for caged hene.</li> </ul>	<li>ii) "behaviour not to be disturbed and adaptability is not to be overcharged".</li>	justification, phasing out of stalls and banning of tethers. Concepts of disturbed behaviour
	iii) ban on tether housing.	and adaptability only applied to pig
iii) recognises advantages of modified cage designs and suggests financial incentive to encourage adoption.	iv) phase out of stall housing.	housing.
iv) monitor overseas developments into alternative systems.	v) examine overseas research into alternative systems.	
v) acknowledges disadvantages of free range systems.		
ANZFAS - Australian and New Zealand Animals; ACEP - Australian Council of E Veterinary Association; APIPC - Australian	Federation of Animal Societies; RSPCA igg Producers; QDPI - Queensland Depi n Pig Industry Policy Council.	- Royal Society for the Prevention of Cruelty to artment of Primary Industries; AVA - Australian

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welfare science has been demonstrated to be a true science (i.e. has good predictive capability), it is to the possible detriment of animal welfare for the committee to attempt to direct research away from certain areas. If scientific creativity is stifled, the "science of animal welfare" may never develop. This science will incorporate a number of disciplines that are proven to be important in predicting/measuring welfare. It is not surprising that in developing this new discipline there is disagreement among scientists on methodologies and interpretation of data. This may make life 'difficult' for politicians but it is an important point not adequately recognised in the report.

#### Assessment of welfare and the concept of suffering

The committee devotes considerable attention to the assessment of welfare and highlights some of the acknowledged problems, particularly in using physiological criteria to assess welfare. The important words here are *acknowledged problems* and most scientists are generally aware of the limitations of the techniques they use (Dantzer *et al.*, 1983; Barnett and Hutson, 1987; Barnett and Hemsworth, 1990).

The stress response has been widely used to assess welfare (Dantzer *et al.*, 1983; Dantzer and Mormède, 1983; Moberg, 1985; Barnett and Hutson, 1987). Most people accept its validity as it is a reasonable belief that if stress increases then welfare decreases. The methodological problems are recognised (see citations above) and while welfare cannot simply be equated with stress, the stress response is a useful tool in assessing welfare. Similarly, the presence of a number of emotional states e.g. frustration, motivation, fear, have been used to assess welfare and again the problems associated with the behavioural methodologies are recognised (Duncan and Dawkins, 1983; Barnett and Hutson, 1987; Ödberg, 1987). However, the Committee feels that it is insufficient to measure stress, pain, conflict, frustration and fear, and instead suffering or well-being should be measured. This is surprising as in a previous report on Animal Experimentation (SSCAW, 1989) the Senate Select Committee, which had some members common to the present committee, stated:

".... The Committee does not find the use of the term 'suffering' to be very helpful. There is presently no agreed definition of suffering that would provide guidance to ethics committees and experimenters. The concerns with animal well-being that are not directly related to pain are more appropriately described by the term 'distress'" (page 46).

At the present state of knowledge, to attempt to measure suffering as an entity would be a total misdirection of limited resources, both in terms of monies and in terms of improving animal welfare; it could lead to similar open-ended arguments that occur in the definition of welfare or well-being and would provide yet another excuse for making only limited gains for animal welfare. Peter Medawar (1986) suggests "there is no limit upon the ability of science to answer the kind of questions that science can answer". The question of animal suffering as an holistic entity is not open to scientific investigation. This is not only a personal view; Duncan and Dawkins (1983) in a chapter of assessing well-being and suffering conclude that "Both well-being and suffering are subjective states which cannot be investigated directly".

However, this does not mean that scientists ignore the concept of suffering; they attempt to break it down into some of its component parts which can then be measured. Hurnik and Lehman (1982) consider some of the components of suffering to be pain, fear and frustration. There are a number of other mental states that could suggest suffering e.g. anxiety, anger, hate, boredom. The physiological consequences of these states are detailed in a tome by Selye (1976) that is based on 100,000 publications that deal with stress, its causes and its consequences. It should also be noted that fear, hunger, pain and rage were the stimuli that Cannon (1914) used in his classic studies on emotional factors capable of stimulating sympathetic nervous activity and the secretion of adrenalin. Other references indicate the important role given to the physiological assessment of mental states over the last 30 years (Bliss *et al.*, 1956; Mason, 1959; 1968; Friedman *et al.*, 1963; Tecce *et al.*, 1966; Dantzer and Mormède,

1985; Moberg, 1985). Some recent texts that the reader may wish to refer are Burchfield (1985) and Gray (1987).

Considerable reference was made in the report on stress and its measurement (much more so than to its contribution to solving problems). Unfortunately, it was not recognised that a pivotal part of the concept of stress is its ability to integrate mental and physical states. Indeed, some authors believe that the principle responses are to neuropsychological states. For example, Dantzer and Mormède have shown for pigs that a psychological stressor (novelty) is as effective as a physical stressor (electric shock) in increasing plasma corticosteroid concentrations (1981) and that when the psychological and physical components of the stress response to temperature change are separated it is the psychological factors that dominate the stress response (1979). Research in pigs has clearly indicated the positive relationship that exists between fear (of humans) and corticosteroid concentrations in this species (Hemsworth *et al.*, 1981, 1986b, 1987; Hemsworth and Barnett, 1991).

Thus, although suffering may be problematic in its definition, the concept has been far from ignored. As with the whole subject of animal welfare, knowledge and understanding have progressed without any agreement on a precise definition; this is particularly the case with emotions. What the scientist does is to substitute measurable parameters such as behaviour and neuroendocrine changes. What is yet to be determined is not the recognition of emotions or suffering in animals but the level of change in emotional states that compromise welfare; this will best be achieved by intensive research on behavioural/physiological correlations.

#### Housing systems for pigs: The recommendations and the literature

There are several recommendations in the report that are relevant to the housing of adult pigs. These are:

".... the maximum recommended stocking densities .... be reviewed ...." (page 216).

".... noting the advantages of stalls and tethers .... future trends in housing the dry sow should be away from individually-confined stalls systems..... The .... tethering of sows be banned" (page 217); and

"On the issue of farrowing crates .... some producer pilot systems to test the viability of designs which will allow sows more freedom of movement and access to a separate exercise area at least some time each day" (page 217).

The above recommendations acknowledge that dry sows are housed both individually (tethers or stalls) and in groups. The thrust of this paper is the scientific input into the recommendations and therefore it is worthwhile to examine the literature in relation to the welfare of dry sows in alternative housing systems. The report's recommendations concentrates on "systems" *per se* while the overall conclusion from the literature is that it is the design of the system rather than the system *per se* that is important to welfare.

In group-housing systems for dry sows some preliminary work has been undertaken on stocking densities (space allowance and group size) with the aim of evaluating recommendations in the code of practice (Australian Bureau of Animal Health, 1983; Hemsworth *et al.*, 1986a; Barnett *et al.*, 1986). Some recent work has also examined design features of group pens for pigs. This work has shown welfare advantages by the provision of partial stalls in group pens to reduce aggression on mixing (Barnett *et al.*, unpublished data) and around feeding (Petherick *et al.*, 1987; Barnett *et al.*, unpublished data) and a trend (which has been confirmed in subsequent work that also indicates the importance of pen shape) for reduced aggression on mixing in pens with a small space allowance (0.96 and 1.4 m<sup>2</sup>/pig) rather than a large (1.96 and 3.0 m<sup>2</sup>/pig) space allowance. This research has obvious implications for developing recommendations for incorporation in the code of practice and emphasises the importance of the design of the housing system for the welfare of pigs.

In relation to individual housing of pigs, in tether-stall housing a design with vertical bars poses risks to welfare; the evidence for this is a chronic stress response
(Barnett *et al.*, 1985, 1987a, 1987b, 1988, 1989, 1991a), suppression of the immune system (Barnett *et al.*, 1987b), changes in nitrogen balance evident of gluconeogenesis (Barnett *et al.*, 1985, 1989), a reduced ability to respond to the acute stressor of transport (Barnett *et al.*, 1985) and a reduced pregnancy rate (Barnett *et al.*, 1991b). The cause of the chronic stress response appears to involve aggression, as changing the design of the stall to reduce aggression by covering the vertical bars with steel mesh, reduces the level of stress to that found in group-housed pigs (Barnett *et al.*, 1987a, 1989). Other studies have shown both evidence of a chronic stress response of pigs to (girth) tethers (Becker *et al.*, 1985; Borell and Ladewig, 1989) and no evidence of a chronic stress response (Friend *et al.*, 1988), although in these studies the design features of the tether stalls were not fully described.

Similarly, in individual cage-stalls, it is again the design of the stall division that affects welfare; there is evidence of a chronic stress response if stall divisions are of horizontal bars compared to either vertical bars or vertical bars covered with steel mesh (Barnett *et al.*, 1989, 1991a).

The recommendation on farrowing-crates is disturbing for the scientist. The literature on piglet survival following the introduction of the farrowing crate is equivocal. While the number of piglets weaned/sow/year has increased during the last 30 years, this has been, in part, due to lower weaning ages, an increase in litters/sow/year and an increase in litter size. English and Morrison (1985) showed that pre-weaning mortality declined in the UK from above 22 % in 1960 to < 12 % in 1982, although Robertson and Clarke (1980) are not convinced of a genuine improvement in the level of piglet survival. Whether or not piglet survival has changed, there is still a concerted effort to reduce piglet mortality levels world-wide. That piglet mortality levels are too high does not appear to be disputed.

Considerable basic behavioural research is underway to determine the maternal behaviours that are important for piglet survival, piglet behaviours that affect piglet survival, the interactions between piglet and maternal behaviour and farrowing crate design to improve piglet survival and growth (e.g. Hutson et al., 1989; Blackshaw and Hagelsø, 1990; Cronin and Cropley, 1991). The situation is obviously complex and it is naive and potentially detrimental to pig welfare to suggest that producers undertake trials to improve the situation. Duncan (1987) indicates how this type of recommendation on farrowing crates could have arisen. "One of the big advantages for a speaker or writer on the subject of behaviour is that most of the audience will recognise the end product -the behaviour patterns; they have witnessed themselves. Compare this with the difficulties encountered by a nutritionist talking about metabolisable energy or a physiologist talking about a certain enzyme activity. However, this very fact is also a disadvantage. Cannot everyone be an amateur ethologist? After all, it is only commonsense, is it not? That animal is grooming itself, therefore, it must be itchy; that one is nosing its feed, therefore it must be hungry, and I hope to demonstrate that this view is false, and that sometimes so on. "commonsense" can be dangerously misleading." In agreement with this view of Duncan, both producers and scientists should apply themselves to their own métier, the producer's is pig production and the scientist's is science. Once the science is understood, on-farm trials may then be appropriate to demonstrate any production consequences to the farming community to speed-up the process of industry adoption of new recommendations.

#### Current and future research based on the report's recommendations

The majority of people who are sufficiently interested to read the report would probably concentrate on the recommendations. The recommendations are both at the beginning of the report and at the end of the relevant sections and a number of them have a clear research component. Presumably their purpose is twofold. Firstly, to indicate further research required in certain areas and secondly, to indicate areas for new research. The latter recommendations which are implying 'new' areas of research generally do not take into account the sometimes considerable work already done and the recommendations on stockmanship, education and training, in particular, appear to be based, in very large part, on the existing situations for pigs rather than any new suggestions. I suggest that the recommendations were based on the extensive information supplied to the inquiry by the pig industry that emphasised the research into stockmanship and the development of training courses. Thus, while most would agree with the recommendations, they tend hot to acknowledge the efforts and achievements already made in this area. The recommendations are:

"The Committee therefore recommends:

- 1. That the subject of animal behaviour be recognised as an integral component of the curriculum in agricultural and veterinary colleges in Australia, especially as a component of welfare.
- 2. The development of certificate training courses for stockpersons in the pig and poultry industries by Technical and Further Education and agricultural college courses.
- 3. Funding initiatives be developed to support skills training of stockpersons unable to gain access to formal training courses.
- 4. The Pig Industry Research Council, the Chicken Meat Research Council and the Egg Industry Research Council give greater priority to welfare-related stockmanship research" (p. 253).

In relation to these recommendations, item (b) is well in hand in the form of training courses for stockpeople (Piggery Attendants Traineeship and national Associate Diploma in Pig Husbandry) and supervisors (Piggery Supervisors Course) in the pig industry. The development of these courses was initiated in mid 1989, in large part by the pig industry, through the National Award Restructuring Committee and the courses should be accepting participants in early 1992. In addition, in relation to item (c), States run a number of training courses at different levels for stockpeople. For example, in Victoria funding was made available in 1989 through the (Victorian) Swine Compensation Fund and the Federal Rural Training Board, to develop a training course for stockpeople who have only limited knowledge of the pig industry. This course has been underway in Victoria for 2 years and in addition to a specific topic in the course being on welfare, welfare is also an underlying theme of the whole course. This course was developed in consultation with the industry and educators, and piggery owners appear willing to pay for their staff to participate. In relation to item (a), this has been carried out at the University of Queensland for 6 years; an expansion of teaching this discipline would have the support of industry and researchers. In relation to item (d) the Pig Research and Development Corporation (then the Pig Research Council) has supported research specific to stockmanship since 1980 and currently spends 21.5 % of its allocation for welfare research in this area (welfare research is funded to the level of 5.2 % of the Corporation's total 1990/91 research budget; Pig Research Council, 1990). This research has determined some of the behaviours of humans that pigs find fear-provoking (Hemsworth et al., 1981; Gonyou et al., 1986), the physiological mechanisms in pigs through which fear can adversely affect production and welfare (Hemsworth et al., 1981, 1986b, 1987) and some of the mechanisms (within humans and pigs) that regulate the level of fear of humans by pigs (Hemsworth et al., 1989, 1990). This research was also instrumental in the development of the Stockperson Training Course in Victoria. Thus, there is clear evidence that the pig industry and its associates have a strong and continuing commitment to stockmanship research.

Similarly, a major recommendation on housing systems implies little research is

# undertaken in Australia. The recommendation is:

".... the Committee recommends that the Commonwealth Government fund a research project in Australia to examine and evaluate housing systems that may be suitable to Australian conditions and that this review:

- 1. Examine overseas research findings into alternative housing systems
- 2. Assess the welfare benefits and any welfare disadvantages of such systems
- 3. Evaluate the economic viability of alternative systems
- 4. Take account of the views of producers, industry service providers, design engineers and specialist ethologists" (p. 218).

The implication is that housing systems research in Australia occurs in isolation of overseas research. This is not the case; the industry, particularly through representatives of the Pig Research and Development Corporation and scientists working in this area of research, are well aware of continuing overseas research into the use of alternative systems such as electronic feeding stations (Dantzer *et al.*, 1988; Jensen and Pedersen, 1988) and the development of the 'family-pen' system based on observations of extensively housed pigs (Stolba, 1983; Stolba and Wood-Gush, 1984; Kerr *et al.*, 1988). Once the latter system has been implemented under commercial conditions and some of the recognised problems associated with electronic feeding stations (Cornes, 1986; Jordan, 1990; Putten and Burgwall, 1990) have been overcome, they are more likely to be adopted in Australia. Some assessment is being made of electronic sow feeders in Australia (Taylor and Clarke, 1988).

As with all areas of research, only limited funds are available and therefore it is a question of priorities. In Australia, rather than duplicate overseas research, the emphasis on housing systems research has been on evaluating existing systems and modifying these to improve welfare. Also, welfare research in Australia has been largely isolated from an economic evaluation of any changes; I believe this was the correct approach at the time as the economic argument can easily dictate research direction. The approach in Australia is similar to that used, with apparent advantages, for the laying hen (ie, cage modifications with perches and solid sides to improve welfare) and has already suggested significant welfare advantages for the pig by modifying existing systems (see previous section).

In contrast to suggesting research in these 'new' areas of research, where considerable work is already being done, the report recommends further research in areas where little is being done. The two recommendations are:

".... further research into the causal factors of tailbiting be undertaken ....." (page 232); and

".... further research be conducted into the underlying reasons for infection that necessitates teeth clipping" (page 232).

While the suggestion of more research into the need for tooth clipping and tail docking is laudable, at the present time these areas would have to be of low priority as the chances of success are currently slim. Although sows can successfully raise piglets when piglets are not tooth clipped (Fraser, 1975; Wilkinson and Blackshaw, 1987), considerable damage to the sow can occur ("4 of 40 sows had teats chewed off and 2 of these lost all teats") (Wilkinson and Blackshaw, 1987). Similarly, tail-biting can cause considerable damage when it occurs (Luescher *et al.*, 1989) and there is considerable research on the underlying causes (eg, Smith and Penny, 1986; Fraser, 1987).

In spite of the above criticisms, there is little doubt that the report identifies important areas for research into pig welfare. It is likely that research into stockmanship and housing systems for pigs will continue and it is probably timely that some economic evaluation be incorporated into some of this work. The structure of the Australian Pig Industry, with a direct contribution by producers to research funding and a somewhat more indirect contribution into research direction leads to a rapid adoption of new ideas/technologies that can be of benefit to both production

## and pig welfare.

## Conclusion

In conclusion, from a scientist's perspective, the report has merit in identifying areas for continuing and future research and thus indirectly recognises the value of research. However, for the welfare of pigs to be improved, care must be taken in the uncritical acceptance of a number of recommendations as the limited scientific knowledge available was often not considered in formulating these recommendations.

## SYMPOSIUM CONCLUSION

### J.K. Blackshaw

The SSCAW (1990) report on "Intensive Livestock Production" is an important document for the pig industry. It represents the views of many sections of the community but the scientific content of the literature presented at the Inquiry has not been adequately assessed. However, the report does identify important areas for research into pig welfare and it encourages continued research into stockmanship and housing systems for pigs.

An interesting development in the UK pig industry was the announcement on Friday, January 11, 1991 from the Minister of Agriculture that stalls and tethers for dry sows are to be banned in all pig units. No new systems will be allowed and all existing systems must be out by 1998. Industry leaders are bitter about the decision and felt that the British Government see the support of the Green vote more important that the welfare of the pig producer (Jones, 1991).

One issue is the replacement of stalls and tethers with a viable alternative. It could be a disaster to replace the individual housing system with a group system which causes problems.

Although Mr. B. Muirhead comments that scientific information should influence decisions more than community concerns, I feel that community concerns expressed by Senator Brownhill are just as valid and, in fact, may carry more weight. It is quite legitimate to change laws if the majority of people feel a practice is unjustified.

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# A COMPARISON OF OPERANT RESPONDING BY FARROWING SOWS FOR FOOD AND NEST-BUILDING MATERIALS

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An important problem in the intensive pig industry is that farrowing sows are confined in crates which frustrate the performance of normal nest-building behaviour. The consequences of this frustration for the welfare and production of sows are unknown. In a previous experiment using operant methods (Hutson, 1988) I found that sows showed low motivation towards the utilization of straw at farrowing, although they readily performed other nest-building behaviour patterns at this time. However, in order to validate this result it is necessary to compare operant responding for nest-building materials with operant responding for a known positive reinforcer, such as food. Matthews and Ladewig (1986) have used an increasing fixed ratio to demonstrate that food was more important to pigs than social contact with a familiar pig.

Sixteen sows were tested individually in a  $1.9 \times 2.5$  m farrowing pen in a room at 20°C. They were introduced to the pen 6 days before the expected farrowing date and trained over 2 days to lift a lever on a fixed ratio of 10 lifts per reinforcement. Sows that did not reach a criterion of a minimum of 4 reinforcements were rejected. Four reinforcement treatments were compared. Lifts on the lever gave 20 sec access to either (1) a box containing 2 kg straw, (2) a box containing 7.2 kg of sticks with a mean diameter of 20 mm and a mean length of 554 mm, (3) an empty box (the control treatment) or (4) unlimited access to a food drop of 2.7 g of pellets. Four sows were tested on each treatment for 4 days immediately before and after farrowing.

There was a significant difference in number of lifts per 8 h day between days and treatments (P<0.001). Lever lifting increased up until farrowing, declined immediately after farrowing, and then increased again. Sows lifted more for food than for the control or nest-building materials (P<0.001). Prior to farrowing the mean number of lifts for food on any day exceeded the mean of the other treatments by a factor ranging from 20 to 68 times. Comparisons among the nest-building treatments showed that there was no difference between lifting for nest-building materials and the empty box (P>0.05), but sows lifted significantly less for sticks than straw (P<0.001).

The results confirm Lawrence and Illius' (1989) conclusion that there is an extreme divergence betweeen food-restricted pigs' motivational need for food and their economically determined food allowances. In the farrowing sow this motivational requirement completely overshadows the potential effect on welfare of frustration in any other motivational system.

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# PRE-FARROWING BEHAVIOUR OF SOWS GIVEN ACCESS TO STRAW AND SPACE

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Sows in modern intensive piggeries are confined to farrowing crates about a week before farrowing. There is concern that this confinement may be detrimental to the sow's well-being. In a previous experiment (Haskell and Hutson, 1989) we found that sows allowed access to space showed a significant increase in locomotion on the day prior to farrowing. The timing of this increase in walking corresponds to the time in which free-ranging domestic sows in the wild leave the herd and walk considerable distances to find a suitable nest site, build a nest, and farrow in it (Jensen, 1986). The purpose of this increase in locomotion remains unclear. Is the sow seeking isolation, nest-building materials, or a suitable nest site? How does the construction of a nest affect the temporal pattern of locomotion?

Two experiments were designed to investigate this behaviour. In the first experiment the effect of provision of straw in the usual farrowing site on prefarrowing locomotion was assessed. Approximately 5.5 kg straw was placed on the floor of a 2 X 2 m home pen, in the corner of a 7 X 7 m test arena. In the second experiment the effect of provision of straw away from the usual farrowing site was assessed. The same home pen and test arena were used, but the straw was placed in a dispenser fixed in the opposite corner of the arena, 8.3 m from the entrance to the home pen. Six sows were used in the first experiment and 12 in the second. Each sow was observed for eight hours a day, from four days prior to the due date of farrowing until farrowing actually occurred. Distance walked by the sow was estimated from her position in the arena in successive 1 min observations.

We found that when straw was provided in the home pen, which was the normal farrowing site, the sows did not show a significant increase in distance walked on the day prior to farrowing, in marked contrast to our previous experiment (P>0.05). All sows used the straw in their nest-building and farrowed in it within the home pen. However, when straw was provided in the dispenser the sows walked significantly further on the day prior to farrowing (P<0.001). Five of the 12 sows carried straw from the dispenser to make a nest. Analysis of distance walked in the 12 hours immediately preceding farrowing showed that there was a significant interaction between carrying and hours (P<0.001). Carrying appeared to alter the temporal pattern of nest-building, with sows who carried straw walking further in the 6 hours immediately prior to farrowing than sows who did not carry straw.

The results indicate that the provision of straw can decrease the sow's motivation to walk, but the straw must be placed in a location that the sow might normally choose to farrow. We suggest that provision of straw to sows farrowing in crates might help reduce restless behaviour induced by confinement.

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# ADRENOCORTICAL ACTH RECEPTORS IN PIGS OF DIFFERING STRESS RESPONSIVENESS

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Since the report that individual pigs showed a large and repeatable difference in adrenocortical response to a standard stressor (Hennessy *et al.*, 1988) our research has been directed towards determining the reasons for those differences in adrenal responsiveness. A recent study showed that much of the *in vivo* difference in adrenocortical response to ACTH administration could be accounted for by differences in the adrenocortical cell mass (Zhang *et al.*, 1990). The present study was to determine whether differences in ACTH receptors exist between pigs of high and low adrenal responsiveness. Receptor affinity for ACTH and receptor numbers were assessed by measuring the equilibrium constant of dissociation (Kd) and the concentration of binding sites (Bmax) on adrenocortical membrane preparations from high-responding (HR) and low responding (LR) pigs.

Six HR and six LR, 12-week-old, male, Large White X Landrace pigs were sacrificed and the adrenal glands collected and stored frozen in liquid nitrogen. Plasma cortisol concentrations 1 h after ACTH challenge (6.25 IU, i/m, at 3 weeks of age) for the HR and LR pigs were  $684 \pm 42$  (mean  $\pm$  SE) and  $179 \pm 16$  nmol/l respectively. Adrenocortical membrane protein was obtained by homogenizing the adrenal glands in a glass-glass homogenizer. The peptide [Phe<sup>2</sup>, Nle<sup>4</sup>]-ACTH<sub>1.24</sub> was iodinated by the chloramine-T method and served as the radioligand. Receptor binding assays were performed by incubating an aliquot of adrenocortical membrane with the radioligand, and Kd and Bmax were analyzed using published statistical methods (Priore and Rosenthal, 1976).

In both high and low responding pigs only one class of ACTH receptor was detected. The receptor affinity for ACTH was similar between high and low responders,  $Kd = 2.57 \pm 0.35 \times 10^9$  M in HR pigs and  $Kd = 1.68 \pm 0.18 \times 10^9$  M in LR pigs. However, the concentration of binding sites in adrenocortical membrane preparations from high responders was significantly higher (P<0.05) than in adrenal membrane preparations from low responders, Bmax =  $1.59 \pm 0.06$  pmol/mg protein and  $1.17 \pm 0.11$  pmol/mg protein for high and low responders respectively.

Thus, in addition to differences in adrenocortical cell mass observed previously, differences in the concentration (Bmax) of adrenocortical ACTH receptors also contribute to the differences in adrenocortical responsiveness to ACTH observed between pigs of the same age, sex, body weight and breed. This evidence further supports our previous suggestion that there are differences in the degree of trophic drive being supplied to the adrenal glands, which results in the differences in adrenocortical responsiveness to stress.

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# THE BEHAVIOUR OF GESTATING SOWS IN ELECTRONIC SOW FEEDING SYSTEMS

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The most common pen arrangements used to facilitate an Electronic Sow Feeder (ESF) are the single pen system (SP) where the sows have continual access to the ESF and the two pen system (TP) where the sows only have the opportunity to use the ESF once daily. The TP system has the advantage of preventing the repeated non-feeding visits to the feeder by dominant sows which can interfere with, or exclude the entry of more timid sows. The SP system requires less space and labour to operate. Both systems are used commercially but as yet no comparison of behaviour has been attempted. A project was set up to compare the behavioural patterns of gestating sows fed by an ESF in a SP or TP system, as a part of this work a comparison of levels of social interactions between the two groups was undertaken.

One group of 20 pregnant Large White sows, parity 1-3, was trained for at least 6 weeks for the SP system and another group for the TP system. Observations were made for each group for two hours morning and afternoon, over a period of 3 days. This was repeated 4 times with 2 days between each repetition. Feeding began at 1500 h and afternoon observations were undertaken during the 30 min prefeeding stage (PF) and in the first 90 min of feeding (F). Records were kept for social interaction on a scale of resistance to displacement used by Hunter *et al.* (1988).

		Strong	Moderate	None	Failed	Retaliation	Attack	Total
Mor	ning							
	SP	0.35	0.55	0.25	0.10	0.05	0.00	1.30
	TP	2.15	1.60	0.50	0.20	0.10	0.02	4.75
	SEM <sup>1</sup>	0.10**	0.12**	0.12	0.05	0.05	0.05	0.20**
After	rnoon							
PF	SP	4.60	5.90	0.75	0.65	0.25	0.10	12.25
	TP	5.85	6.35	2.75	0.40	0.60	0.15	16.10
	SEM	0.52	0.57	0.30**	0.17	0.15	0.07	0.87**
F	SP	7.45	11.50	4.10	2.75	0.85	0.95	26.60
	TP	13.80	13.85	6.65	2.75	0.65	1.85	39.55
	SEM	0.42**	0.47*	0.10**	0.25	0.12	0.17**	0.77**

Table 1. Mean number of social interactions on the basis of resistance to displacement by pregnant sows fed by an ESF using a single pen (SP) or two pen (TP) system (events per half h)

<sup>1\*</sup> P<0.05; \*\* P<0.01.

The group in the two pen system showed a significantly higher level of total number of social interactions than the group in the single pen system, possibly reflecting the more competitive nature of sows when kept in the two pen system. Confirmation work is continuing.

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# THE EFFECTS OF MODIFYING THE FARROWING CRATE ENVIRONMENT ON PIGLET MORTALITY

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Pre-farrowing nesting behaviour may promote the development of maternal behaviour in nulliparous sows (Cronin and van Amerongen, 1991). This paper examines the effects of modifying the farrowing environment to stimulate nesting behaviour, on duration of parturition and piglet survival around parturition.

Parturition was observed in 173 sows (parities 1 to 10) at a commercial piggery during a continuous 48 h watch starting at 1400 h in each of three weeks. Half an hour before each watch, about one-half of the sows (Sawdust Treatment: SD) received 1 to 2 handfuls of sawdust at 30 to 60 minute intervals until they commenced to farrow. Control Treatment (C) sows did not receive sawdust.

Significant treatment effects were only found for the younger parity sows (parities 1 to 3) and only data for these sows are presented (n = 42 and 57 sows for the  $\hat{SD}$  and C treatments, respectively). More (P<0.05) piglets were born alive in the SD than C treatment (means adjusted for total born were 10.53 and 10.04 piglets, respectively;  $LSD_{(P=0.05)}=0.434$ ). The difference in the mean number born alive was largely due to the lower (P<0.01) incidence of intra-partum deaths (IPD: piglets that died just prior to, and during, parturition, Randall and Penny, 1967) in the SD than C treatment (11/475 v 32/584 live piglets, respectively;  $\chi^2_1$ =6.73); this may have been a consequence of the duration of parturition (Sprecher et al., 1975) which was shorter (P<0.05) in the SD than C treatment (back transformed loge) means adjusted for litter size were 133 v 168 minutes, respectively; LSD<sub>(P=0.05)</sub>=0.232). While there was no difference between the treatments in the proportion of litters that had IPD (11/42 v 18/57 litters, respectively), there was a difference (P<0.01) in the proportion of litters that contained multiple IPD (0/11 v 9/18 litters, respectively, for the SD and C treatments;  $\chi^2_1$ =7.98). Further, the proportion of sows that crushed piglets during parturition and the subsequent six hours was lower (P<0.01) in the SD than C treatment (1/42 v 12/57 sows, respectively;  $\chi^2_1$ =7.39).

The results suggest that environmental factors around farrowing have important effects on piglet survival. The mechanism may involve some aspect(s) of nesting behaviour, yet to be determined, leading to improvements in maternal behaviour. An interpretation of the present data is that the application of sawdust, by influencing nesting behaviour, has had consequences for maternal behaviour and piglet survival. The current magnitude of piglet mortality in the industry (16.2% of piglets born alive, Victorian Pig Management Recording Scheme) clearly indicates that this problem warrants further research.

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# THE EFFECT OF SPACE RESTRICTION DURING REARING ON GROWTH AND CORTISOL LEVELS OF MALE PIGS

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Crowding pigs during rearing can reduce growth rate and feed conversion ratio (FCR), but in many studies the effect of space allowance has been confounded with group size. In this experiment we reared 42 male pigs from 25 to 100 kg in 6 pens with adjustable walls (n=7/pen), and provided them with either crowded (C, 4 pens) or uncrowded (UC, 2 pens) conditions by adjusting their space allowance (A, m<sup>2</sup>/pig) weekly according to the equation  $A=kW^{0.66}$ , where W=mean body weight and k is a constant (Petherick, 1983). The value of k for the uncrowded pigs (0.048) allowed all animals in the group to lie fully recumbent at the same time, but k for the crowded pigs (0.025) resulted in a space allowance at which the spatial preference of a group of pigs was just exceeded. At 80 kg, these allowances equate to about 140% and 90% respectively of those recommended in the Code of Practice. In addition to the two space allowances, a third treatment (C+T) was created by supplying 2 of the crowded pens with simple toys (chains, bars, tyres).

Growth rate of individual pigs, and FCR and feed intake on a pen basis were measured over the 15 week experimental period. In week 11, basal plasma cortisol concentration (mean of 10 hourly samples) and plasma cortisol response to 25 iu of ACTH were measured in 4 pigs from each pen.

Table 1. Growth performance from 25-90 kg of pigs reared under uncrowded or crowded conditions in barren pens, or under crowded conditions and supplied with toys (mean  $\pm$  SE)

	Uncrowded	Crowded	Crowded+toys
Growth rate (g/day) <sup>1</sup> FCR	735 <sup>a</sup> ±24 2.73±0.14 1.82±0.11	658 <sup>b</sup> ±21 2.86±0.14 1.84±0.06	637 <sup>b</sup> ±16 3.01±0.14 1.85±0.06
Feed intake (kg/day)	1.82±0.11	1.04±0.00	1.85±0.06

<sup>1</sup>Values with similar superscripts do not differ (P>0.05).

Between 25 and 90 kg, UC pigs grew faster (P<0.05) than either the C or C+T pigs. Feed intake did not differ significantly and FCR tended to be poorer in the crowded treatments. Mean cortisol concentration in the 10 hourly samples taken in week 11 was not affected by treatment (UC =  $9.9 \pm 3.68$ ; C =  $10.6 \pm 2.94$ ; C+T =  $10.3 \pm 2.56$  ng/ml). When the data for C and C+T pigs were pooled, peak cortisol concentration after ACTH was higher for the pigs crowded during rearing ( $80.7 \pm 7.7$  vs  $60.5 \pm 2.65$  ng/ml, t=2.35, P<0.05).

Reducing the space allowance of growing pigs to below their spatial preference while keeping group size constant significantly reduced growth rate. Impaired efficiency of feed utilization due to chronic stress, as evidenced by the crowded pigs releasing more cortisol in response to ACTH, appears to be the mechanism by which crowding depressed growth.

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# MORPHOMETRIC ANALYSIS OF THE PIG ADRENAL CORTEX

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Individual pigs can be classified as high, medium or low stress responders based on their adrenocortical response to exogenous adrenocorticotropin (ACTH) (Hennessy *et al.*, 1988). Individuals classified as high adrenocortical responders (HR) have a greater adrenocortical cell mass than low responders (LR). This greater cell mass, in part contributes to the higher cortisol production in HR pigs in response to standard stressors (Zhang *et al.*, 1990). However, individual adrenal cells from HR pigs produced less cortisol per cell when incubated with ACTH than did cells from LR pigs (Zhang *et al.*, 1990). The present study was to assess the size of the adrenal *zona fasciculata* (ZF) cells of HR and LR pigs.

Adrenal glands were obtained from four HR and four LR, 18-week-old, female, Large White x Landrace pigs of equivalent body weight. Plasma cortisol concentrations 1 h after i/m injection with 25 LU. ACTH, given a few days prior to removal of the adrenal glands were HR,  $622.3 \pm 58.0$  (mean  $\pm$  SE) LR,  $206.3 \pm 29.5$ nmol/l. Under general halothane anaesthesia the adrenal glands were located and perfused *in situ* with glutaraldehyde. Immediately after fixation the pigs were euthanased and the adrenals removed. After dehydration and embedding ultra-thin sections (50-70 nm) were cut from three equidistant and parallel sites in each gland. Each section was photographed under an electron microscope at a magnification of 4,000. About 90 micrographs, which were taken from at least six sections per adrenal gland per pig, were used for the morphometrical analysis using a computer package (Video Image Analysis System, SciCom Computer Consultants, El Cerrito, California).

High responders were found to have a larger total adrenal gland weight than LR pigs ( $6.62 \pm 0.37$  g versus  $5.35 \pm 0.31$  respectively, P<0.05) and a higher relative adrenal weight (Table 1). The size of the nucleus of the ZF cells was similar for both HR and LR pigs. However the cytoplasmic area was smaller in HR pigs, resulting in a smaller total cell size in HR pigs than in LR pigs (Table 1).

Pig type	N° of ZF cells counted	Adrenal wt (mg/kg body wt)	Nuclear area (µm²)	Cytoplasmic area (µm²)	Cell area (µm²)
HR	1832	115.6 ± 6.8	27.4 ± 0.5	256.0 ± 7.3	283.0 ± 7.6
LR	1714	85.4 ± 8.7	$27.8 \pm 0.5$	287.0 ± 7.3	315.0 ± 7.6
Sign.1		. *	NS	**	**

Table 1. Size of adrenal ZF cells in 4 HR and 4 LR pigs (mean ± SE)

<sup>1</sup>NS, non significant, P>0.05; \*P<0.05; \*\*P<0.01.

The acute stimulation of steroid production by ACTH is primarily the result of increased cholesterol availability to the side-chain cleavage enzymes (DiBartolomeis and Jefcoate, 1984). In this respect, smaller ZF cells seen in HR pigs may have smaller stores of cholesterol, and hence produce less cortisol per cell compared to LR pigs.

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# AN EVALUATION OF THE QUALITY OF AUSTRALIAN PORK

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Pork has two main quality defects namely: PSE (pale, soft and exudative) and DFD (dark, firm and dry). If the incidence of PSE and DFD is similar to that found in other countries, these two quality defects are costing the Australian pork industry approximately \$20 million annually (Mitchell and Heffron, 1982).

To improve or even maintain the quality of Australian pork an initial baseline level of quality is necessary. With this in mind, a survey was carried out to determine the processing and eating quality of pork produced at four major Australian pork processing plants at monthly intervals over a twelve month period. At approximately monthly intervals, 24 loins were selected randomly from the previous days kill and 30 cm long samples were removed from the anterior end of each loin. The following analyses were performed on each sample as previously described (Trout, 1991): 1) Visual colour and texture score; 2) Tristimulus La,b values (Minolta and Colormet); 3) Water-holding capacity (High speed centrifugation and filter paper); 4) Protein solubility; 5) Pigment concentration; 6) Fibre optic probe measurements; 7) Ultimate pH; 8) Drip loss (48 hours); 9) muscle cortisol; and 10) Cure uptake and yield. An additional ten 60 cm-long loin samples were taken for sensory evaluation and instrumental texture evaluation.

The results indicated that the processing quality of pork was extremely variable, and was dependent on the plant surveyed and the time of sampling. Depending on these two variables, the incidence of PSE and DFD pork ranged from 0-62% and 4-42%, respectively. Over the period sampled, the average incidence of PSE was 32% and DFD 15%. The drip loss from DFD, normal and PSE pork was 1.3, 2.6 and 5.2%, respectively and the corresponding cured yield was 101, 101, and 93%. Sensory evaluation of the loins indicated that there was little variation in meat aroma, meat flavour, tenderness and juiciness but a marked variation in other aroma and other flavour.

Several of the techniques used in this study have potential as rapid methods for monitoring pork quality. The following methods showed the most potential: 1) A rapid filter paper water-holding capacity method; 2) High speed water-holding capacity technique; 3) Tristimulus L,a,b measurements; and 4) Fibre optic probe. These techniques gave results which were highly correlated (r=0.65-0.81) with the economically important characteristics drip loss and cure uptake and yield. Moreover these techniques will allow closer monitoring of the quality of Australian pork and the subsequent ability to improve the quality.

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# A TEST FOR EVALUATING MEAT QUALITY IN PIGS

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Pig meat quality was determined by a combination of measurements of waterholding capacity (WHC), pH and the amount of  $Ca^{2+}$  released after incubation of *M. longissimus dorsi* (LD) muscle for 45 mins at 39°C.

Immediate post-mortem (<3 mins) LD muscle sample (0.5g), after incubation with an equal volume of 150 mM KCl for 45 mins at 39°C, was finely minced for 2 mins in ice and centrifuged at 12,000 g for 2 mins. The supernatant ("Fluid Volume"), an indicator of WHC of LD muscle, was collected and the volume, pH and  $Ca^{2+}$  concentration were determined. Meat quality was also assessed on LD muscles at 24 hrs post-mortem by pH and fibre optic probe measurements.

A significant difference (P<0.001) in the "Fluid Volume", pH and  $Ca^{2+}$  released was observed between the halothane +ve and halothane -ve British Landrace (B/L) pigs (Table 1). The data suggest that measurements of the "Fluid Volume" and pH could differentiate halothane +ve and -ve pigs, and predict meat quality.

Table	1.	Relatio	onship	betweer	r "Fluid	Volun	ne", Ca <sup>2+</sup>	releas	ed, pl	H and	l mea	t qu	Jality
after	incu	ubation	of LD	muscle	with an	equal	volume	of 150	mM	KCl a	at 39°	Cf	or 45
mins													

Pigs (B/L)	"Fluid Volume" (g/0.5g LD)	Ca²+ Released (ug/g LD)	pH (45 mins)	Meat quality
+ ve	$0.59 \pm 0.03$ (n = 6)	$4.74 \pm 1.19$ (n = 6)	$5.74 \pm 0.08$ (n = 6)	PSE
- ve	$0.35 \pm 0.03$ (n = 8)	$1.19 \pm 0.49$ (n = 8)	$6.77 \pm 0.17$ (n = 8)	Normal

This new procedure for identifying halothane +ve and halothane -ve pigs, and evaluating pig meat quality could be applied to live animals using small (0.3-0.5 g) "Shot Biopsy" (Lahucky, 1987) LD muscle samples. With Landrace x Duroc pigs, a high correlation  $[r = -0.85 \pm 0.19 (P<0.001)]$  was observed between the +ve (n = 32) and -ve (n = 42) pigs in "Fluid Volume" and pH, with PSE-prone pigs showing a "Fluid Volume" greater than 0.45 g/0.5 g wet wt LD (Cheah *et al.*, 1991).

The present data imply that this new procedure can select pigs with a potential of producing pork of good WHC.

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# PREDICTION OF PALE, SOFT AND EXUDATIVE (PSE) MEAT IN THE LIVE PIG FROM "SHOT BIOPSY" MUSCLE SAMPLES

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Since PSE was identified as a serious problem in the pig industry efforts have been made to develop a simple and reliable method for the detection in the live animal, of pigs with a potential for expression of poor meat quality characteristics. Ideally the method should be accurate and allow detection at an early age. Attempts to detect PSE in the live pig, by taking biopsies of the *longissimus dorsi* (LD) under anaesthesia, were found to be time consuming and costly (Schmidt *et al.*, 1972). A "shot biopsy" technique devised by Lahucky (1987) for obtaining small LD samples without general anaesthesia has been shown to be feasible and cost-effective.

The aim of the present trial was to determine the suitability of the 'shot biopsy' technique under field conditions. Sixteen Landrace X Large White pigs - 8 halothane sensitive (Hal<sup>+</sup>) and 8 halothane insensitive (Hal<sup>-</sup>) - were used. All pigs were halothane tested at 10 weeks of age and retested 2 weeks later. Biopsies (0.35 g - 0.55 g) from the LD muscle at the level of the last rib were taken 4 weeks before slaughter in the Hal<sup>-</sup> group and 2 weeks before slaughter in the Hal<sup>+</sup> group. Fluid Volume (FV), a measure of water-holding capacity, and its pH were determined according to the methods used by Cheah *et al.* (1991). The pigs were slaughtered at bacon weight [hot carcass weight =  $80.2\pm1.9$  kg; 74.6 $\pm2.7$  kg and back-fat (P2) =  $14.0\pm0.5$  mm; 15.4 $\pm0.8$  mm for Hal<sup>+</sup> and Hal<sup>+</sup>, respectively]. PSE was characterized as described by Oliver *et al.* (1991).

	FV	pH Biopsy	pH 1 h pm	IR 24 h pm	EC 2 h pm
Hal <sup>+</sup>	0.48±0.02	6.14±0.04	5.85±0.12	40.9±3.9	5.9±1.0
Hal <sup>-</sup>	0.37±0.02	6.48±0.04	6.25±0.07	30.8±1.0	3.0±0.19
t - test	P<0.01	P<0.001	P<0.02	P<0.05	P<0.05

Table 1.	Muscle	biopsy	and	meat	quality	characteristics	from	Hal <sup>+</sup>	(n=8)	and	Hal
(n=8) Lan	drace X	Large W	<b>/hite</b>	pigs (	(Mean ±	SEM)					

Normal values: pH 1 h post-mortem >6.0; IR-Internal reflectance,  $L^* < 32.0$  ( $L^* =$  lightness, Hunter colour space values; Eagerman *et al.*, 1977); EC-Electrical conductivity <5.0 mS/cm.

The results in Table 1 agree with those of Cheah *et al.* (1991) who reported a cut-off point of 0.45 g/0.5 g wet weight (FV) between PSE and normal meat (normal, <0.45; PSE, >0.45). In this study a good correlation was found between the FV and its pH ( $r^2$ = 0.52), and also between FV and IR at 1 hour post-mortem ( $r^2$ = 0.81) in the Hal<sup>+</sup> group. These results indicate the usefulness of the "shot biopsy" as a quick and reliable procedure for obtaining small LD muscle samples for prediction of PSE in the live animal.

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# BODY PROTEIN LOSSES CAN BE MINIMISED DURING LACTATION

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Body fat and protein are often catabolised during lactation to provide energy and protein for milk and, if food intake is restricted, these catabolic losses can be very high (46% of total body fat and 26% of total body protein). Even when high quality diets are available *ad libitum* to gilts during lactation tissues losses can still be high (Mullan and Williams, 1990). If the supply of amino acids to the sow could be increased to conserve body protein then, as King (1987) suggests, subsequent fertility might be improved.

Amino acid supply can be increased by increasing food intake or by increasing the quality and/or amount of protein in the diet. We offered gilts a high-protein diet (194 g/kg crude protein and 14.1 MJ DE/kg, as fed) ad libitum to gilts who started lactation at either 150 or 161 kg bodyweight. At each bodyweight there were two amounts of body protein.

	H1	Hh	Ĺ1	Lh
Number of gilts	27	36	33	39
Post-farrowing				
Bodyweight (kg)	161±2.1	162±2.0	150±1.3	150±1.8
Backfat (P <sub>2</sub> mm)	39.8±1.33	30.2±0.87	36.7±0.97	25.3±0.64
Body fat (kg)	54.7	40.4	50.0	34.9
Body protein (kg)	15.6	19.0	14.4	17.1
Loss during lactation				
Liveweight (kg)	27±2.5	23±2.1	23±2.1	14±1.6
Body fat (kg)	13.7	10.9	11.0	7.4
Body protein (kg)	0.8	0.9	1.3	1.4
Voluntary food intake (kg/d)	2.6±0.18	3.7±0.17	2.8±0.17	4.4±0.14
Piglet growth $(g/d)$ (0-4 weeks)	200±6.1	226±4.3	188±5.6	227±4.9

Table 1. Losses of body components during lactation (30 days) for heavy (H) and light (L) gilts with high (h) or low (l) body protein at the start of lactation  $(\pm SEM)^1$ 

<sup>1</sup>Ten gilts were killed on each treatment and used to predict body composition at farrowing and weaning.

The high protein diet minimized but did not prevent loss of maternal protein. All gilts lost 0.8 to 1.4 kg of body protein during a 30-day lactation. The amount lost on each treatment was remarkably similar despite large differences between the treatments in voluntary food intake (2.6 to 4.4 kg/d) and thus intake of protein (506 to 846 g/d), the mass of protein at the start of lactation (14.4 to 19.0 kg), and in milk output as reflected by the growth of piglets (188 to 227 g/d). The highest intake of protein of 846 g/d is in excess of the amount (833 g/d) calculated by the ARC (1981) that to be needed for maximum milk yield and to maintain maternal protein. These results suggest that either the Agricultural Research Council (1991) underestimated the protein requirements of the lactating sow or that some catabolism of maternal protein is necessary during lactation.

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# THE CONCENTRATION OF CELLULAR METABOLITES IN SOWS' MILK CHANGE DURING LACTOGENESIS

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Lactogenesis 2 in the sow is characterised by an increase in the rate of synthesis of lactose. This increase is primarily responsible for the increased volume of milk produced, since lactose is the most osmotically active component of milk. Lactose is synthesised from glucose by its conversion to glucose 6-phosphate, glucose 1-phosphate, uridine diphosphate glucose (UDPglu) and uridine diphosphate galactose (UDPgal). Glucose and UDPgal are transported into the Golgi apparatus and form lactose and UDP, the later is broken down to uridine monophosphate (UMP) and inorganic phosphate (Pi). We have determined the changes in the concentration of glucose, UDPglu, UDPgal, lactose, UMP and Pi in milk during lactogenesis for three sows. One sow (sow 3) developed the MMA syndrome.

Hind milk samples (0.5 - 1ml) were collected from 1 - 2 glands from three sows approximately 1 h prior to farrowing and at 1 h intervals for 24 h post-partum. Milk samples were stored at -80°C until analysed. Lactose, UDPgal, UDPglu, UMP and Pi were measured spectrophotometrically and glucose was measured luminometrically.

Milk metabolite	e		Time around parturition (h)						
		-1	3	7	10	18	24		
Glucose (µM)	A <sup>1</sup> B	30±4	58±42	68±25	178±110	254±37	258±100		
UDPglu (µM)	A	501±135	553±44	486±29	477±51	389±51	456±75		
UDPgal (µM)	Α	187±61	359±81	666±140	771±102	781±145	999±183		
Lactose (mM)	Α	98±3	99±7	110±10	119±8	131±4	129±3		
	В	126	105	124	120	122	130		
UMP (mM) Bi (mM)	A	$1.4 \pm 0.3$	$1.1\pm0.3$	$103 \pm 0.1$	$1.5 \pm 0.1$	$2.1 \pm 0.1$	2.3±0.2		
11(1101)	A	0.010.4	J.7±0.7	7.4±1.0	9.013.0	13.011.0	12.311.3		

Table 1. Concentration of cellular metabolites (mean  $\pm$  SE) in sows' milk during lactogenesis

 $^{1}A$  Refers to glands from sows 1 and 2 (n=3); B refers to 1 gland from sow 3. Metabolite concentrations not shown for B were similar to A.

In the goat, changes in the concentration of the above metabolites in milk reflected their levels in the mammary secretory cell (Faulkner, 1980). Since the changes in these metabolites in sow's milk are consistent with the goat, the low initial concentration of UDPgal compared to UDPglu suggests that either the synthesis of UDPgal or its transport into the Golgi apparatus was limiting lactose synthesis. Whereas, the increase in the concentration of UDPgal and lactose prior to glucose indicated that glucose was not limiting the synthesis of lactose during lactogenesis. The high initial concentrations of lactose and glucose for sow 3 confirmed Gooneratne *et al.* (1982) observations of premature lactogenesis in sows with MMA, and suggest that predisposing factors can be identified prior to farrowing.

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# PEAKS IN THE SOW'S INTRAMAMMARY PRESSURE OCCUR IMMEDIATELY AFTER RATHER THAN EITHER JUST BEFORE OR AT PIGLET DELIVERY

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Intramammary pressure (IMP rises acutely in response to increases in the plasma concentration of oxytocin (Whittlestone, 1954). The concentration of endogenous oxytocin is elevated in the sow during farrowing (Forsling *et al.*, 1979) and oxytocin is often administered to sows to promote uterine contraction and hence assist the delivery of the next piglet. We have investigated the relationship between increases in IMP in the farrowing sow, the timing of piglet birth, and first milk intake. We monitored IMP using COBE disposable transducers on unanaesthetised, unrestrained sows. We used video recordings to determine the times taken for the piglets to first reach the udder, obtain their first intake of milk and whether the latter coincided with the birth of their siblings. Milk intake was verified by weighing the piglets every 10-15 min over the farrowing interval.

We monitored the IMP of eight sows for a total of 43 piglet deliveries. There was a rise in IMP in the minute prior to four (9%) of the deliveries. The IMP increased at the instant of birth for 13 (30%) of the piglets. The duration of these increases in IMP was  $19\pm88$  (mean $\pm$ SD). The peak amplitude of the increase in IMP was 22.7  $\pm$  10.0mmHg. There was a distinct rise in the IMP within a minute after the delivery of 40 (93%) of the piglets. The duration of the rise in IMP was 28  $\pm$  8s. The peak amplitude of the rise was 25.9 $\pm$ 12.0mmHg. The increase in IMP after birth was of longer duration than that observed during birth (P<0.001, Student's t-test) but there was no statistical difference in the peak amplitudes recorded either before/at birth or after birth.

The time to reach the udder was recorded when the first nose-to-udder contact occurred, and was  $26\pm27$ min (n = 32). The time taken for these piglets to obtain milk was  $44\pm29$  min. Thus, there was a distinct difference between the time taken for the piglets to get to the udder and the time taken to obtain milk. This difference must be accounted for when evaluating behavioural studies of initial milk intake. In only two of these 32 piglets was their first milk intake associated with the delivery of another piglet.

IMP peaks occurred more often immediately after the delivery of a piglet than either just before or at the instant of birth. The results indicate that the endogenous release of oxytocin occurs at a later time than would be expected if oxytocin facilitated the delivery of each piglet. Also, the piglets did not get milk when IMP increased in association with the delivery of another piglet. Hence, oxytocin release in relation to individual births must have another role such as enhancing the maternal behaviour of the sow (Keverne, 1988).

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# MORE CELLS MIGHT LEAD TO MORE MILK

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We have found that lean gilts have four times the concentration of DNA as fat gilts in their mammary tissue at 112 days of gestation (Head and Williams, 1991). This indicates that lean gilts have more cells in their glands and probably more cells in their secretory alveolae than fat gilts.

Quantitative histology was used to estimate specifically the number of cells in the alveolae and to determine whether different feeding regimes affected proportions of the major tissues comprising the gland. Frozen mammary tissue from the seven fat and seven lean gilts described in a previous abstract in this proceedings (Head and Williams, 1991) was taken for histology. It was fixed in formalin, put into araldite resin, cut into 1.5  $\mu$ m sections and stained with haematoxylin and eosin. The proportional volume occupied by each tissue was calculated at 100x magnification using a point counting technique involving 420 fields and a test grid of 42 points for each feeding regime. The numerical density of alveolar cells was determined at a higher magnification (1000x) (Weibel, 1979).

The proportion of the total volume occupied by alveolar, fat and connective tissue was the same in gilts from both nutritional regimes (Table 1). Within the alveolar tissue itself the proportions of wall and lumen were also similar. But the lean gilts had twice as many alveolar cells as the fat gilts.

Table 1.	The	propor	tional	l volume	s of	differ	ent	tissues	and	the	numerical	density	of
alveolar	cells	within	the <b>n</b>	nammary	gla	nd of	gilts	at 112	days	s of	gestation (	(n=7)	

	Fat gilts	SEM	Lean gilts	SEM
Backfat at $P_2$ (mm)	36	0.9	25	0.64
Proportion of gland (%)				
Alveolar wall	40.2	2.55	38.8	1.98
Alveolar lumen	36.6	2.31	31.5	1.87
Adipose tissue	13.1	2.56	15.0	1.62
Connective tissue & other	9.9	0.79	14.3	1.54
Numerical density (million cells				
/g mammary gland)	69.8	2.64	140.7	6.99

The cell counts support our hypothesis that the gilts with a higher concentration of DNA in mammary tissue had twice the number of alveolar cells. This demonstrates that nutrition during pregnancy can control the number of cells with the potential to secrete milk.

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# THE EFFECTS OF OESTRADIOL TREATMENT OF NEONATAL PIGLETS ON ACTIVITY, GROWTH AND SURVIVAL

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The rate of preweaning piglet mortality has been steadily reduced over recent years by the use of various management 'regimes and farrowing pen/crate modifications. To now further reduce mortality rate emphasis needs to be placed on the activity level and suckling ability of the piglet itself. Little is known about these factors although Bate and Hacker (1982) have suggested that piglet oestradiol concentration is positively correlated with activity level in the immediate postpartum period. Therefore, the two studies reported here were designed to investigate the effects of exogenous oestradiol administration to piglets at birth on subsequent activity levels, growth and survival.

Experiment 1 used 25 litters (248 piglets) from Large White x Landrace sows of mixed parities. At birth piglets were injected i.m.alternately with either 1 ml of sterile physiological saline or 1 ml of a solution containing 0.1 mg of oestradiol benzoate in 0.1 ml of peanut oil and 0.9 ml of sterile physiological saline. Experiment 2 used 38 litters (385 piglets) from Large White x Landrace sows of parities 1 and 2. The same two treatment injections were employed as described for Experiment 1, but these were given on a whole litter basis withint 4 hours of birth. Piglet behaviour data were collected via a continyous 3 hour videorecording on day 1 (commencing at the birth of the first piglet of the litter) and a continuous 2 hour videorecording on days 3,7,14 and 21 postpartum for piglets in Experiment 1. The latter recordings were transcribed into proportions of time that piglets were active (suckling, walking, playing, running or standing) or inactive (sleeping or lying). Growth data was also collected for piglets in Experiment 1 and data on piglet survival to weaning was recorded for both experiments.

Oestradiol treatment significantly reduced both the time taken by the piglet from birth to reach the udder (BTU) and the time from birth to first suckling (BTS) in Experiment 1 (Table 1). Subsequent piglet activity level and weight gain to weaning were unaffected by treatment. Preweaning piglet mortality tended to be lower for oestradiol-treated piglets in both Experiment 1 (2.4 vs 7.2%) and Experiment 2 (10.2 vs 13.0%), although neither of these differences was statistically significant.

	Control	Oestradiol	SEM	Significance <sup>1</sup>
BTU	740	596	46.5	*
BTS	1497	1060	90.0	**

Table 1. The effects of oestradiol treatment of piglets on the time taken from birth to first reach the udder (BTU) and suckle (BTS)

<sup>1\*</sup> P<0.05; \*\* P<0.01.

These data support the conclusion of Bate and Hacker (1982) that piglets with higher oestradiol concentrations are more active in the immediate postpartum period. Furthermore our data tentatively suggest that this increase in activity level may result in an increase in piglet viability resulting in reduced preweaning mortality, although such a conclusion must await further studies.

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# SEXUAL MOTIVATION OF BOARS AND THE SEXUAL PARTNER PREFERENCES OF OESTROUS GILTS

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This study examined whether the sexual partner preferences of oestrous gilts were related to differences in the level of sexual motivation of their male partners. Twelve one-year old boars, of similar liveweight (160 to 180 kg), were ranked on the basis of the total number of copulations achieved and the average interval to first mount in three weekly 15 min mating tests (Hemsworth *et al.*, 1978). The four top-ranking and four bottom-ranking boars were assigned to form test pairs, each comprising a high motivation (H) and a low motivation (L) male. The relative preferences of 15-17 ovariectomized oestrous gilts for boars in each of the four pairs were individually studied in 5 min T-maze tests. Data recorded included the time that the gilt spent within 0.5m of each of the two boars as well as bouts of courting behaviours (Hemsworth *et al.*, 1978) and the incidence of salivation exhibited by the boars. Gilts were induced into behavioural oestrus with an intra-muscular injection of oestradiol benzoate. The arms of the T-maze were 3.5m long and 3.0m wide, and at the end of each arm a boar was held in a  $1.3 \times 3.0m$  wire-mesh pen.

A sexual partner preference was found within only two of the four boar pairs. In one case the gilts spent more time within 0.5m of the H boar (mean ( $\pm$ SE) proportion of time near each boar; 0.46 $\pm$ 0.10 and 0.20 $\pm$ 0.14, P<0.01) while the L boar of the other pair was preferred (0.44 $\pm$ 0.03 and 0.30 $\pm$ 0.03, P<0.01). Differences between boars in levels of courting behaviour in the T-maze tests within these pairs corresponded to boar preferences. However, a difference (P<0.01) in the level of courting behaviour of the boars in a third pair was not accompanied by a preference for the more active male. No differences in boar preferences and courting behaviour were observed in the fourth pair. Incidence of salivation by boars was not associated with boar preferences or levels of courting behaviour of the boars.

It was concluded that boars may differ in their ability to attract oestrous gilts. However, this attraction for boars does not appear to be primarily determined by the level of sexual motivation of the boar. Further research to improve our understanding of the boar stimuli that attract the oestrous female may have practical implications for the detection and mating of oestrous female pigs.

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# PREDICTION OF LEAN AND FAT IN SOW CARCASSES

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Sows culled from the breeding herd and sent to slaughter represent a significant proportion of the total pig meat produced. Sow carcasses are usually deboned and the lean meat used for further processing. However, the method of payment in most countries in the world is not based upon the yield of lean meat. Dissections of 204 sow carcasses were therefore conducted to develop prediction equations for lean and fat yield.

Cull sows were allotted to 7 carcass weight classes from <100 to >225 kg, in 25 kg increments and 3 fat classes, lean, average and fat, within each weight class. Weight and fat class limits were based upon a previous survey of sow marketings (Aziz *et al.*, 1990). Depth of fat and loin were measured on the hot carcass between the 3 and 4 last rib 7 cm from the midline using an electronic probe. Fat depth was also measured on the midline at maximum lumbar, 3 and 4 last ribs, and last rib using a ruler. Carcasses were separated into primal, commercial and retail cuts, then into lean, fat and bone (Table 1).

			the second state of the se				
Wt range	<99.9	100 - 124.9	125 - 149.9	150 - 174.9	175 - 199.9	200 - 224.9	>225
N° sows	26	30	30	30	31	29	28
Carcass wt (kg)	89.9	112.4 <sup>,</sup>	138.6	159.8	186.2	211.0	245.1
•	±7.41	±6.99	±7.21	±7.62	±5.87	±6.82	±17.22
Backfat (BE) (mm)	15.1	18.3	22.2	24.2	29.4	37.8	47.7
	±4.26	±7.19	±8.80	±7.69	±9.09	±10.06	±10.95
Retail yield (%)	61.7	61.5	61.1	60.9	58.9	56.7	53.6
•	±2.90	±4.10	±3.87	±3.39	±3.67	±3.97	±2.96
Lean yield (%)	44.4	43.2	42.6	42.5	39.9	37.8	34.6
•	±2.66	±4.79	±4.69	±3.61	±3.91	±4.23	±3.08
Fat yield (%)	14.4	16.2	18.0	18.5	21.3	24.7	28.8
•	±3.67	±5.68	±5.49	±4.03	±4.45	±4.89	±3.95
Bone yield (%)	12.9	11.6	11.0	10.6	10.4	9.2	8.4
-	±1.73	±1.96	±1.86	±1.46	±1.24	±1.20	±1.04

Table 1.	Carcass c	haracteristics	(mean ±	SD) f	for sows	of	various	weig	zhi	t
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Carcass weight (CW) increased as live weight (LW) increased (P<0.001, CW=-6.319+0.837 LW, r=0.991, SE=0.008). When lean and fat yield were predicted using backfat thickness by electronic probe (BE), the equations were: %lean =49.827-0.329BE,  $R^2 = 0.767$ , MSE = 2.448; %fat = 7.754+0.451BE,  $R^2 = 0.860$ , MSE = 2.463. Muscle depth at the same location had the lowest coefficient of determination ( $R^2 = 0.008$ ). Among the ruler measurements, maximum fat depth over lumbar vertebrae (BR) was associated with the most accurate prediction of lean and fat yield: %lean = 49.711-0.252BR,  $R^2 = 0.711$ , MSE = 2.702; %fat = 7.435+0.359BR,  $R^2 = 0.859$ , MSE = 2.450. The weights of lean and fat in the carcass were accurately predicted by BE and CW: kg lean = 5.894 - 0.292BE + 0.192CW,  $R^2 = 0.926$ , MSE = 2.005; kg fat = -8.739 + 0.390BE + 0.085CW,  $R^2 = 0.959$ , MSE = 1.862. Lean and fat yield from sow carcasses can be accurately predicted using either electronic probe or ruler measurements of backfat.

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# EFFECT OF A FEED FLAVOUR IN A HIGH AND LOW QUALITY DIET ON FEED INTAKE AND GROWTH RATE IN THE YOUNG PIG

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Attempts have been made to stimulate post-weaning feed intake by the inclusion of flavours. The addition of an aromatic flavour to sow and piglet feed resulted in improved weaner performance (Campbell, 1976). The effect of including an aromatic flavour (Bigarol Multifruit (BM), Haarmann & Reimer Pty. Ltd.) in two different weaner diets on feed intake is reported here.

In a randomized block design, 32 individually fed pigs received one of four diets: diet A (wheat, meat meal, soyabean meal; 20% CP, 13.8 MJ DE/kg, 0.75g available lysine/MJ DE), diet A plus 200 g/tonne BM, diet B (wheat, full fat soya, fish meal, whey powder, meat meal; 20% CP, 15 MJ DE/kg, 0.75g available lysine/MJ DE) or diet B plus 200 g/tonne BM. These diets were fed from weaning at day 21 to 10 weeks of age. Feed intake was recorded on a daily basis.

					Weeks			
Diet	BM	4	5	6	7	8	9	10
Feed intake (I	kg/pig,	/week)						
A	-	0.804	1.823	3.367	5.220	7.499	9.250	11.173
A	+	0.805	1.918	3.829	6.373	7.585	10.500	11.645
B	-	0.823	2.162	4.269	6.980	8.727	10.155	11.767
B	+	0.910	2.450	4.739	7.245	8.887	10.559	11.520
SE <sup>1</sup>		0.102	0.144	0.204	0.414	0.416	0.512	0.563
Diet		NS	**	**	**	**	NS	NS
BM		NS	NS	**	NS	NS	NS	NS
Growth rate (	(kg/pig	/week)						
A+B	-	-0.038	1.300	2.356	3.099	4.402	5.500	5.656
A+B	+	0.094	1. <b>45</b> 0	2.606	3.625	4.438	5.782	5.500
SE		0.102	0.150	0.204	0.256	0.296	0.316	0.312
BM		NS	NS	NS	*	NS	NS	NS

Table 1.	Effect o	of BM in	two	weaner	diets	on fee	d intake	and	on	growth	rate	pooled
by diet	in 4 to 1	0 week (	old p	igs						-		-

<sup>1</sup>NS, non significant, P>0.05; \* P<0.05; \*\* P<0.01.

The main effects of diet on intake were significant (P<0.01) in weeks 5-8 inclusive and for flavour in week 6, with no significant interactions. After pooling the growth rate data for the two diets, the difference in week 7 was significant (P<0.05). The effect of the flavour on feed intake and growth rate over the 7 week period did not attain statistical significance (P>0.05).

#### References

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# EFFECT OF SEASON AND DIETARY LYSINE LEVEL ON THE PERFORMANCE OF RESTRICTIVELY FED PIGS

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Environmental temperature affects the way nutrients in the body are partitioned between maintenance and accretions of fat and lean (SCA, 1987) and may affect lysine (L) requirements. The effect of environmental temperature on the pig's response to dietary amino acid supply is unclear because of variations in food intake. An experiment was conducted to examine the effect of season when iso-energetic diets varying in L content were fed to pigs on a restricted basis.

In a 4 x 3 factorial experiment, 192 pigs were used to compare four dietary L levels over three seasons. Diets differing in L content (Table 1) were prepared by the serial addition of soyabean meal at the expense of wheat. Thirty two gilts and 32 entire males began test in mid-February (AUT), mid-July (WIN) and late-December (SUM) of 1986. Ambient temperature (°C) was recorded daily at 0900 and 1500 h. Pigs were fed individually twice daily an amount equal to  $0.1 \times \text{liveweight}^{0.75}$  to a maximum of 2.2 kg for pigs above 60 kg; daily feed intakes averaged  $1.39 \pm 0.014$  and  $2.12 \pm 0.004$  kg for periods 25-50 kg and 50-85 kg respectively.

There was no interaction between diet and season (P>0.05). Growth rate (GR), food conversion (FCR) and P2 fat improved with increasing L (Table 1). Live pig performance declined during WIN for the 25-50 kg period and during SUM for the 50-85 kg period. Carcases had less  $P_2$  fat in AUT than in either SUM or WIN (P<0.05).

Table 1.	Effect of	diets	and	season	on	growth,	feed	conversion	and P2	2 fat :	responses	;

Attribute			Diets	1			Season <sup>1</sup>				
					SEM		AUT	WIN	SUM	SEM	
25-50 kg period				•							
DE <sup>3</sup>	14.32	14.32	14.36	14.33	0.049	am²	24.9	17.2	27.1	-	
$L^4$	8.8	9.6	10.5	11.3	-	pm²	29.2	21.5	30.6	-	
GR (kg/d)	0.60c	0.62b	0.64a	0.64ab	0.007	-	0.63x	0.61y	0.64x	0.006	
FCR (g:g)	2.34b	2.25a	2.19a	2.20a	0.026		2.23x	2.31y	2.19x	0.023	
50-85 kg period								•			
DE <sup>3</sup>	12.85	12.86	12.81	12.94	0.088	am²	22.4	22.3	26.3	-	
$L^4$	6.0	6.7	7.4	8.2	-	pm <sup>2</sup>	26.4	25.7	29.4	-	
GR (kg/d)	0.63c	0.67b	0.71a	0.72a	0.007	•	0.70x	0.69x	0.66y	0.006	
FCR (g:g)	3.41c	3.20Ъ	2.99a	2.97a	0.034		3.07x	3.12x	3.24y	0.029	
At 85 kg											
P <sub>2</sub> fat	15.9c	15.0b	14.3ab	13.8a	0.41		16.1y	14.1x	14.2x	0.36	

 $^{1}a,b,c;$  x,y Within main effects, means without a common letter differ (P<0.05). <sup>2</sup>Mean temperature (°C) readings at 0900 h (am) and 1500 h (pm). <sup>3</sup>Dietary digestible energy (MJ/kg). <sup>4</sup>Dietary lysine (g/kg).

Thus the pig's response to dietary L is independent of season. However, small seasonal differences, viz. mean daily temperatures of 26, 22 and 28°C for AUT, WIN and SUM respectively, can affect the performance of restrictively fed pigs.

#### References

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# EXOGENOUS ENZYME SUPPLEMENTATION OF CREEP- • WEANER DIETS

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Exogenous feed enzyme supplements provide a means of improving the digestibility of a range of feed components, including vegetable proteins, starch and non-starch polysaccharides and fat for pigs. The effect of two different multi-enzyme supplements on the weight gain, feed intake and feed conversion ratio (FCR) was evaluated for creep weaner pigs in two separate experiments.

The control diet for both experiments contained (g/kg) wheat 706.3, fish meal 93, meat and bone meal 80, soyabean meal 70, tallow 37, limestone 1.6, L lysine HCl 1.1, vitamins and minerals 1, and was formulated to 15 MJ/kg of digestible energy (DE) and 0.67 g available lysine/MJ DE. In Expt. 2 500 g/t of Fuzone 200 was added. In both experiments, the total amount of enzyme added was similar (2 g/kg), but the nature of the enzymes varied. In Expt. 1, the enzyme supplement (supplied by Alltech) added (g/kg): proteinase 0.25, lipase 0.25,  $\beta$  glucanase 0.5, amylase 0.5 and cellulase 0.5. In Expt. 2 the enzyme supplement (supplied by Novo Nordisk) added (g/kg): Biofeed Plus (containing  $\beta$ -glucanase, hemicellulase, pentosinase and cellulase) 1.0, Biofeed Alpha (amylase) 0.333, Biofeed Pro (proteinase) 0.333 and Lipozyme 10,000L (lipase) 0.333. Twenty four litters, twelve per diet were paired on the basis of initial weight and litter size, in each experiment. There were 164 and 223 pigs used in Expts. 1 and 2 respectively. The creep period was from 21-29 days for Expt. 1 and 14-27 days for Expt. 2.

	Expt. 1				Expt. 2		
Age (d)	Control	+ Enzyme	SEM	Age (d)	Control	+ Enzyme	SEM
Gain (g/d)							
0				14-21	204	204	15.2
21-29	224	223	12.1	22-27	261	248	13.9
30-50	309	319	17.4	28-48	302	272	13.2
21-50	286	289	13.6	14-48	274	253	11.1
Feed intake	(g/d)						
	U			14-21	14	13	1.6
21-29	39	36	5.2	22-27	35	24	6.6
30-50	474	438	26.6	30-48	521	512	42.8
21-50	357	330	20.0	14-48	322	314	26.6
FCR							
				14-21	0.07	0.07	0.001
21-29	0.16	0.18	32.5	22-27	0.14	0.1	0.026
30-50	1.54	1.40	47.0	28-48	1.71	1.87	0.108
21-50	1.23	1.14	37.9	14-48	1.21	1.25	0.065

Table 1.	Performance of	piglets given	enzyme supr	plemented	creep-weaner	diets
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The two multi-enzyme supplements had no effect (P>0.05) on piglet performance (weight gain, feed intake or FCR) when fed with a conventional wheat based creep-weaner diet.

# WHOLE-BODY MINERAL COMPOSITION OF HIGH GENETIC MERIT PIGS

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The factorial method, which includes data on whole-body mineral composition, may be used to determine the dietary mineral requirements of the pig. Only a limited number of studies has been undertaken to determine body mineral composition. The aim of this study was to determine the body content of calcium, phosphorus, potassium, sodium and magnesium for entire-male and female pigs of an improved genotype, depositing body protein at their maximal rates (around 130 and 170 g/d for gilts and boars respectively).

A diet consisting of highly digestible ingredients was prepared with amino acids balanced according to the ARC (1981) recommendations, and with vitamins and minerals provided in excess of requirements. The dietary energy to protein ratio was such that ideal protein was never limiting in relation to energy for whole body protein deposition. That this diet was able to support maximum body protein deposition within the confines of *ad libitum* intake was confirmed in a series of independent conventional nitrogen balance trials.

A serial slaughter trial was conducted involving thirteen pigs of each sex allocated to four slaughter weights (25, 45, 65 and 85 kg live weight). The pigs were offered the experimental diets *ad libitum*. Upon reaching its respective slaughter weight each pig was euthanased, the whole body ground, mixed, and sub samples analysed for minerals. A statistical linear model, which included terms for empty body weight (EBW), sex and EBW x sex was fitted to the data. The effects of sex and EBW x sex were not significant (P0.05) and the relevant data were pooled across sexes. There was a significant effect of EBW (Table 1) for all the minerals, except calcium.

	Mea	Terrel of			
Element	23.7 (n=10)	43.3 (n=6)	62.3 (n=5)	82.6 (n=5)	significance <sup>1</sup>
Calcium	8.7(0.37)	7.1(0.51)	8.3(0.59)	7.4(0.48)	NS
Phosphorus	5.8(0.19)	4.9(0.26)	5.6(0.28)	5.0(0.22)	*
Potassium	2.5(0.03)	2.3(0.04)	2.3(0.06)	2.2(0.5)	**
Sodium	1.1(0.02)	0.9(0.01)	0.8(0.04)	0.8(0.03)	**
Magnesium	0.3(0.01)	0.2(0.01)	0.3(0.01)	0.3(0.01)	*

Table 1.	Mean (±SE)	whole empty	body mineral	contents	(g/kg) for	growing p	oigs at
different	liveweights						

<sup>1</sup>NS, non significant, P>0.05; \* P<0.05; \*\* P<0.01.

These body mineral contents were similar to other published findings, except that calcium levels were consistently lower. The data are limited, however, in that mineral content expressed on a body weight basis is influenced by the degree of fatness. Future analysis will allow examination of the mineral contents expressed per unit fat-free body mass and per unit body protein.

#### References

AGRICULTURAL RESEARCH COUNCIL. (1981). "The Nutrient Requirements of Pigs", p. 67-124. (Commonwealth Agricultural Bureaux: Slough).

# EFFECT OF SUPPLEMENTARY ENZYMES ON ILEAL NUTRIENT DIGESTIBILITY AND POST-WEANING PERFORMANCE OF WEANER PIGS

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In a recent experiment  $\beta$ -glucanase supplementation of a hulless barley-based diet significantly improved live weight gain and protein digestibility in the small intestine of early-weaned pigs, whereas the digestibility of other nutrients was unaffected (Inborr *et al.*, 1991).

An experiment was designed to investigate the effect of adding a multi-enzyme product ( $\theta$ -glucanase, xylanase,  $\alpha$ -amylase) to a pig starter diet based on wheat (35%), barley (35%) and soyabean meal (20%) on performance and nutrient digestibility in the small intestine of early-weaned pigs.

A total of 64 pigs weaned between 21 and 25 days of age were divided into eight uniform (sex, litter origin and weight basis) groups of eight animals and placed in flatdeck cages and fed the experimental diets for three weeks. On day 21 two pigs per flatdeck were slaughtered and the contents of the small intestine (divided into four sections) collected and analysed for its constituent nutrients.  $TiO_2$  was used as an indigestible marker.

Table 1.	Daily	weight	gain	and	feed	conversion	ratios	during	each	week o	f the	21-
day expe	riment	al perio	d						•			

Week	1	[	2	2	3		
Enzymes	-	+	-	+	-	+	
Gain (g/d) FCR	56 ND <sup>1</sup>	64 ND	203 1.20	209 1.36	379 1.37	423 1.34	

<sup>1</sup>ND, not determined.

 Table 2. Dry matter, crude protein and starch digestibilities (%) in the small intestines (SI) on day 21 (mean and SEM)

SI section	2nd quarter		3rd q	uarter	4th quarter		
Enzymes	- +		- +		-	+	
Dry matter Crude protein Starch	24.5±3.0 31.0±5.5 33.2±5.2	22.4±5.2 40.5±1.8 44.1±3.5*	52.6±2.7 61.9±2.9 80.7±1.7	55.0±2.7 64.6±3.2 85.6±3.0	64.3±0.8 76.8±1.8 94.9±0.8	67.9±0.6* <sup>1</sup> 78.4±1.3 97.7±0.6*	

<sup>1</sup>\* P<0.05.

Enzyme supplementation tended to improve daily weight gain during the three week period (Table 1) and improved ileal dry matter (4th quarter) and starch (2nd and 4th quarter) digestibility (P<0.05) (Table 2).

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# PALATABILITY TO PIGS OF SORGHUM GRAIN DIFFERING IN MIDGE RESISTANCE

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The sorghum midge, *Contarinia sorghicola* (Coquillett), is a major pest of sorghum in Australia, costing the industry around \$6-10 M annually. Several midge-resistant sorghum genotypes have been bred (Henzell *et al.*, 1986) but it is not known if selection has also affected the palatability of the grain. A pig experiment was conducted to compare the palatability of grain sorghum hybrids that were either susceptible to midge, Pacific 810 (S1) and DK55+ (S2), with low resistance to midge, Barrier (R1), or with moderate resistance to midge, AQL39/QL36 (R2) and DK470 (R3).

In a seven-week experiment, 16 entire boars and 16 gilts (start and end liveweights of  $28.6 \pm 2.51$  and  $74.1 \pm 6.16$  kg respectively) were individually housed in pens containing two feeding troughs and offered *ad libitum* a choice between two diets. The diets were paired so as to compare S1 with R1, R2 or R3 and S2 with R1, R2 or R3 with two additional comparisons, S1 with S2 and R2 with R3, making eight treatments in all. The positions of the feeding troughs were alternated weekly. Each of the grains was included in the diet at 750 g/kg and all diets were formulated from determined amino acid analyses of the ingredients to contain total lysine, methionine + cystine and threonine contents of 11.22, 5.90 and 6.73 g/kg respectively; DE was estimated to be 14.1 MJ/kg. The condensed tannin content of each of the hybrids was <1 g/kg.

Atribute	S1 & R1	S1 & R2	S1 & R3	S2 & R1	S2 & R2	S2 & R3	S1 & S2	R1 & R2	±SEM
Diet pref. <sup>1,2</sup>	0.37bc	0.46abc	0.55ab	0.64a	0.63a	0.66a	0.29c	0.58ab	0.084
FI (kg/d)	2.27	2.31	2.54	2.38	2.33	2.49	2.33	2.36	0.135
ADG (kg/d)	0.92	0.91	0.95	0.95	0.93	0.98	0.90	0.91	0.029
FCR (g:g)	2.47	2.56	2.70	2.51	2.52	2.53	2.59	2.61	0.110

Table 1. Effect of sorghum hybrid on diet preference and pig growth performance

<sup>1</sup> Proportion of the total food consumed for the first mentioned diet of each pair.  ${}^{2}a,b,c$  - Means without a common letter differ (P<0.05).

The type of diet offered to the pigs did not affect (P>0.05) either feed intake (FI), growth rate (ADG) or feed conversion ratio (FCR) but diet preferences were observed (Table 1). When comparing the pig's diet preference of the three resistant hybrids with each of the two susceptible hybrids, differences were seen only between S1 and S2 diet combinations with the latter clearly being the more palatable (means of 0.46 and 0.65, SEM  $\pm$  0.048 respectively; P<0.05). When S1 and S2 diets were offered as a pair, pigs consumed almost 2.5 times as much of S2 as S1. The results indicate that pigs have distinct preferences for certain sorghum hybrids but that those bred for midge resistance are equally as palatable as those that are not resistant.

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# EFFECTS OF EXTRUSION OF WHEAT ON DRY MATTER AND STARCH DIGESTIBILITY IN THE YOUNG PIG

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Extrusion involves heat, physical shear forces and pressure. These factors vary depending on the extruder type, operating conditions and the material being processed. Ileal digestibility coefficients for dry matter (DM), energy and starch in pigs weighing 78kg were significantly increased by Insta-Pro extrusion (Des Moines, Iowa, U.S.A.) of barley (Fadel *et al.*, 1988). The present study determined the effect of Insta-Pro extrusion of wheat on DM and starch digestibility in piglets.

Twenty male pigs weaned at 14 days of age were divided into two equal groups and fed a mash diet in which the wheat component  $(650g/kg^{-1})$  was either ground or extruded. From 45 days of age, feed containing 2g/kg of Cr<sub>2</sub>O<sub>3</sub> was restricted to 90% of *ad libitum* and fed hourly in equal amounts. At 48 days of age each pig was anaesthetized 15 min after an hourly feed. Digesta samples were taken from the stomach (St), four parts of the small intestine (SI), the caecum (Ce), two parts of the large intestine (LI) and the colon (Co) for DM, starch and Cr determination.

	St	SI1	SI2	SI3	SI4	Ce	LI1	LI2	Co
DM dig. (%)	<u>,</u>	40.0	05.4	54.0	(0.0	<b>T</b> O <b>O</b>	00 (		04.0
Unextd. wheat	0	12.3	25.4	54.8	63.9	79.3	82.6	84.1	84.9
Extd. wheat	2.2	29.7	46.4	69.3	71.3	79.5	84.2	84.8	85.2
Significance <sup>1</sup>	NS	**	**	**	**	NS	NS	NS	NS
Starch dig. (%)									
Unextd. wheat	0	25.1	58.7	79.2	89.9	94.5	97.9	99.0	99.8
Extd. wheat	1.1	62.0	85.2	93.3	95.3	96.3	98.1	<b>99.5</b>	99.9
Significance	NS	**	**	**	NS	NS	NS	NS	NS

Table 1. Effect of extrusion of wheat on DM and starch digestibility at various sites along the digestive tract

<sup>1</sup>NS, non significant, P>0.05; \*\* P<0.01.

The main effects of extrusion and gut location were highly significant (P<0.01). The interaction of extrusion with location showed significant increases in DM and starch digestibility in the small intestine of 6 - 7 week old pigs. Extrusion of wheat facilitated digestion of DM and starch in the small intestine. This should increase the efficiency of energy utilization and may reduce the risk of scouring initiated by hind-gut fermentation of starch that escapes small intestinal digestion.

#### References

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# ENERGY EVALUATION SYSTEMS FOR PIG DIETS

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## Introduction

The cost of feed is 60 to 70% of the total cost of pig meat production with the energy component representing the greatest proportion. Therefore, it is important to estimate precisely the energy value of feedstuffs in order to 1) determine the hierarchy between available raw materials for least-cost diets formulation, 2) to adapt the feed supply to the energy requirements of the pigs, and 3) to predict the amount of body weight gain or pig meat obtained from a given amount of feed. In addition, it is also necessary to assess the energy value of feeds for regulation needs. However, a given diet or ingredient is given different energy values according, firstly, to the considered step of energy utilization by the pig (digestible (DE), metabolizable (ME) or net (NE) energy) and, secondly, to the prediction method used for each step. An energy system corresponds to the combination of one step of energy utilization and one prediction method. The situation is the most complicated for NE since its value is difficult and, in many situations, impossible to measure directly and is therefore predicted from equations. The main difficulty is then to choose between available energy estimates for a given feed, its value compared to other feeds being dependent on the system. From that point of view, NE seems preferable since it takes into account the metabolic utilization of energy and would be representative of the true productive energy. NE is the only system in which energy requirements and diet energy values are expressed on the same basis, values being independent of the feed. Anyway, the "quality" of an energy system will be appreciated through its ability to predict the animal performance with a satisfactory degree of accuracy and the energy value of both raw materials and compound feeds.

Several reviews on this topic have been produced recently (Morgan and Whittemore, 1982; Henry and Perez, 1982; Wiseman and Cole, 1983; Henry *et al.*, 1988; Batterham, 1990), most attention being devoted to DE and ME systems. In this review, our interest will be more focused on NE systems and on the limits and advantages of each system in order to answer the question: which system should be used in practical conditions?

### Methodological aspects

The different steps of energy utilization are given in Figure 1. The DE value of a feed corresponds to the difference between its gross energy content and the energy losses in faeces, both being measured directly by bomb calorimetry of consumed feed and collected faeces. However, DE is not a true measure of the feed energy absorbed from the digestive tract since faeces contain endogenous losses (i.e. digestive secretions and intestinal cell debris). In addition, gas and heat from fermentation processes are produced but not usually measured and then considered as digested energy. Nevertheless, quantification of endogenous and heat of fermentation losses is difficult and has a small practical interest and, surprisingly, energy losses in gas (methane and

List of main abbreviations:

GE: gross energy; DE: digestible energy; ME: metabolizable energy; NE: net energy; HI: heat increment; FHP: fasting heat production; DCe: digestibility coefficient of energy; MEm: ME for maintenance; BW: body weight; CF: crude fibre; NDF: neutral detergent fibre; ADF: acid detergent fibre; CP: crude protein; DCP: digestible crude protein; DFat: digestible crude fat; DCF: digestible crude fibre; NFE: nitrogen-free extract.

hydrogen) are considered in the transformation of DE to ME. The DE concept used in practice corresponds therefore to the apparent DE content of a feed.



Figure 1. Energy utilization in pigs (under thermoneutral conditions).

The ME content of a feed is the difference between DE content and energy losses in urine and gases. Most of the energy lost in gases is due to methane production. While energy content of feed, faeces and urine can be measured with pigs kept in metabolism crates, the measurement of methane production necessitates the pig to be housed in a respiration chamber. Consequently, most ME values reported in the literature and tables ignore energy losses as methane.

NE is defined as ME minus heat increment (HI) associated with metabolic utilization of ME and also to the energy cost of ingestion and digestion of the feed. As illustrated in Figure 2, only total heat production of the pig is measured directly, HI being calculated as the slope(s) of the linear regression of heat production on ME. As discussed below, this slope is not constant over a large range of ME intakes for a given feed. HI is lower below than above maintenance and decreases with the proportion of energy retained as lipids. Consequently, in order to compare different feeds for their NE content, it would be necessary to feed all diets at the same relative energy level (x maintenance) and to keep composition of the product (weight gain in growing pigs, for instance) as constant as possible. Energy balance measurements are



Figure 2. Methods for measurement of net energy (A and B correspond to estimated and "true" fasting heat production, respectively).

complex and time consuming, so that only one feeding level is usually applied for each diet. In addition, in growing animals, energy balance below maintenance is physiologically meaningless. So, the "true" fasting heat production (FHP) is not measured but estimated by extrapolating heat production measured on a series of diets to zero ME intake (Figure 2). This means that the efficiencies of ME for maintenance and for energy gain are supposed to be the same for a given diet. For pigs on a positive energy balance, i.e. when energy retained is considered as NE for production (NE<sub>p</sub>), the NE value of the diet is the sum of estimated FHP and NE<sub>p</sub>. The absolute NE value of a diet is therefore dependent on the estimate of FHP. In order to avoid any bias due to FHP estimate or composition of tissue gain, it is preferable to carry out measurements on pigs from the same genotype, sex and body weight, which are kept in a controlled environment and above their critical temperature and given approximately the same amount of feed. An erroneous estimate of FHP will affect the absolute NE value but not the hierarchy between tested diets.

In practice, DE and ME values of compound feeds and some raw materials are measured by giving only the test feed to the pigs. However, protein or fat sources are usually added to a basal diet and their energy values are calculated by the difference method. In that approach, it is assumed that energy values are additive. More complex methods involve combination of several test ingredients in complete and balanced diets, the inclusion levels of all ingredients being statistically independent. The energy values of the ingredients are then calculated by regression techniques (Noblet *et al.*, 1990). NE is obtained from measurements of either heat production (Schiemann *et al.*, 1972; Noblet *et al.*, 1989a) or energy retention by the comparative slaughter technique (de Goey and Ewan, 1975; Just, 1982d). In the latter case, heat production is calculated as the difference between ME and energy retained. In addition, since it is logical to feed balanced diets to pigs in order to meet their nutritional requirements and maintain their production level, most NE measurements are made by using complete diets; the objective is then to relate, by regression techniques, NE to DE, ME or digestible or crude nutrients content. The obtained equations are then applied to raw materials.

This brief presentation of methods available for energy determinations indicates that apparent DE or ME values of pig feeds can be measured with simple, precise (variation coefficient usually lower than 1%) and reliable techniques. On the other hand, NE determinations require sophisticated equipment (respiration chambers or calorimeters) or complex methods (comparative slaughter technique). They rely on assumptions (especially for FHP), are based on more variable and complex measurements (heat production) and should be carried out with balanced diets, at constant feeding levels. In addition, the NE value of a given feed is related to its final utilization (maintenance, growth or milk production and their combinations). Even if NE meets the final objective for energy evaluation of feeds since it represents the true production value, this concept and NE values must be used carefully.

#### **Energy utilization**

#### Digestive utilization

The digestibility coefficient (DC) of energy (DCe) which corresponds to the ratio (x 100) between DE and gross energy varies between 70 and 90% for most pig diets and between 0 and 100% for raw materials. These variations are associated with differences in faecal digestibility of the nutrients constituting organic matter. With regard to crude protein and crude fat, their (apparent) DC vary between 60 and 95% according to their chemical characteristics and their origin, while soluble carbohydrates (starch and sugars) are highly digestible (95 to 100%). In fact, most of the variation of DCe is associated with the presence and the type of fibre (defined as the sum of non-starch polysaccharides (NSP) and lignin) and, to a lesser extent, the amount of minerals (Table 1). Indeed, DC of dietary fibre is usually low but also very variable.

N°	Feeds	Equation	RSD	Reference		
1	Mixed diets	DCe = 94.46 - 0.0839 x NDF	3.7	King and Taverner (1975)		
2	Mixed diets	DCe = 93.81 - 0.128 x ADF -	1.6	Perez et al. (1984)		
		0.064 x (NDF-ADF)				
3	Mixed diets	DCe = 97.5 - 0.116 x NDF	2.0	Noblet <i>et al.</i> (1989a)		
4	Mixed diets	DCe = 104.9 - 0.136 x Ash -	1.6	Noblet <i>et al</i> . (1989a)		
		0.100 x NDF				
5	Barley	DCe = 91.99 - 0.227 x CF		Perez et al. (1980)		

Table 1. Effect of diet composition (g/kg dry matter) on digestibility coefficient of energy (DCe, %) in growing pigs

For instance, Chabeauti and Noblet (1990) found DC of total NSP in wheat straw, wheat bran, sugar beet pulp and soyabean hulls equivalent to 16, 46, 69 and 79%, respectively. These large variations are related to the chemical composition and the physical form of NSP. In sugar beet pulp and soyabean hulls, NSP contain large proportions of highly digestible (80 to 90%) pectic substances while, in wheat straw, large amounts of lignin linked to NSP prevent their degradation (Chabeauti *et al.*, 1991). According to these observations, the reduction of DCe with dietary fibre addition will vary with the tested fibrous material (Figure 3).



Figure 3. Effect of sugarbeet pulp (SBP), soyabean hulls (SBH), wheat bran (WB) and wheat straw (WS) addition to a basal diet (B) on digestibility coefficient of energy in pigs (from Chabeauti and Noblet, 1990).

Therefore, the amount of total dietary fibre is an inadaquate criterion for predicting DCe; additional information on chemical (neutral sugars of hemicelluloses and cellulose, uronic acids of pectic substances and lignin contents) and physical characteristics would then be necessary. However, methods for quantifying the "quality" of dietary fibre are complicated and are not applicable in routine measurements of feedstuffs. Consequently, prediction of DCe from fibre content still relies on methods which quantify the different fractions or the total amount of dietary fibre. The oldest and most commonly used method is the Weende technique in which the measured residue, so-called crude fibre (CF), corresponds approximately to cellulose and lignin and represents from 25 to 60% of dietary fibre (Carré and Brillouet, 1986). It therefore ignores the hemicellulose fraction. However, composition of dietary fibre (cellulose, hemicelluloses, pectic substances and lignin percentages) is relatively constant in a feed of a given botanic origin; the percentage of dietary fibre recovered in the CF residue and its nutritional significance will therefore be relatively constant within the same botanic origin (Henry and Etienne, 1978). Chemical methods developped by Van Soest and Wine (1967) for forages involve the identification of a first residue, the Neutral Detergent Fibre (NDF), which includes lignin, cellulose and a large proportion of hemicelluloses; but soluble carbohydrates of NSP are ignored. A second residue, the Acid Detergent Fibre (ADF), corresponds to cellulose and lignin and, in most cases, its value is close to the determined CF content. The difference between NDF and ADF provides an estimate of the hemicelluloses fraction. Other methods allow determination of total dietary fibre and its subsequent chemical composition (Southgate et al., 1978; Carré and Brillouet, 1989) but they are not yet applicable to routine measurement.

Limits of analytical procedures and variations in digestive utilization of dietary fibre explain the unsatisfactory and contradictory relationships between DCe and estimate of fibre content. Indeed, the statistical superiority of one estimate is directly related to the type of diets and fibre sources which have been used. In addition, this relative inability of any dietary fibre estimate to predict DCe is due to the fact that none of these chemical methods allows isolatation of the nutritionally significant fractions. However, for practical purposes, CF or NDF and/or ADF represent
reasonable predictors of DCe in complete diets in which several fibrous sources are mixed, with a preference for NDF when it is correctly measured (Henry, 1976; Morgan *et al.*, 1987; Noblet *et al.*, 1989a). Such equations should not be applied to raw materials where specific relationships are to be used.

The depressive effect of dietary fibre on DCe is not only due to its low degradation rate in the digestive tract but also to a negative effect on apparent digestibility of other components of the diet. Indeed, the coefficient obtained in Table 1 for NDF (close to 1) suggests that NDF itself is not degraded while its measured digestibility was estimated to be 40 to 50% (Noblet *et al.*, 1989a). Even if little information is available on this subject (Low, 1985), it is suggested that fibre presence in the digestive tract increases endogenous losses and reduces the mean transit time, especially in the large intestine. Antinutritional factors and various heat treatments affect DCe but this aspect will not be considered here.

DCe is affected by other factors than those related to the diet itself. Firstly, DCe decreases when, at one specific physiological stage, feeding level is elevated. Everts et al. (1986) found that DCe was decreased by about 2% when feeding level changed from 1.2 to 2.4 times maintenance, either in growing pigs or sows; Roth and Kirchgessner (1984) obtained a smaller effect in growing pigs but with diets of higher digestibility. When sows and growing pigs are compared at the same relative energy level, differences in DCe are usually small (Everts et al., 1986) but when the comparison is done at normal feeding levels for pregnant sows and growing pigs, digestibility coefficients are superior in all cases for the sows, the difference being greater with diets or ingredients of lower digestibilities (Etienne, 1987; Fernandez et al., 1986; Figure 4). For fibrous diets or raw materials, the difference may be equivalent to more than 10%. Similarly, as live weight in growing pigs increases, DCe is higher (+1.5%) between 40 and 80 kg (Roth and Kirchgessner, 1984), this difference being more important with fibrous diets (J. Noblet and H. Fortune, unpublished). DCe differences with age or physiological stage and feeding level are explained, to a large extent, by changes in the digestive utilization of fibre and crude protein (Figure 4) which depend on transit time and pig digestive capacity. At similar feeding levels, DCe is not significantly affected by pig genotype or climatic environment (Noblet et al., 1985; J. Noblet et al., unpublished).

# ME:DE ratio

Variations in ME:DE ratio are related to the importance of energy losses in methane and urine. In growing pigs, energy lost in methane is usually relatively low; an average value of 0.4% of DE intake was measured by Noblet et al. (1989) on 41 diets. However, this percentage ranged from 0.1 to 1.2%, the latter value being obtained with diets which contained soyabean hulls or sugar beet pulp (highly digestible fibre). In that particular situation, the methane energy loss represented about 5% of the DE of the raw material. The same authors found a significant relationship between methane production and the amount of digestible NDF, so that methane energy loss represented about 6% of degraded NDF energy. This value is comparable to other literature data obtained in non-ruminant (Muller and Kirchgessner, 1986) or ruminant (Czerkawski, 1980) species. In sows fed at the maintenance level, methane production represents a much higher proportion of DE intake: 1.4 vs 0.4% in growing pigs (Table 2). That three fold increase in methane production in sows would not only be associated with the higher degradation of fibre (+45% in the present situation) but also with a more intensive microbial degradation of other components of the diet. The great difference in faecal DC of nitrogen between sows and growing pigs (Table 2) suggests that energy from proteins may also contribute to the higher methane production in sows.

Energy lost in urine represents a variable percentage of DE since urinary energy is highly dependent on the amount of nitrogen in urine. At a given physiological stage where the amount of nitrogen retained in the body is stable, the urinary nitrogen will mainly depend on the amount of digestible protein and therefore on the crude protein (CP) content of the diet. Consequently, the ME:DE ratio is linearly related to dietary protein content (Table 3). Between physiological stages, protein retention as a percentage of digestible or dietary protein may vary to large extents. Recent data indicate that in extreme conditions (adult sows fed at maintenance vs 45 kg growing pigs fed *ad libitum*), urinary energy represented 6.5 and 3.3% of DE, respectively (Table 2). This difference would be smaller with diets adapted to protein requirements of each group.



Figure 4. Effect of diet NDF content on digestibility coefficient of energy (DCe) in sows fed at maintenance level (O) and growing pigs fed ad libitum ( $\bullet$ ) (from Noblet et al., unpublished).

Tal	ble 2. Compa	rison (	of dig	estil	bility	<mark>/ coef</mark> i	ficien	ts o	btained v	vith 8 d	iets in s	ows	fed
at	maintenance	level	and	45	kg	pigs	fed	ad	libitum <sup>1</sup>	(from	Noblet	et	al.,
un	published)												

	Sows	Growing pigs
Digestibility coefficient (%)		
Organic matter	86.0	77.1
Crude protein	85.5	75.0
NDF	72.1	51.8
Energy	84.3	74.8
ME/DE (%)	92.1	96.3
Energy as methane (%DE)	1.35	0.40
ME (MJ/kg DM)	14.52	13.48

<sup>1</sup>Diets contained 22.1% protein, 20.7% NDF, 32.0% starch, 6.9% fat and 18.72 MJ per kg dry matter.

N°	Equation	Reference
6	ME/DE = 99.7 - 0.0189 X CP	Morgan <i>et al.</i> (1975)
8 <sup>1</sup>	$ME/DE = 99.8 - 0.0202 \times CP$ ME/DE = 99.8 - 0.0200 X CP	NRC (1988) Noblet <i>et al.</i> (1989a)
9 <sup>1</sup>	$ME/DE = 100.7 - 0.0210 \times CP - 0.0050 \times NDF$	Noblet <i>et al.</i> (1989a)

Table 3. Effect of diet composition (g/kg dry matter) on ME/DE ratio (%) in growing pigs

<sup>1</sup>Methane losses included.

In most situations, the ME:DE ratio is considered relatively constant and equivalent to about 0.96. However, that ratio is not acceptable when dietary CP content and/or protein retention are either high or low. But the main problem is to decide how to apply that mean ratio to single feed ingredients. In a recent study (Noblet et al., 1990), ME:DE ratios (methane energy loss included) ranged from 100% for animal fat to 97-98% for cereals, 93 to 96% for protein sources (soyabean meal, peas) and 90 to 92% for fibrous protein sources (rapeseed meal, sunflower meal) (Table 12). Sows on low feeding levels digest energy more efficiently than growing pigs (Table 2). Therefore, that difference is reduced when diets are compared on a ME basis. From mean data reported in Table 2, DE content of diets was 12% higher in sows while the ME content was only 7% higher. In addition, on a ME basis, the difference was negligible for highly digestible diets and equivalent to about 10% for fibrous diets. In other words, the lower urinary and gas energy losses in growing pigs do not compensate for the higher DCe in sows, except for highly digestible diets or raw materials where ME values are comparable in growing pigs and sows. For most ingredients and diets, ME value is higher when it is determined with sows (J. Noblet et al., unpublished).

#### Metabolic utilization of ME

Metabolizable energy is used for meeting different requirements of the pig: maintenance, growth, milk or uterine gain. Below the maintenance level or in animals with a negative energy balance, additionnal ME (ME<sub>m</sub>) is used for preventing body energy losses (NE<sub>m</sub>): the ratio between NE<sub>m</sub> and ME<sub>m</sub> corresponds to the efficiency of utilization of ME for maintenance (Figure 2). This efficiency (k<sub>m</sub>) is in fact apparent since the true efficiency would be equivalent to the proportion of ME trapped in ATP (Armstrong, 1969). When ME intake is higher than ME requirement for maintenance, a proportion of the additional energy supply (ME for production: ME<sub>p</sub>) is retained in the body as protein or fat or exported as milk (NE<sub>p</sub>); the ratio NE<sub>p</sub>:ME<sub>p</sub> corresponds to the efficiency of utilization of ME for growth (k<sub>g</sub>) or milk (k<sub>l</sub>). During growth, energy gain includes protein and fat energy; the efficiency of utilization of ME for energy gain as protein or as fat are defined as k<sub>p</sub> and k<sub>t</sub>, respectively. The difference between ME<sub>m</sub> and NE<sub>m</sub> or ME<sub>p</sub> and NE<sub>p</sub> is equivalent to heat increment (HI) for maintenance (HI<sub>m</sub>) or production (HI<sub>p</sub>), respectively.

ME originates from different nutrients (oses, amino acids, long-chain fatty acids or volatile fatty acids). The main objective in NE evaluation of feeds will then be to quantify the efficiencies of utilization of these nutrients for NE for maintenance or body energy storage or milk production. It is obvious that the same feed will have different NE values according to its final utilization.

On theoretical considerations,  $k_m$  values for glucose, fat, protein and a mixture of amino acids are about 102, 100, 82 and 87%, respectively (Armstrong, 1969). In most cases, there is an excellent agreement between these data and those actually measured in animals infused with pure nutrients (Armstrong, 1969). However, in animals fed mixed diets, the  $k_m$  value is usually lower than the theoretical one which is expected from the composition of the diet. The difference results from the

additional energy costs of ingestion, digestion and activity. For pigs, Breirem's data (1939) still represent the most satisfactory mean  $k_m$  estimate (about 81%) obtained on conventional diets. But, to our knowledge, there is no equivalent value for the different digestible nutrients when used for maintenance in pigs. Therefore, it is impossible to propose a NE system for maintenance in pigs.

Similarly, theoretical calculations indicate that k<sub>p</sub> is 85 to 90% while k<sub>f</sub> ranges from 70 to 85 and 98% when ME is provided by protein, carbohydrate (as glucose) and fat, respectively (Armstrong, 1969; Millward et al., 1976). In vivo values for k, with pure nutrients are in agreement with these estimates; k. values measured with diets range between 70 and 80% and agree also with theoretical estimates (Schiemann et al., 1972). Theoretical and experimental  $k_f$  values in sows when ME is provided by infused volatile fatty acids are in close agreement and vary between 75 and 80% (Roth et al., 1988). On the other hand, "in vivo"  $k_p$  values are more variable (30 to 80%) and, in most cases, much lower than expected estimates. The reasons for this discrepancy have been proposed (Millward et al., 1976). But we have also shown that there is a close relationship between  $k_p$  and the way maintenance requirements are estimated. Indeed, k<sub>p</sub> was about 50% when ME<sub>m</sub> was supposed to be proportional to body weight (BW) exponent 0.75 while, in a more appropriate statistical model where the exponent was close to 0.60, k, averaged 64% (Noblet et al., 1989b). On a larger set of data (Noblet et al., 1991), we propose 80 and 60% as mean estimates of  $k_{\rm f}$  and  $k_{\rm he}$ respectively, in growing pigs fed cereal-soyabean meal diets; these values are higher than the "preferred" literature ARC (1981) estimates (74 and 56%, respectively). Mean values for  $k_1$  (72%) have been proposed by Noblet *et al.* (1990b) in lactating sows fed highly digestible diets.

Under practical conditions, HI of feed is not measured in all nutritional situations, so that NE values are usually a combination of  $NE_m$  and  $NE_p$  (see above). The efficiency of utilization of ME for NE (k) estimated in these conditions is also dependent on diet composition. Among the many papers dealing with this topic on pigs, only the large scale studies in which the objective was to propose a basis for NE evaluation of feeds will be considered. In near mature or mature pigs depositing predominantly fat, Schiemann et al. (1972) obtained efficiencies (i.e. for maintenance+fattening) of 52, 63, 98 and 73% when ME was provided by digestible protein (DCP), digestible crude fibre (DCF), digestible fat (DFat) and digestible nitrogen-free extract (NFE equivalent to organic matter-(protein+crude fibre+fat)), respectively. The coefficients can be calculated from equations reported in Tables 5 and 9. In growing pigs (i.e. for maintenance+growth), the efficiency of utilization of ME increased when more fat was included in the diet (Just, 1982a) and decreased with increased protein (Holmes et al., 1980; Just, 1982b; Noblet et al., 1987) or crude fibre (Just, 1982c; Gadeken et al., 1988). From his first experiments (Just, 1975 and 1982d) and all his experiments (Just et al., 1983a), Just and co-workers proposed overall relationships (Tables 4 and 9) indicating that the efficiency of utilization of ME was related to the energy concentration of the diet. Noblet et al. (1989a) obtained a series of relationships (Table 4) which indicated a positive contribution of dietary starch and fat and a negative effect of crude protein and fibre on the efficiency of ME utilization in growing pigs (maintenance+growth). The average efficiency measured on 41 diets was 74% but nutrients were used differently: 95, 85, 54, 57 and 0% for fat, starch, protein, hemicelluloses and cellulose, respectively. Even if the coefficients reported by these groups differ (see "Energy systems" section), they all indicate that digestible fibre is poorly utilized; this result is associated with the low efficiency of utilization of end-products of fibre digestion (volatile fatty acids) (Armstrong, 1969). This result is also consistent with the reduced efficiency observed when a larger proportion of the substrate is fermented in the hindgut (Just et al., 1983b; Noblet et al., 1989a). The depressive effect of crude protein on k is in agreement with the lower theoretical and experimental efficiencies of dietary protein either for maintenance or for fat gain and also with the low values reported for  $k_p$ .

Table 4. Effect of diet composition (g/kg dry matter) on the efficiency of utilization of ME for net energy (k, %) in growing pigs

N⁰	Equation	Reference
10	k = 66.7 + 0.031 x Fat - 0.052 x CF	Just et al. (1983a)
11	$k = 49.9 + 0.20 \times ME$ , % of Gross energy	Just <i>et al.</i> (1983a)
12	k = 67.4 + 0.051  x Fat + 0.019  x Starch - 0.018  x CP	Noblet et al. (1989a)
13	$k = 63.0 + 0.068 \times Digestible fat + 0.021 \times Starch$	Noblet <i>et al</i> . (1989a)

Many experiments (Thorbek, 1975; Gadeken *et al.*, 1988) indicate a close relationship between k and the proportion of energy retained as fat. This result is consistent with the difference between  $k_p$  and  $k_f$ . With regard to NE evaluation of feeds for growing animals, it is therefore important to control the partition of retained energy between protein and fat. Finally,  $k_g$  is apparently increased (up to 100%) when pigs are kept below their thermoneutral zone (Verstegen *et al.*, 1973; Noblet *et al.*, 1985 and 1989c), since the HI of feeding is partly used for thermoregulatory purposes. HI and its variations with diet composition will then be reduced under sub-optimal or cold climatic conditions (Noblet *et al.*, 1985 and 1989c). The practical consequence of this effect of environmental temperature on k is that NE measurements of feeds should be carried out under thermoneutral conditions.

Table 5. Prediction equations of DE and ME content of feeds (MJ/kg feed) from digestible nutrient contents (g/kg feed) in pigs

N⁰	Energy system	DCP	Regre coeffi	ession cients	Starch	DRes <sup>1</sup>	CV (%)	Reference
	, 		Dfat	DCF				
14	DE	0.0242	0.0394	0.0184		0.0170	1.1	Schiemann et al. (1972)
15	DE	0.0239	0.0363	0.0210		0.0167	0.8	Just (1982)
16	DE	0.0230	0.0394	0.0115		0.0175	0.6	Noblet et al. (1989a)
17	DE	0.0230	0.0394	0.0115	0.0175	0.0176	0.6	Noblet <i>et al</i> . (1989a)
18	ME	0.0208	0.0366	0.0143		0.0170	1.3	Schiemann et al. (1972)
19	ME	0.0215	0.0377	0.0197		0.0173	0.4	Just (1982)
20	ME <sup>2</sup>	0.0197	0.0397	0.0081		0.0174	0.8	Noblet <i>et al.</i> (1989a)
21	ME <sup>2</sup>	0.0200	0.0398	0.0083	0.0175	0.0168	0.8	Noblet <i>et al</i> . (1989a)

<sup>1</sup>DRes equivalent to digestible organic matter minus other nutrients considered in the equation. <sup>2</sup>Energy losses as methane included.

#### **Energy systems**

As described in the first section, an energy system results from the combination of one step of energy utilization (DE, ME and NE) and one prediction method within each step. DE, ME and NE available systems will be presented successively. Our attention will be focussed on most recent proposals.

#### Digestible energy

The DE content of a diet can be obtained directly on pigs kept in metabolism cages from determination of amounts of dietary and faecal energy. This method has been widely used for measurement of DE of raw materials reported in feeding tables (NRC, 1988; INRA, 1984; SCA, 1987). However, that approach cannot be used in routine measurements on a large number of samples and with a short response delay. Apart from taking mean values given in tables for raw materials, alternative methods have therefore been proposed.

For raw materials, a first approach is to relate the DE of a feed to its content of digestible nutrients. This method involves measurement of gross chemical composition and estimation of digestibility coefficients of nutrients, the DE content being predicted from regression equations (Table 5). Tables giving DC of nutrients according to the Weende procedure (CP, CF, Fat and NFE) are available (DLG, 1984; CVB, 1988). In comparison with mean tabulated DE values for raw materials, this method takes into account the variations in chemical composition of some ingredients but it assumes that DC are constant irrespective of the nutrient level in the feed and the presence of other nutrients (interactions). In addition, some DC are insufficiently accurate. A second approach is to use specific equations for prediction of DE value of ingredients of highly variable composition. Some published equations are presented in Table 6. However, their number is insufficient and for many ingredients, no equation is available.

DE content of compound feeds can be obtained by adding the DE contributions of ingredients and assuming no interaction. When the actual composition of the feed is unknown, the only possibility is to use prediction equations based on chemical criteria. Some of the numerous proposed equations are presented in Table 7. According to the authors, only a few predictors are considered (ie. equations with intercept) or all the chemical fractions representing the dry matter. The accuracy of these equations vary according to the experimental conditions (number and type of diets, analytical procedures, etc); in the best situations, the residual standard deviation and the coefficient of variation are about 250 KJ and 2%, respectively. The criteria to be considered for selecting the best equations when laboratory analytical variation is also taken into account have been discussed by Morgan *et al.* (1987). An additional criteria would be the cumulative cost of analytical procedures.

In all equations, fibre has an important effect on the accuracy of the prediction, with a superiority for NDF, in comparison with the classical CF (King and Taverner, 1975; Morgan et al., 1987; Noblet et al., 1989a). But as pointed out in the first section, the main limitation of such equations is their inability to consider the nature of fibre and, to a smaller extent, the composition of fat. The main consequence is that, according to the fibre source, the DE value of the diet when predicted from such equations will be either overestimated when fibre is poorly digestible or underestimated when fibre is highly digestible. The latter situation has been observed by King and Taverner (1975) with lupin meal or by Perez et al. (1984) and Noblet et al. (1989a) with diets containing sugar beet pulp or soyabean hulls. In all equations not including gross energy as a predictor, ash content has a significant and negative contribution to DE. However, the coefficient assigned to ash is usually higher than what would be expected from the dilution effect of ash (Just et al., 1984; Perez et al., 1984; Morgan et al., 1987; Noblet et al., 1989a) (Table 7). No clear physiological explanation for this result can be given; one hypothesis would be the increase in endogenous losses associated with the presence of minerals in the digestive tract.

In the first section, we have indicated that DCe is affected by feeding level, weight of the animals, etc. The validity of the DE predictive equations or their application to other situations depend therefore on the differences between the experimental conditions when the equations were established and those to which they will be applied. Consequently, it is logical to obtain systematic differences between predicted (from literature equation) and measured DE values of diets (Noblet *et al.*, 1989a). However, in similar experimental and analytical situations, the validity of one equation is satisfactory. For instance, the equation proposed by Noblet *et al.* (1989a) and based on ash, crude protein, fat and NDF (Table 7) predicts with a negligible difference the DE value of the 43 diets used by Perez *et al.* (1984).

From a practical point of view, the main question refers to the comparison of DE values of compound feeds when calculated by additivity of tabulated ingredients DE values (DEt; INRA, 1984), estimated from equations (DEe) and measured (DEm). Noblet *et al.* (1990a) showed that with 17 complex diets prepared from 13 ingredients and fed to 45 kg pigs given 90 to 95 % of their *ad libitum* intake, the agreement

Table	e. Prediction	equation	s of DE	content	(MJ/kg D)	M) of som	ie raw mate	erials for p	igs from	chemical	characteristics (g/kg DM)
z	Feed	İstuff				Eq	uation			R <sup>2</sup>	Reference
2	Barley			DE = 17.0	14 - 0.046 x	Ľ.				0.92	Perez et al. (1980)
ន	Cereals			DE = 15.6	60 + 0.0079	× CP + 0.	0244 × Fat	- 0.0356 × /	ADL	0.82	Wiseman & Cole (1980)
24	Cereals			DE = -7.5	2 + 1.36 x	Gross ene	rgy - 0.012	x NDF		0.85	Batterham et al. (1980a)
52	Wheat			DE = -4.3	5 + 1.17 x	Gross ene	rgy - 0.052	×G		0.94	Batterham et al. (1980a)
26	Wheat by-pr	oducts		DE = 17.7	75 - 0.042 ×	ADF	i			0.76	Batterham et al. (1980a)
27	Wheat by-pr	oducts		DE = 18.1	9 - 0.053 ×	ម្ល				•	INRA (1984)
28	Meat and bo	ne meal		DE = -4.6	3 + 0.021 >	<pre>&lt; CP + 0.0</pre>	48 × Fat			0.81	Batterham et al. (1980b)
5	Rapeseed me	al		DE = 17.2	28 + 0.020	x Fat - 0.0	30 × CF			0.77	Bourdon (1986)
8	Sunflower m	eal		DE = 16.8	35 - 0.033 ×	ß				0.92	: Perez et al. (1986)
31	Cassava mea	1		DE = 18.4	(1 - 0.046 ×	CF - 0.01	8 x Ash			'	INRA (1984)
Tabl	27. Prediction	equation	s of DE	content	of feeds ()	MJ/kg DN	1) from che	mical com	position	(g/kg DN	1) in pigs
å				Regres	sion coeffi	cients			$\mathbb{R}^2$	RSD	Reference
	Constant (	GE A	\sh	Ð	Fat	NDF	Starch	Res <sup>1</sup>			
32	-4.54 1.	177				-0.0168		2	0.94	0.44	King and Taverner (1975)
34	5.75 0.1	740 -0.1	0299			-0.0177			0.87	0.28	Perez et al. (1984)
35	17.66	- Q	0480	0.0090	0.0161	-0.0138			0.88	0.27	Perez et al. (1984)
37	5.62 0.4	692 -0.1	0223	0.0040		-0.0163				0.34	Morgan et al. (1987)
36	17.50	Ĩ	0325	0.0078	0.0157	-0.0149				0.32	Morgan <i>et al.</i> (1987)
38	18.50	Ŷ	0435	0.0056	0.0153	-0.0161			0.95	0.25	Noblet <i>et al.</i> (1989a)
39		Ŷ	0247	0.0241	0.0337	0.0024	0.0185	0.0184		0.26	Noblet <i>et al</i> . (1989a)
<sup>1</sup> Res	ie. Residue) coi	responds	to the	difference	e between	dry matte	r and the si	um of other	r nutrient	s conside	rred in the equation

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between DEe (from equation 38 in Table 7) and DEm was satisfactory. The average values were identical and the maximum difference between both values was 220 KJ per kg feed dry matter. On the other hand, average DEt overestimated by about 4.5% the average DEm, with overestimations ranging from 0 to 10% for the individual diets. The analysis of differences between DEm and DEt indicates they were correlated with the presence of high fibre feedstuffs (rapeseed and sunflower meal, corn gluten feed in our study) or the NDF content. Similarly to the conclusions of Morgan *et al.* (1987), DEm and DEt were equivalent in simple diets based on cereals and soyabean meal. Consequently, the additivity rule represents a simplification that is not acceptable when fibrous ingredients are included at high levels or in combination in diets for growing pigs.

One objective of the DE prediction equations is to estimate, for control or regulatory purposes, the energy value of diets when only chemical characteristics can be measured. It is impossible to give a preference for one published equation. For one author, it would be logical to favour the equations with the lowest residual standard deviation and practically applicable (repeatability and cost of analysis). Between authors, more attention should be paid to equations established with a large number and chemically variable diets, each diet being analyzed by different laboratories. Those proposed by Morgan *et al.* (1987) or by INRA (Perez *et al.*, 1984; Noblet *et al.*, 1989a) fulfill these conditions. Anyway, even if the accuracy of such equations is not high ( $\pm 0.5$  and 0.75 MJ per kg of feed with a 80 and 95% probability of success (Morgan *et al.*, 1987)), they are able to rank correctly, according to their DE content, available complex diets under similar analytical conditions, unless they contain fibre (and fat) sources with extreme digestibilities. In addition, these equations take into account possible negative interactions occurring with some diets. However,

in any case, they can predict the DE value of raw materials (Noblet et al., 1989a).

#### Metabolizable energy

The approaches for predicting ME value of pig feeds are similar to those described for DE. ME contents of raw materials are given in feed tables (NRC, 1988; DLG, 1984; INRA, 1984) but since direct ME measurements are not carried out routinely, tabulated values have been calculated from DE values with a ME:DE ratio either constant or, preferably, related to the protein content of the diet (INRA, 1984; NRC, 1988). ME is also predicted from equations relating ME to digestible nutrients content (Table 5). Digestibility coefficients are given in feeding tables for raw materials (CVB, 1988; DLG, 1984). Finally, like DE, ME content of mixed diets can be estimated from chemical composition (Table 8). In this case, the main difference between the corresponding equations for DE and ME concerns the coefficient of CP and, to a smaller extent, the coefficient of fibre (in equations where methane losses are considered). The limits for using DE equations also apply to ME equations.

Different corrections are subsequently applied to measured or calculated ME values, i.e. for a zero nitrogen balance (de Goey and Ewan, 1975), in order to take into account the energy loss as urea in urine or for the amount of fermented carbohydrates (DLG, 1984) on the assumption that the metabolic efficiency of fermented energy is lower. These corrections represent attempts to standardize evaluation conditions or to get closer to the estimation of NE. However, these correction factors are questionable, not always relevant and insufficient in order to estimate the true energy value of the diet.

#### Net energy

As mentioned above, no NE system for maintenance is available for pigs. The published systems combine the utilization of ME for maintenance and for growth (Just, 1982d; Ewan and co-workers; Noblet *et al.*, 1989a) or for fattening (Schiemann *et al.*, 1972). The most important equations are given in Table 9.

Following the earlier works of Kellner and Nehring, the research group of the Oskar Kellner Institute in Rostock (Germany) measured by indirect calorimetry the

Table	8. Predict	ion equ	lations of	ME cont	ent of fe	eds (MJ/k	g DM) fr	om chen	nical com	position	ı (g/kg	dry #	latter) in pigs
å				R	gression	coefficient	S				$\mathbb{R}^2$	RSD	Reference
	Constant	GE	Ash	Ð	Fat	CF	NDF	Starch	Sugars	Res <sup>1</sup>			
<b>4</b>	5.53	0.720	-0.0323				-0.0165				0.89	0.25	Perez et al. (1984)
41	17.78		-0.0479	0.0018	0.0166		-0.0135				0.89	0.26	Perez et al. (1984)
42	17.50		-0.0340	0.0059	0.0160		-0.0153						Morgan et al. (1987)
432	18.93		-0.0420		0.0156		-0.0164				0.95	0.25	Noblet et al. (1989a)
442			-0.0251	0.0213	0.0342		0.0024	0.0185		0.0179		0.25	Noblet et al. (1989a)
45				0.0203	0.0252	-0.0178				0.0162	0.76		Just et al. (1984)
46				0.0226	0.0319	-0.0129		0.0166	0.0184	0.0097		0.37	Kirchgessner & Roth (1983)
<sup>1</sup> See 7	able 7. <sup>2</sup> M	lethane	losses incl	uded.						-			
TaDIt	2. FIEUICI	חחוו באר		זובו בזובז	gy curren	BN/INI II	מושות לוח	rd in /ra	rr siarn ș	מחו כוובה	נוזרקו ה	in boo	MUDII BAR my maner
å					Equat	tion					CV (%		Reference
47	NE,=0.01	DCI × 60	TP + 0.036	1 × DFat	+ 0.0090	x DCF + 0	.0125 × D	NFE			3.8	х Х	chiemann et al. (1972)
48	NE=0.75	× ME -	1.88								2.3	Ŋ	st (1982)
49	NE=0.81	× ME -	2.20								3.6	<u> </u>	st et al. (1983)
50	NE6=0.01	104 × DC	CP + 0.037	0 × DFat	+ 0.0148	x Starch -	0.0041 ×	DCF + 0	.0118 × D	DRes <sup>1</sup>	2.2	Ž	oblet <i>et al.</i> (1989a)
51	NE6b=-1.	52 + 0.0	1125 × DCI	P + 0.039	18 x DFat	+ 0.0165 ×	Starch +	0.0141 x	: DRes <sup>1</sup>		2.0	Ž	oblet <i>et al</i> . (1989a)
52	NE19=0.6	663 × DI	3 - 0.0039	x CP + 0	.0095 × Fa	at - 0.0056	× CF + 0	.0032 × S	tarch		1.8	Ž	oblet <i>et al</i> . (1989a)
53	NE20=0.6	398 × M	E - 2.23								2.8	Ž	oblet <i>et al</i> . (1989a)
2	NE26=0.8	327 × M	E - 0.0058	× CP + 0	$0.0040 \times F$	at - 0.0044	к Ч				2.0	Ž	oblet <i>et al</i> . (1989a)
55	NE45=12	.11 - 0.0	282 × Ash	+ 0.0197	′ × Fat - 0	.0126 × NI	DF + 0.00	33 x Star	ch		2.5	Ž	oblet <i>et al.</i> (1989a)

<sup>1</sup>DRes= Digestible organic matter - (DCP + DFat + DCF + Starch).

energy retained (almost exclusively as fat) in 95 to 185 kg BW pigs which received 67 different diets (Schiemann et al., 1972). Most of the variation in the chemical composition of diets concerned the carbohydrate fraction since CF content (% of dry matter) ranged between 3.6 and 14.1%; the fibre sources were mainly forages and barley. A few diets contained high amounts of CP (six had more than 20%) and only one diet had more than 4% crude fat. The DE content varied between 12.2 and 15.8 MI/kg dry matter. Feeding levels were not constant (from 454 to 1180 KI DE per kg  $BW^{0.75}$ ). NE corresponded to the sum of retained energy and FHP (assumed as proportional to  $BW^{0.75}$  and estimated by regression to 280 KJ/kg<sup>0.75</sup>). The equation obtained from these measurements and based on Weende digestible nutrients (Table 9) does not take into account the heterogeneity of the NFE fraction. Later calculations (Hoffmann and Schiemann, 1985) and statistical analysis we have carried out on data of Schiemann et al. (M. Beyer, L. Hoffmann and W. Jentsch, 1990, personal communication) indicate a small interest, according to the type of diets they used, to partition the digestible NFE component into digestible starch and digestible residue (ie. digestible NFE-digestible starch) (Table 10). But their data were better described when an other exponent (0.60 from non-linear regression analysis) was used to calculate FHP, then equivalent to 630 KJ per kg BW<sup>0.60</sup>. Changes in the coefficients affected to the digestible nutrients according to the fractionation method or the estimate of FHP are described in Table 10. Using the coefficients given in equations 56 and 60, the efficiencies of utilization of ME for NE are then equivalent to 58, 100, 61, 76 and 76% when ME is provided by CP, fat, CF, starch and residue, respectively. In connection with the chemical characteristics of the diets, the accuracies of the coefficients for fat (±5.9 KI) and, to a smaller extent, for CF (±3.3 KI) in the NE equations, are low. Finally, the possible bias due to the large variations in feeding levels on the coefficients of the equations are difficult to appreciate. The equation proposed by Schiemann et al. (1972) is routinely used (after some adaptation) in several countries and feeding companies in Europe.

Studies conducted in Denmark by Just and co-workers were carried out on growing pigs between 20 and 90 kg liveweight, energy retained over that period being measured by the comparative slaughter technique. Feeding levels were comparable across the tested diets within a given series of experiments. A first prediction equation of NE was proposed in 1975 (Just, 1975 and  $\overline{1}$ 982d) from data obtained with 20 diets where most of the variation in chemical composition was obtained by changing the content of CF from cereals. A second equation, based on a larger number of diets, was published in 1983 (Just et al., 1983a) (Table 9). Several estimates of FHP were proposed for calculating NE: the preferred value suggested by the authors was 340 KJ per kg BW<sup>0.75</sup> or 618 KJ per kg BW<sup>0.60</sup> (Just et al., 1983a). Although these authors measured a negative effect of fibre and protein and a positive contribution of fat on the efficiency of utilization of ME in growing animals, they proposed a simple equation by only considering the effect of energy concentration (ME per kg dry matter). The negative intercept means that diets with high ME content (containing fat or starch) are better utilized. ME content is predicted from digestible nutrients content of the diet (Equation 19 in Table 5).

At Iowa State University, Ewan and co-workers measured the NE of ingredients using the comparative slaughter technique and the difference method (de Goey and Ewan, 1975). The tested ingredient was added at two or three feeding levels to a daily basal diet meeting the requirements for amino acids, minerals and vitamins. Feeds were given to piglets kept at about 26°C. Their initial BW was 5 to 7 kg and each experiment lasted four weeks. Estimate of FHP (291 KJ per kg BW<sup>0.75</sup>: Ewan, 1982) was obtained by regression of energy gain on ME intake and extrapolation to zero ME intake. ME is assumed to be used as efficiently for maintenance as for energy gain. So far, NE values have been estimated for about 20 ingredients (some of them are reported in Table 11) with an attempt to combine all data in a more general model (Ewan, 1989). Apart from the animal model whose digestive physiology is probably different from that in growing-finishing pigs or sows (especially with regard to

Table 10 to differ	l. Predicti ent calcula	ion equat ition metl	ions of n hods (fror	et energy n original	content I data and	(MJ/kg dr I personal	y matter) communio	of pig cation b	diets from Schiemann <i>et al.</i> (1971) data, according y Beyer, Jentsch and Hoffmann) <sup>1</sup>
å	Energy		R	egression	coefficien	ts		S	Reference
	system	DCP	Dfat	DCF	Starch	Sugars <sup>2</sup>	DRes <sup>3</sup>	(%)	
56	ME	0.0208	0.0352	0.0170	0.0172		0.0162	1.2	From authors' personal communication
57	BE	0.0109	0.0361	0600.0			0.0125	3.8	Schiemann et al. (1972)
58	NE	0.0110	0.0341		0.0129	0.0106	0.0120	3.7	Hoffmann and Schiemann (1985)
26	BE	0.0121	0.0365	0.0084			0.0129	3.3	From authors' personal communication
60	NE	0.0121	0.0354	0.0105	0.0131		0.0123	3.3	From authors' personal communication
<sup>1</sup> Fasting multiple matter av	heat prod linear and nd other d	uction wa d non lin igestible 1	is estimat ear regres nutrients c	ed as 280 ssions). <sup>4</sup> considerec	KJ per k Only mor 1 in the ex	g <sup>0.75</sup> in equ nosaccharic quation.	iations 57 des. <sup>3</sup> DRe	and 58 s corres	and 630 KJ per kg <sup>0.60</sup> in equations 59 and 60 (from ponds to the difference between digestible organic

digestive and metabolic utilization of fibre), the main limit of these studies concerns the generalization of the results for prediction of NE of ingredients whose chemical composition differs from the studied ingredient or, a fortiori, of not studied ingredients or diets.

The NE prediction equations established by Noblet *et al.* (1989a) are based on measurements carried out with 45 kg Large White boars fed balanced diets in order to maximize protein deposition and to simulate fast and lean modern pigs. The chemical composition (on a dry-matter basis) of the 41 investigated diets varied widely (11 to 27% for crude protein; 4 to 24% for NDF; 1 to 11% for crude fat and 30 to 62% for starch), the chemical characteristics being as independent as possible. Each diet was fed at two feeding levels (2.30 and 1.55 MJ ME per kg BW<sup>6.60</sup>) to four pigs kept in respiration chambers. NE was calculated as the sum of retained energy at the highest feeding level and FHP. Retained energy corresponded to ME intake minus heat production. FHP (750 KJ per kg BW<sup>0.60</sup>) was obtained by regression analysis of energy retained at both feeding levels on digestible nutrients intake. The relationship was not affected by weight of gut fill and activity of the pigs. Three approaches were used for NE prediction.

Table 11. Digestive and metabolic utilization of energy of some raw materials in piglets (see text for methodology) (from Ewan and co-workers, Iowa State University)

Feedstuff	DE (MJ/kg DM)	DCe (%)	MD:DE (%)	NE:ME (%)	Reference
Corn	16.07	86.8	96.8	69.0	de Goey and Ewan (1975)
Wheat	16.15	86.9	95.6	66.9	Wu and Ewan (1979)
Oats	13.43	68.6	95.9	50.9	de Goey and Ewan (1975)
Rice	15.48	84.5	96.8	70.1	Robles and Ewan (1982)
Rice bran	16.23	83.4	95.6	54.7	Robles and Ewan (1982)
Soyabean meal	17.53	88.9	94.6	55.1	R.C. Ewan, unpubl. data
Soyabean oil	31.63	80.5	96.3	75.4	Phillips and Ewan (1978)
Yeast protein	16.36	83.7	93.4	49.0	Pearson <i>et al</i> . (1978)

The first one (see equations 50 or 51 in Table 9) was comparable to the technique used by Schiemann *et al.* (1972) but different fractionation methods of digestible nutrients were compared. The second one (see equations 52, 53 and 54 in Table 9) compares with the approach of Just *et al.* (1983a) with DE (or ME) and chemical characteristics as predictors. The objective of the third one (see equation 55 in Table 9) was to predict NE of a diet from its chemical characteristics. All prediction equations were reported by Noblet *et al.* (1989a); the most important or directly applicable (from available feeding tables) ones are presented in Table 9. Equations resulting from the first two approaches can be used for raw materials and diets while it is not realistic to consider the equations based on crude nutrients for predicting NE value of raw materials. Compared to the previous studies of Schiemann *et al.* (1972) and Just *et al.* (1983a), our data demonstrate the advantage of a more appropriate fractionation of carbohydrates and particularly the importance of starch.

# Comparison of energy systems

#### DE, ME and NE systems

From equations 52 and 54 reported in Table 9, it is obvious that the hierarchy between diets obtained in the DE or ME systems will vary in the NE system with their specific chemical composition. The equations indicate that, relatively to the DE estimate, NE is reduced when protein and fibre contents are higher while it increased when more energy is provided by fat or starch. Since NE represents the best estimate of the "true" energy value of a diet, this means that the energy value of protein or fibrous feeds is overestimated when expressed on a DE (or ME) basis. On the other hand, fat or starch sources are underestimated in a DE system. This conclusion is clearly demonstrated in Tables 12 and 13 where DE, ME and NE values of some ingredients studied by Noblet *et al.* (1990a) are presented. The extreme example is given when animal fat and soyabean meal are compared: on a DE basis, animal fat energy content was equivalent to 1.8 the value of soyabean meal; on a NE basis, the ratio is 3.5.

Table 🗆	12.	Measured	digestive	and	metabolic	utiliza	tion (	of	energy	in	some	raw
materia	uls w	when incorp	orated in o	comp	lex diets (	adapted	from	N	oblet et	al.,	1989a	and
1990a)												

Feedstuff	DCe (%)	ME:DE (%)	NE:ME (%)
Wheat	87.5	97.7	76.8
Barley	82.1	97.8	78.0
Cassava meal	92.1	98.3	82.8
Peas	87.3	96.6	70.5
Soyabean meal	82.3	93.5	52.5
Animal fat	75.6	99.2	99.1

Table 13. Measured and calculated energy values of some raw materials when incorporated in complex diets (adapted from Noblet *et al.*, 1989a and 1990a)<sup>1</sup>

Feedstuff	DE	NE	NEr	NEj	NE6	NE19
Wheat (MJ/kg dry matter) As a percentage of wheat	16.17	12.14	11.04	9.97	12.29	12.33
Wheat	100	100	100	100	100	100
Barley	93	95	94	92	93	94
Cassava meal	98	106	104	98	106	104
Peas	100	91	96	99	91	91
Soyabean meal	101	66	80	96	65	72
Animal fat	184	241	244	204	225	237

<sup>1</sup>DE and NE are measured values while NE<sub>r</sub>, NE<sub>y</sub> NE6 and NE19 are calculated from digestible nutrients contents or DE and chemical characteristics (see Table 9).

In the study of Noblet *et al.* (1989a), the ratio NE:DE (mean: 71%) varied between 64 and 76%, the extreme values being obtained in unusual pig diets. With more realistic diets, the range would be smaller (from 68 to 73%). In other words, the advantage and the consequences of a NE system are much more important for the choice of ingredients (least-cost formulation) than for evaluating conventional pig diets (Table 13). However, economical or technical considerations are likely to produce diets of extreme composition. The superiority of a NE system is then clear. Finally, with regard to the comparison of ME and NE systems, the conclusions are similar to those given for DE and NE with the bias due to protein content being slightly attenuated.

#### Net energy systems

As described above, several equations (and therefore systems) for prediction of NE of diets which have been obtained in different situations (animal model, fractionation of nutrients, estimate of FHP, etc) are available. In order to evaluate these systems, the NE values measured on 41 diets (Noblet *et al.*, 1989a) and some ingredients (Table 13 from Noblet *et al.*, 1990a) whose DE, ME and digestible nutrients contents were measured, have been compared with the calculated NE values from

Schiemann *et al.* (1972) (NE<sub>r</sub>), Just *et al.* (1983a) (NE<sub>r</sub>) and Noblet *et al.* (1989a) (NE6 and NE19). Results are presented in Tables 12 and 13 for raw materials and Table 14 for diets.

Table 14. Relationship between calculated NE values (MJ/kg dry matter) according to different energy systems (NE<sub>rr</sub> NE<sub>jr</sub>, NE6, NE6b, NE19: see Table 9; calculations from measured digestible nutrients or DE and chemical characteristics of diets) and measured NE (NE) on 41 diets (from Noblet *et nl.*, 1989a)

N⁰	Equation	CV (%)	RSD
60	NE <sub>r</sub> =2.22 + 0.728 x NE	2.4	0.24
61	$NE = 0.68^{ns} + 0.821 \times NE$	2.3	0.23
62	NE6=1.24 + 0.883 x NE	1.9	0.20
63	$NE6b=1.00 \times NE$	1.9	0.21
64	NE19=1.00 × NE	1.7	0.18

The mean calculated NE values for the 41 diets were 9.95, 9.40 and 10.60 MJ per kg dry matter in the NE<sub>r</sub>, NE<sub>i</sub> and NE6 systems, respectively, while the measured mean value was 10.60 MJ. The mean difference between measured and calculated NE value is mainly due to differences in the estimate of FHP. However, the difference is not proportional to the energy content of the diet, especially for NE, (Table 14). In addition, the analysis of differences between measured and calculated NE of the diets indicates that, for NE,, the difference increases with increase in starch content and is reduced when the fibre content of the diet is increased. For NE<sub>v</sub> the difference is related positively to fat and starch contents of the diet and negatively to crude protein content (Noblet et al., 1989a). As expected, no difference between NE6, NE6b or NE19 estimates and measured NE was obtained; however, there was a tendency for overestimation of low energy diets with the NE6 equation. No bias was observed with NE19 and NE6b equations. These conclusions are accentuated when calculated NE values of raw materials are compared (Table 13). In other words, compared to measured NE values in 45 kg BW growing boars, NE, underestimates the energy value of starch rich feeds and overestimates the energy content of fibrous and, to a smaller extent, protein rich feeds. Similarly, NE<sub>i</sub> underestimates high fat or starch feeds and overestimates protein rich feeds. The discrepancy between NE<sub>i</sub> and measured NE is not so surprising if we consider the biologically limited significance of the overall correction factor applied to ME in the  $NE_i$  equation (Table 9); the consequences are quantitatively important when the NE, system is applied to feedstuffs. With regard to NE, the difference can be due to some limits in the experimental design adopted by Schiemann et al. (1972) and also to differences in the animal model (see above).

# Validation of energy systems

NE equations are obtained from energy balance measurements conducted under specific conditions (animals, body gain composition, experimental design, analytical procedures, statistical models, etc). Each system should then be tested under practical conditions in order to validate the proposed equations. The NE measurements carried out by Just and co-workers were correlated with growth performance since the comparative slaughter technique was applied over the total growth phase. But in such circumstances, energy retention measurements are probably less accurate and affected by environmental conditions which cannot be kept constant, or at least comparable, over successive series of experiments. Since a rather high proportion (90% according to Noblet *et al.*, 1989) of the variance in NE is explained by the DE (or ME) of the diet, we can assume that the relative inaccuracy of their balance measurements did not allow inclusion of any significant criteria in addition to ME. Even if the simplified NE<sub>i</sub> equation is acceptable for standard pig diets, it is totally inadequate for raw materials.

A validation experiment of the NE<sub>r</sub> system was conducted in the Netherlands

(Borggreve *et al.*, 1975) which indicated that the energy value was underestimated for high starch diets. This conclusion would agree with those obtained in the comparison of NE, and measured NE on diets or raw materials (Noblet *et al.*, 1989a).

With regard to the NE equations proposed by Noblet et al. (1989a), a first validation was given when measured and calculated NE values of feedstuffs were compared. As illustrated in Table 13 (from Noblet et al., 1990a), the agreement was satisfactory between both values for most ingredients. A second validation was carried out recently (J. Noblet et al., unpublished data) by measuring growth performance (growth rate and body composition at slaughter) in 540 pigs kept in 5 different locations and fed, according to an energy feeding scale, in order to obtain similar growth performance between diets. Eight diets which differed widely in their energy content and their chemical composition were tested. Measurements of DE, ME and digestible nutrient contents at 45 and 70 kg BW allowed calculations of NE values of diets. The feed energy:BW gain ratio of each diet was calculated for each energy system (DE, NE., NE., NE6, NE19 and NE56: see Table 9) and adjusted for a similar BW gain and composition (carcass muscle percentage) of the gain between diets. The efficacy of a system was reflected in both the low variability (coefficient of variation) of the feed:gain ratio and the abscence of any systematic bias related to composition of diets. Mean daily BW gain and feed; gain ratio were 730 g and 2.59 kg dry matter per kg gain, respectively. Results indicate that the between diets coefficient of variation of the feed energy: gain ratio was 1.9, .9, 1.6, 1.2, 1.2 and .8% for DE, NE., NE, NE6, NE19 and NE45, respectively. In the NE, system, the ratio was negatively correlated to the energy concentration of the diet. In the other systems, no significant correlation was observed. The results of this growth trial confirm that DE and NE values are not accurate predictors of growing pig performance while, in the NE, system, diets with high energy concentration are underestimated. Surprisingly, NE predicted from gross chemical composition (NE45) was the best compromise. This last result would mean that the results of the digestibility trial which were obtained under specific conditions (feeding level, BW, animals, etc) are not representative of the actual mean digestive utilization in the growth trial. On the other hand, NE45 ignores the variations due to animals and feeding conditions.

#### Conclusions

The information reported in this review shows the limits and inadequacies of all energy systems. None of them is able to predict the "true" energy value of a diet and, subsequently, the performance of the pigs. However, NE systems should be preferred, especially for assessing the energy value of raw materials. In addition, the recent system proposed by Noblet et al. (1989a) which considers more information on chemical characteristics seems able to predict NE values of both ingredients and diets. The NE value is satisfactorily correlated to pig performance. The main limit for predicting correctly the energy value of a diet remains the estimation of its DE or digestible nutrients content. Indeed, variation of digestibility coefficients with feeding level or physiological stage and negative interactive effects have been indicated. Consequently, the commonly accepted concept that DE content of a diet is only related to its composition cannot be further accepted since animal and other dietary factors modify the hierarchy between diets or ingredients. For some ingredients, a range for most probable DE (and ME and NE) values could then be suggested. These observations and the tendency for preparing more complex pig diets (including more by-products) emphasize the importance of future studies on digestive interactions.

DE, ME and NE of feeds when only chemical composition information is available can be predicted from equations with a satisfactory degree of accuracy. Such relationships could be proposed for legislation purposes but they cannot be applied to predict the energy value of raw materials. However, the accuracy of the prediction relies on the adequacy of analytical procedures. Since fibre is the main variation factor of digestive utilization of the diet, more emphasis should be given to routine techniques that identify the nutritional and physiological "quality" of dietary fibre.

For least-cost formulation purposes, the most important objective is to predict correctly the nutritional (mainly protein, energy and "technological") value of raw materials. With regard to energy content, it is clear that NE systems should be preferred. In addition, the results of formulation are directly dependent on the energy system which is adopted since relative energy values of ingredients differ between systems. For instance, diets formulated on a DE basis contain more protein than on a NE basis (D. Sauvant *et al.*, unpublished). In practical conditions, NE value of raw materials can be calculated either from DE (or ME) values given in feeding tables (INRA, 1984; SCA, 1987; NRC, 1988) (equation NE19, Table 9) or from digestible nutrients content (equation NE6, Table 9) estimated from tables (DLG, 1985; CVB, 1988) and where digestible nitrogen-free extract is divided into starch (assumed to be 100% digestible) and digestible residue.

When energy values of feeds are expressed on a NE basis, net energy requirements of pigs are theoretically independent on the diet composition. This conclusion assumes that a given diet is similarly used in all physiological situations. Literature on NE values for pigs consider exclusively growing or fattening animals; no information is available for maintenance even though it amounts to about 80% of energy requirements of pregnant sows. Nutrients and, consequently, raw materials, are probably used differently for maintenance and for growth. In addition, growth is not a stable process since the ratio between protein and fat in the gain and the proportion of energy intake used for maintenance vary continuously. Therefore, complementary studies are in progress in order to analyze the adequacy of the NE system proposed by Noblet *et al.* (1989a) to various physiological situations. Most of our attention is focussed on ME utilization for maintenance and also according to composition of BW gain. Because of methodological difficulties, ME utilization during lactation can be assumed to be comparable to that obtained during the growing phase.

Finally, feed industry requires rapid and accurate prediction methods for evaluating the nutritional value of feeds. Measurements with animals have interest only in research or for calibration purposes. Use of values in feeding tables or predicted from equations give a reasonable estimate of the nutritional value of ingredients. However, available analytical procedures are often insufficient. Most of the progress in rapidity and reliability of nutritional value estimates of pig feeds will come from proposals concerning the application of in vitro methods or more sophisticated physico-chemical techniques. The main interest of studies on animals will then concern the applicability and the limits of such estimates. Finally, on a long term basis, progress in pig nutrition will come from mechanistic approaches where both the animals requirements and dietary nutrients utilization will be considered in a biochemical and regulatory approach.

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# **IDEAL PROTEIN: ITS VARIABLE COMPOSITION!**

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The concept of 'ideal protein' has been promulgated widely in recent years for the formulation of pig diets. The advantage as stated by Fuller and Wang (1990) is that it allows specification of "the complete amino acid requirements of the pig in one variable; ie. 'ideal protein'". This statement implies, and often has been interpreted to mean, that there is only one amino acid composition for 'ideal protein' in the pig. Although this interpretation was not initially intended, its adoption is widespread and can produce inaccuracies in diets formulated to meet animal requirements.

The composition of 'ideal protein', ie. the relative proportions of required amino acids, varies because the amino acid profiles needed for each body function (body protein synthesis, milk protein synthesis, inevitable amino acid catabolism and net endogenous amino acid losses into the gut) are different, and the relative contributions of each function to total requirements change with level of feeding, stage of growth, genotype and reproductive phase of the animal. As an example, the AUSPIG model is used to illustrate the effect of genotype, stage of growth and composition of the diet on the required ratio of available threonine to lysine (Figure 1). Growth from 30 to 60 kg was simulated for entire male pigs of a highly selected modern strain (1990) and a strain typical of smaller piggeries ten years ago (1980). The initial diet contained 83% wheat grain with the remainder from soyabean meal, blood meal, meat and bone meal, lysine and methionine. Wheat was replaced by barley in the diet introduced at 40 kg and 25% lucerne meal was included in the diet introduced at 50 kg. The digestible energy (MJ/kg) and neutral detergent fibre (%) contents of the diets were, respectively for the three diets, 14.3, 13.0; 12.8, 19.0; 11.2, 23.9.

The required threonine to lysine ratio increased for both genotypes as body weight increased, was higher for the 1980 than for the 1990 strain and increased with the fibre content of the diet. In each case, the higher ratio was associated with a higher proportion of the total requirement coming from endogenous losses and reflects the larger proportion of threonine in ileal amino acid secretions compared with that in body protein. This demonstrates the variable nature of 'ideal protein' and shows that the concept of 'ideal protein' is irrelevant when accurate simulation models can be used.



Figure 1. Predicted threonine to lysine requirements for highly selected (1990) and older (1980) strain pigs fed diets varying in fibre content.



# UTILIZATION OF ILEAL DIGESTIBLE TRYPTOPHAN FROM DIFFERENT PROTEIN SOURCES BY GROWING PIGS

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Previous work indicated that values for the ileal digestibility of lysine (Batterham *et. al.*, 1990) and threonine (Beech *et. al.*, 1991) in heat-processed proteins were unsuitable in formulating diets as they did not reflect the amount available to the pig. The aims of this experiment were to determine 1) whether tryptophan was similarly affected and 2) the retention of ileal digestible tryptophan by growing pigs.

The ileal digestibility of tryptophan in cottonseed meal (CSM) (0.81), meat and bone meal (MBM) (0.65) and soyabean meal (SBM) (0.90) was determined with pigs fitted with simple T-piece cannulas. Tryptophan utilization was then determined in a second experiment with three sugar-based diets containing 225 g/kg CSM, 275 g/kg MBM, or 167 g/kg SBM as the only source of protein. Diets were formulated to 0.065 g ileal digestible tryptophan/MJ DE. Additional free tryptophan (0.47 of ileal digestible tryptophan supplied) was needed in the diet containing MBM because increased inclusion of MBM would have resulted in excessive calcium. Other essential amino acids were added to ensure a 29% surplus relative to tryptophan. An additional three diets were supplemented with free tryptophan to demonstrate that tryptophan was limiting in the first three diets. The pigs were fed 3 hourly, at 3 x maintenance  $(0.5W_{kg}^{0.75})$ , over the 20-45 kg growth phase. The pigs were then slaughtered and the tryptophan content in the empty bodies determined. Tryptophan was determined by Degussa AG, Germany.

 Table 1. Performance of pigs given diets formulated to 0.065 g ileal digestible

 tryptophan/MJ DE over the 20-45 kg growth phase

	Pı	CEM		
	CSM	MBM	SBM	SEM
Liveweight gain (g/d)	393	531	437	27.7
Feed conversion ratio	2.9	2.4	2.4	0.07
Crude protein deposition (g/d) Tryptophan retention:	. 54	75	63	3.5
ileal digestible tryptophan intake	0.46	0.45	0.38	0.010

Growth and protein deposition of the pigs given the diet containing MBM were significantly greater (P<0.05) than those fed SBM and CSM, whilst the retention of ileal digestible tryptophan was higher in pigs fed CSM and MBM, relative to SBM (P<0.05).

The superior growth of pigs fed MBM indicates that either a) the ileal digestible value for tryptophan in MBM underestimated availability, or b) free tryptophan had greater growth promoting ability than the ileal digestible tryptophan from the other two protein sources. Tryptophan was less retained than what has been observed for lysine (0.75, Batterham *et al.*, 1990) and threonine (0.64, Beech *et al.*, 1991) in soyabean meal.

Overall, these results indicate that ileal digestible values for tryptophan are not effective in dietary formulations as they do not reflect the amount of tryptophan that is available to the pig.

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# *IN VITRO* DIGESTIBILITY OF NITROGEN - A PRACTICAL APPROACH TO THE ASSESMENT OF APPARENT ILEAL DIGESTIBILITY IN MIXTURES AND RAW MATERIALS FOR PIGS

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A rapid and inexpensive laboratory method for the estimation of N digestibility in pigs has been developed. The method, which intends to simulate digestive processes in the pig, is based on incubation of feed samples with industrially purified enzymes (HCl/Pepsin and Pancreatin) in a two step process.

The *in vitro* estimate of N digestibility, however, reflects true rather than apparent *in vivo* ileal digestibility. Therefore, *in vitro* values were adjusted by calculating the endogenous contribution (Nend) as a linear function of the indigested dry matter (IDM): Nend = k \* IDM

The value of "k" was preliminarily obtained by assuming that the difference between *in vitro* and *in vivo* estimates of digestible N (g/kg feed DM) reflected the endogenous excretion (g/kg feed DM).

Eight samples of common feedstuffs, previously assayed with ileum-cannulated pigs (Just *et al.*,1985) were analysed *in vitro* (Table 1).

			Digestibility (%)				
	N in feed (g/kg DM)		In vitro				
_		True	Adjusted	Apparent			
Barley	19.0	85	66	70			
Rye	18.2	87	73	65			
Wheat	23.2	91	76	74			
Oats	18.2	89	67	61			
Soybean meal	29.1	92	79	78			
Rapeseed meal	31.2	83	68	69			
Sunflower meal	31.4	90	73	73			
Grass meal	14.2	74	36	35			

Table 1. In vitro digestibility of N compared with in vivo apparent ileal values

The results show a satisfactory agreement between *in vitro* and *in vivo* values (r=0.96). In this study, however, the value of "k" was not generated completely independent of the test material. A larger number of samples is therefore currently being analysed.

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# SYMPOSIUM: NEW DEVELOPMENTS IN THE UNDERSTANDING OF PROLIFERATIVE ENTERITIS

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# Symposium introduction

Throughout Australia many pig producers add an antibacterial agent to their grower and finisher diets; contributing as much as \$2.70 to the cost of producing each pig. While the practice may result in some benefits in growth rate and feed efficiency, many producers make the expenditure solely in the hope and expectation of controlling proliferative enteritis, a disease which causes diarrhoea or acute deaths in grower and finisher pigs. Pig producers call the disease 'campylobacter'; a hangover from the recovery of *Campylobacter* spp bacteria from the intestine of affected pigs.

Historically, the disease has attracted many names. It is not a new disease. Biester and Schwarte (1931) described it as an intestinal adenoma and since then the terms proliferative haemorrhagic enteropathy, necrotic enteritis, regional ileitis and terminal ileitis have been added, as people seek to define the disease. Four terms describing the range of syndromes which occur in proliferative enteritis are used here. They are porcine intestinal adenomatosis (PIA), proliferative haemorrhagic enteropathy (PHE), necrotic enteritis and regional ileitis.

Proliferative enteritis is a fickle disease. It is expressed differently on different farms. On some, production without prophylactic medication is precarious. These farms risk substantial growth rate collapse in the face of an outbreak of PIA. On other farms disease outbreaks occur infrequently, and respond quickly to treatment. Similar uncertainty follows PHE. Introduction of breeding stock to farms may be a simple process, but it can be marred by acute episodes of the characteristic haemorrhagic disease of the small intestine and death in the introduced stock. The link between these two apparently different forms of proliferative enteritis and the others is the basic lesion - the presence of adenomatous intestinal crypt epithelium containing *Campylobacter*-like organisms.

The precise nature of the *Campylobacter*-like organisms (CLO) is unclear. Observation of numerous curved organisms in silver stained histological sections of affected gut and recovery of *Campylobacter mucosalis* and *C. hyointestinalis* originally led to the belief that these bacteria were intimately associated with the disease. While they may have some role in the pathogenesis of the disease it is now clear that they are not the cause of proliferative enteritis. Researchers have not been able to satisfy Koch's postulates regarding these two organisms and the disease (Roberts *et al.*, 1980; McCartney *et al.*, 1984).

More recently, McOrist *et al.* (1989a) have demonstrated that the curved shaped CLO, while apparently associated with the disease, were not known *Campylobacter* spp bacteria.

The challenge to discover the cause of the disease has intensified because the organism has not been cultured in artificial media. Serological tests require large quantities of antigen. Currently the only form of antigen available is a partially purified filtrate of affected intestinal mucosal homogenate that is laborious to prepare (McOrist, 1988; Monckton *et al.*, 1991).

Despite the uncertainty of the aetiological diagnosis and a poor understanding of the disease process the disease is easily diagnosed at autopsy. More surprising for such an apparently complex syndrome is that it is easily and relatively cheaply treated in the field. Clinical episodes of the disease, with the exception of the more chronic forms (necrotic enteritis and regional ileitis), respond well to a range of antibacterial agents.

Problems arise when producers, because of the disease's apparent variability,

prefer to take a measure of disease control insurance by continuously feeding their pigs low level antibiotics. This adds to the cost of production and also the residue risk. The latter assumes increasing importance with regard to consumer attitudes against detectable residues. Therefore, as antibiotics and bacteriostatic agents are removed from diets in compliance with community concerns, export conditions and attempts to reduce the cost of production, the importance of proliferative enteritis will increase.

This symposium examines the disease on the three fronts representative of the thrust of the Bendigo group's research, field and diagnostic efforts.

The clinical nature of the disease and its epidemiology on farms and throughout Australia is presented by Dr P. Holyoake. This paper summarises the distribution of the disease, the forms which occur, case histories and draws together some possible risk factors.

In "The Pathology of Proliferative Enteritis", Dr L. Sims has pieced together aspects of the nature of the lesion and the underlying disease processes. Dr R. Monckton has dealt with the problems of detecting the CLO and preparing diagnostic reagents in the absence of a readily available supply of antigen. It is from developments in the identification and culture of the CLO that the next breakthroughs in knowledge of this disease will occur.

The key to an understanding of proliferative enteritis rests with the isolation of the CLO. The sequence of events is likely to involve satisfying Koch's postulates, establishing via serological tests the distribution of the disease both within populations on-farm and nationally, and developing more cost effective and epidemiologically sound methods of control. The development of a vaccine would be the next logical step.

# THE EPIDEMIOLOGY OF FIELD CASES OF PROLIFERATIVE ENTERITIS

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#### Introduction

Proliferative enteritis (PE) (Lomax *et al.*, 1990; Chang *et al.*, 1984) is an enteric disease of grower-finisher pigs. Its major effect is to decrease productivity through growth rate depression and diarrhoea. A sudden death syndrome also exists.

This paper discusses the aetiology, clinical signs and epidemiology of PE. Statistics on prevalence obtained from Australian veterinary diagnostic laboratories, abattoirs and farms are presented. Possible risk factors are outlined with particular reference to medication strategies available for disease treatment and control. Elements of case histories documenting the herd impact of PE are presented.

## Aetiology

Proliferative enteritis has attracted a range of names in an attempt to describe the disease. They include intestinal adenomatosis complex (Barker and van Dreumel, 1985), porcine intestinal adenomatosis (PIA), necrotic enteritis (NE) (Rowland and Lawson, 1986), proliferative haemorrhagic enteropathy (PHE) (Love and Love, 1977), regional ileitis (RI) (Jönsson and Martinsson, 1976), haemorrhagic bowel syndrome (O'Neill, 1970) and porcine proliferative enteropathies (Rowland and Lawson, 1986). More recently the disease has been known by the misnomer "*Campylobacter spp* induced enteritis" (Straw, 1990) due to the irregular isolation of various *Campylobacter* species from proliferative lesions. These included *C. mucosalis* (Lawson *et al.*, 1981) and *C. hyointestinalis* (Gebhart *et al.*, 1985). However oral challenge of susceptible pigs with these organisms do not produce disease (Roberts *et al.*, 1980; McCartney *et al.*, 1984).

Organisms morphologically similar to *Campylobacter* spp bacteria can be detected by Warthin-Starry or fluorescent monoclonal antibody staining within infected enterocytes accompanying proliferative lesions (Lawson *et al.*, 1985). These *Campylobacter*-like organisms (CLO) are antigenically distinct from known species of *Campylobacter* and are therefore thought to be either a different bacterial species, or an unidentified *Campylobacter* (McOrist *et al.*, 1989). CLO-infected enteric scrapings and mucosa produced disease in susceptible animals when introduced via stomach tube. No *Campylobacter* species could be cultured from the inoculate (McOrist, 1988; Mapother *et al.*, 1987), adding further weight to the theory that CLO may be involved in the aetiology. However, the CLO are elusive. Despite repeated attempts, culture of the CLO by routine techniques has been unsuccessful.

#### Clinical signs

Proliferative enteritis manifests in two main forms: a non-haemorrhagic form represented by PIA, RI and NE; and PHE, the haemorrhagic form of the disease. In the non-haemorrhagic form of the disease (NHPE), young grower pigs, typically within the 6 to 20 week old age group may show a reduced growth rate. In the more severe cases, a mild scour, anorexia, weight loss and occasionally death are evident (Roberts *et al.*, 1979; Jönsson and Martinsson, 1976).

Subclinical infection may be present within a herd but unless growth rates are monitored closely or an unthrifty pig appears, it will not be detected. One of the major concerns of the pig farmer is that the disease may appear suddenly causing widespread stock losses and illthrift, only to disappear as unexpectedly (Love, 1981). Roberts *et al.* (1979) evaluated the weight gain of pigs in an hysterectomy-derived herd by the cumulative sum technique. They found that seven of the nine animals selected by this technique for poor growth were affected with porcine intestinal adenomatosis. No clinical signs were obvious amongst selected pigs.

In a trial involving 96 pigs on a minimal disease piggery, marked illthrift occurred affecting 19 (19.8%) pigs within the experimental group. Growth rate decline commenced at 31kg body weight, affecting performance for a period of 1-2 weeks. The daily rate of gain over a 6 week period in unthrifty pigs was an average of 77.3% (range 17-98%) of the weight gain of unaffected counterparts. PE was diagnosed within the herd on the basis of intestinal pathology (Gogolowski *et al.*, in press). This reduction in rate of weight gain may also affect the reproductive performance of the gilt herd by delaying maturity and therefore the age of first mating. Infected pigs may also have reduced litter size as a consequence of being mated at a lower weight (Holyoake, unpublished).

The other form of PE, proliferative haemorrhagic enteropathy (PHE) affects older pigs, usually from 20-30 weeks of age. Disease in pigs of this age manifests as dysentery with production of a black tarry scour. Affected animals succumb rapidly, usually within 24 hours of the onset of scouring. Anaemic carcases are evident at slaughter or post mortem examination (Love *et al.*, 1977; Jeng *et al.*, 1987).

PE has an incubation period of about 21 days. Love *et al.* (1977) found that clinical disease occurred in susceptible pigs as early as three week after entry into an infected breeding unit, but more frequently in the period four to ten weeks after their introduction. Experimental challenge with CLO resulted in clinical signs 21 and 37 days after inoculation. Untreated pigs housed in contact with inoculated pigs exhibited clinical signs after a lag period of 12 days (Holyoake, unpublished). Gebhart *et al.* (1985) found that hamsters developed grossly thickened ilea with cryptal hyperplasia and intracellular *Campylobacter*-shaped organisms three weeks post challenge with intestinal scrapings from pigs with PE.

# Epidemiology

# The cost of PE

There is little information on the economic importance of PE. Cutler and Gardner (1988) estimated that PE costs the Australian pig industry \$26 per sow per year in stock losses and control by medication. This estimate was based on a broad assessment of the impact of PE on the Australian herd. The estimate suffers from the paucity of information regarding herd prevalence, frequency of occurrence and severity.

# Prevalence

# Abattoir prevalence

Herd prevalence as determined by abattoir monitoring varies widely between states. In Victoria, infection is present on 4% of farms surveyed (Cutler, unpublished), whilst in other states prevalences ranging from 3% to 37% in South Australia (Pointon, 1988; Pointon, 1989), 19% in Western Australia (Mercy and Skirrow, personal communication) and 29% in Queensland (Marr, 1986) are recorded.

How prevalence data collected during abattoir surveys relates to clinical disease on-farm is not widely documented. Pointon (1988) reported that disease associations existed between the presence of typical pathology at slaughter and the occurrence of clinical disease on 67% of 21 farms surveyed. He suggested that if lesions were detected at slaughter and acute deaths had not yet occurred, it was likely that clinical PE would follow soon after in finisher pigs.

In our studies, investigations were carried out on seven pig farms experiencing PE to determine the relationship between the presence of gross intestinal pathology typical of PE at slaughter and the signs of disease on-farm. The cases of PE were diagnosed through the clinical signs on farms confirmed by pathological examination. Five of the seven farms demonstrated proliferative lesions in follow-up abattoir checks conducted within two weeks of the farm outbreak. On two farms with clinical disease, follow up abattoir checks failed to detect lesions. These results are summarised in Table 1.

Three additional herds in which pigs demonstrated gross changes typical of PIA at slaughter were investigated clinically. Only one farm reported scours. Approximately 10% of its 11 to 14 week old grower pigs were affected. No finisher pigs were affected on any of the 3 farms. Within-herd lesion frequency ranged from 3 to 18% at the abattoirs.

The results from Table 1 indicate that the 'on-farm' status of PHE in older pigs is more likely to be reflected on post mortem diagnosis at an abattoir than in cases of NHPE in grower-aged stock. Pointon (1988) found that the presence of intestinal pathology typical of PE at slaughter may be an indicator of an impending PHE outbreak on-farm. Because of the speed at which the intestinal mucosa turns over, spontaneous recovery may occur in the gut of the NHPE-infected grower pig before it reaches bacon weight.

Lesions associated with NHPE are often mild, despite the drop in growth rate and scouring which may occur in affected stock. It is also possible that these mild lesions may be missed at slaughter or may be confused with normal peristaltic contractions of rigor mortis. Detection sensitivity may therefore be lower in cases of NHPE.

At slaughter, although lesions typical of PE may be detected in bacon-aged pigs, disease is not always obvious on-farm because it may be manifesting only as a depression in growth rate without obvious scouring, ie. a subclinical disease. Compensations in electrolyte and fluid reabsorption may be occurring in the large intestine.

We can conclude from these investigations that although PE is present on a farm, disease may not be detected at slaughter. Although abattoir health checks are a

useful aid to PE diagnosis, results should be used in conjunction with an investigation of the disease "on-farm" to determine the extent and severity of PE.

Farm number	Age range of pigs (weeks)	Form of PE <sup>1</sup> in pigs	Prevalence at abattoir (%)	Number of pigs examined
1	6-20	NHPE	nil	40
2	6-15	NHPE	nil	40
3	18	NHPE	18	11
4	20-30	PHE	3	60
5	25-35	PHE	7	27
6	20-30	PHE	15	40
7	16-24	PHE	39	130

Table 1.	Comparison	between	PE	detected	at	abattoirs	and	on	seven	farms	in	1990
(Holyoak	e, unpublishe	ed)										

<sup>1</sup>Form of PE: NHPE = non haemorrhagic proliferative enteritis; PHE = proliferative haemorrhagic enteropathy.

#### Field prevalence

For such an apparently common disease, very little information is available on the prevalence of PE in pig herds. Rowland and Lawson (1986) reported that 0.25% of stock coming through gilt and boar testing stations in the UK had PHE. Other estimates of herd prevalence include 2.5% (Roberts *et al.*, 1979), 0.89% (Jackson and Baker, 1980) and a range of 1 to 10% (Lomax *et al.*, 1990).

It is difficult to assess the prevalence of PE. Data collected from veterinary diagnostic laboratories in Australia indicate that PE is diagnosed at a low but variable frequency. Over the six year period from 1985 to 1990, there were no diagnoses in South Australia, 7 in Tasmania, 12 in Queensland, 49 in Victoria and 184 in New South Wales. (Holyoake, unpublished data).

This low frequency may be a reflection on the low prevalence of disease, or it may indicate that farmers and veterinarians diagnose the disease in the absence of laboratory confirmation.

The frequency of diagnosis increased from 31 cases in 1985 to 83 cases in 1990. This could be due to an increase in the number of pig submissions or an increase in the actual number of PE cases occurring.

All laboratories based their diagnosis on a combination of gross examination and histopathology. Warthin Starry stains were also commonly used to demonstrate the presence of CLO within infected enterocytes. Eleven of the 18 laboratories surveyed attempted culture for *Campylobacter* spp despite evidence (McOrist *et al.*, 1987) that they are not associated with the aetiology of the disease.

The two syndromes NHPE and PHE occurred with almost equivalent frequency. PHE was diagnosed in both 6 week old pigs and parity 6 sows whilst NHPE affected pigs from 6 to 52 weeks of age. (Table 2). Ward and Winkelman (1990) proposed that pigs less than 4 weeks of age are protected from infection by passive colostral immunity acquired from the dam. They also state that stock greater than one year of age are resistant to infection due to previous exposure to the organism. If this is so, our data indicate some sows either escape infection, have a reduced or succumb to exterme challenge by the CLO.

#### Farm surveys

Clinical signs of PE were observed on 40 farms drawn from a random sample of 71 farms surveyed retrospectively during the period 1988 to 1990. Seventy five percent of the cases occurred in the grower house and 25% in the finisher/breeder herd. A diagnosis made by a veterinarian based on a combination of clinical signs, gross pathology with or without histopathology was made on 28% of farms. It would appear that on the basis of a 4% prevalence level seen at Victorian abattoirs, compared with the 28% prevalence for veterinary diagnoses in the field, many PE lesions heal by the time the animal reaches bacon weight or lesions are too subtle to be detected. Clearly, PE is a common disease with a prevalence of at least 28% and possible as high as 56%, although some of the clinical signs may have been those of swine dysentery, which was not differentiated by the farmers.

Laboratory	Total PE cases (1985-1990)	Total PHE cases	Age group (weeks)	Total NHPE cases	Age group (weeks)
Bendigo	40 <sup>1</sup>	22	18 - parity 6 sow	12	8-24
Hamilton	5	2	14-36	3	8-16
Attwood	1	0	0	1	_2
Melbourne Uni	3	3	12-32	0	-
Orange	31	-	6-52	-	6-52
Camden	1	0	0	-	-
Armidale	39	less frequent than NHPE	-	more frequent than PHE	-
Wollongbar	22	9	7-16	13	5-44
Mt Pleasant	7	2	-	5	-
Total		38		35	

Table 2.	The	diagnosis	of PHE	and	NHPE	at	Australian	veterinary	laboratories	and
the age g	roup	of affected	d pigs							

<sup>1</sup>Form of PE (PHE/NHPE) not stated in six cases; -<sup>2</sup> Information not supplied.

#### Epidemiological variables associated with PE

CLO are thought to be shed through faecal contamination, however its infectivity, survival time in the environment or the role of carrier animals such as rodents in the spread of disease are unstudied. The disease does exist in rodents (Vandenberghe *et al.*, 1985) and has been produced in hamsters using porcine origin ileal lesions (McOrist and Lawson, 1987). No test is available to detect carrier animals although fluorescent monoclonal antibodies may prove useful.

The factors which predispose a herd to infection are largely undetermined. Environmental factors such as herd size, feed type, seasonality, rodent population and source of stock may all contribute to the occurrence of outbreaks.

In the retrospective farm survey (Holyoake, unpublished), herds with more than 100 sows experienced 83% of the reported outbreaks of PE (P = 0.037) (Table 3). Inspection of 196 South Australian pig herds between 1985 and 1987 revealed a significant relationship between herd size and the prevalence of PE lesions at first inspection at slaughter (Pointon *et al.*, in press).

There appears to be a link between herd size and PE diagnosis as made by a veterinarian, but the link could be tenuous because veterinarians are more likely to visit large farms.

Jackson (1980) reported an increased incidence of PE over summer but this is at odds with Ward and Winkelman (1990), who report no seasonality to the disease, and

our own experience with 21 outbreaks of the disease during 1989-1990.

•			
Less than 100	101-200	200 +	Total
30 <sup>1</sup>	9	12	51
5	6	9	20
35	15	21	71
	Less than 100 30 <sup>1</sup> 5 35	Less than 100         101-200           30 <sup>1</sup> 9           5         6           35         15	Less than 100101-200 $200 +$ $30^1$ 912569351521

Table 3. The effect of herd size on prevalence of PE

<sup>1</sup>Prevalence of PE based on those herds in which a diagnosis was made by a veterinarian.

The source of stock may have a large influence on the likelihood of PE occurrence on a farm. Penny (1984) reported that hysterectomy-derived or specific pathogen free herds are more susceptible to infection possibly due to an imbalance in gut microflora. Ward and Winkelman (1990) expand on this view, claiming that PHE occurs more commonly on SPF and closed herds whilst NHPE occurs more often in commercial herds. They emphasise the importance of poor management in disease causation, especially such factors as crowding, early weaning, chilling and poor quality feed. In our studies of 17 outbreaks of the disease, 8 occurred on recently stocked farms (Table 4).

Zinc deficiency has also been implicated as one of the factors which may contribute to PE susceptibility (Schugel, 1990; Daniels, 1990). These authors claim increased performance in terms of growth rate and feed conversion efficiency in response to feeding a zinc methionine supplement to grower and finisher pigs challenged with PE-infected intestinal material compared to a non-supplemented control group.

Pen effluent disposal method (partially slotted floors versus open drain) for finisher pigs had no influence on disease occurrence between PE infected and non-infected farms (Pointon, 1989).

The occurrence of PE varies depending on whether antibacterial medication is used. In-feed medication may act to prevent infection and therefore prevent development of immunity to PE, with the result that disease occurs when medication is stopped or changed.

Antibiotics form the basis of treatment and control regimes for PE. Individual animal treatment (tetracyclines and penicillins) is mostly used in PHE where the disease is rapidly fatal and the affected population is more valuable. More commonly, a combination of high and/or low level in-feed medication is used (Love, 1981). Examples of high/low rate treatment and control regimes include a 10-14 day course of antibiotic at a high dose rate (oxytetracycline at 200mg/kg, chortetracycline at 200mg/kg or olaquindox at 100mg/kg), followed by low rates (nitrovin at 50mg/kg, olaquindox at 30mg/kg or furazolidone at 100mg/kg) (Pointon, 1989).

Decisions regarding the success of treatment are made empirically. Inexpensive products with minimal or no withholding periods such as olaquindox (25-50 g/tonne) or furazolidone (100-200g/tonne) can be used throughout the life of the pig until marketing. Alternatively, exposure of pigs to infection followed by medication after 21 days to terminate infection before severe clinical disease occurs is effective (Love and Love, 1977).

Of 29 properties found through a retrospective survey to have experienced PE infection, 62% of those with signs typical of NHPE in their grower herd responded by adding medication. Eighty nine percent reported a positive response in terms of clinical improvement. Clinical signs of PHE in the breeder herd resulted in 56% of farmers treating with antibiotics. All farmers reported a positive response (Holyoake, unpublished data).

Paradoxically, herds with PE grew faster (573 versus 549 grams per day)

(P<0.05) than herds in which no diagnosis had been made (Holyoake, unpublished). This would most likely be due to the implementation of control programs when a diagnosis of PE was made but could also be linked to the greater awareness and technical responsiveness of those producers who knew they had the disease.

Eight of 21 outbreaks investigated occurred on properties using no medication (Table 4). Ten outbreaks occurred in stock which had been off medication for at least four weeks. Three outbreaks occurred despite the use of medication within the ration of infected stock. Disease on farm F was controlled through the use of Tylosin at a rate of 100 g/tonne of feed in the weaner, grower and finisher ration. Clinical disease

Unit	Herd size (N° sows)	Form of disease <sup>1</sup> (NHPE/PHE)	Medication <sup>2</sup>	Age (weeks)	Restocked or new farms
Α	300	PHE	nil	30-38	Yes
		PHE	nil	21-24	
		PHE	nil	22	
		PHE	nil	24	
		NHPE	nil	11	
В	250	PHE	nil	30-38	Yes
C <sup>3</sup>	135	PHE	nil	32	No
$D^3$	214	PHE	nil	20-26	Yes
Ε	grower unit	PHE	nil	22-33	Yes
F	500	PHE	100ppm Monensin	16-30	No
G³	1530 growers	NHPE	nil	18-21	. Yes
H <sup>3</sup>	236	NHPE	nil	21	Yes
I3	30	NHPE	nil	8-16	No
J	53	NHPE	nil	6-15	No
К	50	NHPE	nil	7-14	No
L	40	NHPE	nil	6-20	No
M <sup>3</sup>	45	NHPE	nil	18	Yes
N <sup>3,4</sup>	450	NHPE	nil	9-10	No
O <sup>3,4</sup>	1100 growers	NHPE	200ppm Terramycin	11-14	Yes
P <sup>4</sup>	5000	NHPE	100ppm Furazolidone	24	No
Q4	1200	NHPE	nil	24	No

 Table 4. Results of investigations of proliferative enteritis outbreaks on farms (Holyoake, unpublished)

<sup>1</sup>Form of disease: NHPE = non haemorrhagic proliferative enteritis; PHE = proliferative haemorrhagic enteropathy. <sup>2</sup>Medication: in-feed present up to 4 weeks prior to the outbreak. <sup>3</sup>Farms in which stock had been off medication for a minimum of 4 weeks. <sup>4</sup>Cases diagnosed at slaughter through herd health checks; other cases were diagnosed through field evidence of disease.

was not obvious on farm O, despite the presence of typical adenomatous lesions in 5 of 50 carcases examined at slaughter. For this reason, the farmer chose not to alter his medication strategy. No deleterious effects followed. The disease was controlled on farm P by doubling the rate of furazolidone from 100 g/tonne to 200 g/tonne of feed.

Nineteen farms with pathology typical of PE detected at abattoir were subjected to different medication regimes (Pointon, 1989). Over a 10 month period, five herds continued to have gross lesions detected at the abattoir. Four of these used furazolidone (100mg/kg) in finisher rations; one of the four in combination with dimetridazole (200g/tonne). The remaining herd changed from halquinol (120mg/kg) to nitrovin (100mg/kg) and then to olaquindox (50 g/tonne).

The other 14 herds suffered no recurrence of lesions. Four of these used a 10-14 day course of high dose rate antibiotic followed by a low dose rate. The remaining 10 herds used various medications at low concentration. There were, however, insufficient herds in this study to be able to determine the efficiency of high-low versus low level medication regimes in eliminating gross intestinal lesions.

#### Conclusions and practical implications

PE is a complex disease, both in terms of aetiopathogenesis and epidemiology. It is a common disease; unmedicated groups of pigs appear to be at greatest risk; and outbreaks of the disease can be associated with substantial, but variable reduction in growth rate. Consequently, if replacement stock are affected, reduced liveweight at first mating may reduce litter size. The disease is easily treated and controlled by selected antibiotics, but the increasing consumer interest in residue issues makes this method of control in the long term tenuous. Hence greater understanding of the disease is essential.

# CAMPYLOBACTER-LIKE ORGANISMS IN PORCINE PROLIFERATIVE ENTERITIS

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The term proliferative enteritis (PE) embraces four related disease syndromes. They are known as porcine intestinal adenomatosis (PIA), necrotic enteritis, regional ileitis and proliferative haemorrhagic enteropathy (PHE). The four conditions all affect the ileum causing lesions in the enteric crypt epithelial cells, resulting in diarrhoea, growth retardation (Rowland and Lawson, 1986) and in PHE, bloody diarrhoea and often death. PE occurs worldwide and has assumed increased importance in recent years as producers become less reliant on sustained antibiotic use in pig rations. Estimates of its economic significance are hard to obtain, but revenue losses of \$26 per sow per year have been given (Cutler and Gardner, 1988). Pointon (1989) estimated that between 5 and 20% of South Australian herds are affected. Our recent evidence suggests that the disease is wide-spread in Victoria.

# The cause

It has long been recognised that bacteria were associated with the proliferative lesions characteristic of this disease. Spiral or curved rod-shaped organisms were observed by silver staining of ileal tissue sections. Various *Campylobacter* spp were isolated from cases of PE. In particular *Campylobacter hyointestinalis* and *C. mucosalis* were nominated as likely causes of the disease (Lawson and Rowland, 1974; Gebhart *et al.*, 1983). However, numerous attempts to produce disease by oral inoculation of *C. mucosalis* or *C. hyointestinalis* and other *Campylobacters* have failed (Andress *et al.*, 1968; McCartney *et al.*, 1984; Boosinger *et al.*, 1985). Oral inoculation of homogenates of

affected ileal mucosa have resulted in various degrees of PE (Roberts et al., 1977; Lomax et al., 1982; Mapother et al., 1987a,b; McOrist and Lawson, 1989a).

More recently, work on the identity of the curved rod-shaped organism has revealed that the organism, whilst having a shape and morphology similar to *Campylobacter* spp, is in fact quite different. An intracellular *Campylobacter*-like organism (CLO) was described by McOrist *et al.* (1987) and McOrist and Lawson (1989) using specific monoclonal antibodies for CLO. They showed that intracellular CLO are not ubiquitous in the pig population, and that clinical disease arises only when this organism infects susceptible animals. CLO prepared from filtered mucosal homogenates from clinical cases of PE have induced microscope lesions in hamsters and gnotobiotic piglets (McOrist *et al.*, 1987; McOrist and Lawson, 1989).

It has become clear from our own work in the last two years that CLO is intimately associated with PE, and that other *Campylobacters* isolated are unlikely to have any primary relevance to the disease.

# **Detection of CLO**

The diagnosis of PE is made on the basis of gross and histopathological lesions within the ileum of affected pigs. To augment the histopathological diagnosis, we have used fluoroscein-labelled monoclonal antibodies specific for CLO (supplied by Dr GHK Lawson) on mucosal smears, histological sections of ileum or faecal smears. Mucosal smears (Figure 1) stained with these antibodies reveal of numerous curved bacilli. Identical staining procedures on smears of *C. hyointestinalis*, *C. mucosalis*, *C. coli* or *C jejuni* failed to reveal fluorescence.

Sections of paraffin embedded ileum similarly stained revealed the presence of numerous fluorescing curved bacilli present throughout the enterocytes of the villi (Figure 2). CLO appear to be heavily clustered around the crypts (Figure 3). Examination of paraffin embedded ilea from a variety of PE cases collected over the last 10 years, reveal CLO to be present in all cases where PE has been diagnosed histopathologically.

We have extended this technique for use on faecal smears. The monoclonal antibody can be used to indicate the presence of CLO or CLO within enterocytes shed from the intestine within the faecal smear. In addition, where histological diagnosis of PE is made and CLO are present in mucosa, CLO appear also in faecal smears. On this basis it is anticipated that this technique could, in future, be used to detect subclinical cases of PE. However, this assumes CLO are only detected in association with disease.

### Characterisation of CLO

It has not been possible to culture CLO by any conventional bacteriological means. As a consequence, the diagnosis and understanding of the disease has been severely restricted. Nevertheless, relatively pure preparations of CLO can be prepared from the ilea of pigs with PE and purified by filtration and gradient centrifugation (Monckton *et al.*, 1991).

Electron micrographs of purified material reveal a small  $(2\mu m \text{ in size})$  spiral or curved organism. CLO are considerably smaller than other *Campylobacter* spp but similar in appearance, except for the absence of flagellae (Figure 4).

Further clarification of the identity of CLO has been undertaken by extracting the DNA from purified preparations. Whole genomic DNA has been cut with restriction enzymes and the restriction endonuclease profile confirms the result that the CLO is a unique organism. Preparations of DNA from the other *Campylobacter* spp are quite dissimilar to restricted CLO DNA.



Figure 1: Campylobacter-like organisms in homogenates of affected mucosa stained with monoclonal fluorescent antibodies (x 1000).



Figure 2: A section of intestinal villi showing CLO within enterocytes. Monoclonal fluorescent antibody staining (x 400).

# DNA probes for CLO

Alternate diagnostic methods have emerged in recent years. These include DNA probes which rely on DNA unique to an organism as the marker for identification. This technique has particular suitability in this case, as CLO are unable to be cultured. Consequently, genetically engineered strains of *E. coli* containing CLO DNA were prepared for use as probes for the organism. These probes can be used to specifically differentiate CLO from other *Campylobacter* spp (Monckton, unpublished).

Most recently, we have developed the DNA probes for use on histological sections. The results confirm the presence of CLO clustering in the crypt epithelial cells.

DNA probes have some advantages over fluorescent antibodies. Because of their highly specific nature, they can be used to routinely examine large numbers of specimens for the presence of the organism. These techniques are being explored for routine diagnostic use on faecal specimens with a view to using them for whole herd monitoring programs.

DNA probes and their associated technology can also be exploited to create proteins or antigens for use with other CLO diagnostic assays or ultimately in vaccine use.



Figure 3: A section of intestine showing rings of fluorescent bacteria at the base of crypts. Monoclonal antibody staining (x 400).



Figure 4: Electron micrograph of CLO stained with 0.5% uranyl acetate.

# **CLO ELISAs**

ELISAs are a technique used to detect the presence of antibodies to microorganisms. They can be adapted to screen thousands of sera to gain an understanding of a disease patterns, when and how infection occurs, the level of immunity within populations, and the distribution of PE.

Developing ELISAs for CLO monitoring is vital to the epidemiological aspects of our studies. However, CLO ELISAs pose a special problem. The antigen for preparing ELISAs would normally come from pure cultures of the organism. As CLO cannot be cultured, we have prepared CLO ELISAs using semi-purified CLO extracted from affected mucosa. It is a time consuming and tedious process which has limited our ability to screen the thousands of sera which would normally be screened. Nevertheless, we have developed ELISAs to measure Immunoglobulin M (IgM), to monitor levels of the antibody passed from the sow to the piglet and to attempt to assess the level of antibodies following infection.

Preliminary results of a direct ELISA, based on coating plates with dilutions of our semi purified preparation reveal that serum IgM antibodies can be measured. Preliminary results show that antibody is, as expected, passed maternally to the piglet. High ELISA absorbance values are detected prior to weaning. These decline at weaning and then begin to increase again thereafter, suggesting that infection is probably taking place after weaning. Serial bleedings of a group of pigs from 2 to 24 weeks of age indicated low ELISA value which gradually increase after weaning (Figure 5).

Further experimentation is required to allow accurate interpretation of antibody levels and the process of infection. We are continuing to develop ELISAs to measure Immunoglobulin G (IgG). These ELISAs will allow a more accurate interpretation of the pattern of infection with CLO and to make more meaningful statements about protective levels of antibody or otherwise in the natural population.

However, major improvements in the availability of CLO antigen for ELISA are needed to develop an accurate and reliable antemortem test for routine diagnostic use. The culture of the organism or the production of antigen by genetic engineering methods are necessary prerequisites for a constant reliable supply of antigen.

#### Culturing the CLO

The traditional diagnosis of infectious disease demands the culture of an organism. Attempts to culture bacteria have clearly resulted in confusion over the cause of PE. Attempts to identify a *Campylobacter* spp as the aetiological agent have led to the identification of *Campylobacter mucosalis, hyointestinalis* or *coli*. (Lawson and Rowland, 1974; Gebhart *et al.*, 1983). It is clear that these organisms can be cultured from the scraped mucosa of PE affected pigs. Indeed, our own efforts have also resulted in these organisms being cultured from both affected and unaffected pig intestine. In particular, *Campylobacter hyointestinalis* is recoverable from a wide age range of pigs of all types leading us to believe that this organism may be a normal commensal species in the pig. Whether there is a role for these *Campylobacters* is still not determined. They may play a role in secondary invasion of affected mucosa.



Figure 5. ELISA absorbence values for Immunoglobulin M (IgM) to CLO, for a series of 10 pigs over a range of 24 weeks. ELISA values are given as Multiple of Normal Absorbence (MONA) values. Bars show standard deviations.

Several problems arise in attempting to culture CLO. Firstly, any material recovered from the intestinal mucosa is likely to be contaminated by a number of different bacteria. Some of these grow aerobically, some anaerobically and they may then grow preferentially on an agar plate. *Campylobacter* spp particularly, swarm and tend to quickly overwhelm a plate so that competition from any other organism which doesn't swarm or is slower growing would not be achieved. This can be overcome to some extent by selected antibiotics, but as CLO cannot be grown, the antibiotic sensitivity of this organism can only be surmised.

Secondly, CLO is an intracellular organism. The requirements for its growth may differ markedly from those of other traditional enteric organisms whose normal growth is extracellular. It is only by careful experimentation and trial and error that the specific growth requirements for CLO can be discovered.

It remains clear that until CLO can be cultured, progress with diagnosis and

understanding of the disease will be restricted.

## Vaccination against PE

Vaccines are normally based on killed or attenuated whole organisms. We are attempting to develop a culture method for the conventional production of CLO, which may then be adapted to production of whole cell vaccines. However, even if CLO can be grown, we do not know if vaccination will be successful. The only other means of preparing CLO antigens will come from extended work on DNA cloning. CLO DNA genetically engineered into other expression bacteria, (eg *E. coli*) may be able to produce CLO antigens which can then be used to vaccinate susceptible animals. Indeed, it is possible that these vaccines, may be considerably greater in efficacy than whole cell vaccines because specific antigens are targeted.

# Conclusions

There are a lot of unknowns with PE. Clearly, CLO appear to be the organism involved but until recently we have been looking at the wrong organism. To develop diagnostic tools for CLO is difficult, especially when traditional methods such as culture are not available. Nevertheless, molecular biological techniques have meant that we can circumvent some of these problems. We have been able to use monoclonal fluorescent antibodies and, more recently, DNA probes to begin to track down the aetiology of disease. With our ability to monitor CLO in mucosal or faecal samples we can begin to understand how and where infection takes place in the herd. Likewise, our ongoing research into the DNA of the organism will lead to proper taxonomic classification which then may help us to culture the organism itself. DNA methods will enable us to monitor large numbers of specimens and get more accurate pictures of the level of infection within herds. Similarly, the DNA work could lead us to alternative vaccines for PE control. The development of accurate ELISAs and the understanding of immunity may have to wait in part for the routine availability of purified antigen, but some understanding of the infection process is now possible.

There are still many questions. What is the accurate antibiotic sensitivity of CLO? Why do they invade enterocytes? How are CLO cultured? Are there different serotypes causing the different forms of the disease? Despite these questions, the prospects for PE control look considerably better than was the case only a couple of years ago.

# THE PATHOLOGY OF PROLIFERATIVE ENTERITIS

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# Introduction

Proliferative enteritis is a collective term used to describe a group of related diseases of pigs characterised by adenomatous intestinal crypt epithelium containing *Campylobacter*-like organisms. Many different names have been applied to the various diseases and conditions encompassed in this group and this leads to some confusion when comparing case reports in the literature (Ward and Winkelman, 1990).

Of the many titles used to describe the manifestations of proliferative enteritis, four are worthy of retention, even though there is some overlap between them. These are intestinal adenomatosis, proliferative haemorrhagic enteropathy, necrotic enteritis and regional ileitis. Each of these conditions can be differentiated on the basis of characteristics other than the basic adenomatous lesion. Throughout this review I will use proliferative enteritis as the group name but will also refer to, and define, the specific conditions making up this group.
Proliferative enteritis has been chosen in preference to another group name, intestinal adenomatosis complex (Barker and Van Dreumel, 1985). The term proliferative enteritis is now in common use in the veterinary literature and it is somewhat less cumbersome. Also, its use avoids confusion with one of the four specific manifestations of the disease - intestinal adenomatosis.

There is much that still needs to be determined about proliferative enteritis. The reasons for the different manifestations are not understood and there is still work needed on the identity of the causative organism. Much of the work undertaken so far is descriptive and few studies have been devoted to the pathogenesis of the lesions. In this review the pathology of proliferative enteritis will be discussed and possible pathogenetic mechanisms explored.

## The basic lesion

Pigs with proliferative enteritis have a characteristic microscopic change in the intestine - the presence of multiple abnormal immature crypts. These crypts are composed of undifferentiated epithelial cells with basophilic cytoplasm. Often the epithelial cells are pseudo-stratified (Figure 6). Examintion of silver stained sections reveals the presence of numerous curved *Campylobacter*-like organisms (CLO) within the cytoplasm of the crypt epithelial cells, concentrated mainly in the apices. Abnormal crypts can extend beyond their normal anatomic boundaries and may be found through the entire thickness of the mucosa - from the lamina muscularis mucosae to the luminal surface (Lomax and Glock, 1982). Extension of crypts into the sub mucosa, usually in lymphoid follicles, is also seen. Occasional reports of metastasis of crypts to mesenteric lymph nodes can be found (Emsbo, 1951; Lomax and Glock, 1982).

When present, the lesion is most likely to be found in the ileum and distal jejunum and may involve the distal 4 to 6 metres of the small intestine (Love *et al.*, 1977, Sims, unpublished). In some cases lesions extend into the caecum and colon and, although reported (Senk *et al.*, 1990), it is rare to find adenomatous changes in the large intestine without accompanying lesions in the small intestine.

The extent of adenomatous proliferation is variable. In early cases occasional immature crypts are seen and the remainder of the intestinal mucosa appears to be relatively normal. Usually these abnormal crypts are found above Peyer's patches in the ileum (Lomax and Glock, 1982). As disease progresses the number of immature crypts increases and an admixture of normal and abnormal crypts is present (Figure 7). In advanced cases there can be a complete absence of normal crypts and the mucosa is composed of undifferentiated proliferating intestinal epithelial cells. Crypts become elongated and branched (Lomax and Glock, 1982) and there is a loss of normal villus structures. Invariably there are aggregates of granulocytes in the lumen of many affected crypts forming "crypt abscesses". In human pathology it has been proposed that these may be the result of secondary infection of an already damaged mucous membrane (Morson and Dawson, 1979).

Adenomatous crypt epithelium in proliferative enteritis differs from that of normal crypts (Eriksen *et al.*, 1990). Enzymatic studies have demonstrated that the cells in adenomatous intestine do not possess the enzyme activity of their normal counterparts. In particular they have a complete or partial lack of Mg-ATPase activity in the basolateral borders of cells. There is also an absence of intracellular acid phosphatase activity (presumably in the lysosomes of normal cells). In normal crypts there is a transition in enzyme activity from crypt to villus corresponding to the maturation from the undifferentiated crypt cells to the mature villus cells. This is not apparent in gut sections of pigs with severe proliferative enteritis and the enzyme activity of crypt and surface epithelium is the same. Connective tissue surrounding crypts is also altered in adenomatous intestine with the pericryptal fibroblast sheath displaying enzyme profiles suggestive of immaturity. Laminin, an intercellular glue, is also deficient in the sub epithelial area of adenomatous intestine, another indicator of cell immaturity (Eriksen *et al.*, 1990).

Villus atrophy is a feature of most pigs with proliferative enteritis (Barker and Van Dreumel, 1985). In this disease there is a marked increase in the undifferentiated cell



Figure 6: Pseudostratified immature crypt epithelium in the ileum of a pig with proliferative enteritis. Some neutrophils present in the crypt lumen.



Figure 7: Mixture of normal and immature crypts in an early case of proliferative enteritis.

component but a reduction in the number of functional cells. This suggests that many of the immature cells are being lost prematurely. The kinetics of cell proliferation in the ileum of pigs with proliferative enteritis has not been studied. Of interest, however, is the observation that the number of mitoses in crypts does not appear to be increased in all cases (Sims, unpublished). This may indicate that, rather than being produced at a greater rate, the crypt cells are unable to migrate up the villi or to differentiate.

# Campylobacter-like organisms (CLO) - their role in the pathogenesis of proliferative enteritis

The presence of *Campylobacter*-like organisms in enterocytes is an integral feature of proliferative enteritis (Rowland and Lawson, 1976; Lomax and Glock, 1982). Although the number of organisms seen may vary from case to case they are always detected whenever adenomatous epithelium is present. The precise nature of these intracellular organisms is still unknown. Nevertheless, it has been shown that they are distinct from *Campylobacter mucosalis* and *C.hyointestinalis*, the two previous candidates as the cause of the disease (McOrist *et al.*, 1989a). Despite this evidence, there is still limited information on how this organism actually produces disease or how it gains entry into crypt epithelium.

It is generally presumed that the organisms gain entry into the crypt epithelium by direct penetration of immature cells in the crypts. Successful production of proliferative enteritis has been reported using intestinal homogenates from naturally occurring cases (Roberts *et al.*, 1977; Lomax *et al.*, 1982a,b; Mapother *et al.*, 1987a; McOrist and Lawson, 1989) but convincing evidence of attachment of bacteria to, or penetration of, enterocytes was not presented in any of these trials. Similarly, in experimental reproduction of transmissible ileal hyperplasia of

hamsters, a condition closely related to proliferative enteritis (McOrist and Lawson, 1987), active penetration of epithelial cells by intraluminal bacteria was not detected (Johnson and Jacoby, 1978). Membrane bound organisms were found just below the microvillous border in one study in hamsters but no relationship between organisms in crypts and those in cells could be established using immunofluorescent techniques (McOrist *et al.*, 1989b). Therefore, the possibility of penetration by other routes warrants further examination. The phagocytic M cells overlying lymphoid tissue in the ileum are used as the point of entry by other bacteria (and viruses) (Owen *et al.*, 1986; Wassef *et al.*, 1989) and CLO could enter via this route. The localisation of early lesions over Peyer's patches provides some support for this, but the means by which subsequent reinvasion of crypt epithelium occurs needs to be determined.

An interesting feature of proliferative enteritis is the ability of the CLO to survive and multiply inside cells. Neither the methods used by CLO to evade host defences nor the defence mechanisms which lead to the elimination of CLO from the body are known. However, it is worth examining some of the recent findings relating to other intracellular organisms because it is likely that similar host/bacteria interactions occur in proliferative enteritis.

Intracellular bacteria gaining entry into cells must be capable of surviving in an aerobic environment. In phagocytes they must survive an oxidative burst which results in the generation of potentially lethal reactive oxygen intermediates including superoxide anion, hydroxyl radical, hydrogen peroxide and singlet oxygen. To combat these, some bacteria possess (or can be induced to produce) enzymes such as superoxide dismutase and catalase which convert these to less reactive substances. Mutant strains which do not possess these enzymes are not able to survive in macrophages and are generally non-pathogenic (Franzon *et al.*, 1990).

Oxygen independent destruction of bacteria also occurs (via lactoferrin, lysozyme, reduced intracellular pH and other mediators) and resistance to this is also required for intracellular survival. Methods used by intracellular organisms to avoid destruction include inhibition of phagosome-lysosome fusion, escape from phagosomes into the cytoplasm, or into cisternae of rough endoplasmic reticulum, where they avoid contact with lysosomal enzymes, or resistance to lysosomal enzymes (Wells and Rikihisa, 1988; Detilleux *et al.*, 1990). Electron micrographs of mucosal epithelial cells in proliferative enteritis reveal that the organisms are free in the cytoplasm, suggesting that this may be important in CLO survival.

Immunological defence mechanisms also play a role in eliminating or limiting the effects of intracellular organisms. Cytokines, including  $\gamma$  interferon, produced by a variety of cell types, have been shown to reduce the capacity of intracellular organisms to proliferate (Rose *et al.*, 1991; Newman *et al.*, 1991). Tumour necrosis factor (TNF) has been shown to be cytotoxic to fibroblasts infected with bacteria, suggesting a role for this cytokine in the control of diseases where intracellular organisms are found (Klimpel *et al.*, 1990). It is likely that in the immune response to CLO (or possibly to altered enterocytes) in proliferative enteritis, activated T cells and macrophages would produce TNF and other cytokines and that these may have a role in controlling the disease.

Host factors play a part in the initial resistance to infection with *Campylobacter*-like organisms. In all experimental trials reported so far there is variation in the number of pigs which actually develop lesions following inoculation with infected gut scrapings. Only occasionally has this approached 100%. The natural disease is seen in immature pigs with disease in older (>1 years old) breeding animals being extremely uncommon. This may reflect an age related resistance but is more likely due to immunological events. In the first reported outbreak of CLO-related disease in Australia, sows of all ages were affected (Love *et al.*, 1977). Subsequent outbreaks on the same units involved younger animals only, providing support for the development of acquired immunity. It has also been suggested that controlled exposure to the organism, by the use of strategic medication, can provide protection (Love *et al.*, 1977).

It is probable that acquired resistance to infection with CLO will involve a range of lymphoid cells. Recent experimental work involving the treatment of mice previously exposed to coccidia with a cytotoxic monoclonal antibody against effector T cells has demonstrated the importance of these cells in the protection against reinfection (Stiff and Vasilakos, 1990).

Secretory and circulating antibodies present in these mice were not sufficient to prevent reinfection. Although this work applies to a protozoan infection, it may be of considerable significance in the search for a successful vaccine against the CLO.

Circulating antibodies are produced against CLO but these are found in animals with active infection. Given the intracellular location of the organisms, the persistence of infection in the face of these antibodies is not surprising. However, their presence does indicate that some CLO antigen must penetrate beyond the local mucosal immune system. CLO have been seen in macrophages in the lamina propria of intestine in cases of proliferative enteritis (Love and Love, 1979) and these could transfer antigen to other sites.

The presence of secretory antibody specifically directed against CLO has not been reported although in one study large amounts of IgA were detected within the apical cytoplasm of infected crypt epithelial cells (Lawson *et al.*, 1979). Normal crypt epithelial cells do contain and secrete IgA but the levels in these pigs were abnormally high. It was proposed that the epithelial cells were immature and could not discharge the IgA (which was not specific) or that the IgA was specific to the intracellular organisms and was bound to them. It is highly likely that CLO-specific IgA is produced and would afford some protection against invasion by "immune exclusion" (Stokes *et al.*, 1975). This mechanism has been demonstrated to act against a range of bacteria and other antigens.

It is well established that antibiotic therapy of herds with proliferative enteritis results in a rapid clinical response. It is presumed that this response corresponds to the death of the bacteria which then removes the stimulus for abnormal cell growth. Formal studies examining the regression of lesions in treated pigs have not been undertaken. However, it is likely that the abnormal cells are sloughed off and uninfected crypt epithelium returns to the task of providing functional enterocytes to line the villi. A review of some of the factors which control division of crypt epithelium in the "normal" gut may help shed some light on how the CLO induce changes in enterocytes.

# Some factors controlling cell proliferation in the gut

In normal gut there is a balance between the loss of functional epithelial cells which slough from the tips of villi and replacement of these cells by dividing and maturing crypt epithelium which migrate up the villi. Clearly there must be mechanisms to control this and there are a number of factors which are known to influence this process. These have been reviewed recently (Townsend *et al.*, 1988).

Attention has been focused on a group of substances referred to as growth factors. These hormones are usually produced and act locally (so called paracrine hormones as distinct from the circulating endocrine hormones). These factors include platelet derived growth factor, epidermal growth factor and transforming growth factors (TGF)  $\alpha$  and  $\beta$ . Specific or shared receptors for these are found on a wide range of cells. Attachment of growth factors to a receptor leads to a complex biochemical cascade which results in the activation of protooncogenes and induction of the cell into the cell cycle. Cell division also can be suppressed by certain growth factors and recent work in liver cells has demonstrated the role of TGF  $\beta$  in regulating cell proliferation (Fausto and Mead, 1989). A control loop was identified with production and local action of TGF $\alpha$  by hepatocytes stimulating cell reproduction and regulation of this by adjacent endothelial cells which produced TGF  $\beta$ . Whether these events occur in gut epithelium is not known but colonic cancer cells have been shown to produce TGF-like substances and to have receptors for them (Coffey *et al.*, 1986).

Whether the CLO produce disease by stimulating proliferation of crypt epithelium or inhibiting cell differentiation is not yet known. However, if they do increase the rate of proliferation then this could arise by the production of a growth factor-like substance, by the production of one or more of the intermediaries in the growth signalling pathway, or by blocking the effect of cell growth inhibitors such as TGF  $\beta$ .

In the parasite infected gut it has been shown that crypt hyperplasia precedes villus atrophy. T cell dependent responses have been suggested as the cause (Manson-Smith *et al.*, 1979). This form of villus atrophy results from the premature sloughing of developing crypt epithelium. It has been proposed that this mechanism may be responsible for the crypt

hyperplasia in proliferative enteritis (Barker and Van Dreumel, 1985), but it seems unlikely that this would explain the uncontrolled crypt proliferation and local invasion seen in this disease.

## The four forms of proliferative enteritis

On the basis of pathology there are four main forms of proliferative enteritis. Overlap occurs between some of the categories.

#### 1. Intestinal adenomatosis

Intestinal Adenomatosis (IA) is the uncomplicated form of proliferative enteritis and this descriptor should be reserved for cases in which necrosis and haemorrhage are not apparent macroscopically. Gross lesions may not be seen but, when present, are restricted to the terminal ileum and, on occasions, the caecum and colon. The serosa of the ileum may develop accentuation of the reticular pattern producing a "cerebriform" appearance (probably due to some oedema of the submucosa and to the mucosal proliferation). There may be slight thickening of the mucosa, the development of irregular folds or even focal thickened plaques.

The mucosa of the terminal ileum in many normal pigs is slightly thickened and folded so the proper assessment of these gross changes depends on histological examination. In intestinal adenomatosis, the characteristic proliferative crypt epithelium will be present. The extent of this change varies but in advanced cases most crypts are affected, villus atrophy is present and the surface epithelium is immature. Focal fibrin exudation and necrosis may be seen (Barker and Van Dreumel, 1985). The tunica muscularis is normal.

Clinical signs may be absent in these pigs but growth retardation and mild diarrhoea can The pathogenesis of diarrhoea in intestinal adenomatosis has not been studied. occur. However, it is likely that the balance between secretion and absorption in the affected portion of gut would be altered due to the increase in secretory crypt cells and loss of mature absorptive enterocytes. Therefore, increased fluid would be presented to the large intestine. The large bowel has the capacity to reabsorb an oversupply of fluid from the small intestine if undamaged so it is unlikely that this is the only means by which diarrhoea develops. In humans, it has been proposed that bile acids may play a role in the production of diarrhoea in diseases of the ileum (Field et al., 1990). The terminal ileum is the site of reabsorption of bile acids and absorption of these may well be reduced in pigs with proliferative enteritis. Unabsorbed bile acids which move to the large intestine are unconjugated by colonic flora and can insert themselves into the lipid phase of the plasma membrane. Through their detergent action, membrane permeability is increased and they also promote fluid secretion by increasing the activity of adenylate cyclase in mucosal epithelium. In other forms of proliferative enteritis where the damage to the wall of the intestine may be more extensive, other mechanisms, such as osmotic diarrhoea, may be more important.

#### 2. Proliferative haemorrhagic enteropathy

Proliferative haemorrhagic enteropathy (PHE) is the most spectacular form of proliferative enteritis. Clinically, it is characterised by the development of severe dark or bloody diarrhoea, usually in finisher pigs or replacement breeding stock. Gross lesions are found in the distal small intestine and may involve both the ileum and the jejunum, extending much further proximally than the lesions in intestinal adenomatosis. The wall of the intestine is usually thickened and the serosa has the characteristic cerebriform folds described above, but far better developed. The lumen of the intestine contains large amounts of fresh blood often in the form of a haemorrhagic cast loosely adherent to the mucosa. The mucosa is congested, but is not always thickened or folded. The basic proliferative crypt lesion is seen histologically but, despite the extensive haemorrhage seen grossly, there may be no obvious site of blood loss; haemorrhage appearing to be due to capillary leakage. Focal mucosal necrosis is seen in some areas. An interesting feature of many cases of this disease is the presence of well formed villi (Lawson et al., 1979; Love and Love, 1979), suggesting a shorter time course for this condition compared with the other forms of this disease. It is also noteworthy that the proximal extent of blood in the lumen corresponds almost exactly to the areas of adenomatous change (Sims, unpublished).

The pathogenesis of PHE has not been resolved satisfactorily. The microscopic inflammatory changes are similar to those seen in other acute bacterial infections. It has also been suggested that these changes may be the result of a Type 1 hypersensitivity reaction (resulting from exposure to an antigen which is normally intracellular - the CLO) (Love and Love, 1979). What is difficult to explain with these two proposals is the massive haemorrhage in PHE, suggesting some other factor(s) may be involved. We have already speculated that TNF and other cytokines may play a role in elimination of infection from cells and they might also be involved in the development of lesions. In mice, injection of recombinant TNF leads to endothelial damage, villus necrosis and extravasation of red blood cells in the small intestine (Remick *et al.*, 1988), lesions similar to those seen in PHE. Local production of TNF by activated macrophages in the lamina propria of affected gut could explain the haemorrhage in PHE.

If TNF is the 'cause' of the haemorrhage then an explanation for the different response in PHE compared with the other forms of proliferative enteritis is required. Theorising again, this may be 'dose related' as the proliferative lesion in PHE often involves several metres of intestine and usually is more extensive than in the other forms.

# 3. Necrotic enteritis

Necrosis of adenomatous intestine in proliferative enteritis is a common finding. When this is obvious macroscopically the condition should be referred to as necrotic enteritis (NE). The gross appearance of NE is variable, depending on the age of the lesion, but necrosis of the mucosal surface and replacement with a fibrinous pseudomembrane is evident. The intestine is thickened and the serosa folded. A fibrinous cast may be present in the lumen. There may be oedema of the mesentery associated with affected gut. Lesions may extend several metres proximally from the ileocaecal junction and may involve the caecum and colon. Usually, pigs with this disease are in poor condition.

Microscopically the lesion may vary from focal areas of superficial necrosis and inflammation to severe, often full thickness, necrosis of the mucosa. Islands of normal and adenomatous crypts may be found amongst the necrotic debris which contains large numbers of bacteria (Barker and Van Dreumel, 1985). In longer standing cases granulation tissue forms in severely damaged areas. Coagulation necrosis of mucosal tissue may be seen.

As with the other forms of proliferative enteritis the exact pathogenesis of the lesions is not known. It has been proposed that the lesion is the result of damage by anaerobic 'faecal/colonic-type' bacterial flora which injures an altered epithelial surface. Sections of intestine do contain large numbers of bacteria amongst the necrotic debris and microaerophilic culture yields large numbers of *Campylobacter* spp which are not present in the normal pig ileum (McOrist *et al.*, 1989b). Reduced amounts of mucus overlying the mucosa, due to the failure of goblet cells to develop, may also allow easier attachment of organisms to epithelial cells (Rowland and Hutchings, 1978). Thrombi are seen in vessels in the submucosa in some cases indicating that anoxia may play a role in the development of lesions. Mucosal necrosis is also a feature of PHE (Love *et al.*, 1977), which may indicate that PHE and NE share a common pathogenetic pathway.

The poor condition of pigs with NE can be explained in part by the loss of fluid and protein through a severely damaged gut. It is possible that TNF may also play a role in the development of cachexia. TNF is also known as cachectin and (among its many actions) can produce anorexia and weight loss when injected into laboratory animals (Beutler and Cerami, 1987).

# 4. Regional ileitis

Regional ileitis (RI) is a chronic disease which is regarded as the fourth form of proliferative enteritis. In this condition there is marked muscular hypertrophy of the terminal ileum accompanied by mucosal ulceration or necrosis. A direct link between this condition and proliferative enteritis is yet to be established because adenomatous change has not been demonstrated in pigs with this disease. However, epidemiological evidence suggests a link and it is possible that it could develop following resolution of proliferative lesions and repair of necrotic ulcerated mucosa.

In regional ileitis the terminal small intestine is markedly thickened and firm ('hose pipe' gut). There may be oedema of the mesentery and roughening of the mucosa. Histologically, the mucosal lesions resemble those of necrotic enteritis, with granulation tissue prominent. Both muscle coats are hypertrophied. The absence of adenomatous change causes problems when trying to establish the identity of the disease because not all pigs with thickened ileal musculature necessarily have proliferative enteritis. Some of the published descriptions of regional ileitis have included cases in which there was marked granulomatous inflammation of the mucosa (Embso, 1951). Cases with muscular hypertrophy without mucosal involvement have also been described (Neilsen, 1955). These are not considered to be part of the proliferative enteritis group.

## Conclusions

This review has considered the range of changes seen in proliferative enteritis. It is clear that our knowledge of the pathogenesis of the disease is still rudimentary. This paper proposes some mechanisms which may be involved in the production of disease and these warrant further investigation.

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# EFFECT OF AN ACIDIFIER/DIRECT-FED MICROBIAL COMBINATION PRODUCT ON FAECAL HAEMOLYTIC E. COLI IN WEANER PIGS

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The use of certain organic acids in either the diet or drinking water of young pigs may improve growth and reduce the proliferation of coliform bacteria (Cole, 1968). The basis of these effects relates predominantly to the relative physiological deficiency in gastric hydrochloric acid production in the young pig which may be exacerbated by certain dietary ingredients which have a high acid-binding capacity.

The present study investigated the effect of Acid-Pak 4-Way (Alltech Inc. Kentucky, USA) in the drinking water (1 g/l) with four levels of whey powder (0, 40, 80, 120 g/kg) in the diet. The experiment was therefore a 2 x 4 factorial with four replicates each of 36 pigs (equal numbers of male and female pigs per treatment). Pigs were weaned at 21 d of age and the treatments were applied over the following 25 d. Growth performance and the excretion of haemolytic E. coli (faecal swabs at day 6 after weaning) were measured. Results are given in Table 1.

Whey (g/kg)	Acid-Pak 4-Way	Growth rate (g/d)	FCR	Haemolytic E. coli (% of total)
0	-	314	1.33	67
	+	262	1.40	37
40	-	318	1.28	84
	+	279	1.40	41
80	-	326	1.34	65
	+	324	1.36	32
	-	339	1.35	70
120	+	331	1.30	25
SEM		32	0.04	29

Table 1. Growth performance and haemolytic *E. coli* excretion in pigs in the postweaning period (21-46 d of age) given diets with and without Acid-Pak 4-Way

There were no significant (P>0.05) effects of Acid-Pak 4-Way on growth or FCR. However, Acid-Pak 4-Way reduced (P<0.001) faecal haemolytic *E. coli* output. Although haemolytic *E. coli* (% of total) is only a qualitative measure of the microflora status of the gastrointestinal tract, the results confirm (Johnson and Campbell, 1991) that Acid-Pak 4-Way may significantly reduce haemolytic *E. coli* in weaner pigs.

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# LYMPHOID CELLS IN THE UTERUS OF PREPUBERTAL AND CYCLING GILTS

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In a recent study presensitization of the uteri of prepubertal gilts with killed semen antigens or seminal components resulted in a significant improvement in litter size by 1.5 - 2.0 piglets following a fertile mating at the second oestrus (Bischof and Hughes, 1990). To determine whether the local immune system of the uterus plays a role in enhancing fertility, a preliminary study was carried out to establish the cellular basis for the local immune response in the porcine uterus.

At 165 days of age twelve prepubertal gilts were randomly allocated to four equal groups. Three gilts were slaughtered at the prepubertal stage while the remaining nine gilts received daily 20 minutes of boar exposure and were inspected for signs of pubertal oestrus. After reaching puberty (designated day 0 of the oestrous cycle) groups of three gilts were slaughtered at the early- (day 3), mid- (day 10) and late- (day 18) stages of the first oestrous cycle. Uterine tissues were collected for routine light microscopy and immunohistochemical studies. Frozen sections were stained by the indirect immunoperoxidase technique using a panel of monoclonal antibodies to pig leukocytes to examine the subpopulations of lymphoid cells in the uterine endometrium.

In the prepubertal uterus the most predominant lymphocyte phenotype was CD2<sup>+</sup> (pan T cell marker) followed by occasional CD4<sup>+</sup> and CD8<sup>+</sup> cells. Lymphocytes as well as numerous neutrophils were localised at the basal region of the uterine epithelium with some of these forming clusters in the upper regions of the stroma. Also at this stage numerous MHC Class II\* cells with extensive cytoplasmic processes were observed beneath the uterine and glandular epithelium and in the stroma. In early oestrus CD2<sup>+</sup> lymphocytes were the predominant cell type, localised mainly at the base of the epithelium and in the subepithelial region. Some of these cells were CD4<sup>+</sup> while others were CD8<sup>+</sup>. At mid-cycle MHC Class II<sup>+</sup> cells were predominant, the majority of these were spindle to stellate shaped and were found beneath the uterine and glandular epithelium and in the stroma. The late stage of the oestrous cycle was characterised by an extensive infiltration of neutrophils into the subepithelial stroma often forming clusters along the basal region of the uterine epithelium. Clusters of CD2<sup>+</sup> cells were observed at the base of the uterine epithelium and within and around blood vessels, particularly in the subepithelial region. Some CD4<sup>+</sup> and CD8<sup>+</sup> cells were also observed in these areas.

The results of this preliminary study indicate that the oestrous cycle influences the distribution and migration of leukocytes in the porcine uterus. The leukocyte phenotypes found in the porcine endometrium suggest that a local immune response could be elicited.

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# THE INTESTINAL SPRIOCHAETES: GENETIC DIVERSITY AND DISEASE ASSOCIATIONS

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Intestinal spirochaetal bacteria from pigs have been divided into two main species: Serpulina (Treponema) hyodysenteriae is strongly beta-haemolytic and causes swine dysentery (Stanton et al., 1991); Serpulina (Treponema) innocens is weakly beta-haemolytic and is considered non-pathogenic (Kinyon et al., 1977). This simple correlation between extent of beta-haemolysis and virulence can however be misleading. Avirulent isolates of *S. hyodysenteriae* have been recovered from pigs (Lysons et al., 1982), a pathogenic weakly beta-haemolytic "biotype" of *S. hyodysenteriae* has been described (Binek and Szynkiewicz, 1984), and certain weakly beta-haemolytic isolates of "*S. innocens*" have been thought to cause "spirochaetal diarrhoea" (Taylor et al., 1980). This confusion over the description and disease-associations of the intestinal spirochaetes has led us to examine their genetic relationships in the current study.

A total of 190 intestinal spirochaetes were examined using the technique of Multilocus Enzyme Electrophoresis. The electrophoretic mobilities of 15 constituent enzymes were equated with allelic states. A phenogram identifying genetic groupings was then constructed.

Three broad genetic groups were identified, with 88 electrophoretic types (ETs). One group contained all 99 *S. hyodysenteriae* isolates, as well as 18 weakly beta-haemolytic isolates. *S. hyodysenteriae* formed a distinct subgroup, with 32 ETs, but two isolates were less closely related to the others. Seven spirochaetes which had biochemical similarities to *S. hyodysenteriae* but which were weakly beta-haemolytic were located immediately adjacent to the main *S. hyodysenteriae* subgroup. Other more typical *S. innocens* made up the rest of this group.

All isolates in the other two genetic groups were weakly beta-haemolytic. Isolate P43/6, the original isolate from "spirochaetal diarrhoea" (Taylor *et al.*, 1980), fell in the larger of these two groups. Very similar Australian isolates from pigs with diarrhoea were identified. Other isolates resembling "*S. innocens*" were present in both groups.

This study demonstrates that the intestinal spirochaetes are genetically diverse. It suggests the existence in Australia of weakly beta-haemolytic spirochaetes which may be a "biotype" of *S. hyodysenteriae*, together with quite distinct weakly beta-haemolytic spirochaetes which may cause spirochaetal diarrhoea. The pathogenic potential of selected isolates from these groups is being tested. Results from this study will improve our understanding of intestinal spirochaetal infections in Australia.

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# INFLUENCE OF VITAMIN U (S-METHYLMETHIONINE SULPHONIUM CHLORIDE) ON PARS-OESOPHAGEAL GASTRIC ULCERS

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Certain vegetables, in particular cabbage, contain a nutritional factor with reported anti-ulcer properties. This factor, S-methylmethionine sulphonium chloride has also been called vitamin U (from the Latin ulcus) although its status as a vitamin has not as yet been accepted. Studies in Eastern Europe (Solntsev and Filipovich, 1978) have suggested that vitamin U supplementation of pig diets helped to reduce the number of animals exhibiting ulcerative lesions. Similarly, Tamas et al. (1986) observed a reduction in ulcer severity scores at abattoirs when pigs were given vitamin U. In Australia, King (1990) reported a field case where a dramatic increase in mortality occurred due to gastric ulcers. Diet changes appeared to resolve the problem and when diets that were originally used were re-issued with vitamin U, there was no recurrence of the ulcer problem. Sixty pigs from a SPF herd with continuing gastric ulcer problems were endoscopically assessed (Kopinski and Fogarty, 1991) for initial ulcer status. Forty-eight pigs were selected and allocated to replicated treatments in a 2x2 factorial experiment for the objective assessment of vitamin U on the prevention or therapy of gastric ulcers. All pigs were fed a commercial pelleted diet of the following composition: DE, 14 MJ/kg; lysine:DE, 0.625 g/MJ; and crude fibre, 41 g/kg.

	No ulce	r group	Ulcer	group	
	_	Vitamin U	(mg/kg alet)		
	0	200	0	200	SEM
Initial ulcer score <sup>1</sup>	1.0	1.0	3.0	3.0	0.06
Final ulcer score <sup>1</sup>	1.83	2.08	2.67	2.25	0.222
Daily gain (g/d)	755	765	795	843	38.7
FCR (g/g)	3.33	3.21	2.97	3.17	0.15
P <sub>2</sub> Backfat (mm)	14.3	15.1	13.0	14.6	0.76

 Table 1. Mean performance and ulcer results assessing the benefit of vitamin U supplementation of pigs with or without ulcers

<sup>1</sup>(0 - no abnormalities, 1 - hyperkeritinzation, 2 - erosion, 3 - ulcers).

From the results shown in Table 1, vitamin U supplementation did not affect pig performance. Where ulcer status was initially good, vitamin U did not effectively prevent ulcer occurrence but tended to enhance ulcer development. Vitamin U failed to exhibit significant effects on existing ulcers, with only a trend towards improvement of existing ulcers. At current cost, unless the slight ulcer improvement results in dramatic reduction of ulcer mortalities, vitamin U supplementation is not justified.

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# GASTRIC PROTEASES IN 0-15 DAY-OLD PIGS

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In previous experiments, protease activity in the different regions of the gastric mucosa was determined as milk-clotting activity (MCÅ) (Foltmann *et al.*, 1987; Vavala and Cranwell, 1988), or only measured in the fundic region (Sangild *et al.*, 1989). The aim of present study was to investigate, in 0-15 day-old Large White x Landrace pigs, the distribution and concentration of MCA and general proteolytic activity (GPA) in the fundic (F), cardiac (C) and antral (A) regions of the glandular mucosa in the stomach.

Stomachs were obtained from 34 pigs (0-15 days old). Tissues from the three regions of the stomach were homogenized and extracted (1g tissue: 2-4ml H<sub>2</sub>O) in an ice bath (0°C). The GPA and MCA in the extracts were measured using radial diffusion techniques (Samloff & Kleinman, 1969; Lawrence & Sanderson, 1969). Porcine pepsin (Sigma) was used as the standard in both assays.

Table 1.	Protease	activity	in the	cardiac,	fundic	and	antral	regions	of the	stomach	ı in
0-15 day	-old pigs	(Mean ±	SEM)					-			

Age _		Milk	-clotting activ	vity <sup>1,2</sup>	General proteolytic activity <sup>1</sup>			
(ď) <sup>n</sup>	Cardiac	Fundic	Antral	Cardiac	Fundic	Antral		
0	4	1.34±0.51	5.11±1.47	2.30±0.82	0.36±0.15	0.22±0.05	0.09 <u>+</u> 0.03	
1	8	0.46±0.11	8.88±1.89	$2.04 \pm 0.41$	0.23±0.10	0.35±0.08	0.19 <u>+</u> 0.06	
2-5	10	0.53±0.18	7.79±1.66	3.02±0.62	0.39±0.06	$0.59 \pm 0.11$	0.35+0.24	
7-8	5	0.48±0.19	15.69±2.46	3.78±0.62	0.33±0.02	1.21±0.33	0.17+0.10	
11-15	7	0.14±0.01	$5.36 \pm 0.49$	2.54±0.18	0.40±0.03	0.86±0.13	0.41 <u>+</u> 0.03	

<sup>1</sup>mg pepsin per gram of tissue. <sup>2</sup>Except for 0 days, F>A>C; P<0.05.

Fundic tissues from all pigs contained high MCA and low GPA which is in agreement with the findings of Foltmann *et al.* (1987) and Sangild *et al.* (1989). However, in the present study maximal MCA occurred at 7-8 days, not at birth. Similar results were observed for antral tissues but in cardiac tissues there was little difference between MCA and GPA. At each age, except for 0 days, the MCA in fundic tissue was greater than that in antral tissues which in turn was greater than that in cardiac tissues (Table 1). The GPA was similar in the three regions during the first week; in the second week it increased in fundic tissue but not in cardiac or antral tissues. The results confirm that in newborn and sucking pigs the fundic mucosa is the main site of synthesis of gastric protease zymogens. The results also indicate that the stomach of the newborn pig is well equipped to clot colostrum and milk but its capacity for general proteolytic activity is limited during the first two weeks after birth.

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# MAINTENANCE OF VILLOUS HEIGHT AND CRYPT DEPTH IN THE SMALL INTESTINE OF WEANED PIGLETS

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The small intestine of the piglet undergoes villous atrophy, crypt hyperplasia and reductions in specific disaccharidase activity after weaning (Hampson, 1986). Many hypotheses have been put forward to account for these changes, but they may simply reflect the interruption to nutrient intake and/or a lack of specific nutrient(s) in the lumen of the gut. Souba *et al.* (1985) highlighted the vital role glutamine plays in maintaining mucosal integrity following catabolic stress so, in this experiment, we fed whole milk (plus milk fortified with L-glutamine) to weaned pigs to test the hypothesis that maintenance of luminal nutrition after weaning will prevent villous atrophy and crypt hyperplasia.

Thirty-two piglets weaned at  $28\pm0.4$  days and weighing  $8.9\pm0.34$  kg were allocated to one of four treatments: (i) unweaned controls killed at weaning (U/W); (ii) starter diet (15 MJ DE/kg, 1.4% total lysine) fed *ad libitum* (Starter); (iii) ewes' liquid milk (EM); and (iv) ewes' liquid milk plus 2% L-glutamine (EM+Gln). Piglets were offered fresh milk every two hours in a feeding regime that increased from 1.2 1/pig on day one up to *ad libitum* intake on days four and five. On day five all pigs were killed and samples were taken at 25%, 50% and 75% along the small intestine. Samples were fixed in 10% phosphate-buffered formalin, and 5 µm sections were cut and stained with haematoxylin and eosin. Measurements of villous height and crypt depth were made on 10 well-oriented villi.

Group	% of intestine	U/W	Starter	EM	EM+Gln
Villous height (µm)	25	550°(10.C)	356 <sup>b</sup> (30.4)	545ª(57.6)	596 <sup>a</sup> (32.0) <sup>1</sup>
	50	496°(22.1)	354 <sup>b</sup> (26.5)	406ª <sup>b</sup> (50.9)	455 <sup>a</sup> (26.5)
	75	323°(16.0)	285 <sup>a</sup> (19.6)	291ª(30.6)	338 <sup>a</sup> (30.9)
Crypt depth (µm)	25	118ª(6.4)	200 <sup>ь</sup> (6.6)	168°(11.7)	155°(9.4)
	50	130ª(7.3)	200°(9.5)	165 <sup>ь</sup> (11.3)	144 <sup>ab</sup> (10.2)
	75	104ª(4.7)	185°(9.6)	142 <sup>ь</sup> (12.0)	125 <sup>ab</sup> (9.1)

Table 1. Villous height and crypt depth at three sites along the small intestine of pigs killed five days after weaning (mean  $\pm$  s.e by one-way ANOVA; n=8/group)

<sup>1a,b,c</sup>Within rows, means not followed by a common superscript differ (P<0.05).

Feeding ewes' milk arrested the post-weaning decline in villous height along the small intestine of weaned piglets. Crypt depth increased in the EM and EM+GIn treatments at the 25% site, but at 50% and 75% crypt depth in piglets offered EM+GIn did not differ from U/W piglets. In contrast piglets offered the starter diet had shorter villi at the 25% and 50% sites and greater crypt depth at all sites along the small intestine. Villous atrophy and crypt hyperplasia can be prevented if the nutritional stress of interrupted intake at weaning is ameliorated.

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# USE OF THE ENDOSCOPE FOR THE DETECTION OF GASTRIC ULCERS IN THE YOUNG PIG

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The main stomach ulcer occurring in pigs is the oesophago-gastric ulcer which occurs in the non-glandular pars oesophagea. Little data exists on the herd prevalence of gastric ulcers, although examinations at slaughter have shown erosive lesion occurrence in over 50% of pigs from a number of commercial piggeries. In one very large piggery, gastric ulcers were the most significant cause of mortality in grower pigs (Driesen *et al.*, 1987). Aside from sporadic outbreaks of gastric ulcer mortalities and the related income loss, little is known about any adverse effects of ulcers on pig performance and production efficiency.

At present the aetiology of the syndrome is unknown, although it appears that gastric ulceration is closely correlated with the feeding of finely ground diets. A number of predisposing factors have been suggested as influencing the occurrence of ulcers:- dietary, stress-related, hereditary and microbial. Studies in the 1970's examined these factors by comparison of ulceration incidence recorded at slaughter. The value of these studies is doubtful because of the variable occurrence of ulceration and the non-reproductibility of the syndrome.

Kowalczyk *et al.* (1968) published the first comparative study on the use of gastroscopy and gastro camera photography for diagnosis of pathological changes in pig stomach, especially gastric ulcers. Subsequently it has been used for the examination of chemical induction of oesophago-gastric ulcers. The benefits of the technology as an experimental and diagnostic tool have not been fully exploited.

In a trial investigating gastric ulcers, 60 pigs from as small as 30 kg were endoscopically assessed for ulcer status. The procedure involved initial feed withdrawal followed by pre-medication with atropine and azaperone. Anaesthesia was induced with intravenous thiopentone sodium and following intubation, maintained with halothane. A protective mouth guard was placed in the pigs mouth to protect equipment from damage. An endoscope (Olympus GIFP3) was passed approximately 750mm down the oesophagus into the stomach. The optic eye of the endoscope was retroverted to allow observation and photography of the pars oesophageal region of the stomach, illumination being provided by a Olympus CLE light source connected to the endoscope. An in-built flushing/suction mechanism allowed maintenance of a clear viewing region.

The ease of gastric endoscopy at 30 kg combined with stomach examination at slaughter, has the potential to allow objective assessment of the value of treatments in the therapy of existing ulcers or in the prevention of new ulcers. Endoscopy, combined with photography, allows serial examination and study of the pars oesophageal tissue with reduced subjective assessment of normality or abnormality.

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# COPPER RESISTANCE IN ENTERIC BACTERIA ISOLATED FROM PIGS IN THE UK

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Tetaz and Luke (1983) showed that copper-resistant strains of *Escherichia coli* isolated from piggery effluent carried a conjugative plasmid, pRJ1004. This plasmid confers resistance to cupric salts due to plasmid-borne copper resistance (*pco*) genes (Rouch, 1986), and is compatible with other plasmids which carry pathogenic determinants for adhesion and enterotoxin production (Tetaz, 1981). The presence of *pco* determinants in some pathogenic strains of *E. coli* has been reported (Morgan *et al.*, 1987).

A recent study of enteric isolates from three piggeries in the UK has shown that determinants very closely related to *pco* are present in a number of *Escherichia*, *Citrobacter* and *Salmonella* strains. *Proteus* and *Providencia* spp. isolated from the same sources probably have a different copper tolerance mechanism. In each of the three piggeries, in Shropshire, Humberside and Lothian, copper-supplemented rations were fed to the weaner pigs from which samples were taken. These piggeries were geographically distant from each other and hence it would appear that samples from each could be regarded as unrelated.

The presence of pco related determinants in species other than E. coli raises questions as to how transferable these determinants are within the intestinal flora of the pig, particularly to potentially pathogenic strains. Experiments using the pco-like copper resistance determinants from the UK have shown that these determinants are not as readily transferable to standard recipient strains as is the original pRJ1004 plasmid. Differences in transfer behaviour may result from differences in the plasmid upon which the determinants are carried, or from differences in the nature of the host organism which may modify not only transfer but expression of pco. Differences in MIC and inducibility are evident within the pco-like bearing strains.

Investigations are continuing to clarify the genetic basis for copper resistance in enteric organisms and the significance of copper supplementation to its incidence in piggeries.

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# FIXED OR FLEXIBLE DOSE REGIMES FOR DETACH

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Detach (Ciba Geigy) is an orally-dosed protease that is designed to be active in the piglets' small intestine. Intestinal surfaces that have been exposed to high levels of proteolytic activity have reduced capacity for attachment of microbial adhesins and toxins associated with diarrhoeal diseases; apparently because intestinal receptor molecules are inactivated (Chandler and Luke, 1987). Detach is registered for treatment of diarrhoeal diseases in sucker and weaner piglets.

Trials described in this paper were conducted on a large commercial piggery near Bendigo between October 1990 and April 1991. On this farm sows (150/week) were farrowed in sheds managed on an 'all-in all-out' basis. Similar numbers of Detach-treated and untreated (control) litters were selected from south eastern aisles of each shed on the Friday of three successive farrowings; for each of two dose regimes. Litters were chosen to provide similar numbers of age- and weight-matched piglets. Piglets were eartagged and weighed individually. They were weighed again 21 days later and those which could not be located in the shed were recorded as mortalities. Shed management routines for foster mothering and medication remained unaltered throughout the trials.

The two dose regimes were: (A) a fixed dose regime in which all piglets were dosed with Detach on days 2, 5 or 6, and 12 or 13 (these were days immediately prior to when diarrhoea was considered likely to occur, based on previous experience at the piggery); and (B) Detach treatment given to all piglets on day 2, with follow-up doses given as required to litters with poor growth performance or signs of diarrhoeal disease. Total Detach use was recorded. Mortality and weight-gain data for the two dose regimes are shown in Table 1.

Experiment	Treatment	N° of piglets	N° of litters	Gain (g/day)	Mortality <sup>1</sup> (%)
A	Untreated	298	28	179(5) <sup>2</sup>	8.7(2.4) <sup>2</sup>
Α	+ Detach	289	27	186(4)	3.4(1.9)
В	Untreated	254	· 26	171(4)	8.9(2.4)
В	+ Detach	266	26	194(4)	5.5(0.9)

Table 1. Performances of piglets treated with (A) a fixed dose regime, or (B) a flexible dose regime of Detach

<sup>1</sup>Days 2 to day 21; <sup>2</sup>SEM in brackets.

Both dose regimes resulted in higher mean weight gains and lower mortalities, however, only weight gain obtained with the flexible dose regime was significant (P<0.05). Thirty percent fewer doses of Detach were used with the flexible dose regime, indicating a more effective match of dose administration with disease incidence.

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# PORCINE LEPTOSPIROSIS IN AUSTRALIA: DIAGNOSIS AND CONTROL

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More than 200 serovars of the genus *Leptospira* are recognized worldwide, some of which can cause reproductive losses in female pigs. *L. interrogans* serovar *pomona* is widespread in Australian pigs, and causes leptospirosis among abattoir employees and meat inspectors. Control of human leptospirosis in abattoirs is best achieved by controlling leptospirosis in pigs.

Kidneys with corresponding serum samples were collected in Victorian abattoirs from 368 pigs originating from 42 farms (Chappel *et al.*, 1991). The kidneys were not a random sample but were designed to provide a representative cross-section both of kidneys with visible lesions suggestive of leptospirosis ("white spots") and of visibly normal kidneys. Kidneys with white spots (102) were therefore over-represented compared with the general population. Forty-four pigs were considered to be infected because their kidneys were culture-positive to *pomona* (27 pigs) or positive by immunogold silver staining (IGSS) (23 pigs), or because of microscopic agglutination test (MAT) titres to *pomona* of  $\geq$  1024 (42 pigs). Infection was diagnosed in 23.5% of pigs with white spots on their kidneys and in 7.5% of pigs with visibly normal kidneys. Using these two figures, and on the assumption that 10% of kidneys from pigs slaughtered in Victoria have white spots, it can be estimated that 9% of pigs slaughtered in Victoria are infected with serovar *pomona*.

The sensitivities of a number of diagnostic tests for leptospirosis relative to the above definition of infection were estimated to be: MAT (at a titre of 1024) 95%; IgM enzyme immunoassay (EIA) 82%; culture 61%; IGSS 52%; Warthin-Starry silver staining 20%. Lower MAT titres (32 to 256) were widespread, and their significance is uncertain, as are some IgM EIA reactions. Although only 23.5% of 102 pigs whose kidneys had white spots were infected, 48% of 27 pigs whose kidneys had large white spots (1 cm or greater) were infected.

Pigs typically become infected with leptospires at 15 to 20 weeks of age, after the decline of maternally-derived antibody (Davies *et al.*, 1991). Vaccination of grower pigs (with or without the use of antibiotics) is a necessary part of any control program, and vaccination at 10 and 14 weeks of age following the administration of tetracyclines in feed has been shown to eradicate leptospirosis from one herd. Control programs are being evaluated in several other herds.

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# WHICH PIGLETS PERFORM THE BEST AFTER WEANING?

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Low voluntary food intake after weaning imposes a significant production penalty in many piggeries, with some pigs failing to re-establish their pre-weaning growth curve by 14 days after weaning (Fowler and Gill, 1989). The major difficulty in studying this problem is knowing which pigs in a pen eat and, if they do, how much then consume. It is reasonable to assume that those piglets affected least by the stressors (mixing pigs, changing diet, moving pens) imposed on them at weaning will grow the quickest. In this experiment we tested two hypotheses. First, that heavier pigs tolerate the stressors of weaning best and will have the least interruption to their growth and, second, that pigs will grow faster if offered food from a single-spaced feeder because it gives more protection from aggressive penmates than conventional multi-space feeders.

A total of 189 piglets weaned at  $29.8\pm0.30$  days and weighing  $9.1\pm0.14$  kg were allocated to three feeders: (i) a single-space feeder connected to water; (ii) a single-space feeder not connected to water; and (iii) a conventional multi-space feeder. Piglets were penned in groups of nine on mesh floors providing 0.18 m<sup>2</sup> per pig. Weaner diets (15 MJ DE/kg, 1.35% total lysine) were offered *ad libitum* from day 14 of lactation to 28 days after weaning. Data were analysed by ANOVA and simple linear regression.



Figure 1. Relationship between weaning weight and (a), live weight [y=0.67+1.03x; r=0.88, P<0.001]; and (b), growth rate [y=50.55+9.28x; r=0.13, P>0.05) seven days after weaning.

Weaning weight explained 78% of the variation in live weight one week after weaning. However, there was no relationship between weight at weaning and growth rate in the following week. This refutes our hypothesis that heavier piglets tolerate the stressors of weaning best. The design of the feeder was of no consequence, as pigs ate the same amount of food from either multi-space or single-space feeders. To support an acceptable growth rate after weaning of 300 g/day piglets need to consume 470 g/day of a diet containing 15 MJ DE/kg (Black *et al.*, 1986). This level of food intake was not achieved in this experiment until the end of the second week after weaning, suggesting that it is low voluntary food intake that remains the major obstacle to improving pig performance after weaning.

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# ENVIRONMENTAL FACTORS AFFECTING FEED INTAKE IN PIGS

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## Symposium introduction

The voluntary food intake of pigs is now of greater significance to the efficiency of pig production than hitherto. Prior to the 1970's it was common for growing pigs greater than 50 kg bodyweight to be fed at a restricted level of intake to minimize the depth of subcutaneous fat at slaughter. The intensive selection of animals for a combination of rapid growth rate, efficient feed conversion and low backfat thickness under *ad libitum* feeding conditions means that improved strains will now only express their full potential for deposition of lean if feed intake is maximized (Dunkin, 1990). While in 1979 67% of herds fed grower pigs at a restricted level, six years later this figure was 44% (MLC, 1987). The emphasis in selection programs on efficiency of food use and carcass leanness has, however, inadvertently selected pigs with reduced voluntary food intake (Webb, 1989), such that food intake is now a limiting factor at several stages of the production cycle.

Intensification within the pig industry has increased the degree of control that we now have over the pig's immediate environment but has limited the animal's ability to adapt to a change in environmental conditions. For example, the common practice of housing pregnant sows in individual stalls means that in a cold environment they are unable to huddle to conserve body heat, while in a warm environment they are unable to wallow to maximize evaporative heat loss via the skin. This means that we need to understand first the pig's requirements and, then, to manipulate the pig's environment to meet these requirements.

Environment can be defined as the external surroundings in which an animal lives and can be broadly classified into that pertaining to the thermal (e.g. ambient temperature, wind speed, relative humidity), structural (floor type, feeder design) or social (group size, stocking density, stockmanship) environment. In considering the effect that the environment has on food intake, we need to consider the following aspects:

- 1. The mechanism by which environment influences food intake and hence performance, and to predict and measure the response of the animal to a change in environment
- 2. Interactions between the various factors that influence the pigs environment
- 3. Economic and welfare implications of a change in environment
- 4. Requirements of management and the stockperson

The following papers consider many of these issues. The first two papers are primarily concerned with the animal's social and structural environment with particular reference to how feeder design affects food intake. These are followed by an examination of how the thermal environment influences food intake of the growing pig and breeding sow.

# THE DESIGN OF THE FEEDING ENVIRONMENT FOR PIGS

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#### Introduction

The design principles of pig feeders have in the past focused primarily on aspects of hygiene, economy, durability and the ease of management. Little attention has been paid to the physical and social requirements of the pig. Scientific studies have considered basic issues such as whether trough feeding is better than floor feeding, whether pigs should be fed as a group or individually, and the effect of the form of feedstuff (e.g. meal vs pellet, wet vs dry). Unfortunately most of this information is peripheral to the design of a feeding system which will maximise food intake and food conversion efficiency of the growing pig.

In this paper I will discuss the principles of designing a feeder to maximize food intake and minimize food wastage.

#### Background

#### Floor vs trough feeding

During the 1960's, when pig production in the United Kingdom was undergoing a period of rapid intensification, it was thought that a pig feeder occupied valuable floor space and, that if the feeder was removed and food was dropped on the floor, then the stocking rate could be increased. In a large coordinated experiment, Braude and Rowell (1966) concluded that growth rate decreased (3.8%) and food conversion efficiency was reduced (4.6%) if pigs were fed meal on the floor rather then from a trough. If pellets were used there were no differences in either growth rate or feed efficiency, indicating that the difference in performance was attributable to a greater wastage of food when meal was fed on the floor.

Kennerly (1983) has recently examined the social aspects of floor versus trough feeding. With floor feeding, the uneven distribution of food over the floor was exploited by the socially dominant pigs, and social rank was found to be significantly correlated with the density of food at the pig's chosen feeding sites. Trough-fed pigs ate faster (21%) but showed more aggression (61%) than the floor-fed pigs. Therefore, if we accept that pigs are to be fed *via* a trough to minimize wastage, then the challenge is to design a system which will minimize aggressive behaviour.

#### Level of feeding

Prior to the introduction of superior genotypes it was necessary to restrict the intake of pigs once they attained approximately 50 kg liveweight to avoid excessive deposition of body fat. Modern genotypes have greater potential to deposit body protein and their voluntary food intake is lower (Webb, 1989). Consequently most pigs can now be fed *ad libitum* for all the time prior to sale at 80 to 100 kg liveweight. The requirement is for a feeding system which will maximise voluntary food intake, especially in the young pig when the relative potential to deposit body protein is at its greatest.

#### Feed wastage

Feed is by far the major cost item to the pig producer. As a percentage of total costs, feed accounts for 75% of the total in the United Kingdom (Meat and Livestock Commission, 1990) and 62% in Australia (Australian Pig Industry Reference Manual, 1989). Therefore any factor that reduces feed usage without affecting performance will have a significant effect on efficiency and profitability.

Published estimates of food wastage vary considerably depending upon the

design of feeders, size of pig and feed type (meal or pellets). Gill (1964) reported waste of between 4 and 25% depending on feed type whereas Hovarth and Elliott (1964) recorded wastage from 1 to 20% for a range of feeder designs. High levels of feed wastage are not always obvious; Gill (1966) observed only "slight amounts of food" on the floor during an experiment but this was later recorded as 33% of total food allocated. In these and other published experiments, insufficient details were given of, for example, feeder design, to enable general conclusions to be made about design features contributing to feed wastage. Nevertheless, the high levels of feed wastage that have been recorded is of concern, especially since in commercial practice it is difficult to estimate feed wastage when most is invariably lost in the effluent system.

#### Principles of feeder design

The design of any feeding system must consider both the physical and social requirements of the animal itself, as well as the management objectives of the enterprise. The space required for an individual pig to feed comfortably needs to be described in relation to the size of that animal and its posture during feeding. With group-feeding, the space required between pigs to minimize aggression also needs to be included.

#### Preference for height of feeders

Intuitively one could envisage a pig being able to feed throughout a range of feeding heights. Since the pig is a rooting animal it may prefer to feed at or slightly below floor level rather than, for example, at a level above its own shoulder height. Pigs can reach beyond this range if they are coaxed but food intake is reduced (Heitman and Bond, 1962).

In a study of feeder height preferences, single pigs were given access to seven feeders side by side in a stepped arrangement, offering feed at the full range of heights which the pigs had previously been found to reach. In effect, this ranged from just below floor level (- 32 mm for an 80 kg pig) to slightly less than shoulder height (274 mm). During the course of the preference testing, pigs were observed to feed from all the different heights but showed a clear preference for feeder heights slightly above floor level. Of the total observation time, only 5% was spent at the feeder below floor level and a further 5% at each of the two highest feeders. By contrast, 30% of the time was spent at each of the two feeders just above floor level (19 to 70 mm for an 80 kg pig) and this does not appear to be influenced by liveweight.

### Shape and size of pigs whilst feeding

Any feeder must be of sufficient size and of an appropriate shape to allow the pigs access to the food. In principle, pigs should be able to feed in a posture which they would adopt naturally. Petherick (1983) has determined the relationship between the width of the shoulder (mm) and the weight (W) of the pig as  $61W^{0.33}$ . This relationship is shown in Figure 1 as well as various estimates from a number of European and North American countries (Brent, 1986; BSI, 1981; Kraggerud, 1960; MAFF, 1971; NRC, 1970; Van der Voorde, 1968; Whittemore, 1980). The lower range of recommendations matches well with pig shoulder width and, as a practical guide, 1.1 times shoulder width is a reasonable estimate of minimum trough space allocation.

To determine the profile of a feeding pig, Baxter (1986, 1989) photographed pigs of different liveweights whilst feeding from a shallow feed pan, which could be raised or lowered in relation to the height of the floor. An example of the profile for a pig of 160 kg feeding at a range of feeding heights is shown in Figure 2. On the basis of these measurements, regression equations were used to predict body dimensions for any weight of pig feeding at any height off the floor. These dimensions could then be combined to describe a space envelope which the interior of a feeder must provide to allow pigs to feed in their preferred posture. The results of this work have been



applied in the design of a range of *ad libitum* feeders for weaner, grower and finisher pigs.

Figure 1. Trough-space allocations recommended in the published literature (from Baxter, 1986).



Figure 2. The outline of a pig weighing 160 kg feeding at a range of feeder heights (Baxter, 1986).

# Group feeding behaviour

#### Social space requirements

Ninety percent of all aggression by pigs occurs during feeding (Ewbank and Meese, 1971). This is clearly a bigger problem with restricted feeding but experiments have shown high levels of aggression, tail-biting and unequal food intake even with ad libitum feeding if there were too few feeding places. In an experiment conducted by Baxter (1986), pigs weighing 50 kg were fed to appetite once a day in groups of six individuals and their feeding and social behaviour were recorded. It was observed that a pig withdrew from the trough immediately following an aggressive incident once every two minutes during feeding. Of all the aggression observed during feeding, 65% was initiated by a pig at the trough against an approaching animal, while much of the remainder was between pigs feeding simultaneously. Pigs which were approaching the trough did not initiate aggression, even if the nearest pig at the trough was subordinate. Overall, subordinate pigs initiated aggression towards a more dominant animal in 34% of all observed aggressive incidents. It would appear, therefore, that defence of their feeding place is a major reason for aggression between pigs and this needs to be considered in the design of a feeding system since acts of aggression are likely to affect performance and animal welfare.

In an attempt to establish the effect of this social behaviour on requirements for feeding space McGlone *et al.* (1983) video recorded pigs (22 kg bodyweight) feeding from an experimental trough which could be varied in length. The space required to allow 1, 2 and 3 pigs to feed simultaneously was thus determined. A single pig of this size required approximately 86 mm, but the space required for either two or three pigs was proportionally greater (387 and 688 mm, respectively). It can be calculated from this work that a buffering zone of approximately 210 mm was required between animals to avoid competitive behaviours.

It can be interpreted from the work of McGlone *et al.* (1983) that providing sufficient space for the body size of the pig plus an allocation for social space should thus be sufficient to minimize aggression during feeding. To test this hypothesis, Baxter (1986, 1989) conducted an experiment in which he incorporated three space allocations based on multiples of the standard feeding space provided for pigs (1.1 x shoulder width) (Figure 3).



Figure 3. Experimental treatments in study of social behaviour during feeding.

In addition, there were three different types of trough barrier between each feeding position, all at a space allocation of  $1.1 \times shoulder$  width. A nose barrier consisted of a single bar placed across the trough, a *head barrier* was a small frame to separate the pigs up to head height whilst they were feeding, while the *head and shoulder barrier* was a three-quarter length feeding stall. There was no significant reduction in aggression with either increased feeder space or the provision of the *nose barrier*, however, there was a significant reduction when the *head barrier* was in place and almost all acts of aggression were eliminated with the *head and shoulder barrier* (Figure 4).

Feed wastage in this experiment was greatest with the largest allocation of trough space (10% of intake) and least (1%) when the *head barrier* was incorporated (Figure 5). It is suggested that with low space allocations and no barriers, acts of aggression contribute to feed wastage whereas at the higher space allocations pigs could stand in the trough and thus some feed was wasted. Having the *head* or *head and shoulder* barrier minimized aggression and forced the pigs to stand perpendicular to the trough with their feet on the floor, thereby minimizing feed wastage.



Figure 4. Aggression at six different feeder types.

In conclusion, social factors are an important determinate of the pigs' requirement for feeding space. Group feeding at a simple trough requires substantially more space than the sum of the individual pigs space requirements. The most effective approach to group feeding is the provision of structural divisions between feeding places since this increases the security of each feeding place and reduces the need for defensive behaviours by pigs at the feeder.

### Time-sharing feeder space

Bayer (1929) first observed that an apparently satiated chicken would recommence eating in the presence of an actively eating companion and would continue to eat 25 to 30% more than its original intake. Later work with rats (Harlow, 1932), dogs (Ross and Ross, 1949) and monkeys (Harlow and Yudin, 1933) established this as a widespread behavioural phenomenon. Hsia and Wood-Gush (1982) studied the feeding behaviour of a previously satiated pig after the introduction of a hungry pig. If the satiated pig was the dominant of the two it fed for about 2 out of the 10-minute test period, whereas if it was the subordinate then it did not continue to feed for more than a few seconds.



Figure 5. Feed wastage from six different feeding situations.

Hsia and Wood-Gush (1983a) also showed that social stimulation of feeding tended to synchronise feeding bouts between pigs in adjacent pens. In another experiment it was also demonstrated that moderate competition for food stimulated food intake of pigs by means of social facilitation; four pigs each weighing 35 kg eating at a 1.2 m trough ate 15% more than similar pigs feeding either in isolation or with only 0.3 m of trough space (Hsia and Wood-Gush, 1983b). A number of other studies have reported a reduction in food intake as the number of animals per feeding station is increased but the results are far from conclusive (Rippel, 1960; Blackshaw, 1981; Lindemann and Kornegay, 1984; Walker, 1990a).

There is little scientific evidence on which recommendations can be made about the feeding space needed for pigs fed *ad libitum*. However, I suggest that for simple feeders with no barriers between the feeding positions, no more than four pigs should be allocated per feeding place. The installation of feeding barriers gives isolation to the pig whilst feeding and the number of pigs per place can be increased to around 10. Provision of water in the feeder may increase the rate of intake allowing the number of pigs per place to increase to between 12 and 15. Further increases require a high level of management and the chance of aggressive behaviour is increased. There does not appear to be any advantage in providing extra feeding space for newly-weaned piglets (Lindemann et al., 1987; Baxter, unpublished) even though it might by hypothesized that piglets would prefer to feed simultaneously as they have done prior to weaning.

#### Guidelines for the design of a feeding system

On the basis of experimental studies and field observations the following set of requirements for the design of a feeding system for the pig have emerged:

- 1. Pigs should be fed from a feeder rather than on the floor because of the reduction in feed wastage and feed spoilage, and the more even distribution of intakes between individuals.
- 2. Adequate trough space per pig, equivalent to 1.1 x shoulder width, should be provided when pigs are ration fed. The variability in individual intakes, feed wastage and aggressive behaviours can all be reduced by the inclusion of trough barriers.
- 3. Mild competition, by limiting the number of feeding places, may increase food intake when pigs are fed *ad libitum* due to social facilitation. There appears to be no advantage in providing extra feeding space for newly-weaned pigs.
- 4. Providing a trough profile closely fitted to the shape of the pig should allow the pig to feed comfortably and to reach the food in a preferred feeding posture. A high lip at the front of the feeder and barriers between the feeding positions would minimize feed wastage.

## The development of an ad libitum feeding system

On the basis of the above guidelines, an *ad libitum* feeder has since been developed (Figure 6). Experimental evaluation of this prototype feeder indicates that, compared to a conventional trough, feed wastage was reduced from 10.2% to 0.3%, and the incidence of aggression was reduced by 60%. Further improvements to this initial design included curving the feeder lip around the curve of the pig's throat and curving the barriers beyond the front of the feeder to prevent pigs from fouling the feeder whilst not taking up any extra lying space within the pen. In the manufacturer of this feeder, a material called polymer concrete was chosen because of its high durability and resistance to corrosion, yet it is a cast material suitable for producing the complex curves required.

A trial comparing the new *ad libitum* feeder with a 'conventional round rotating feeder' was conducted by Neal and Rahmena (1991) using 198 pigs in eight pens.



Figure 6. The ACO ad limitum feeder for pigs (Baxter, 1989).

Of the feed consumed, there was a trend for the feed from the new feeder to be converted more efficiently, probably due to the reduction in feed wastage. In addition, there was a trend for an increase in average daily gain by those pigs on the new feeder which suggests that perhaps food intake was stimulated, due possibly to improved access to the feed and reduced social competition.

 Table 1. Comparison of new ad libitum feeder and conventional rotating round feeder (Neal and Rahmena, 1991)

	Ad libitum	Conventional	Significance <sup>1</sup>
Average daily gain (g)	641	596	NS
Feed disappearance (kg/d)	1.82	1.75	NS
Feed/gain	2.84	2.93	NS

<sup>1</sup>NS, non significant (P>0.05).

Two trials have compared the new feeder with single space wet and dry feeders. Yacentiuk (1991) in Canada reported a reduction in average daily gain with the new feeder but improved feed conversion efficiency. Walker (1990a,b) from Northern Ireland also found reduced growth rate from the new feeder, no difference in feed conversion efficiency but increased thickness of backfat from the wet and dry feeder. The addition of water in the feeder increases food intake by between 7 and 10% (Yacentiuk, 1991; Walker, 1990b) and when the water was switched off in the experiment of Walker (1990a) there was no difference between the two types of feeder. The evaluation and further development of this feeding system is currently in progress.

### Conclusion

In the past the design of feeding systems for the growing pig has concentrated more on the structural requirements of the manufacturer and only a visual assessment of what is required by the pig. Both physical and social aspects need to be considered if food intake and hence average daily gain is to be maximized. This paper has described an approach whereby the dimensions of the pig during feeding were documented and this, together with the results of behavioural observations, were incorporated into the design of a new feeding system for the growing pig. Further studies are required to investigate the true effect of these feeders on performance, especially in regard to the effect on reducing aggression since the decrease in social interaction may in itself reduce food intake. The same approach to design should also be used in developing a feeder suitable for the lactating sow.

# THE EVALUATION OF SINGLE-SPACE AND WET-AND-DRY FEEDERS FOR THE AUSTRALIAN ENVIRONMENT

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### Introduction

The development of single space wet and dry (SSWD) feeders, where feed is available to only one of a group of pigs at a time, represents a major departure from the traditional concepts of feeding pigs. The method by which the pig accesses the feed at the trough may vary, but features common to SSWD feeders are a small hopper located above a trough which is normally about 250-350 mm wide for grower and finisher pigs and contains a nose or bite operated drinker positioned to prevent pigs drinking directly from it. This method of feeding constitutes an extreme restriction on feeding space but, despite this, its benefits have been widely acclaimed especially in Europe. An increase in feed intake, improved growth rates and feed conversion efficiency, leaner carcases, reduced wastage of feed and water, and less aggression between pigs when feeding have all been attributed to the use of SSWD feeders.

Single-space wet and dry feeders were introduced into Australia about four years ago. At that time little experimental evidence was available to support the above claims. For example, information on the optimum number of pigs per feeder, the position of the feeder in the pen and the necessity for independent drinkers had not been adequately determined. There were also some negative reports from the United Kingdom that the use of SSWD feeders had resulted in an increase in carcase backfat and an increase in the fouling of pens.

Australian producers showed considerable interest in SSWD feeders and many purchased a limited number of feeders for evaluation on their units before installing them in any quantity. This paper reports on the evaluation of this new feeding system in respect to the Australian pig industry. On-farm experiences in the use of SSWD feeders, documented during a Pig Research and Development Corporation funded study tour of 18 commercial and 3 research pig units conducted in September 1990 (Payne, 1991), together with experimental data from Australia, is presented.

# **Commercial observations**

#### Number of pigs per SSWD feeder

Experiments conducted in several European countries indicate that between 10 and 15 is the optimum number of pigs per SSWD (Peerlings, 1986; Peet, 1989; Walker, 1990a). In Australia, suppliers of SSWD feeders generally recommend a limit of 15 pigs per SSWD feeder, and the majority of units visited ran between 10 and 15 pigs per SSWD feeder. When group size exceeded 20 pigs two SSWD feeders were provided, positioned on opposite sides of the pen.

#### Position of SSWD feeder in the pen

Pigs will not choose to rest in areas subject to commotion and disturbance such as around self-feeders (Baxter, 1989). Therefore a position half to three quarters of the way down the side of fully-slatted pens, or on the edge of the lying area adjacent to the dunging area in solid-floored or part-slatted pens, has been recommended because it minimizes the likelihood of aggressive behaviour (Peet, 1989).

In commercial practice, however, the position of the feeder was usually determined by constraints of the feed delivery system, and the need for easy inspection, adjustment and cleaning rather than on the basis of the pigs social environment. There were no obvious problems with the SSWD feeders being located at the front of the pen by most producers but some reported an increase in fouling of the pen which was subsequently corrected by felocating the feeder to the edge of the lying area. Most producers were aware of the recommendations of Peet (1989), however, few were prepared to depart from existing practices without more evidence of production, hygiene and welfare advantages.

## Provision of independent drinkers

There is experimental evidence that independent drinkers, besides that located within the SSWD feeder, are not necessary and that their omission can substantially reduce water wastage (Plagge, 1989; Walker, 1990a). Concern that the water supply in the SSWD feeder might be inadequate during warm conditions and that the drinker within the SSWD feeder might become blocked, especially when meal diets were used, were reasons given for independent drinkers being provided on all units visited. Potable water is a scarce commodity in some parts of Australia and perhaps the necessity of independent drinkers needs to be investigated under local environmental conditions.

## Problems associated with the use of SSWD feeders

#### Increased carcase backfat

Higher feed intake from SSWD feeders can lead to an increase in carcase backfat in some genotypes of pigs to the extent that the benefits from growth efficiency are negated by loss in carcase value, especially under stringent grading schemes (Peet, 1989). Increased carcase backfat was thought to be a problem by about half the producers visited, with the change from floor-feeding to SSWD feeders causing the greatest increase. These observations suggest that such a feeding system is most appropriate for producers with superior genotypes since these animals will give the best response to an increase in food intake.

A variety of strategies was employed by producers whose pigs became unacceptably fat after the introduction of SSWD feeders. In some cases adjustments were made to dietary specifications to ensure the use of optimum amino acid to energy ratios for the age, sex and genotype of pig. Simulations using the AUSPIG model (Black *et al.*, 1986) indicate that significant reductions in P2 could be achieved in some circumstances by the re-formulation of diets (Mullan and Payne, unpublished). Other producers altered their marketing policy so as to counteract the increased number of fat pigs.

SSWD feeders are applicable primarily to *ad libitum* feeding regimens but some of the benefits from their use are attractive to producers who restrict feed in the finisher phase. English *et al.* (1988) reported work by Hanrahan who restricted access to conventional *ad libitum* feeders to 6 hours daily apparently without unacceptable variation in growth and carcase quality. This approach is currently being examined at the Intensive Industries Research Centre, Medina, Western Australia, where the performance of groups of 8 pigs given continuous access to SSWD feeders in the grower phase, then restricted to 6 hours daily in the finisher phase, is being compared with that of pigs with no restriction. Preliminary results show that apparent feed intake and growth rate in the finisher phase were about 6% lower on the restricted treatment, with no differences in feed conversion efficiency or P2 backfat. During summer apparent feed intakes were similar for both treatments in some replications, indicating a possible seasonal effect.

### Pen fouling

Some units experienced an increase in pen fouling after installing SSWD feeders, and it would appear that the location of the feeder in relation to the use pigs make of pen space for lying, defecating and other social activities was important. This in turn depends on the shape of the pen, the thermal environment at pig level, the type of barrier and the provision and position of other drinkers (Baxter, 1986). It may be that the availability of water in the feeder creates a wet area around the feeder which triggers a change in dunging and urinating behaviour. Reducing the flow rate to, and water pressure at, the water outlet in the feeder to reduce spillage was found by producers to correct pen fouling, as did disconnecting the water supply. Alternatively, it may be the increase in feed intake or changes in feeding behaviour that causes the increase in pen fouling.

## Practical considerations

The need for a greater input of labour to inspect, adjust and clean SSWD feeders was seen as a disadvantage especially on larger units. These feeders were also considered unsatisfactory for pigs under 15 kg liveweight as young pigs appear slow in learning to use the dispensing systems and tend to play with the drinkers, resulting in excessive spoilage and wastage of feed.

#### Australian research results

# On-farm studies

Few of the results from on-farm trials collected during the study tour had been statistically analysed and many did not have the required number of replications. However, the growth rate of pigs on SSWD feeders was the same or better than with conventional dry self feeders in 16 of the 17 on-farm trials examined and this could be directly attributed to an increase in voluntary feed intake in most cases. In some instances there was an apparent decrease in feed intake but an increase in growth rate, and this was probably due to the combined effect of an increase in feed intake together with a greater reduction in feed wastage with the new feeding system.

In an attempt to quantify the response from using SSWD feeders, the performance of pigs fed from SSWD as a percentage for those fed from existing dry feeders was calculated for those trials which included all of the necessary data (Table 1). The overall increase in growth rate when pelleted diets were used can be explained by the corresponding increase in voluntary feed intake. In the trial where growth rate was depressed by the use of SSWD feeders there was also a reduction in apparent feed intake. When meal diets were used there was an increase in growth rate despite no change in apparent feed intake, but the improvement in feed:gain suggests that there had been a reduction in feed wastage with the SSWD feeders.

	Pelleted		Meal		
	Average	Range	Average	Range	
	(%)	(%)	(%)	(%)	
Number of trials	3 (1084 pigs)		7 (516 pigs)		
Apparent feed intake	105	93-113	101	32-109	
Growth rate	104	87-115	108	101-122	
Feed:gain	102	97-107	93	92-102	

Table 1. The change in average performance of pigs fed from SSWD feeders as a percentage of those fed from dry self feeders in 10 on-farm trials

#### Research results.

Under controlled conditions at the Northfield Pig Research Unit, Adelaide,

South Australia, SSWD feeders with meal diets increased growth rate by about 5% without significant changes in backfat (Cecil, 1990). At the North Coast Agricultural Institute, Wollongbar, New South Wales, also with meal diets, growth rate and apparent feed intake were increased by about 8% and 6%, respectively, and there were small but non significant increases in backfat (Taylor and Clarke, 1990).

At the Intensive Industries Research Centre, Medina, Western Australia, the apparent feed intake of pigs fed meal diets from SSWD feeders was about 10% higher than those on either single space feeders used dry or conventional multi-space feeders. As a consequence their growth rate was about 6% faster and the depth of backfat increased (Table 2). There were no differences between any of the treatments in the feed conversion efficiencies and there were no differences in any parameters between the two dry feeding systems.

	Multi-space	Single-space		SEM <sup>1</sup>	
	Dry	Dry	Wet		
Mean feed intake (kg/d)	2.06	2.00	2.21	0.059	
Liveweight gain (g/d)	777ª	779ª	827 <sup>b</sup>	9.5	
Backfat (P2, mm)	16.1ª	16.3ª	17.5 <sup>ь</sup>	0.42	

Table 2. The performance of growing pigs (20-90 kg liveweight) fed meal diets *ad libitum* from multi-space or single-space feeders (60 pigs/treatment)

<sup>1</sup>Within rows, means with different superscripts are significantly different (P<0.05).

These results suggest that any differences in growth rate between pigs on the SSWD and conventional multi-space feeders are largely attributable to the provision of water at the feed trough stimulating an increase in voluntary feed intake. The feed wastage appeared to be similar for both types of feeder. These results agree with the findings of Walker (1990a) in Northern Ireland who showed that feed intake and growth rate of pigs fed meal diets from SSWD feeders connected to a water supply increase by 7% and 10% respectively compared with pigs fed dry from either single or multi-space feeders. There was a non significant increase of 3% in feed conversion efficiency and P2 backfat increased from 13 mm to 14 mm. AUSPIG simulations using data from the Medina herd suggest that the differences in performance can be accounted for by the increase in feed intake (Mullan and Payne, unpublished).

#### Summary

The Australian pig industry has cautiously accepted SSWD feeders as a means of increasing the voluntary food intake and hence performance of grower and finisher pigs. The results of experiments conducted on farm and at research centres indicate that SSWD feeders will increase the intake of meal diets, with a corresponding increase in growth rate and possibly carcase backfat. Feed conversion efficiency can be expected to improve due primarily to a reduction in feed wastage. When pelleted diets are used the response is less predictable; it appears that there will be some increase in feed intake and growth rate but the savings in feed wastage will be less compared to meal diets and hence there may not be a significant improvement in feed conversion efficiency. These results are in general agreement with results published from overseas.

There are, however, a number of issues which warrant further investigation before the widespread use of these feeders can be recommended. For example, the effects of season, the need for supplementary drinkers and the environmental factors which contribute to an increase in pen fouling all require further study. Many of the concepts of social behaviour, for example those described by Baxter (1986), have also not been considered during the development of this and other new feeding systems and this lack of communication within the pig industry needs to be addressed.

# VOLUNTARY FOOD INTAKE IN GROWING PIGS AT AMBIENT TEMPERATURES ABOVE THE ZONE OF THERMAL COMFORT

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## Introduction

High ambient temperatures pose a major constraint on pig production in Australia principally through a reduction in voluntary food intake (VFI) (Standing Committee on Agriculture, 1987). Other factors, including skin wetness (Vairabukka et al., 1987), air movement (Bond et al., 1965), relative humidity (Morrison et al., 1969) and the amount and composition of the diet (Stahly and Cromwell, 1979) interact with air temperature to determine the extent of heat loss from the pig and the magnitude of the depression in pig performance. Acclimatization to hot conditions may also affect the animal's response to its environment over time (Morrison and Mount, 1971). Because of the complexity of these interacting factors, it is unlikely that simple empirical relationships between VFI and ambient temperature (reviewed by Close, 1987), will provide an accurate prediction of VFI for pigs raised under commercial conditions. A reliable prediction of the changes in VFI with climate requires an accurate assessment of the heat exchange between an animal and its environment, knowledge of the physiological mechanisms involved in altering the rate of heat exchange and description of the associations between these physiological responses and VFI. Such an approach has been used by Black et al. (1986) to integrate the physiological function of pigs into a whole-animal simulation model, AUSPIG, which predicts the effects of climate, genotype, diet and stocking rate on the energy and amino acid utilization of pigs from birth to maturity. Although the influence of raised ambient temperature on several behavioural (Mount, 1968) and physiological (Ingram, 1974) characteristics have been studied, there is insufficient information relating these changes to VFI to be certain that the concepts adopted within the Auspig model adequately reflected reality.

A series of experiments was conducted to examine the associations between physiological changes, VFI and heat production of finisher pigs exposed to high ambient temperatures when other environmental factors were controlled. Heat production was measured continuously as oxygen consumption from the product of cardiac output and the difference between arterial and mixed venous blood oxygen content (Fick principle) as described by Giles *et al.* (1989, 1990). The purpose of this paper is to outline the physiological mechanisms found to be associated with changes in VFI and heat production of finisher pigs when exposed to ambient temperatures above the zone of thermal comfort.

### Regulation of heat exchange with the environment

The pig is able to regulate its heat exchange with the environment over a wide range of climatic conditions. This is achieved by various mechanisms including shivering, altering the rate of blood flow to the skin, changing posture, huddling, regulating evaporative heat loss from the lungs and skin and changing food intake. As ambient temperature falls below the zone of thermal comfort, the animal has to increase heat production to maintain body temperature. The temperature at which this occurs is known as the lower critical temperature (LCT). Once this temperature is reached, the animal has done all it can in terms of vasoconstriction, huddling and change of posture to minimize heat loss from the body.

When air temperature rises above LCT, the animal first controls body temperature by mechanisms that require little effort. These include a change in posture so that more skin is in contact with the floor when it is cooler than air, a change from vasoconstriction to vasodilation so that more heat is lost from the skin and a change in huddling behaviour so that there is reduced contact with other pigs. However, with further increases in temperature, thermoregulation can be achieved either by a substantial increase in evaporative heat loss from the lungs and skin or by reduction in VFI. The air temperature at which evaporative heat loss increases markedly was termed evaporative critical temperature (ECT) by Black *et al.* (1986). The range in ambient temperatures between LCT and ECT, within the thermal neutral zone, is called the zone of thermal comfort.

Evaporative heat loss in pigs is increased mainly through an increase in respiration rate. Whilst the pig is able to elevate respiration rate up to six fold above the zone of thermal comfort, heat loss from the respiratory tract is quite inefficient compared to other animals, such as the dog. Pigs have little capacity to increase evaporative heat loss from the skin because they do not have true sweat glands; in their natural environment, they wallow and evaporate water from mud on the skin. In commercial practice, it is rare to find completely dry pigs in a hot environment. Even pigs held on a slatted or wire-mesh floor are able to wet some skin with drinking water, saliva, dung and urine in a deliberate attempt to wet the skin surface.

The upper critical temperature (UCT) is the temperature at which the animal's maximum rate of heat loss coincides with heat input from the diet and the environment. Between ECT and UCT, the pig will allow body temperature to rise, but at some point between ECT and UCT, the pig will reduce VFI because it has few thermoregulatory mechanisms left. If ambient temperature rises above UCT, a dramatic rise in body temperature is likely to occur, often followed by death.

Confusion exists in the literature about the pig's response in heat production as ambient temperature increases above the zone of thermal comfort. Mount (1974) implies and Holmes and Close (1977) predicted that heat production increases at the upper end of this zone. This concept does not agree with the observations of Nienaber *et al.* (1987) who found a continuous decline in VFI and heat production of growing pigs with increase in ambient temperature up to 30°C. Part of this conflict arises from uncertainty about the upper end of the zone of thermal comfort (or ECT). In addition, the conflict could be explained possibly by the differences in feeding regimes used in the different studies. When a restricted food intake is offered to pigs, heat production will rise at ambient temperatures above thermal neutral because of increased energy expenditure associated with raised respiration rate. However, when pigs are offered food *ad libitum*, the increase in energy expenditure associated with respiration rate may be more than compensated for by a reduction in VFI and activity.

## Short-term responses to ambient temperature above the zone of thermal comfort

To obtain information on the interaction between physiological changes, VFI and heat production at ambient temperatures above the zone of thermal comfort, an experiment was conducted (Giles et al., 1990), with four female pigs, mean live weight 89kg, which were housed in metabolism crates and prevented from wetting the skin surface. Each pig was exposed in turn to 25, 28 and 31°C for 48h with four days at 22°C between each temperature treatment. Mean VFI and oxygen consumption declined at ambient temperatures above 22°C in close association with a rise in body temperature and respiration rate (Table 1). After 48h at each temperature treatment, body temperature had stabilised but mean respiration rate declined at 31°C from 159 respirations per minute during the first 24h to 112 respirations per minute by day 2. The main components responsible for the decline in oxygen consumption were a decline in cardiac output and an increase in mixed venous blood oxygen saturation, reflecting reduced VFI and activity of pigs at an ambient temperature above 22°C. Pigs were observed to be less active above 22°C and this was indicated by an increase in mean daily mixed venous oxygen saturation from 55.5% at 22°C to 60% at all other temperature treatments.
Ambient temperature (°C)	22.7	25.9	28.5	31.4
Voluntary food intake (g/d)	2846	2340	1888	900
	(275.5)	(256.3)	(218.6)	(223,5)
Cardiac output (1/min)	9.3 (1.27)	9.5 (1.09)	8.4 (1.05)	7.5 (1.41)
Mixed venous $O_2$ saturation (%)	55.5	60.0	60.1	59.5
	(1.16)	(1.92)	(1.70)	(1.67)
$O_2$ consumption (ml/min)	511	464	433	371
	(82.5)	(33.5)	(58.8)	(72.0)
Respiration rate (per min)	27	51	85	112
	(2.0)	(4.0)	(2.3)	(15.3)
Body temperature (°C)	39.0	39.1	39.5	40.4
	(0.04)	(0.11)	(0.15)	(0.17)
Skin temperature (°C)	33.9	35.1	37.0	37.9
	(0.13)	(0.42)	(0.29)	(0.31)

Table 1. Effect of ambient temperatures above 22°C for 48h on the mean 24h results (day 2) of four female pigs (average 89 kg live weight) with standard errors in brackets

The results presented in Table 1 indicate that when growing pigs are fed *ad libitum* and prevented from wetting the skin surface, ECT is between 22 and 25°C. The decline in VFI and heat production occurs at an ambient temperature closer to ECT than to UCT as suggested by Mount (1974) and Black *et al.* (1986). Beyond ECT, the growing pig appears to reduce heat production by a simultaneous reduction in VFI and activity. Concomitant with these changes there occurs firstly, an increase in respiration rate to increase evaporative heat loss from the lungs; and secondly, there is an increase in body temperature and blood flow to the body surface which will increase sensible heat loss as shown by the increase in skin temperature (Table 1).

# Acclimatization to ambient temperature above the zone of thermal comfort

There is little information on the changes in physiological characteristics and VFI over time when growing pigs are maintained at high ambient temperature. In a study conducted with two female pigs, (mean live weight of 91 kg), housed in metabolism crates and maintained at 22°C on day one and thereafter at 31°C for 11 days (Giles *et al.*, 1991), it was found that respiration rate and oxygen consumption declined steadily over time but body temperature declined only slightly and remained above 40°C. Conversely, VFI declined to 1.0 kg/day within 24h and remained unchanged throughout the study (Figures 1 and 2). These results support the findings of Morrison and Mount (1971) who found that VFI remained unchanged with pigs of 50 kg live weight maintained at 33°C for 28 days.

The results suggest that VFI is more closely associated with body temperature than to respiration rate. However, the decline in oxygen consumption was related closely to the decline in respiration rate. This suggests that evaporative heat loss per individual breath becomes more efficient over time, thus reducing oxygen consumption while VFI remains unchanged.

# Responses to daily fluctuations in ambient temperature

The previous studies were conducted at constant daily temperatures to identify associations between changes in physiological characteristics and VFI. Because ambient temperature fluctuates hourly in commercial piggeries, another series of experiments was conducted with fluctuating daily temperatures to determine if VFI responded similarly to changes in the physiological characteristics. If so, VFI should be predictable for pigs exposed to any fluctuations in ambient temperature. Furthermore, it was important to know what length of time per day was required at a temperature within the zone of thermal comfort to overcome the depression in VFI caused by a period of high ambient temperature.

Physiological changes, VFI and oxygen consumption were measured with two female pigs (mean live weight, 87.3 kg) exposed to  $22^{\circ}$ C for one day followed by eight days of 12h at 31°C (9.00 to 21.00h) and 12h at  $22^{\circ}$ C (21.00-9.00h) (Giles *et al.*, 1991). The similarity in VFI found for both pigs at a constant temperature of  $22^{\circ}$ C (2.8 kg) and during the fluctuating temperature treatment (2.5 kg) suggests that 12h in a thermal neutral environment is sufficient to overcome 12h at 31°C in the absence of skin wetness. Interestingly, during the first four days of the fluctuating temperature treatment the pigs transferred an increasing portion of their food intake at 31°C to the cooler 22°C period of the day without affecting total VFI (Figure 3). Mean daily body temperature remained above 39.5°C at an ambient temperature of 31°C (Figure 4) which was associated with a depression in VFI during the 12h at 31°C to less than 500 g by day 4. Oxygen consumption declined with time from 450 ml/min on day 1 at 22°C to 300 ml/min by day 6 in association with a decline in respiration rate during the 31°C period of the day.

To determine the dynamic relationship between body temperature and VFI, Lorschy *et al.* (1991) examined the hourly VFI and body temperature of five female finisher pigs (mean live weight 79.3 kg), housed in metabolism crates for seven days and exposed to a daily fluctuating ambient temperature environment of either 16 or 20h at 31°C with the remainder of the day at 22°C. When exposed to an ambient temperature of 31°C, body temperature increased gradually from 39°C to more than 40°C within 12h, but when ambient temperature was reduced to 22°C, body temperature returned to less than 39.5°C within 2h. Whereas, mean hourly VFI was less than 100g during the 31°C period of the day, hourly VFI increased to greater than 250g once body temperature declined to less than 39.5°C at an ambient temperature of 22°C.

# Conclusion

It is now clear from several studies described in this paper that an increase in body temperature above 39°C may have a primary role in the control of VFI in growing pigs. However, other factors, such as the pig's behavioural response, the rate of change in body temperature and energy deficit may also be involved. The decline in VFI appears to coincide closely with ECT. However, under conditions in commercial piggeries, the varying extent of skin wetness, air movement, relative humidity, live weight of pig's and feeding conditions will cause ECT to change from near 22°C as measured under the experimental conditions described in this paper. However, accurate predictions of critical temperatures can be obtained using simulation models such as AUSPIG.

Information is now available on the physiological mechanisms associated with the decline in VFI over a wide range of climatic and animal conditions encountered within the pig industry. This information is essential to the further refinement of mechanistic models such as AUSPIG. The application of techniques to monitor body temperature of a sample of growing pigs within a commercial environment using technology such as ear-canal sensors and telemetry (Jorgensen *et al.*, 1986), would allow the control of sprinklers and air movement and the maintenance of VFI in high temperature environments.

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Figure 1. Respiration rate 0-0 and  $O_2$  consumed 0-0 by two pigs maintained at 31°C.

Figure 2. VFI 0---0 and body temperature •--• of two pigs maintained at 31°C.





Figure 3. VFI of two pigs at 31°C ●---● (0900-2100h) and 22°C 0---0 (2100-0900h).

Figure 4. Body temperature of two pigs at  $31^{\circ}$ C  $\bullet$ — $\bullet$  (0900-2100h) and 22°C 0—0 (2100-0900h).

# THE RESPONSE OF THE BREEDING SOW TO THE CLIMATIC ENVIRONMENT

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# Introduction

The design of a management strategy for sows includes setting reproductive targets and then calculating nutrient allowances to meet these specified targets (Williams and Mullan, 1989). Environmental factors that influence either voluntary food intake (VFI) or the partition of ingested nutrients will determine whether these allowances, and hence reproductive targets, are achieved. The following paper considers the impact that some of the major climatic factors have on the performance of sows during gestation and lactation.

# Gestation

The recommended gains in maternal bodyweight during gestation can be achieved at levels of intake considerably less than *ad libitum*. For example, Friend (1971) recorded intakes of 67 MJ DE/day by sows fed *ad libitum* during their first pregnancy, whereas the Standing Committee on Agriculture (SCA) (1987) recommended daily energy intakes of between 21 and 27 MJ DE. Therefore, the effect of environment on feed intake *per se* is of little consequence to the pregnant sow. However, since the energy cost of maintenance represents 75 to 85 % of the total energy requirements of gestation (Aherne *et al.*, 1991), environmental factors which affect maintenance requirement for energy will affect the partition of nutrients during gestation with subsequent effects on performance. Of primary concern are those factors which influence the lower critical temperature (LCT), which is the temperature below which the animal must utilize metabolisable energy to increase heat production in order to maintain body temperature (Giles and Black, 1991).

# Lower critical temperature

There are a number of factors which will influence the LCT and these have been the subject of several reviews and research papers (Holmes and Close, 1977; Geuyen *et al.*, 1984; Close, 1987; Kemp *et al.*, 1987). Of particular importance and quantifiable to the pregnant sow are:

- 1. *Wind speed,* which disrupts the thermal insulation provided by the boundary layer of air around the animal and is the major determinate of convective heat loss.
- 2. *Group versus single housing,* thereby determining the ability of animals to huddle and their physical activity.
- 3. Bedding and floor type, since proportionately about 0.20 of the animal's body can be in contact with the floor and this influences the degree of conductive heat loss.

The combined effect that level of feeding and any of the above three factors has on determining the LCT for a pregnant sow is illustrated in Figure 1. Lower critical temperatures were determined using AUSPIG, a computer simulation model which takes account of factors such as the amount and composition of food eaten, climatic conditions, genotype, stage of reproduction and body composition to predict, on a daily basis, energy and amino acid utilization in the sow and piglet (Black *et al.*, 1986). Sows fed 1.5 times their maintenance requirement (24 MJ DE/day for a 140 kg sow), group-housed in a shed with minimal air speed (0.15 m/sec) and with access to straw for bedding would have a LCT of 15°C, whereas the LCT for the same animal housed individually, in draughty conditions (0.6 m/sec) and on a concrete floor would be 25°C. While this illustrates the large range in LCT present in commercial dry sow accommodation, what is more important is whether ambient temperature is above or below the animal's LCT.



Figure 1. Effects of environment on LCT of pregnant sows.

# Effects of low ambient temperature on performance

The relationship between ambient temperature and LCT, and its effect on the partition of nutrients is demonstrated by the results of AUSPIG simulations presented in Table 1. To achieve a target gain during pregnancy of 30 kg maternal bodyweight and 6 mm of backfat (P2), a sow weighing 120 kg at mating with 20 mm of backfat would require 25 MJ DE/day, similar to that recommended by the SCA (1987). This is provided, however, the animal is housed within its zone of thermal comfort, which is the temperature range between LCT and evaporative critical temperature (ECT) (Giles and Black, 1991).

If there is a change in LCT, for example an increase from 18 to 23°C due to a change in the system of housing as portrayed in Table 1, then despite ambient temperature remaining the same it is now less than the LCT. The animal must increase heat production to maintain body temperature thereby increasing the energy required for maintenance and leaving less dietary energy available for tissue deposition; the principal effect is a 70% decrease in the deposition of body fat. Nitrogen retention and hence protein deposition is affected to a much lesser degree. These results are supported by the findings of Hovell *et al.* (1977) and Kemp *et al.* (1987) from experiments conducted to examine the effect of environmental temperature on energy and protein metabolism in the pregnant sow. For example, in the experiment of Kemp *et al.* (1987), fat deposition by only 20% (from 94 to 76 g/day) when ambient temperature was reduced from 21°C to 15°C whilst the LCT remained at 18°C.

Ambient temperature (°C)	20	20	20
LCT (°C)	181	23²	23²
Feed intake (kg/d)	2:0	2.0	2.3
DE intake (MJ/d)	25.0	25.0	28.8
Weight gain during gestation (kg) <sup>3</sup>			
Liveweight	30.0	23.0	32.0
Fat	11.0	3.4	11.6
Protein	4.9	4.8	4.9
			•

Table 1. The relationship between ambient temperature and lower critical temperature (LCT) and its predicted effect on the composition of weight gain during gestation

<sup>1</sup>Group-housed sows with straw bedding; <sup>2</sup>individually housed sows on concrete floors, and <sup>3</sup>sows were 120 kg at mating with 20 mm P2 backfat.

The importance of body condition to the response of animals to cold environments is well illustrated by the work of Holmes and McLean (1974) and Hovell *et al.* (1977). The former calculated that tissue insulation of fat sows was 40% greater than that for sows with half as much body fat. This effect could exacerbate the problem of some modern genotypes that are already too lean when they commence breeding, since not only will they be less able to tolerate cold environments (Holmes and Close, 1977) but they will also deposit less body fat during gestation if they are given the same amount of nutrients as sows in better body condition. In the case of sows being housed outdoors, suitable accommodation must be provided or additional dietary energy supplied to compensate for the reduction in energy retained.

A situation where ambient temperature is less than LCT could be corrected by a change in management and/or shed design to modify the animal's immediate environment and hence increase LCT. Whether this is cost effective will depend on the extent and duration of the problem. The alternative would be to increase feed intake, thus compensating the animal for the extra energy required to maintain body temperature. For the example described in Table 1, where ambient temperature was  $3^{\circ}$ C less than LCT increasing energy intake by 3.8 MJ DE per day corrected the situation.

# Lactation

The management of sows during lactation should ensure that the intake of nutrients is sufficient to wean an adequate number of piglets of an acceptable body weight, with minimum variation, yet without utilizing excessive body reserves to prejudice subsequent reproductive performance (Mullan *et al.*, 1989). The inability of sows to consume adequate quantities of food during lactation is a common problem (Cole, 1990). Sows will mobilize body reserves to support milk production when food intake during lactation is low and, if the loss of body reserves is excessive especially with young sows, reproductive performance may be impaired (King and Williams, 1984; Mullan and Williams, 1989). Environmental temperature is one of a number of factors which may influence the VFI of the lactating sow (O'Grady *et al.*, 1985) and consequently may have a major impact on the performance of the lactating sow.

# Evaporative and upper critical temperature

The LCT of the lactating sow is approximately 10 to 12°C lower than that for a pregnant sow housed under similar conditions. For example the LCT of a lactating sow is 12°C whereas the LCT of a pregnant sow of similar weight and body condition is 22°C when both sows are housed on a concrete floor with minimal air movement.

This is primarily due to the sow being fed at a level close to *ad libitum* during lactation, thus consuming 3 to 4 times its energy requirement for maintenance. Therefore in most circumstances the LCT for lactating sows is of little consequence whereas the upper range of the zone of thermal comfort is of greater importance.

The evaporative critical temperature (ECT) signifies that point at which thermoregulation can only be achieved by an increase in evaporative heat loss from the lungs and skin (Giles and Black, 1991). The temperature at which evaporative heat loss is maximal and body temperature begins to rise is the upper critical temperature (UCT). Between ECT and UCT body temperature begins to rise and at some stage above ECT the pig will reduce VFI.

# Effects of high ambient temperatures on food intake and performance

The detrimental effect that an increase in ambient temperature has on voluntary energy intake during lactation has been investigated in a number of experiments (Figure 2). From the results of these 9 experiments it could be concluded that each °C increase in ambient temperature above 20°C daily voluntary energy and food intake declines by 2.4 MJ DE and 0.17 kg respectively. However, only one of these experiments included intermediate temperatures and it would seem unlikely that the response between ambient temperature and food intake is linear over this range of temperatures. Rather, as eluded to by Giles and Black (1991), the decrease in intake may depend on the extent to which ambient temperature exceeds the animal's ECT. The extent to which high ambient temperatures may be affecting VFI in Australian piggeries can be gauged by data from a commercial piggery during summer (Figure 3). Temperature sensors were positioned approximately 1 m above floor level and away from the influence of radiant heaters. Mean temperature at sow level for the month was 26°C (range of 17 to 39°C) and hourly temperatures being frequently greater than the animal's ECT of 25°C. However, mean daily food intake may not be affected if the sow responds in a similar way to the growing pig to diurnal temperature fluctuations (Giles and Black, 1991) provided there is sufficient food available for the sow to eat during the cool of the day.

The effect of high ambient temperatures on VFI has important consequences for reproductive performance. In an attempt to maintain milk production sows will mobilize body reserves when food intake is low. Mean data from four experiments indicates that while sows housed at between 17 and 21°C had a bodyweight loss of 6.7 kg during a 28-day lactation, at higher ambient temperatures (26 to 30°C) the loss of bodyweight was increased to 20.1 kg in response to the effect of high temperature on VFI (Lynch, 1977; Stansbury et al., 1987; Yen and Cheng, 1990; Vidal et al., 1991). There is some evidence that high ambient temperatures during lactation cause a decrease in LH pulse frequency and that this is then responsible for a failure to rebreed after weaning (Barb et al., 1991). However, as eluded to by Barb et al. (1991), since there is also evidence that low nutrient intakes during lactation cause an increase in the weaning-to-mating interval of first-parity sows (King and Williams, 1984; Mullan and Williams, 1990), and that this is in part due to a disruption to the normal secretory pattern of LH (Mullan and Close, 1989), then high ambient temperatures per se may not be the direct cause of poor fertility but instead be via its effect on nutrient intake. Feed intake in the experiment of Barb et al. (1991) was reduced from 6.1 to 2.9 kg/day due to the effect of high ambient temperature.

The reduction in the growth rate of piglets suckling sows maintained at high temperatures has been assumed to reflect a reduction in milk yield. This conclusion has been confirmed by recent data of Schoenherr *et al.* (1989b) and Vidal *et al.* (1991) who recorded decreases in milk yield of 10 and 35 %, respectively, when ambient temperature was increased by  $10^{\circ}$ C (Table 2). However, as with the effect of environmental temperature on reproductive performance, it is not known whether this reflects a direct effect of high temperature on milk synthesis and/or whether the decrease in the supply of substrates due to the low VFI of the sow is responsible for the decrease in milk production.



Figure 2. The effect of ambient temperature on the voluntary energy intake of the lactating sow (Barb et al., 1991; Cole, 1990; Lynch, 1977; Lynch, 1989; Schoenherr et al., 1989a,b; Stansbury et al., 1987; Vidal et al., 1991; Yen and Cheng, 1990).



Figure 3. Ambient temperatures inside a farrowing shed - Shepparton, Victoria; February 1991 (Mullan and Maxwell, unpublished).

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Reference <sup>1</sup>	1	1	2	2
Ambient temperature (°C)	20	30	22	30
Length of lactation (days)	22	22	27	27
Sow				
Feed intake (kg/d)	5.9	3.36	7.72	4.95
Change in bodyweight (kg)	-2.6	-15.9	-6.4	-21.0
Change in backfat (mm)	NA	NA	+2.2	-3.0
Milk yield (kg/d)	8.34	7.47	10.27	6.64
Piglet				
Daily gain (g)	206	182	226	167
Litter weight gain (kg/day)	1.88	1.64	2.21	1.53

Table 2. The effect of ambient temperature on voluntary food intake and milk production of the sow

<sup>1</sup>1, Schoenherr et al. (1989b); 2, Vidal et al. (1991).

Simulating the effects of ambient temperature on sow productivity

A great challenge to the piggery manager is presented by the very different thermal comfort zones of the lactating sow and piglet with important ramifications for the efficiency of production if not correct. This is well demonstrated by the results of AUSPIG simulations for the situation where ambient temperature is set within the zone of thermal comfort for either the sow or for the piglet (Table 3). When ambient temperature is set at 20°C, VFI is not constrained by environmental factors and the sow consumes sufficient nutrients to maintain bodyweight during lactation. However, the LCT for the piglet is 29°C and at an ambient temperature of 20°C, even though some supplemental heating is supplied, the growth rate of the piglet is less than the 194 g/day that is achievable if the piglet is housed within its zone of thermal comfort. An ambient temperature of 33°C is more suitable for the piglet but is, however, too high for the sow with the result that VFI is depressed, body reserves are mobilized and the interval between weaning and re-mating is extended. Piglet growth rate is only marginally improved even though the piglet is now accommodated within its zone of thermal comfort, because milk yield of the sow has been reduced due to the low intake of nutrients by the sow. The effect on piglet growth rate in this example is of similar magnitude to the results in Table 2 and from the combined data of Lynch (1977), Stansbury et al. (1987), Yen and Cheng (1990) and Barb et al. (1991) (from 209 to 181 g/day).

The effects of high ambient temperatures on VFI can be overcome if the sow is able to increase evaporative heat loss via an increase in the proportion of skin that is wet. McGlone et al. (1988) reported that at ambient temperatures above 29°C, drip cooling decreased the weight loss of sows from 27 to 9 kg during a 28-day lactation and improved litter weight gain from 1.47 to 1.85 kg/day. The provision of drip cooling systems are now common in farrowing accommodation and the effect on the sow and hence piglet growth is depicted by the results of an AUSPIG simulation (Table 3). Despite ambient temperature remaining at 33°C, sows with access to drip cooling were able to increase the proportion of their skin that was wet and hence increase the amount of heat loss via evaporation and, although voluntary food intake was not sufficient to provide all of the substrates required for milk production, these were adequately supplied from body reserves with minimal effects on reproductive performance. First-parity sows in the commercial piggery referred to in Figure 3 had access to a drip cooling system and consumed 4.2 kg/day, lost 8 kg of bodyweight during a 28-day lactation and recorded piglet growth rates of 170 g/day, a result similar to that simulated by AUSPIG. The final treatment in Table 3 demonstrates the extent to which heat loss via evaporation must increase for voluntary food intake, and hence reproductive performance, not to be affected by high ambient temperature.

Treatment	Thermal comfort (dry)۔		Proportion wet skir	
	Sow <sup>1</sup>	Piglet <sup>2</sup>	15 to 30 <sup>3</sup>	1004
Temperature (°C)				
Ambient	20	33	33	33
ECT	22	26	25	21
UCT	29	33	33	36
Sow				
Feed intake <sup>5</sup> (kg/d)	5.26	2.61	4.31	5.26
DE intake (MJ/d)	71	' 35	58	71
Weight change (kg)	+1.8	-29.1	-9.6	+1.4
Weaning-to-mating interval (days)	5.0	19.1	9.6	5.0
Latent heat loss of evaporation				
from the skin (MJ/d)	10.9	10.5	16.0	25.9
Piglet				
Daily gain (g)	152	168	193	194
Weight at weaning (kg)	5.64	6.08	6.79	6.80

<sup>1</sup>Sow within its zone of thermal comfort; <sup>2</sup>piglet within its zone of thermal comfort; <sup>3</sup>simulating the effect of drip cooling increasing the proportion of wet skin for the sow from 15 to 30%; <sup>4</sup>simulating the situation where the proportion of wet skin for the sow is up to 100%, and <sup>5</sup>does not include feed wastage.

Ambient temperature in all of the AUSPIG simulations described in the above was set at a constant value. This is an unrealistic assumption given the daily variation in ambient temperature illustrated in Figure 3 but there is insufficient information presently available to include such effects in simulation models for the lactating sow.

# Measurement of the climatic environment

The calculation of critical temperatures for each class of animal, in this case sows of varying bodyweights and levels of nutrition, is greatly facilitated by the use of computer models. In view of the significant effects that climatic variables such as temperature and wind speed have on voluntary food intake and performance, knowledge of ambient temperature, relative humidity and wind speed is obviously important if the impact of environment on productivity is to be simulated. While each of these can be measured the task involved is usually not practical. However, Down (1990) described a computer program which predicts temperature, air moisture and ventilation rates according to external weather conditions, management and design of the building, and details of stock. Integration of such programs with models like AUSPIG will assist in ensuring that the productivity of the breeding herd is optimised.

# Conclusion

The effect that either low or high ambient temperatures have on the performance of sows during gestation and lactation, while quite different, does have important implications for the management of the breeding sow. While the response of sows to the major environmental factors has been reasonably well documented and can be simulated using models such as AUSPIG, further investigations are required to examine if temperature has any direct effects on such processes as milk production and fertility rather than *via* a change in food intake. It is important to be able to calculate critical temperatures quickly, using computer simulation techniques, and to understand the relationship between these and ambient temperature so that the economic implications of sub-optimal temperatures can be properly evaluated.

# SYMPOSIUM CONCLUSION

# **B.P.** Mullan

There are many environmental factors that may influence the feed intake of pigs, and hence the ability of that animal to attain its potential for deposition of lean tissue. In this symposium we have considered only two of the factors, the design of pig feeders and the thermal environment.

The design of feeding systems for the pig industry has entered a new era. Previous designs have largely neglected the physical and social requirements of the pig but this has now be addressed as described in this symposium. Feeders designed on the physical and social needs of pigs have had significant effects on reducing feed wastage and aggressive behaviour; factors important to the profitability of pig production. There is, however, an urgent need for well designed experiments, at both an applied and basic level, to be conducted before this and other types of feeders, such as the single-space and wet-and-dry feeders, can be openly recommended for adoption within the pig industry.

It is well known that environmental temperature can have a significant influence on the voluntary food intake and/or the partition of nutrients of the pig. However, this is frequently considered in isolation rather than in combination with other climatic factors which are equally important such as relative humidity, wind speed and the behavioural response of the pig. Given the objectives of management, it is also necessary to consider the thermal environment in context with the thermal requirements of the particular class of animal. This complex array of variables is difficult to consider simultaneously without the use of simulation modelling techniques. The development of techniques, such as that described previously to monitor the physiological response of the pig to its thermal environment, will improve our understanding of how animals respond to sub-optimal environments and improve the predictive power of models such as AUSPIG. Future research should be aimed at understanding mechanisms rather than simple determination of empirical relationships.

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# PREDICTING THE SHED ENVIRONMENT

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In order to quantify the effect of the climatic environment on pig performance and health in commercial sheds, and in order to improve the design and operation of these buildings and plant, it is necessary to be able to determine this environment.

A computer program entitled Pig Housing Internal Climate Simulation (PHICS) has been written to predict the environment in commercial buildings (Down, 1990). The program performs a time-based simulation of the environment on a one hour time step. It calculates the air dry-bulb temperature, moisture content and the air velocity over the animals. In addition, it calculates the ventilation rate, and the carbon-dioxide concentration.

The inputs required are the dimensional and constructional details of the building and plant, details of the size and feed intake of the stock, and hourly data of the external ambient climate in the format of the National Climate Data Bank. The size of the stock is taken to be an average liveweight of the stock in the shed, which remains constant over time.

The program can handle naturally ventilated sheds, and can simulate manual and automatic ventilation controllers. Spray cooling can also be simulated.

Validation of the program has been undertaken using environmental data measured in a commercial grower shed, for 2838 hours over a nine month period. The correlation co-efficient relating the predicted values of air temperature to the measured values was 0.76, and the average error of predicted temperatures -0.61°C.

There are five input factors which are unable to be accurately determined. These are the degree to which the building is sheltered from the wind; the degree to which the animals in the pens are exposed to air movement effects in the buildings; the proportion of the area of the skin of the animal which is wet when the animal is dry; the wetted area when the animals are being spray cooled; and the amount of water in the shed available for free evaporation, as a proportion of the water evaporated from the skin of the animals.

The major sources of error are considered to be the lack of data of external pressure co-efficients for the calculation of ventilation due to wind; prediction of the flow pattern in the building; the simple model of evaporation of free moisture in the building; and the assumption of steady-state heat transfer through the thermally thick building elements, especially the floor.

The program could be further developed by including simulation of fan ventilation systems, and heating and cooling plant in the shed; and by including simulation of lactating and dry sows, and boars.

The program described here can be used to quantify the environment in naturally-ventilated sheds, particularly for use with other programs, eg AUSPIG. Advantages over measurement of the environment include: speed of data generation; ability to simulate proposed sheds, or proposed modifications to sheds; and generalization of results to a standardized external climate.

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# ESTIMATING THE BENEFIT OF CHANGING THE ENVIRONMENT IN PIG GROWER SHEDS

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Pig producers need to be able to estimate the benefits of proposed changes to the thermal environment of a piggery. For example, a frequent and apparently simple question asked by producers is "What benefit will I get from insulating my grower shed?" The usual answer is that the grower shed will be warmer in winter and therefore the pig's FCR will be improved. It is surprising that there is no statistically justifiable method of demonstrating this.

In practice, there are five approximate methods used:

A comparative study of production and environment in contract piggeries using identical stock, feed and buildings has been made in Belgium but in Australia there is little possibility of trying to relate thermal environment and performance of pigs between different farms because of the large number of confounding variables such as genetics, health status, diet, housing and stockmanship.

A longitudinal study can be made to record the seasonal performance of a piggery. This gives some idea of the relationship between climate and pig performance in the piggery being studied. The effects of a changing thermal environment are confounded by changes in health status, feed composition, genetic variation, stocking density and stockmanship.

A degree-day summation can be used to estimate the feed requirements of dry sows housed in different temperature regimes. This approach is not applicable to grower/finisher pigs, which have relatively higher energy intakes than sows, since grower pigs housed below the LCT might not eat additional feed, but may partition the feed energy surplus to maintenance differently.

A neighbour comparison is used by consultants who have a number of clients involved in a recording service. The consultant identifies an area of poor performance on the piggery, and if it is attributable to an environmental cause, comparison with other piggeries gives an upper limit to the expected benefits. This gives a guide to the maximum worthwhile investment.

Before-and-after studies are commonly used in advertisements for environmental control equipment and animal health products. In practice, the effects of a change are often masked by a change in management due to the producer's (unconscious) desire to see the investment justified. It is also debateable whether the effects can be generalised to other piggeries.

Alternatively, simulation modelling may be used to account for the many variables. A pig growth simulation model (AUSPIG V2.03) was used to re-examine a case study of insulating a grower shed. The model can be tuned to agree with the observed reduction in winter feed conversion ratio of the pigs, but the economic consequences were unexpected. The model predicted that the warmer pigs would have a higher body fat content and that the savings in feed costs were negated by a reduction in sale prices.

Although this prediction is specific to a particular genotype, diet, climate and cost regime, it highlights the fact that estimating the cost of environmental changes is not trivial. It demonstrates the complexity of a piggery system and thus the importance of using a combined physical/financial model like AUSPIG to analyse a particular pig enterprise.

# THE USE OF SELF-REGULATING HEATING MATS TOGETHER WITH AN INFRARED LAMP AS A HEAT SOURCE IN FARROWING PENS

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A previous comparison between self-regulating heating mats (Pigmats, MM Cables) and bar heaters showed a higher mortality rate with the mats during the first 72 hours of life, when temperatures dropped below 15°C (Keenan *et al.*, 1990). The present trial tested the mats under similar conditions with the addition of limited supplementary heating at one of two different positions during the first 72 hours of life. The trial was carried out in the University of Queensland, Gatton College piggery with room temperatures varying between 13-30°C.

Sows due to farrow were sequentially allocated to one of the following three treatments until nineteen sows had reared a litter in each: farrowing pens with 700 W bar heaters operating continuously for the first three days and then between 1700 hr - 0800 hr; farrowing pens with self regulating heating mats operating continuously plus 150 W infrared lamp operating for the first 72 hours, 500 mm above the floor, placed at the front over the mats or, alternatively, at the back 500 mm above the area where the piglets were born.

Data collected included: the number and weight of piglets born and weaned; number and cause of mortality; and the power consumed by the heat source to each farrowing pen. The results are summarised in Table 1.

Method of heating	Weight gain (kg)	Mortality (%)	Power consumption (KW-hr) <sup>1</sup>
Infrared bar heaters	6.0	20.3	359.5ª
Mats with infrared lamp at front	6.4	20.3	76.5 <sup>⊾</sup>
Mats with infrared lamp at back	6.3	20.4	74.1 <sup>ь</sup>
SEM	0.21	3.23	11.49

Table 1. Weight gain, percentage mortality and power consumed with different heating methods

<sup>1</sup>Means with different superscripts differ at P<0.001.

The results show that the mats used in conjunction with a supplementary heat source, at either the front or back of the pens for the first three days of life, provide an economical and effective environment for raising piglets in farrowing pens.

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# THE HOURLY PATTERN OF VOLUNTARY FOOD INTAKE IN FINISHER PIGS MAINTAINED AT A FLUCTUATING AMBIENT TEMPERATURE

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High summer temperatures depress pig performance in Australia because of a reduction in voluntary food intake (VFI). Previous studies (Giles *et al.*, 1991) with pigs held at constant ambient temperature showed that VFI declined from 2.8 kg/day at 22°C to 1.0 kg/day at 31°C. However, when daily temperature fluctuated from 22°C to 31°C every 12 hours, VFI was 2.5 kg/day with 80% of the consumption occurring at 22°C. The objective of the current study was to examine the effect of shorter daily periods at 22°C on VFI and its association with physiological parameters.

Five female pigs (mean live weight 79.3 kg) were housed in metabolism crates at 22°C and fed *ad libitum* a pelleted commercial diet estimated to contain 14 MJ DE/kg and 0.9% total lysine. The pigs were exposed for seven days to a daily fluctuating ambient temperature of either 16h (0900-0100h) (n=2) or 20h (0900-0500h) (n=3) at 31°C with the remainder of the day at 22°C. Hourly VFI was recorded and body temperature was measured continuously from an Oximetrix catheter placed in the pulmonary artery. Measurements were also made on each pig for 24h at a constant temperature of 22°C on the day before and on the fifth day after the fluctuating temperature regime.

Mean daily VFI declined from 2.66 kg at 22°C to 1.78 and 1.62 kg respectively, for the 16/8h and the 20/4h regimes. Hourly VFI was depressed during the 31°C period but rose steeply once body temperature was less than 39.5°C following the reduction in ambient temperature to 22°C (Figures 1 & 2). However, mean hourly VFI at 31°C was higher during the 20/4h than the 16/8h treatment (83g and 25g respectively, P<0.001).



Figure 1. Body temperature 0-0 and VFI  $\bullet--\bullet$  of pigs exposed to 16 h at 31°C and 8 h at 22°C.



Although body temperature above 39°C may have a primary role in the control of VFI in pigs, these results indicate that other factors such as temporal behavioural responses, rate of change in body temperature or energy deficit also may be involved.

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# MONITORING PIG HOUSING ENVIRONMENTS

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Sub-optimal environments in Australian piggeries are estimated to cost the industry in excess of \$50 million annually. On an individual grower basis the actual environmental regimes occurring in piggeries are not accurately known. There are significant problems associated with measuring environmental variables in pig housing. A simple, low-cost electronic data logging system was developed and used to monitor environments in over 20 grower sheds (mainly in Queensland). Approximately 15 years of environmental data was collected from these sheds over a 2.5 year period including environmental data immediately external to each of the sheds. Based on a *physical analysis* of this data, seasonal trends and clear differences in shed performance were apparent.

For thermal data, *maximum-minimum* regimes, 'average-day' analyses, degree-hour summations and frequency analyses were performed. Also the average differences between internal and external air temperatures were calculated giving shed lift and drop characteristics over the range of environmental temperature. Frequency analysis and lift/drop curves clearly illustrated differences in shed performance and the beneficial effects of insulating sheds was apparent.

At one site *floor temperature* measurements were made. In general the average daily floor temperature equalled the average daily internal air temperature, however, the daily range of floor temperature was only 2-3°C. The floor acted as a temperature buffer during the hot and cold periods of the day.

Summer cooling effects were evident in some sheds on days when ambient air temperatures exceeded about 30°C. Internal temperatures were significantly cooler than external temperatures during the hot period of the day. An explanation for this phenomenon was not apparent. In sheds with *spray cooling*, measured daily air temperatures were not significantly less than in sheds without spray cooling. This is not to say that spray cooling has no benefit. Most of the time the air temperature sensors did not get wet and did not experience any of the evaporative cooling which the (wet) pigs were experiencing.

Patterns of *relative humidity* were more site specific than for temperature making individual shed effects hard to identify. Whilst sheds impose some modifying effect, it is often the external relative humidity regime which largely dictates patterns of internal relative humidity. Due to warmer conditions inside sheds, internal relative humidities are generally 10-20% lower than ambient.

Delicate and expensive instruments are required for the measurement of *airspeed* at the low levels which occur in piggeries (0.15-2.0 m/s). In naturally ventilated sheds patterns of airspeed were largely stochastic in nature. In one shed where fans were used to assist internal air movement, airspeed was largely independent of external conditions when the fans were on.

Carbon dioxide concentrations were used to predict *ventilation rates* in a shed with automatic control of the natural ventilation. Concurrent patterns of airspeed, ventilation rate and the difference between internal and external air temperature were used to identify modes of ventilation (i.e. wind driven versus stack effect driven).

A number of environmental indicators of thermal performance were determined for each shed. The pig-floor interaction requires further evaluation since the floor acts as a temperature buffer. Further work to provide an explanation for the cooling effect phenomenon in some sheds during summer may have implications for shed design. The collected data may be used to assess the cost-benefit of environmental changes using a physical/financial simulation model like AUSPIG. Unfortunately attempts to do this were unsuccessful due to modelling limitations.

# REMOVAL OF NITROGEN FROM PONDED PIGGERY WASTES BY LOW-RATE AERATION

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The control of nutrients, principally nitrogen (N) and phosphorus, in liquid piggery wastes is becoming a significant issue for the Australian pig industry. A study by Payne (1990) found that the common waste treatment practice of ponding was highly effective at removing phosphorus from the liquid phase (averaging 92% removal), however, N was less effectively removed (46%). With this finding in mind, a technique has been developed to enhance nitrogen removal from ponding systems.

The technique was trialled at a pilot level at the Department's piggery at Medina using two 20 kL tanks set up to simulate an anaerobic/facultative pond system and a third 20 kL tank fitted with a surface aerator. Various flow patterns were trialled in order to optimize the system. All trials were based on a daily effluent input of 1 kL (representing the output of 50 pigs) and a continuous aerator power of 75 Watts.

Figure 1 shows the flow pattern that produced the highest N removal. Effluent was added daily to the first tank along with 4 kL of nitrified liquor recycled from the aeration tank, producing in turn a flow of 5 kL into the second tank from which 4 kL was recycled for aeration and 1 kL was discharged. Nitrogen removal under this regime averaged 82% (568 g N/day input, 100 g N/day output) as compared with 49% removal achieved prior to the initiation of recycling and aeration.





Aeration caused the oxidation of ammonium to nitrate, which was subsequently reduced to nitrogen gas and other volatile N species upon its re-entry into the first tank. This, in combination with the sedimentation and volatilization processes typical of pond systems, led to the removal of 375 g and 105 g of N from tanks one and two respectively.

As the trial was conducted over winter, during which low liquor temperatures (11°C min.) likely suppressed nitrification and denitrification, there is the prospect of higher removal rates or decreased power demand during the warmer months.

It is concluded that this technique offers an effective means of reducing the N content of piggery effluents being released in environmentally sensitive locations.

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# UTILISATION OF ILEAL DIGESTIBLE LYSINE FROM HEAT-TREATED FIELD PEAS BY GROWING PIGS

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Studies into the utilisation of ileal digestible lysine from different protein sources have revealed that, for cottonseed meal, only 36% of the ileal digestible lysine is retained in the pig, compared with 75% for soyabean meal (Batterham *et al.*, 1990). The interpretation of these results was that, with heat processed meals, a considerable proportion of the lysine is absorbed in a form(s) that is(are) inefficiently utilised. The aim of this study was to confirm heat treatment as the cause of low utilisation of ileal digestible lysine in protein concentrates.

Field peas (var dundale) were heat treated at either 110°, 135°, 150°, or 165°C using forced-air dehydrators. The ileal digestibility of lysine in the field peas was determined first, in pigs fitted with simple T-piece cannulae, to yield values of 0.75 for the raw peas, and 0.82, 0.78, 0.75, and 0.62 for the 110°, 135°, 150°, and 165°C treatments respectively. Lysine utilisation was then measured using five sugar-based diets incorporating field peas as the sole lysine source. These diets were formulated to contain 0.36 grams of ileal digestible lysine/MJ of digestible energy (DE). All other essential amino acids were added to ensure a 30% surplus relative to lysine. An additional two diets containing raw peas and peas heated to 165°C respectively were supplemented with free lysine to confirm that lysine was limiting in the first five diets. The pigs were fed frequently (3 hourly) on a three times maintenance ( $0.5W_{kg}^{0.75}$ ) feeding regime (adjusted weekly) over the 20 to 45 kg growth phase. They were then slaughtered and the crude protein of the empty body determined. Lysine deposition in the whole empty body of pigs slaughtered at 45 kg liveweight was estimated using 6.5 g lysine/16 g nitrogen for all treatments (Batterham *et al.*, 1990).

		Treatment				
Response	Raw	110°C	135°C	150°C	165℃	SEM
Gain (g/d)	498	482	477	450	314	12.91
Feed conversion ratio (FCR)	2.58	2.72	2.75	2.91	4.49	0.120
Estimated lysine deposited:ileal digestible lysine intake (g/g)	0.72	0.65	0.64	0.58	0.42	0.021

Table 1.	Performance	and	estimated	lysine	deposition	of	growing	pigs	fed	diets
containing	g raw and hea	t-trea	ted field p	eas (en	npty body b	asi	s			

The results show a linear decrease in gain/day (P<0.01), increase in FCR (P<0.01), and a decrease in lysine deposition as a proportion of digestible lysine intake (P<0.01).

The magnitude of differences in lysine deposition observed with heat-treated field peas are similar to those observed between soyabean meal and cottonseed meal (Batterham *et al.*, 1990). This confirms that the application of heat to protein concentrates renders lysine in a form that is inefficiently utilised by the growing pig. Values for the ileal digestibility of lysine are also shown to be unsuitable for diet formulations when dealing with heat-processed meals.

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# THE EFFECT OF FEED INTAKE ON ENDOGENOUS ILEAL NITROGEN AND LYSINE EXCRETION FOR THE GROWING PIG

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A new method involving peptide alimentation has been proposed (Moughan *et al.*, 1990) for determining endogenous amino acid excretion at the terminal ileum of animals. It has been applied to the rat (Butts *et al.*, 1991) and the pig (Butts, unpublished) and it has been shown that endogenous amino acid loss at the terminal ileum is enhanced following peptide feeding. This approach provides a more accurate estimate of ileal endogenous loss than the protein free method. This study aimed to determine the effect of feed intake on endogenous ileal nitrogen and lysine excretion of the growing pig using this approach.

Sixteen Landrace x Large White male pigs, each fitted with a simple T-cannula at the distal ileum, were fed an hydrolysed casein-based semi-synthetic diet at 8 different levels of intake. The starch-based diet contained 5% cellulose and 10% hydrolysed casein. The experiment was designed as two 4x4 Latin squares. The feed intakes were 0.06, 0.08, 0.10 and 0.12, and 0.05, 0.07, 0.09 and 0.11 metabolic liveweight  $(W^{0.75})$  day<sup>-1</sup> for the first and second Latin squares, respectively. Chromic oxide was included in the diet (0.6%) as an indigestible marker to allow correction of the ileal flows to total daily flows. The diet was fed to the pigs at each intake for 8 days. On the 5th and 8th day, ileal digesta were collected continuously via the cannula for 24 hours. Pooled digesta from each pig were sub-sampled and freeze-dried, and the nitrogen, lysine and chromium contents determined.

	Regression equation <sup>1</sup>	Coefficient of determination (R <sup>2</sup> )	Level of significance <sup>2</sup>
Nitrogen	y=2.4x+1087	75.78	***
Lysine	y=0.5x+267	79.40	***

Table 1. Relationships between endogenous ileal nitrogen and lysine excretion and feed intake determined after feeding growing pigs an hydrolysed casein-based diet

 $^{1}y$  = endogenous excretion at the terminal ileum (mg/day); x = dietary intake (g/day).  $^{2}$ Overall significance of the regression \*\*\* P<0.001.

There were significant (P<0.001) linear relationships between endogenous ileal nitrogen and lysine excretion and feed intake (Table 1). The results show that either dry matter intake *per se* or the intake of a feed component or components strongly influences endogenous excretion from the ileum. Although the amount of endogenous amino acids excreted from the ileum at a given level of dietary dry matter intake is likely to vary for diets with different ingredient compositions, the present relationships, which were derived under physiologically normal conditions, provide preliminary data on the magnitude of small intestinal amino acid losses.

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# METHIONINE ABSORPTION FROM THE HIND-GUT OF PIGLETS

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Amino acids are not considered to be absorbed in significant amounts from the large intestine of growing pigs (Just *et al.*, 1981). However, *in vitro* studies (Sepulveda and Smith, 1979) have identified uptake of methionine into the colonic mucosa of piglets.

This experiment determined if significant amounts of methionine were absorbed from the large intestine of three-week-old pigs. Eight Landrace x Large White piglets, surgically fitted with catheters which allowed infusion into the large intestine, were fed a diet balanced for all amino acids required for growth, except for methionine and cysteine which were at 40% and 60% of the required levels (ARC, 1981) respectively. Four piglets were infused with an isotonic solution of methionine for six days (20.5 mg/kg bodyweight/day), while saline was infused into the other four piglets. The treatments were reversed for a further six days, followed by a final six day period during which time the piglets were given the deficient diet but with supplementary methionine added to the diet (102.5 mg/kg bodyweight/day). Urine was collected over the final three days of each six day period.

Table 1. Mean daily urinary metabolite excretion rates for piglets (n=8) given a diet deficient in the sulphur amino acids and after infusion of saline or synthetic methionine (met) into the large intestine, or after oral administration of synthetic methionine

	Infused saline	Infused met	Oral met	Overall SE	Level of sign. <sup>1</sup>
Total nitrogen (mg/kg <sup>0,75</sup> /day)	207.6ª	208.0ª	160.1 <sup>ь</sup>	9.03	**
Urea nitrogen (mg/kg <sup>0.75</sup> /day)	164.7ª	169.8ª	<b>97.0</b> ⁵	8.29	**
Total nitrogen/creatinine nitrogen	14.5ª	15.3ª	10.9 <sup>ь</sup>	0.81	**
Urea nitrogen/creatinine nitrogen	11.6ª	12.5°	6.6 <sup>b</sup>	0.73	**

<sup>1</sup>Means with different superscripts are significantly different (P<0.01).

Feeding piglets a diet deficient in methionine and cysteine resulted in urinary total nitrogen (N) and urea N excretions which were greater than when the diet was orally supplemented with synthetic methionine (Table 1). Nitrogen from amino acids that were in excess of the needs for protein synthesis, set by the level of methionine in the diet, was excreted in the urine. If methionine infused into the large intestine had been absorbed in nutritionally significant amounts, the dietary amino acid balance should have improved, body protein deposition increased and daily urinary metabolite exrections decreased. It can be concluded that as there were no significant differences (P>0.05) in urinary total N or urea N excretion between infusions of methionine or saline, methionine was not absorbed in significant amounts from the large intestine of the three-week-old pig.

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# DETERMINATION OF ILEAL AMINO ACID DIGESTIBILITY IN GROWING PIGS USING CANNULATED ANIMALS OR THE SLAUGHTER METHOD

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The study aimed to compare apparent ileal amino acid digestibility in pigs using cannulation and slaughter techniques. Sixteen 45 kg liveweight entire-male pigs (Large White X Landrace) were allocated at random to two methods of digesta collection. Eight pigs were each fitted with a T-piece cannula at the terminal ileum, approximately 15 cm anterior to the ileo-caecal valve, while the remaining 8 animals remained intact. The pigs were given *ad libitum* access to a 12% crude protein corn-starch based diet in which the sole protein source was meat and bone meal (MBM), for a single 3 h (08.30-11.30 h) period each day of the study. Chromic oxide was included in the diet as an indigestible marker. The cannulated pigs received the diet for 14 days with hourly collection of digesta over 10 h (09.30-18.30 h) on each of the final 2 days. The intact animals were also given the diet for 14 days and were killed by intracardial injection of a barbiturate, 9 h after the commencement of feeding at 17.30 h on the 14th day. Digesta were flushed from the terminal 20 cm of ileum.

There was no significant (P>0.05) effect of digesta collection procedure on the apparent ileal digestibility of nitrogen or amino acids (Table 1). This is consistent with the observations of Moughan and Smith (1987) that cannulation of the terminal ileum of the pig, at least with a simple T-piece cannula, has little effect on amino acid digestion and absorption anterior to the end of the small intestine. Neither did cannulation affect the apparent faecal estimates of nitrogen and amino acid digestibility which lends further support for the minimal distrubance caused by a simple cannula implanted in the terminal ileum.

_	-	Ileal digestibility			Faecal digestibility		
Item	(I)	(C)	SE <sup>1</sup>	(I)	(C)	SE <sup>1</sup>	
Nitrogen .	72.9	73.1	1.9	83.5	83.8	1.1	
Lysine	81.7	81.6	1.6	90.7	90.0	0.8	
Threonine	72.9	73.0	1.3	85.8	86.0	1.2	
Histidine	75.1	75.9	2.0	84.1	84.7	1.5	
Methionine	83.6	84.0	2.1	91.0	91.5	1.7	

 Table 1. Comparison of the mean apparent ileal and faecal digestibility coefficients

 (%) between eight intact (I) and eight cannulated (C) pigs (mean liveweight 45 kg)

<sup>1</sup>All differences were non significant (P>0.05).

It is important to note that the slaughter method is technically simple and involves minimal interference to the process of digestion. When sampling is carried out with care, it provides data which are no more variable than obtained following cannulation. When applied to large animals, however, the slaughter technique is relatively expensive and the present results give some confidence in using cannulated animals for a more routine and possibly less expensive means of collecting digesta.

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# COMPARISON OF FAECAL SAMPLING TECHNIQUES ON THE DETERMINATION OF THE DIGESTIBILITY OF ENERGY AND NITROGEN IN RAW AND HEAT-TREATED PEAS

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The type of marker and mode and frequency of sampling of faeces can have a significant impact on digestibility studies (Moughan *et al.*, 1991). The aim of this study was to investigate the feasibility of partial (i.e. a daily grab sample of faeces voided) and rectal sampling of faeces during cannulation and ileal dissection experiments respectively, to provide an estimate of the digestible energy (DE) and nitrogen (N) digestibility of field peas. These values were compared with those obtained after total collection of faeces.

The field peas were either raw or heat-treated at 110°C, 135°C, 150°C, and 165°C for 15 minutes using dry heat. These were incorporated in sugar-based diets at a level of 40%. The cannulation experiment involved five Large White male pigs (35-40 kg) fitted with T-piece cannulas. The treatments were arranged in a 5x5 latin square design with eight day experimental periods. Partial collection of the faeces occurred on the last two days of each period. Rectal sampling of faeces was made at the time of surgery during an ileal dissection experiment. This experiment involved six pigs (35-45 kg) per treatment. Chromic oxide was the marker in both trials. A total 7-day collection of faeces was made on four pigs per treatment in a standard metabolism study. In all experiments, samples were bulked and stored at -20°C until analysis.



Figure 1. DE of raw and heat treated field peas assessed by total collection, or by partial or rectal faecal sampling.

Table 1. N c	ligesti	bility a	ssesse	d by
total collection	1, or b	oy parti	al or	rectal
faecal samplin	g			

Protein	N	digestibil	lity	SEM	
source	Total	Partial	Rectal	JEIVI	
Raw	0.81	0.79	0.72	0.015	
110°C	0.76	0.84	0.72	0.022	
135°C	0.75	0.81	0.69	0.022	
150°C	0.72	0.78	0.64	0.022	
165°C	0.52	0.52	0.48	0.022	

All techniques revealed a significant decrease (P<0.001) in the DE of field pea with an increase in heat treatment, however, the rectal collection technique produced significantly lower results (P<0.001) for all treatments. No sampling method x heat treatment interactions were detected. The three collection techniques gave a similar decrease in N digestibility, but with no consistency. These results show that the partial sampling technique is as efficient as the total collection method for the determination of DE in protein concentrates, but not for the determination of N digestibility.

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# ILEAL INDIGESTIBLE DRY MATTER AS AN INDICATOR OF ENDOGENOUS EXCRETION OF PROTEIN AND AMINO ACIDS IN PIGS

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Endogenous protein excretion (EPE) has been shown to vary with the method of determination and with the chemical composition of the diet. These findings indicate that EPE is related to the individual diet and therefore a readily available method for endogenous excretion assessment is needed. Boisen and Fernandez (1991) proposed an *in vitro* method by which EPE was related to the indigestible dry matter (IDM). Consequently, a study was undertaken to ascertain whether ileal IDM determined *in vivo*, could be used as an indicator of ileal endogenous excretion of protein and amino acids.

Diets were formulated by combining barley with sucrose/dextrose and barley with either soybean meal or casein in different proportions, such that a wide range of protein levels were achieved. Ileal digestibilities of dry matter, protein and amino acids were determined with ileal cannulated pigs (35-55 kg liveweight). The relationship between indigestible protein and protein intake and IDM was calculated by the multiple regression technique. EPE was subsequently estimated as the indigestible amount at zero protein intake and apparent digestibilities adjusted accordingly. The same procedure was applied to each amino acid (Table 1).

	Endogenou	s excretion	Ileal dige	Ileal digestibility			
	(g/kg DM intake)	Relative	Apparent (%)	Corrected (%)			
Protein	36.0	100.0	67	92			
Lysine	1.0	2.8	79	95			
Methionine	0.4	1.1	84	98			
Threonine	1.2	3.3	72	93			

Table 1. Mean endogenous excretion and ileal digestibility of protein and amino acids

EPE and its relative content of amino acids were very similar to those of de Lange *et al.* (1990). Furthermore, corrected values of ileal digestibility were more consistent with respect to each other, compared to apparent values. These results indicate that IDM can be used to account for the endogenous component of individual estimates of protein and amino acid digestibility.

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# A SYMPOSIUM - POTENTIAL IMPACT OF BIOTECHNOLOGY ON PIG MEAT PRODUCTION

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### Symposium introduction

Advances in biotechnology have opened up new and unforeseen possibilities for increasing the efficiency with which pig meat is produced. By far the greatest attention has been focussed on the use of exogenous porcine somatotropin (pST), commonly referred to as porcine growth hormone (pGH), to increase lean tissue deposition in the finishing pig. Although the stimulatory effects of crude GH preparations on lean tissue deposition have long been recognised (Turman and Andrews, 1955; Machlin, 1972), it was not until the advent of genetic engineering that highly purified recombinant pGH became available in commercial amounts. Since then the efficacy of daily injections of pGH for increasing growth and improving carcass composition has been well established (Evock *et al.*, 1988; Campbell *et al.*, 1988; 1989; 1991). This area of research has been extensively reviewed (see van der Wal *et al.*, 1989) and will not be a focus for this symposium.

One of the drawbacks of exogenous pGH for improving performance is that because it is a naturally occurring peptide hormone it is digested if given in the feed. Therefore, administration has to be via either daily injection or as a slow-release device. Considerable efforts have been exerted in developing an appropriate slowrelease device but as yet there are no systems suitable for application. Therefore, research efforts have been aimed at developing alternative means of altering pig growth performance through manipulating other aspects of the GH/IGF-I axis. Prominant among these are transgenesis, exogenous IGF-I and immuno-manipulation of GH activity.

The potential for transgenesis as a means of introducing new genes, particularly GH, into the pig germ line was reviewed at our inaugural conference (Seamark, 1987). Since then, this area has expanded rapidly and this technology now appears to be fulfilling some of the initial high expectations. Therefore, it appears appropriate to again review the progress in the production of transgenic pigs and this has been comprehensively done by Steele *et al.* (1991). The other two technologies covered by this symposium (exogenous IGF-I, Walton *et al.*, 1991; immuno-enhancement of GH, Aston *et al.*, 1991) are still very much in their infancy with respect to application to the pig, but both offer exciting potential for our industry.

# DEVELOPMENTS IN MANIPULATING PIG PERFORMANCE: GENE TRANSFER

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# Introduction

The practical application of new genetic manipulation techniques to animal agriculture offers tremendous potential for the future. However, we need to recognize that the full potential will only be reached through improved understanding of the intricacies of animal physiology from the molecular level to the holistic level. In this context, it is not surprising that the research thrusts into mammalian transgenesis during the past few years have contributed far more through expanding the knowledge base in mammalian biology than through the development of commercially valuable transgenic animals.

Research on transgenesis of livestock is presently splitting into two diverging areas of application. One thrust would utilize livestock species to produce valued pharmaceuticals. Since this area is more likely to provide monetary return in the near term, companies with a strong molecular biotechnology base are investing their research funds in this thrust area. The other thrust more applicable to improved livestock gene pools is funded by government institutions and livestock-oriented companies that have greater interest and experience in long-term benefits that could alter global demographics of animal production.

# Methods of transferring genes

# **Microinjection**

The predominant method used to transfer cloned genes into animals is direct microinjection into the pronuclei of one-cell ova or nuclei of two-cell ova. Although microinjection of mouse pronuclei is readily performed, direct application to other species was impeded by the opacity of the egg cytoplasm. Pronuclei of rabbit and sheep eggs can be seen readily using differential interference contrast (DIC) microscopy. However, pig and cow eggs must be centrifuged at 15,000 g for 5 min to induce stratification of the cytoplasm before the pronuclei are visible with use of DIC microscopy (Wall *et al.*, 1985).

The mechanism by which injected DNA integrates into a chromosome is unknown. Usually the DNA integrates in a single site, but multiple integrations can occur. Approximately 70% of transgenic mice produced by microinjection carry the transgene in all cells and the remaining 30% are mosaic, presumably because integration of the gene occurs sometime after the first cleavage stage following microinjection (Wilkie *et al.*, 1986). Frequently, injected DNA integrates at a single locus but with multiple copies of the gene arranged in either head-to-tail or head-to-head array (Gordon and Ruddle, 1985; Hammer *et al.*, 1985a; Simons *et al.*, 1988).

# Retroviral insertion

Embryos have been successfully infected with retrovirus in several species (Jaenisch, 1976; Jahner *et al.*, 1985; van der Putten *et al.*, 1985; Petters *et al.*, 1987; Salter *et al.*, 1987; Bosselman *et al.*, 1989). Retroviral infection is receiving considerable attention because in certain applications it offers several advantages over microinjection. Principle advantages are: (a) single copies of the gene integrate without rearrangement at the site of integration; and, (b) retroviral DNA integrates into a high percentage of embryos. In chickens, infection can be induced by exposure to high concentrations of viral stock, by coculture with infected cells *in vitro*, or by microinjection into the blastodisk. The disadvantages of retroviral vectors are: (a) a retrovirus carrying the transgene is time consuming to construct; (b) only small genes can be inserted into the retrovirus; (c) resulting transgenics exhibit a high incidence of mosaicism, which necessitates extensive outbreeding to establish pure transgenic lines; and, (d) level of expression of the transgene is relatively low (Jaenisch, 1988).

# Embryonic stem cell

The third method of introducing genes into the germ line involves transfection of embryonic stem cells in culture and then incorporating these transgenic stem cells into an embryo. The advantage of this procedure is that a particular genotype can be selected *in vitro* before introduction of the stem cells into the embryo. This technique is thus far the only one that provides the ability for site-specific insertion of a transgene by homologous recombination (Capecchi, 1989). Thus far, only transgenic mice have been produced by this method.

# Sperm cell mediated foreign DNA transfer

Preincubation of sperm cells with the pSV2CAT plasmid for 30 min. prior to *in vitro* fertilization has recently been reported to be an effective method of producing transgenic mice (Lavitrano *et al.*, 1989) and pigs (Gandolfi *et al.*, 1989). The ability to carry out sperm cell mediated transfer of foreign DNA would have numerous advantages over microinjection, but Brinster *et al.* (1989) have been unsuccessful in attempts to produce transgenic mice by this technique.

# **Considerations of transgenesis**

# Components of fusion genes

Selection, construction, cloning, and evaluation of genes to enhance growth and improve feed efficiency in animals have been costly and difficult because these techniques were so poorly understood when the effort was begun. Enhanced growth in a transgenic animal was first reported in mice bearing a fusion gene consisting of mouse metallothionein (MT) promoter ligated to the rat growth hormone (rGH) structural gene (Palmiter *et al.*, 1982). The rGH structural gene was selected largely because few other growth-related sequences were available or characterized at that time. The MT promoter was also one of the few promoters available at the time that was known to express structural genes *in vivo*. The MT promoter normally responds to elevations in heavy metals in the body by increasing the secretion of MT, which helps clear the heavy metal from circulation (Palmiter *et al.*, 1983; Hammer *et al.*, 1985a and 1985b). The possibility of inducing the MT promoter with zinc in the diet was also a consideration. We now believe that the level of expression of MTGH transgene does not require stimulation by exogenous heavy metal. More recently, numerous other gene constructs have been studied in food-producing animals (Table 1).

# Integration efficiency

The efficiency of integrating growth-regulating transgenes into the genome of farm animals has been quite low. Our studies with pigs (Pursel *et al.*, 1990a) show that overall only 8.3% of all transferred microinjected pig ova resulted in offspring at birth (Table 2). Integration efficiency varied from 0.3% to 1% when expressed as the percentage of injected ova that became transgenic pigs. Our studies with sheep (Rexroad *et al.*, 1989) produced similar numbers; 6.6% of transferred microinjected ova became lambs and only 0.2% to 2% were transgenic. Others report similar efficiencies of gene integration for pigs (Brem *et al.*, 1985, 1988b; Ebert *et al.*, 1988) and sheep (Simons *et al.*, 1988). The efficiency of transgene integration is higher for mice. About 10 to 15% of the microinjected eggs develop to term and about 25% of weaned mice are transgenic for an overall efficiency of about 3% (Brinster *et al.*, 1985). Some of the important parameters that influence the frequency of integration have been described for mice (Brinster *et al.*, 1985; Gordon and Ruddle, 1985; Hogan *et al.*, 1986).

## Copy number

The number of copies of the fusion gene that integrates into the genome following microinjection has varied widely, ranging from less than 1 to 490 in pigs (Hammer *et al.*, 1985a and 1985b). Usually, little relationship is found between number of copies integrated and level of expression of the gene product. However, Miller *et al.* (1989) detected a slight but significant positive correlation of plasma bGH with copy number in pigs transgenic with MTbGH (Figure 1).

# Locus and arrangement of integrated genes

Integration of the foreign gene appears to occur at random sites along the chromosome. Frequently several copies of the gene fuse together prior to integration (Palmiter *et al.*, 1982). Integration of the resulting head-to-tail or head-to-head concatamers is the usual reason transgenic animals possess more than one copy of a gene per cell. The functional activity of the gene copies in the middle of the

concatamer may be considerably reduced or absent (Palmiter *et al.*, 1982). Only about 10% of mice possessing more than one copy of a foreign gene have more than one integration site (Wilkie *et al.*, 1986). The proportion of transgenic farm animals with multiple integration sites is unknown, but the transmission of MThGH, MTbGH and MTh-growth hormone-releasing factor (MThGRF) from founder pigs to progeny suggests a high proportion of single site integrations (Pursel *et al.*, 1989a).



Figure 1. Each line indicates the concentration of bovine growth hormone (bGH) in plasma for a MT-bGH transgenic founder pig (after Miller et al., 1989).

# Expression of the integrated gene

About 70% of mice and pigs transgenic with structural genes consisting of genomic DNA have expressed the transgene (Hammer *et al.*, 1985a and 1985b; Palmiter *et al.*, 1982 and 1983; Pursel *et al.*, 1987). Too few transgenic sheep have been produced to calculate a representative value (Rexroad *et al.*, 1989; Simons *et al.*, 1988).

# Non-expressing transgenic animals

Whether or not an animal expresses its transgene may depend upon the site of integration. Regulatory elements adjacent to the foreign gene may suppress transgene expression (Lacy *et al.*, 1983; Palmiter and Brinster, 1986). Expression has generally been poor when the structural gene consisted of a cDNA sequence, rather than genomic DNA. Unknown elements within the introns of genomic DNA may be required for proper expression of the transgene (Brinster *et al.*, 1988).

# Level of transgene expression

The level of gene expression varied greatly among pigs transgenic with MThGH and MTbGH, ranging at birth from 3 to 949 ng hGH/ml and 5 to 944 ng bGH/ml, respectively (Hammer *et al.*, 1985c; Miller *et al.*, 1989). The concentration of GRF in plasma of pigs transgenic with MThGRF or albumin hGRF (ALBhGRF) ranged from 130 to 380 pg/ml and 400 to 8000 pg/ml, respectively (Pursel *et al.*, 1989a and 1989b). Although these values for GRF are 10- to 500-fold higher than the concentrations found in littermate controls, the biological activity of the transgene product was apparently compromised. Plasma contained a 3-44 amino acid metabolite rather than the native 1-44 GRF peptide, which probably explains why expression of GRF did not

Gene constructs (regulatory-structural)	Abbreviation <sup>1</sup>	Animal	Reference
Immunologically Related Genes Avian Leukosis Virus Bovine Papilloma Virus	ALV BPV	Chicken Cattle	Salter <i>et al.</i> (1987) Roshlau <i>et al.</i> (1988)
Immunoglobulin Heavy Chain- <u>c-myc</u> Reticuloendotheliosis Virus	rbEµ-rb <u>c-myc</u> REV	Rabbit Chicken	Knight et al. (1988) Bosselman et al. (1989)
Mammary Expression Genes ß-Lactoglobulin-Factor IX	oßLG-hFIX	Sheep	Clark <i>et al.</i> (1989)
ß-Lactoglobulin-α 1 Anti-trypsin	oßLG-ha1AT	Sheep	Simons et al. (1987)
Whey Acidic Protein	mWAP	Pig	Pursel et al. (1990c)
Whey Acidic Protein-Tissue Plasminogen Activator Growth Related Genes	mWAP-hTPA	Goat	Ebert & Gordon (unpub.)
Mammary Tumor Virus-Growth Hormone	MTV-bGH	Cattle	Roshlau et al. (1989)
Metallothionein-Growth Hormone	mMT-hGH	Pig	Brem et al. (1985)
	. Pig.	Rabbit, Sheep	Hammer et al. (1985b)
	)	Fish	Brem et al. (1988a)
	mMT-bGH	Pig	Pursel et al. (1987)
		Sheep	Rexroad et al. (1989)
	oMT-oGH	Sheep	Murray et al. (1989)
	hMT-pGH	Pig	Vize et al. (1988)
Metallothionein-Growth Hormone Releasing Factor	mMT-hGRF	Pig	Pinkert et al. (1987)
		Pig	Brem et al. (1988b)
		Sheep	Rexroad et al. (1989)
Metallothionein-Insulin-like Growth Factor-I	mMT-hIGF-I	Pig	Pursel et al. (1989b)
Albumin-Growth Hormone Releasing Factor	mALB-hGRF	Pig	Pursel <i>et al.</i> (1989a)
Cytomegalovirus-Growth Hormone	CMV-pGH	Pig	Ebert et al. (1990)

Table 1. Gene constructs integrated into the genome of farm animals (Pursel et al., 1990a,b)

Table 1 (continued)			
Moloney Leukemia Virus- Growth Hormone	MLV-rGH	Pig Dig	Ebert et al. (1988) Ebort et al. (1980)
Phosphoenolpyruvate Carboxykinase-Growth Hormone Prolactin-Growth Hormone	PEPCK-bGH	۲: ۲: ۲: ۲: ۲: ۲: ۲: ۲: ۲: ۲: ۲: ۲: ۲: ۲	DUELT ET 41. (1770) Wieghart et al. (1988) Polos et al. (1980)
Skeletal actin-Estrogen Receptor Transferrin-Growth Hormone	ASK-hER mTF-bGH	Cattle Cattle Cattle	Torge et al. (1990) Massey et al. (1990) Bondioli & Hammer (unmuh.)
		Pig	Pursel et al. (unpub.)
		Sheep	Rexroad et al. (1990)
	mTF-hGH	Pig <sup>-</sup> .	Pursel et al. (unpub.)
<sup>1</sup> Lower case letters designate species from which DNA se	quence was derived:	b, bovine; h, h	uman; m, murine; o, ovine; p, porcine; r, rat.

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result in elevated plasma pGH (Pursel *et al.*, 1989a). Evidence suggests that the GH produced by MThGH and MTbGH in transgenic pigs possesses biological activity. Pigs expressing the hGH transgene had extremely low plasma levels of pGH (Miller *et al.*, 1989), which indicates hGH influenced the pGH negative feedback mechanisms. They also had 2- to 7-fold elevated plasma levels of IGF-1 (Miller *et al.*, 1989) which indicates that IGF-1 synthesis was activated by GH-receptor. stimulation.

In mice bearing hGH or bGH structural genes and the heavy metal responsive MT promoter, zinc in the drinking water increased plasma hGH or bGH 3- to 100-fold (Palmiter *et al.*, 1983; Hammer *et al.*, 1985a). In contrast, in pigs transgenic with MTbGH, zinc added to the feed only increased plasma bGH about two-fold (Pursel *et al.*, 1990b). It is unknown whether the MT promoter is less responsive to heavy metals in the pig or whether the unsupplemented diet of the pig contains sufficient zinc to cause near maximal expression of the structural portion of the transgene.

Fusion gene	Ova injected	Recipient gilts	Gi preg	ilts mant	Offs	pring	Trar	nsgenic	Expres transg	sing ene
	N°	N°	N°	(%)	N٩	(%)	N٥	(%)	N⁰	(%)
MT-hGH	2035	64	37	58	192	9.4 <sup>1</sup>	20	0.98 <sup>2</sup>	11/18	61 <sup>3</sup>
MT-bGH	2330	49	24	49	150	6.4	9	0.41	8	89
MT-hGRF	2236	66	35	53	177	7.9	7	0.31	2	29
ALB-hGRF	968	32	20	63	108	11.2	5	0.52	3/3	100
MTo-hIGF-I	387	13	5	38	34	8.8	4	1.03	1/2	50

Table 2. Efficiency of transferring growth-regulating genes into swine (Pursel et al.,1990b)

<sup>1</sup>Percentage of injected ova resulting in offspring. <sup>2</sup>Percentage of injected ova resulting in a pig with gene integration. <sup>3</sup>Percentage of transgenic pigs expressing the fusion gene.

#### Site of transgene expression

In transgenic animals, the MT promotor tended to cause tissue-specific expression of mRNA. For example, elevated levels of bGH and bGH mRNA in liver, kidney, adrenal, and pancreas of two lines of pigs transgenic with MTbGH are shown in Table 3.

# Transmission of transgenes to progeny

# Mendelian

If transgenic livestock are to be useful in the livestock industry, they must transmit the transgene to progeny in Mendelian fashion. Breeding studies showed successful transmission to one or more progeny in 7 of 10 pigs possessing one of three transgenes (Table 4). Sheep possessing non-expressing transgenes also transmit non-expressing transgenes to their progeny (Rexroad *et al.*, 1989). Sheep producing pharmaceuticals in their milk also transmit the transgene to progeny as simple Mendelian dominants (Simons *et al.*, 1988).

# Non-mendelian

The two pigs that failed to transmit the transgene to their progeny (Table 4) were probably mosaic as was the transgenic boar that only transmitted to 1 of 33 progeny (Pursel *et al.*, 1987). Mosaicism for the microinjected gene would be expected if integration occurred after the first mitotic cell division. Delayed integration could cause some tissues of the body to contain the transgene while others might be devoid. The transgene might be present in only a few or none of the germ cells, yet expression

by transgenic cells in non-gonadal tissue could cause elevated GH in circulation.

# Progeny have similar expression characteristics

While wide variation was observed in plasma level of hGH and bGH among founders (Miller *et al.*, 1989), there was a striking similarity in levels between a founder and its offspring (Pursel *et al.*, 1990a). Transgenic pigs in the MTbGH 31-04 line maintained plasma concentrations of about 1,000 ng bGH/ml over three generations, while pigs in the MTbGH 37-06 line maintained levels of about 85 ng bGH/ml of plasma over four generations. In addition to exhibiting Mendelian inheritance, these transgenes were stably integrated into the genome of the pig.

# A model: The growth hormone transgenic pig

# Performance and physiological characteristics

The enhanced growth rate and increased body size of transgenic mice that expressed foreign GH genes (Palmiter *et al.*, 1982 and 1983) provided both the impetus for transfer of similar fusion genes into other species and the expectation that the rate of growth might be greatly enhanced. This expectation was not realized in the founder population of MThGH and MTbGH transgenic pigs and MTbGH transgenic lambs, even though convincing evidence indicates the transgenic animals produced a biologically active form of GH. Transgenic pigs expressing the hGH gene product rarely had detectable concentrations of plasma pGH (Miller *et al.*, 1989), which indicates a negative feedback mechanism on GH secretion was functioning. Furthermore, IGF-1 concentrations were 2- to 7-fold higher in pigs transgenic with hGH, bGH, or rGH than in littermate control pigs (Ebert *et al.*, 1988; Miller *et al.*, 1989), which also indicates that foreign GH exerted a biologic effect by binding to GH receptors of hepatocytes to stimulate IGF-1 synthesis.

The MThGH and MTbGH founder transgenic pigs were fed a diet containing 16% protein, a recommended level for normal pigs, which may not have provided sufficient protein for transgenic pigs expressing high levels of GH. Recent studies using pigs injected with exogenous pGH indicate that maximal growth rate is attained only if the diet contains adequate protein, particularly lysine (Campbell *et al.*, 1991). Insubsequent growth studies with transgenic pigs, the levels of dietary protein and lysine were increased and weight gain from 30- to 90-kg of MTbGH transgenic pigs was 16.5% faster than sibling control pigs (Pursel *et al.*, 1988). In addition, Vize *et al.* (1988) reported that a transgenic pig expressing MTpGH gained 492 g per day faster than did littermate control pigs during the 20- to 90-kg growth period.

Several recent studies of pigs treated with exogenous pGH have revealed that appetite depression accompanies elevated GH levels in pigs (Campbell *et al.*, 1988 and 1989; Evock *et al.*, 1988). This finding may explain why growth rates of GH-treated pigs are not increased as dramatically as in transgenic mice or in rats with a GH-secreting tumor, which have enhanced feed intake (McCusker and Campion, 1986). Compared with littermate or sibling controls, feed intake was depressed 20% in MTbGH founder and 17% in MTbGH second generation transgenic pigs fed *ad libitum* (Pursel *et al.*, 1989a). These results are comparable, respectively, to a 14 and 17% depression in feed intake reported for pigs injected with pGH (Campbell *et al.*, 1988).

MTbGH founder transgenic pigs were 16% more efficient and MTbGH second generation transgenic pigs were 18% more efficient in converting feed into body weight gain than were littermate or sibling controls (Pursel *et al.*, 1989b). Similar improved feed efficiencies of 23% (Campbell *et al.*, 1988) and 25% (Evock *et al.*, 1988) were reported for pigs injected with exogenous pGH compared with controls.Elevated concentrations of GH in pigs expressing MThGH and MTbGH transgenes produced marked repartitioning of nutrients away from subcutaneous fat and into other carcass components, including muscle, skin, bone, and certain organs. Ultrasonic estimates or slaughter measurements of backfat thickness at the tenth rib of hGH and bGH

Table 3. Plasma b(	<b>3H and bGH mRNA</b>	values for seve	ral organs of	two lines of p	igs expressin	g a MT-bGH	transgene (Pu	ursel <i>et al.</i> , 199(	( <b>9</b> (	
		Plasma			g GI	H mRNA <sup>1</sup> (mo	lecules/cell)			
auri	Cenerauon	non (m/ml)	Liver	Kidney	Heart	Gonad	Lung	Adrenal	Panci	eas
LINE 37-06										
37-06	ទ	45	0	150	360	20	0	0	0	
67-06	ច	111	45	0	0	0	0	14	45	
20-29	ច	20	31	0	0	55	0	13	28	10
136-01	5	183	8	0	0	NA <sup>2</sup>	70	0	65	
136-02	ទ	88	43	ន	0	NA	0	14	22	
136-03	5	29	0	0	0	NA	12	0	55	
137-03	5	39	0	0	0	21	20	S	12	
140-02	3	53	0	0	0	0	14	0	0	
LINE 31-04									I	
115-14	ច	1089	1720	2320	18	500	0	975	20	
182-07	8	822	069	069	NA	203	140	965	510	~
185-03	G	2138	860	1170	15	300	57	2250	215	
Freion		U	Gene	Progen	٨	Born	Transge	mic	Expressir	ള
gene	Founder	Sex	opies Nro	Litters	°N	°N	ů	(%)	²	(%)
>			Ž	expressing						
MT-hGH	3-02	M	330	No	9	52	17	33	7	12
	10-04	F	33	No	4	12	5 S	42	0	0
	11-02	M	1	Yes	1	13	б	33	б	100
	16-03	F	e S	°N	ო	33	0	0	0	0
	21-04	М	1	No	9	52	1	2	0	0
	25-04	н	2	No	1	80	5	63	0	0
MT-bGH	29-01	М	م	Yes	4	36	0	0	0	0
	31-04	М	28	Yes	ო	19	6	32	9	100
	37-06	M	ŵ	Yes	1	11	8	73	8	100
MT-hGRF	86-04	M	100	Yes	Ю	20	11	55	10/10	100

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transgenic pigs at approximately 90 kg body weight averaged 7.0 and 7.9 mm, respectively, whereas littermate control pigs averaged 18.5 and 20.5 mm, respectively (Hammer *et al.*, 1986; Pursel *et al.*, 1989a). Similar reductions in backfat were found for PEPCKbGH transgenic pigs (Wieghart *et al.*, 1990).

Additionally, the backfat measurements do not adequately reflect the reductions of subcutaneous fat in the MTbGH transgenic pigs because total carcass lipid averaged only 3.7% compared with 19.8% for sibling controls (V. G. Pursel and M. B. Solomon, unpublished data).

Ebert *et al.* (1988) reported that by 9 months of age, a transgenic boar with MLVrGH was 26% heavier, and its linear bone growth of fore and hind limbs was greater than that of a littermate control boar. In contrast, at 8 and 10 months of age, MThGH and MTbGH transgenic pigs had not grown to a larger body size, nor were femur, tibia, or humerus longer in four MTbGH transgenic pigs than in sibling controls (Pursel *et al.*, 1989a). Additional investigation is required to determine whether this difference is due to structural differences of bGH and rGH that affect binding to GH receptors in epiphyseal chondrocytes or to some other physiologic factor, or whether the single MLVrGH transgenic boar represents a unique occurrence.

# Health problems

It should come as no surprise that continuous hypersecretion of a biological compound with broad metabolic actions might adversely impact the health of an animal, especially if secretion of that compound cannot be controlled by the animal.

Transgenic pigs and sheep expressing high levels of GH have experienced numerous health-related problems (Pursel *et al.*, 1987; Pursel *et al.*, 1989b; Rexroad *et al.*, 1989; Ward *et al.*, 1989). Pigs expressing MTbGH or MThGH experienced lameness, susceptibility to stress, peptic ulcers, parakeratosis, lethargy, anestrus in gilts, and lack of libido in boars. A transgenic pig expressing rGH (Ebert *et al.*, 1988) also showed signs of joint disfunction, which was diagnosed as osteochondritis dissecans. Joint pathology was also observed in some pigs injected with exogenous pGH for only 57 days (Evock *et al.*, 1988).

The adverse impact on health seems to result from the greatly elevated levels of GH, rather than from damage caused from inserting foreign DNA into a chromosome or other causes. Non-expressing transgenic pigs bearing the MTbGH or MThGH transgene seemed just as healthy as normal control pigs (Pursel *et al.*, 1987). The level of expression of the GH transgene also appears to be an important factor influencing the health of transgenic pigs. No adverse impact on health was observed in PRLbGH transgenic pigs expressing low levels of GH (Polge *et al.*, 1989).

The reproductive capacity of transgenic pigs expressing GH at high levels was seriously impaired. None of the gilts expressing MTbGH or MThGH exhibited estrus (Pursel *et al.*, 1990b) and at necropsy all of their ovaries were devoid of corpora lutea or corpora albicans. In addition, their uteri were small and infantile in appearance, indicating the lack of prior estrous cycle activity. Boars in these same lines lacked libido. However, we have been able to obtain spermatozoa from these boars by either electro-ejaculation or flushing the epididymis at necropsy. The resulting spermatozoa were used in artificial insemination to demonstrate germline transmission of the transgene (Pursel *et al.*, 1987).

The reason for reproductive problems is not fully understood. In control boars, plasma concentrations of estrone sulphate increases about three-fold between 80 and 125 days of age. In contrast, boars expressing MTbGH showed no change during this period (Guthrie *et al.*, 1989). Profiles for follicle-stimulating hormone and luteinizing hormone in plasma over this time period were similar for transgenic and sibling control gilts or boars, suggesting reduced libido in boars may involve steroid metabolism more than pituitary function. Other factors that should not be overlooked in attempting to understand poor libido in boars is that they were often lame or appeared tender on their feet and experienced other health problems. It is interesting that spermatozoa were produced by MTbGH and MThGH transgenic males, but ova
were not produced by females of these same lines.

Transgenic pigs expressing MThGH and MTbGH were moderately hyperglycemic, averaging 10 to 40 mg/dL above littermates, and insulin concentrations in fasted MTbGH transgenics were elevated about 20-fold above those of siblings (Table 5; Pursel *et al.*, 1989a). Pigs injected daily with exogenous pGH had average increases in serum glucose ranging from 8 to 48% and concentrations of serum insulin that were two to sevenfold higher than that of control pigs (Campbell *et al.*, 1988 and 1989; Evock *et al.*, 1988). In comparison, a MLVrGH transgenic pig had glucosuria and consistently had serum glucose levels more than threefold higher than normal (Ebert *et al.*, 1988).

Item	N°	Control (mean±SEM)	N°	Transgenic (mean±SEM)	P<
Glucose <sup>1</sup> (mg/dl)	10	72±5	10	109±13	0.011
Cortisol <sup>2</sup> (ng/ml)	6	39±9	8	37±7	0.84
Insulin <sup>1</sup> (pg/ml)	10	24±4	10	480±118	0.001
Prolactin <sup>1</sup> (ng/ml)	10	3.9±0.5	10	2.3±0.4	0.021
$T_{3}^{2,3}$ (ng/ml)	6	1.2±0.3	7	$1.3 \pm 0.2$	0.68
$T_4^{24}$ (ng/ml)	6	49±4	7	29±3	0.003

Table 5	5.	Glucose	and	metabolic	hormone	concentrations	in	plasma	of	MT-bGH
transge	nic	and litte	rmate	e control pi	igs (Pursel	et al., 1990b)		•		

<sup>1</sup>Blood collected after overnight fast. <sup>2</sup>Blood collected from cannulated pigs.  ${}^{3}T_{3} =$  triiodothyronine.  ${}^{4}T_{4} =$  thyroxine.

### Environment

The nutritional requirements for a transgenic animal producing greatly elevated levels of a potent biologically active compound such as GH are not the same as those developed for animals secreting physiological levels of GH. The normal metabolic mechanisms of the transgenic animals can only respond to GH-directed signals for muscle deposition if adequate nutrients are available for that purpose. Recently, Steele and Pursel (1990) have presented a rationale for the nutrition of growth-modified, including transgenic, swine.

Pigs do not respond to the GH-dependent stimuli for an accelerated rate of muscle deposition by simply eating more feed. To the contrary, the appetite of transgenic pigs expressing bGH or hGH or pigs injected with pGH is actually depressed by about 15 to 20% (Campbell *et al.*, 1988; Evock *et al.*, 1988; Pursel *et al.*, 1990b). Reduced feed intake may, in part, explain reduced backfat thickness and improved feed efficiency. Reduction of intake may be the only means available to the animal attempting to regulate the effects of elevated GH.

# Where to from here?

As mentioned in the introduction, transgenic swine, and other species, could be used to produce pharmaceuticals as well as for far more efficient production of meat. The applications of transgenic technology may be easier to solve as compared to acceptability by consumers due to the rather phenomenal negative public image of genetically engineered germplasm of both plant and animal origin.

### Priority: Public education

Successful evolution of research from the laboratory to the marketplace relies on appropriate consideration by regulatory authorities to avoid negative public backlash. Specific guidelines should be in place early in the regulatory process. Recent events in the US with respect to BST can be used as an example. While the Food and Drug Administration is still deliberating on the details regarding registration of BST as an animal health product, legislation at a State level is banning the use of this product even before such approval. This senario is very dangerous not only for BST, but for technology in general. In this example, vocal special interest groups have effectively promoted their point of view to local legislators without considering the benefit the new technology may have for producers and consumers.

The need for education of both the public-at-large and the regulators will require a proactive scientific community to effectively communicate information to nontechnical audiences (Baile and Krestel-Rickert, 1988). Unfortunately, publicly funded research structures such as universities and federal laboratories rely on administrative leadership to set policy such that researchers can, with assurance, speak candidly. If the potential benefits from biotechnology, including transgenic germline development, are to be realized, a well publicized policy, preferably national in scope, needs to be established and a commission, even if serving on a colateral duty status, should be in place to buffer the public inquiries from the bench scientist. Considering that commercialization of a biotechnology product such as BST or PST requires a company to invest many millions of dollars, failure to gain registration approval because of negative public image could temper future industrial interest in supporting basic research.

# Priority: Applications to improve production efficiency

As employees of agricultural science, the first obligation is naturally to the constituents of this segment in the economy. As noted by Bolt *et al.* (1990) the initial research program utilizing the MThGH construct in farm animals was not founded on the practical goal of improving livestock productivity with this gene, but rather the gene was selected by necessity since few constructs were available at that time. As lines of animals are developed, increased characterizational research will be necessary. The following considerations will rely on classical approaches to livestock production:

- 1. The nutritional requirements for a transgenic animal producing greatly elevated levels of a potent biologically active compound such as GH are not the same as those developed for animals secreting normal, physiological, levels of GH. The normal metabolic mechanisms of the transgenic animals can only respond to GH-directed signals for muscle deposition if adequate nutrients are available for that purpose.
- 2. Develop appropriate performance testing programs to evaluate founder animals with respect to genetic worth. In that each transgenic animal created by current methods represents a uniquely different member of the specie, with success in creation of transgenic germlines comes the necessity for maintaining large animal populations. With assistance from animal breeders, expeditious performance selection procedures will require development.
- 3. Characterizational analyses of transgenic meat products will be required to rule out the possibility that the foreign structural gene (i.e., GH) insertion and expression does not alter the properties of myofibrillar protein in a detrimental manner. Additionally, the degree to which collagen deposition may be altered in GH transgenic swine has not been reported.

# Priority: Increase basic research support

In addition to diets better suited for their accelerated metabolic demands, transgenic pigs have been produced using genes constructed with promoters that respond to specific metabolic signals (Wieghart *et al.*, 1990). A good example is phosphoenolpyruvate carboxykinase promoter (PEPCK) fused to bGH structural gene. This approach of using regulatory elements may offer an advantage in animals with growth regulating transgenes.

As inferred, continued research investment into the exploration of desirable

regulatory gene sequences, investigations to control the extent and timing of foreign gene expression and methods to improve the efficiency of transgene insertion and expression will be required. These issues may be overcome in the near future.

Currently, success of transgenesis in livestock has been restricted to application of structural genes involved in the GH axis (GH, GRF and IGF 1). An alternative approach might be derived from the discipline of immunology (Aston *et al.*, 1991). In mice, Holder *et al.* (1985) has reported that growth rate and longitudinal bone growth were improved by passive immunization with a specific hGH/hGH monoclonal antibody. Pell *et al.* (1989b) has reported that milk production by sheep treated with a bGH/oGH monoclone produced significantly more milk as compared to sheep treated with exogenous oGH. Characterization of the molecular properties of the desired monoclonal antibody library may provide a construct desirable for transgenic manipulation. The advantage to this technique is that species specificity with respect to the structural GH construct utilized may be overcome. Additionally, health problems associated with supra-physiological concentrations of the transgene may be overcome.

### Priority: Transgenesis and human health

Progress in the mapping of the human genone will evolve a multitude of opportunities to develop biological strategies to improve human health and well-being. Currently, biologicals of great economic value in human medicine are viewed as the likely candidates for commercialization via both recombinant DNA techniques and mammalian transgenesis. Clearly interferon and blood clotting factors are in the market "pipeline". Fundamental understanding of both the major and minor histocompatability complex may create an extremely valuable niche for swine in the biomedical marketplace for organ donation to the human population. Possibilities are limited basically only by imagination.

# INSULIN-LIKE GROWTH FACTOR ANALOGUES: POTENTIAL IMPACT ON PORK PRODUCTION

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# Introduction

The advent of Biotechnology has realized several exciting and promising strategies for enhancing the efficiency of pork production via peptide hormones. Treatment of pigs with growth hormone (GH) and growth hormone-releasing factor, as well as the development of transgenic pigs expressing these hormones, are examples of strategies that are currently under commercial development. An alternative approach is via the somatomedins, otherwise known as the insulin-like growth factors (IGFs). The utility of IGFs as performance enhancers in meat animals has received little attention under the shadow of the dramatic effects observed in response to GH treatment. The reason for this lies in the dogma that IGFs are "wimpy" growth factors, based on a limited number of growth studies performed using rodents. Recently, we have discovered, patented, and commenced producing potent IGF analogues that have allowed us to test the effects of IGFs on growth and metabolism. Therefore the objectives of this paper are firstly to describe the recently discovered, potent analogues of IGFs; secondly to lay to rest the concept that IGFs are "wimpy" growth factors by presenting evidence that IGFs, and in particular recombinant forms of the potent IGF analogues, can stimulate growth and muscle accretion in animals; and thirdly to discuss the potential impact of this biotechnology on pork production.

### Insulin-like growth factors

Salmon and Daughaday's (1957) "somatomedin hypothesis" proposed that the growth-promoting actions of pituitary growth hormone are mediated by a separate class of compounds called the somatomedins. We have since discovered that these somatomedins, more widely known as the insulin-like growth factors, do mediate many, but not all of the actions of growth hormone. The extensive literature on IGFs, their biological actions, receptors and binding proteins has been reviewed elsewhere (Froesch et al., 1985; Baxter and Martin, 1989; Sara and Hall, 1990; Walton et al., 1990a). For the purpose of this paper it is necessary to note that there exist two forms of IGFs, i.e., IGF-I and IGF-II. These are peptides of 70 and 67 amino acids, respectively. IGF-I and IGF-II share substantial homology with proinsulin (40-50%) and thus they belong to the insulin family of peptides (which also includes relaxin and nerve growth factor). The IGFs act via three types of receptor on cell surface membranes: the type I and type II IGF receptors, and the insulin receptor. IGFs stimulate biosynthesis, cell division and differentiation, and inhibit catabolism in a variety of target cells, apparently via the type I IGF receptor. IGF-I and IGF-II are carried in blood and other fluids tightly bound to IGF binding proteins (IGFBP). When bound to IGFBPs, IGF-I and IGF-II are not available to target cell receptors and are therefore in a biologically inactive form. Six separate, but structurally related IGFBPs have been identified (Kiefer et al., 1991). One of these, IGFBP-3, carries most of the IGF in blood (Baxter and Martin, 1989).

Porcine IGF-I and IGF-II were isolated and structurally characterized in our laboratory (Francis *et al.*, 1989). Porcine IGF-I is identical to human IGF-I, whereas porcine IGF-II differs in only one amino acid compared to human IGF-II. Because of this structural homology, the human and porcine IGFs are indistinguishable in radioimmunoassays and radioreceptor assays (Francis *et al.*, 1989). In addition, human IGFs bind to porcine IGF receptors and IGFBPs (Gopinath *et al.*, 1989; Evock *et al.*, 1990) and are biologically active on porcine target tissues (Walton *et al.*, 1989a, b).

Using assays established for human IGFs, the status of IGFs in the pig has been examined. Like all species examined to date, circulating IGF-I levels are regulated by GH. Treatment of pigs with a dose of porcine GH (pGH) known to elicit a maximal effect on growth performance caused a two-fold increase in blood levels of IGF-I and a corresponding increase in levels of the major IGF binding protein in blood, IGFBP-3 (Walton & Etherton, 1989). In the same study, markedly reduced levels of IGF-I and IGFBP-3 were observed in hypophysectomized pig plasma (Walton & Etherton, 1989). Growth hormone does not increase IGF-II levels, in fact a decrease in IGF-II levels in pGH-treated pigs has been reported (Owens et al., 1990). This is consistent with the dogma that IGF-I, rather than IGF-II, is the major somatomedin regulating postnatal growth. There are also sex differences in IGF-I levels in pigs, with males having approximately 40% higher concentrations than females (Owens et al., 1990). IGF levels exhibit little or no diurnal variation in pigs (Walton and Etherton, 1989; Walton et al., 1989a; Owens et al., 1991). However, as shown in Figure 2, concentrations of IGF-I in plasma increase as pigs become heavier (R. G. Campbell, B. Luxford, P. C. Owens and P. E. Walton, unpublished data). Note that there is a precipitous decline in IGF-I levels at weaning; this is most likely due to the fasting associated with weaning, and is consistent with lower IGF-I levels observed with fasting or nutrient intake-restriction in other animal species (Clemmons and Van Wyk, 1984; Tomas et al., 1991). Also note that higher levels of IGF-I are observed in the faster growing breed at the heavier weights (Figure 2), a result consistent with earlier reports showing correlations between body size and IGF-I levels in pigs and other species (Froesch et al., 1985; Buonomo et al., 1987). Although there is a paucity of data on IGF-I actions in pigs, it is reasonable to assume that IGF-I is a major regulator of growth. It is also reasonable to assume that exogenous IGF-I will stimulate growth in pigs.



Figure 2. Plasma IGF-I levels in gilts between birth and 45 kg live weight. Two breeds of pigs were examined, ie, Hampshires ( $\bullet$ ) and Large Whites (O). The Large Whites were the faster growing breed, attaining 45 kg, on average, eight days earlier than the Hampshires. Values are means  $\pm$  S.E.

# **Potent IGF-I analogues**

Recently, researchers in our group at the CSIRO Division of Human Nutrition and the University of Adelaide discovered des(1-3)IGF-I, a potent form of IGF-I in bovine colostrum (Francis et al., 1988). Des(1-3)IGF-I is a truncated IGF-I that lacks the first 3 amino acids and is several fold more potent than IGF-I in vitro and in vivo (Francis et al., 1988; Ballard et al., 1991a; Tomas et al., 1991). Systematic studies of synthetic variants of IGF-I lead to the discovery that the glutamic acid residue at position 3 in IGF-I is crucial for binding of IGF-I to IGF binding proteins, and that removal or substitution of this glutamic acid with a residue of different charge produces IGF-I analogues that exhibit markedly reduced binding to IGFBPs. Because these analogues do not bind to inhibitory IGFBPs, they also exhibit enhanced potency compared to IGF-I in any biological system containing IGFBPs (Ballard et al., 1989; Walton et al., 1990b). A further class of IGF-I analogue, designed to allow highly efficient and inexpensive production using E. coli, is a fusion peptide comprising a portion of porcine growth hormone and IGF-I with an arginine substitution at position 3; thus named long R<sup>3</sup>IGF-I (LR<sup>3</sup>IGF-I). These IGF-I analogues, depicted in Figure 3, were patented and are now being commercially produced for cell culture and research applications by an Adelaide-based biotechnology company, GroPep Pty. Ltd.

The enhanced potency of des(1-3)IGF-I and LR<sup>3</sup>IGF-I compared to IGF-I in stimulating protein synthesis in cultured rat muscle cells is shown in Figure 4. Note that the IGF-I analogues are five-tenfold more potent than IGF-I at stimulating protein synthesis in cultured muscle cells. This enhanced potency is associated with a markedly lower affinity of the IGF analogues for the IGFBPs produced by the cultured muscle cells (Figure 4).



Figure 3. Structures of IGF-I and LR<sup>3</sup> IGF-I. The first three residues in IGF-I, i.e., G-P-E as shaded, are absent in des(1-3)IGF-I. Substitution of a glycine or an arginine residue for the glutamate at position three in IGF-I as indicated also yields potent IGF-I analogues. LR<sup>3</sup>IGF-I is a patented, fusion peptide that comprises IGF-I with an arginine substitution at position three, and a 13-amino acid extension peptide containing a portion of pGH.

# Animal growth trials with IGF-I analogues

The enhanced potency of the IGF-I analogues in vitro provided evidence that they will stimulate muscle growth and other anabolic processes in animals, and thus may be useful as anabolic agents for clinical, veterinary and animal production purposes. To test this, an extensive series of growth trials in growth-restricted rats have been performed (Ballard et al., 1991a; Read et al., 1991; Tomas et al., 1991). The initial objective of these studies was to test IGF-I and its analogues in rat models of trauma or nutritional stress as preclinical trials for applications in treating human polytrauma (ie. muscle wasting associated with burns, cachexia, surgery, multiple fractures, kidney disease, etc.). This series of growth trials has addressed the effects of IGF-I analogues on weight loss in the following growth-restriction models: 1) dietary nitrogen restriction; 2) glucocorticoid-treatment; 3) streptozotocin-induced diabetes; 4) intestinal resection (ie. 80% of the small intestine removed); 5) partial nephrectomy; and 6) genetic dwarfism (i.e., lit/lit mice). With the exception of the last model, all of these studies were performed on laboratory rats. Although this series of studies did not include normally growing animals (these will be done), the dramatic effects on growth observed demonstrate the potential of this class of compounds for enhancing pork production. In the current paper we will present selected data from one of the glucocorticoid-treatment experiments in which rats were treated with IGF-I, des(1-3)IGF-I or LR<sup>3</sup>IGF-I.

### Experimental Protocol

Two osmotic pumps (Alza model 2001) were surgically implanted s.c. into male Hooded Wistar rats (approximately 150 g body weight) under light anaesthesia. One pump delivered 20  $\mu$ g of dexamethasone sodium phosphate per day, the other pump delivered insulin-like growth factors, i.e., IGF-I, LR<sup>3</sup>IGF-I or des(1-3)IGF-I at the doses indicated in the *results* for seven days. Body weight gain, nitrogen retention, feed



Figure 4. Upper panel: Stimulation of protein synthesis in cultured L6 rat muscle cells by IGF-I ( $\bullet$ ), des(1-3)IGF-I ( $\blacktriangle$ ) and long R<sup>3</sup>IGF-I ( $\blacksquare$ ). Des(1-3)IGF-I and long R<sup>3</sup>IGF-I are fivefold more potent than IGF-I. Lower Panel: Binding of radiolabelled IGF-I to IGF binding proteins produced by cultured L6 rat muscle cells. Higher concentrations of des(1-3)IGF-I ( $\blacktriangle$ ) and long R<sup>3</sup>IGF-I ( $\blacksquare$ ), compared to IGF-I ( $\bullet$ ) are required to displace radiolabelled IGF-I from IGFBPs, thus they exhibit substantially lower binding affinities for IGFBPs.

intake, muscle protein synthesis and degradation, organ weights and carcass composition were determined using methods described elsewhere (Tomas et al., 1991).

### Results

Figure 5 shows the net changes in body weight and in cumulative nitrogen retention in rats treated for seven days with IGF-I or the IGF-I analogues. Dexamethasone treatment of rats caused severe losses in body weight and resulted in negative nitrogen balance, evident by comparison with either pair-fed or *ad-lib*-fed controls. All IGF treatments ameliorated the body weight losses and resulted in positive nitrogen balance. However, LR<sup>3</sup>IGF-I and des(1-3)IGF-I were two to fivefold more potent than IGF-I in eliciting these effects.



Figure 5. Body weight changes (upper panel) and cumulative nitrogen accumulation (lower panel) in dexamethasone-treated rats following seven day's infusion with IGF-I ( $\bullet$ ), des(1-3)IGF-I ( $\bullet$ ) or LR<sup>3</sup> IGF-I ( $\bullet$ ). Significance from dexamethasone-treated rats receiving no IGF, tested using one-way ANOVA and LSD is indicated by: a, P<0.05; b, P<0.01; c, P<0.001. Ad libitum-fed ( $\Diamond$ ) and pair-fed ( $\Box$ ) normal controls are also indicated. Values are means  $\pm$  S.E.

To examine the mechanism of these effects on nitrogen balance, muscle protein synthesis rate at slaughter, and cumulative myofibrillar protein degradation (as 3-methylhistidine excretion) were measured (Figure 6). Consistent with the observed reduction in nitrogen retention, muscle protein synthesis and degradation rates were substantially reduced and increased, respectively, in dexamethasone-treated rats compared to pair-fed or *ad-lib*-fed controls. IGF-treatment not only significantly increased muscle protein synthesis, but also substantially decreased muscle protein degradation. Again, LR<sup>3</sup>IGF-I and des(1-3)IGF-I were approximately two to threefold more potent than IGF-I, particularly at lower dose rates.



Figure 6. Final muscle protein synthesis rate (upper panel) and average muscle protein breakdown as cumulative urinary excretion of 3-methylhistidine (lower panel) in dexamethasone-treated rats following seven day's infusion with IGF-I ( $\bullet$ ), des(1-3)IGF-I ( $\blacktriangle$ ) or LR<sup>3</sup> IGF-I ( $\blacksquare$ ). Significance from dexamethasone-treated rats receiving no IGF, tested using one-way ANOVA and LSD is indicated by: a, P<0.05; b, P<0.01; c, P<0.001. Ad libitum-fed ( $\Diamond$ ) and pair-fed ( $\Box$ ) normal controls are also indicated. Values are means  $\pm$  S.E.

No significant differences in carcass composition were observed due to IGF treatment. It is especially important to note that IGF-I and the IGF-I analogues did not stimulate fat accretion, indeed a tendency to decrease carcass fat was observed (Figure 7). This result is consistent with other studies performed by our group in which IGF-I analogues stimulated protein accretion with no concurrent increase in fat in diabetic rats, whereas exogenous insulin markedly stimulated fat accretion (Ballard *et al.*, 1991a). Thus in eliciting their anabolic actions, IGF-I analogues are not merely acting as insulin mimics.

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Figure 7. Carcass fat in dexamethasone-treated rats following seven day's infusion with IGF-I, des(1-3)IGF-I or LR<sup>3</sup> IGF-I. Ad libitum-fed and pair-fed normal controls are also indicated. Values are means  $\pm$  S.E.

A significant observation during this series of experiments was that IGF-I and the IGF-I analogues stimulated the growth of gut tissue in a dose-dependent manner by 60% above that of the control groups, including control animals not receiving dexamethasone (Figure 8). Growth occurred throughout the gut, including the stomach, small intestine and colon, and was a result of an increase in cross-sectional area rather than gut length, as reported elsewhere (Read *et al.*, 1991). LR<sup>3</sup>IGF-I and des(1-3)IGF-I were more potent than IGF-I in eliciting these effects. The functional relevance of this increase in gut in terms of the overall anabolic actions of the IGF analogues requires further study. However, this outcome suggests that IGF treatment may result in an enhancement of net nutrient uptake by the gut.

In summary, the IGF-I analogues, LR<sup>3</sup>IGF-I and des(1-3)IGF-I, are anabolic in dexamethasone-treated rats. In eliciting these effects, these analogues are substantially more potent than IGF-I. Earlier reports of treatment of rats with exogenous IGF-I have failed to demonstrate dramatic effects on growth. This was probably due to employment of inadequate doses or inappropriate modes of administration. Given a constant infusion of an adequate dose, exogenous IGF-I and its analogues will stimulate growth. A constant infusion is necessary because des(1-3)IGF-I and LR<sup>3</sup>IGF-I given as a bolus injection, are rapidly cleared from the circulation as a consequence of poor binding to blood-borne IGFBPs (Ballard *et al.*, 1991b; Walton *et al.*, 1991).

# Application of IGF-I analogue biotechnology to pigs

There are three potential applications for IGF-I analogues in pork production:

- 1. As an anabolic agent to stimulate performance in grower and finisher phase pigs. That is, as a direct competitor to porcine GH to elicit enhanced growth, reduced carcass fat, and increased efficiency of feed conversion.
- 2. As an adjunct treatment to porcine growth GH.

3. As a treatment for stress-induced weight loss, e.g., associated with weaning, scours, febrile states, etc,.



Figure 8. Gut weight in dexamethasone-treated rats following seven day's infusion with IGF-I ( $\bullet$ ), des(1-3)IGF-I ( $\blacktriangle$ ) or LR<sup>3</sup> IGF-I ( $\blacksquare$ ). Ad libitum-fed ( $\Diamond$ ) and pair-fed normal ( $\square$ ) controls are also indicated. Values are means  $\pm$  S.E.

To directly compete with pGH, IGF-I analogues will need to be an equivalent product in terms of cost per dose, efficacy and safety, or should be superior to pGH in some aspect of their application. In light of the paucity of any data on IGF effects on growth in pigs, it is premature at this point in time to consider in detail the issue of dose and efficacy. However, based on the doses employed in the current series of rat experiments in which the highest IGF-I dose effectively doubled circulating IGF-I levels, the substantially lower circulating IGF-I levels in pigs compared to rats (Owens et al., 1990; Ballard et al., 1991b), and the markedly enhanced potency of LR<sup>3</sup>IGF-I, it is not unreasonable to expect LR<sup>3</sup>IGF-I to stimulate growth at doses comparable to pGH, i.e., as low as 100 µg per kg body weight. The cost of producing recombinant IGF-I analogues, particularly LR<sup>3</sup>IGF-I, is equivalent or even less than that needed to produce recombinant pGH on the same scale. Whilst the safety of IGF-I analogues as anabolic agents in meat animals requires further study, comprehensive IGF-I toxicity studies performed to support submissions to the U.S. Food and Drug Administration for the use of bovine GH in dairy cattle (bGH causes increased milk IGF-I levels), indicate that IGF-I analogues should pose no potential hazards (Juskevich and Guyer, 1990).

There may be potential advantages of IGF-I analogues compared to pGH. Four obvious examples are: 1) route of administration. IGF-I, circulating in relatively high constant levels in blood rather than following an episodic diurnal pattern, is a particularly appropriate hormone to administer via an implanted, constantly infusing delivery device; 2) IGF-I analogues may stimulate growth in the grower phase, during which pGH is relatively impotent; 3) IGF-I analogues may act without the side-effects attributed to pGH, e.g., reduced feed intake and osteochondrosis (Evock *et al.*, 1988); and 4) during nutritional stress and/or febrile states it is unlikely that GH will be effective at ameliorating weight loss since levels of circulating GH are high and levels of IGF-I are low in such conditions (Clemmons and Van Wyk, 1984).

### The future

To transfer this biotechnology to pork production we must begin by testing these products in appropriate animal models. Studies currently under way and/or in the final stages of planning to evaluate the efficacy of LR<sup>3</sup>IGF-I in normal rodents and in growing pigs will address the testing phase. Our rodent studies performed to date provide encouraging evidence that LR<sup>3</sup>IGF-I will prove to be an effective anabolic agent in pigs. The further development of a LR<sup>3</sup>IGF-I-based product for the pork industry will require the collaborative input of a large pharmaceutical company.

# IMMUNOLOGICAL ENHANCEMENT OF GROWTH HORMONE ACTIVITY: APPLICATION TO PIG PRODUCTION

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### Introduction

During the past ten years significant emphasis has been placed on developing recombinant growth hormone (GH) for the improvement of animal production. However, the general use of hormones in animals is coming under increasing pressure from the regulatory authorities, consumer groups and the public. As a consequence of this a number of laboratories have focused their attention on developing non-hormone based methods towards improving animal production parameters (Flint, 1987; Holder et al., 1991). Among such studies have been a number of immunological approaches which include: vaccination against somatostatin (Smt) (Spencer et al., 1983; Petitclerc et al., 1988; Dubrueil et al., 1989), adipocytes and growth hormone as well as hormone like anti-idiotypic antibodies (Morrison, 1986). Unlike the administration of hormone, most immunological methods are aimed at employing the animal's immune system to alter physiological status. Perhaps the simplest example of such a product is the recently developed immunocastration vaccine against luteinizing hormone releasing hormone (LHRH) (Hoskinson et al., 1990). As expected, immunization of animals against LHRH results in gross physiological and tissue changes leading to reversible loss of fertility. Along similar lines, approaches such as immunization against adipocytes or Smt were anticipated to result in animals with improved carcass composition and growth.

The observation that certain site-directed monoclonal antibodies could result in significant enhancement of GH activity in vivo paved the way for the identification of the regions of the GH molecule associated with this phenomenon, and consequently the development of a corresponding peptide vaccine. The frequent association of antibodies with the neutralization of infective agents (viruses, bacteria etc.) or indeed with the inhibition of hormonal activity (eg LHRH) has historically overshadowed the less common observation that antibodies can have precisely the opposite effect: that is, potentiation of hormonal or enzyme activity. Perhaps the earliest demonstrations that antibodies could enhance hormonal activity were those relating to the treatment of animals with antisera to gonadotrophic hormones (Thompson, 1937; Rowlands, 1939). Although the authors of these early experiments often interpreted the enhancement effect as being mediated by inhibition of hormone-release-inhibitory factors, more recent studies indicate that the active components were the hormone-antibody complexes themselves (Cole et al., 1975). Along similar lines, Ferguson (1954) showed that repeated treatment of sheep with crude GH preparations resulted in a delayed enhancement of wool growth; the effects became apparent many weeks after ceasing

the GH treatment and were consistent with the time course of an immune response to the hormone. The systematic analysis of hormonal enhancement by particular antisera was not undertaken until the early eighties. This process was greatly facilitated by the preparation of panels of monoclonal antibodies (MAbs) recognizing non-overlapping epitopes on the GH molecule. This review is primarily aimed at summarizing the more recent developments of the immunoenhancement of growth hormone activity and discussion of its potential application to animal production.

### Enhancement of growth hormone activity

The treatment of hypopituitary mice with GH in complex with MAbs directed towards particular epitopes results in substantial enhancement of hormonal activity (Holder *et al.*, 1985; Aston *et al.*, 1986, 1987, 1989). The extent of the enhancement effect, over the equivalent dose of free hormone, ranges from between 3-7-fold as determined by incorporation of radioactive sulphate into costal cartilage. By weight gain, animals have been shown to increase in growth by up to 30% over hormone only treated groups. Similarly, in order to mimic the growth improvement achieved by GH-MAb complexes, the dose of free hormone required will generally correspond to 10-50-fold the dose employed in complex with antibody (Aston *et al.*, 1986,1987). Complexing GH with MAbs of non-enhancing specificity results in either inhibition of hormonal activity or no effect at all (ie equivalent to free hormone). In contrast, complexes of GH with pairs of MAbs recognizing non-overlapping specificities always results in reversal of the enhancement phenomenon; in the presence of particular MAb pairs, inhibition of GH activity is observed (Aston *et al.*, 1989).

### Mechanisms

In order to account for the dramatic improvement in hormone activity associated with complexing hormone with antibody a number of mechanisms have been considered, these include: (i) facilitated cross-linking of receptors through antibody bivalency (Schechter et al., 1979a,b); (ii) Fc-region mediated targeting of the hormone to the liver (Leclerc et al., 1984); (iii) slow release of the hormone from circulating antibody (Dixon et al., 1975); (iv) induction of more potent conformations of GH and (v) restriction (or targeting) of the hormone to a particular subclass of GH receptor (Aston et al., 1991). The demonstration that univalent antibody fragments (Fab'), which lack the antibody-Fc region, are equally efficacious as the intact antibody in hormonal enhancement makes mechanisms (i) and (ii) unlikely candidates (Aston et al., 1987). Previous work with insulin and epidermal growth factor has shown that dramatic enhancement of hormonal activity can be achieved through antibody This mechanism is based on the concept that the complexes formed bivalency. between receptors and the corresponding MAb-hormone complexes are more stable and of higher affinity. Furthermore, the bridging effect of the antibody-hormone complex between pairs of receptors facilitates the crosslinking of receptors; this process is known in itself to be an important parameter in the mechanism(s) of hormone action. Indeed, bivalent antibodies to insulin receptor are known to mimic insulin activity in the absence of hormone (Khan et al., 1978, 1982).

At the present time it is not possible to completely exclude the possibility that the enhancement of GH activity by MAb is mediated, at least in part, by more optimal delivery of the hormone (ie. slow release). Indeed significant enhancement of GH activity is achieved by continuous or pulsatile administration as compared with a single bolus injection (Clark *et al.*, 1985). The three main arguments against this mechanism are that: (i) enhancement of endogenous GH activity can be achieved in the absence of exogenous GH by passive administration of antibody or active vaccination with peptide (it is presumed that the pituitary release is near optimal); (ii) it has been shown that particular pairs of MAbs with identical effects on GH half-life *in vivo* can have completely contrasting effects on growth hormone activity (ie. inhibit and enhance GH activity *in vivo* respectively); (iii) the antibody mediated enhancement

phenomenon has been demonstrated to occur in non-systematic models for GH As for the 'slow release' mechanism, the activity in vivo (Aston et al., 1986). demonstration that favourable conformational changes are occurring following MAb binding to hormone are difficult to demonstrate. Perhaps the strongest argument in favour of the latter hypothesis is the observation that cleavage of the GH sequence in the region 136-149 results in dramatic enhancement of GH activity in vivo (Singh et al., 1974; Lewis et al., 1975). Coincidentally, the enhancing region of GH includes this The 'restriction' hypothesis for antibody-mediated sequence (see next section)! hormonal enhancement {mechanism (v) above} encompasses a number of different possible mechanisms: essentially, it is predicted that there are at least two different GH receptors which are distinguished by the nature of their interaction with GH. Furthermore, enhancing antibodies inhibit binding of GH to one class of receptor but not the other, thus the antibody-hormone complex is targeted. Although little in vivo data is available to support this hypothesis, it is clear that GH receptors do differ in hormone specificity and that enhancing antibodies enable binding of the hormone to one class of receptor but not the other (Aston et al., 1989).

### Location of the enhancing region on growth Hormone

Despite the availability of numerous MAbs to bovine and human GH, it has not been possible to map the epitopes or peptide regions on the hormone that these bind to. This has been mainly due to the conformational nature of most MAb epitopes and our consequent inability to represent them with short peptides. The location of the enhancing and non-enhancing sites on GH have been identified by extensive peptide synthesis and subsequent examination of the corresponding peptide antisera *in vivo* (Aston *et al.*, 1991).

The identification and selection of peptides for synthesis has been primarily based on examination of secondary, and more recently tertiary structure of GH. Peptides were generally limited to no more than 20 residues although occasionally a Cys-Ala carboxy-terminal was added to provide an additional conjugation route. For the growth studies summarized in the following section peptides were conjugated to ovalbumin with glutaraldehyde (Aston et al., 1991). Overlapping peptides spanning the majority of the GH molecule have been synthesized and tested in target species for their antigenicity (a representative selection of these is presented in Aston et al., 1991). Identification of enhancing antisera was undertaken by co-administering the corresponding antiserum (from sheep) with GH to hypopituitary dwarf mice. The growth rates of animals treated with various anti-peptide antisera is shown in Figure 9. Two overlapping peptide sequences (120-140 and 134-154) consistently yielded antisera in sheep which when administered to dwarf mice in complex with GH resulted in enhancement of growth rate. A further region (35-53), which is sequentially displaced from the other two peptides (124-140 and 134-154) was also capable of generating growth enhancing antisera (Bomford and Aston, 1990). This can easily be accounted for by examination of the three dimensional structure for GH (Figure 10); it can be seen from the Figure that although peptides 35-53 and 134-154 are sequentially distant, in topographic terms they are adjacent. Further studies with the non-enhancing shorter peptide (32-46) reveal that the relevant region for enhancement is between residues 45 and 53. Indeed, it can be seen from Figure 10 that it is the carboxy-terminal portion of peptide 35-53 that is topographically proximate with the loop linking helices 3 and 4 and encompassing the 134-154 peptide.

# Growth enhancement with passive antibody administration or active vaccination

The two major challenges during the evolution of this work were the achievement of successful auto-immunization of target species with GH peptides and the demonstration of enhancement of endogenous GH in animals. At the present time, we have successfully shown that it is possible to auto-immunize up to 80% of animals (pigs and sheep) following three vaccinations. The demonstration of

enhancement of endogenous GH with antibody has been pursued on two fronts: (i) enhancement of GH activity by passive administration of murine monoclonal antibody or anti-peptide (134-154) antiserum from sheep and (ii) active vaccination of sheep and pigs with peptide 134-154.



Figure 9. Enhancement of bovine GH activity in dwarf mice by site directed antisera. The growth response of hypopituitary dwarf mice to injected GH is associated with incorporation of  $^{35}$ S into costal cartilage (Aston et al., 1987). Growth rates in response to bGH (50 µg) in the presence of control or anti-peptide antisera are shown as means of groups of 6 animals. Control Ig was derived from ovalbumin immunized sheep. Statistical significance was determined by Student's T-test, P<0.005 for peptide antisera to sequences 35-53, 120-140 and 134-154 as well as for MAb 14.

# Enhancement of IGF-1 levels

The treatment of lactating sheep with GH or enhancing MAb (OA11) has been shown to result in significant increases in circulating IG1-1 levels (Table 1) (Pell *et al.*, 1989b). Similarly, animals treated with complex (GH+OA11) produced correspondingly greater IGF-1 levels than GH only treated animals. Treatment of hypopituitary rats with complexes between bGH and MAbs also results in significant elevation of serum IGF-1 (Wallis *et al.*, 1987). In the latter studies, there were differences in the peaking and persistence of IGF levels depending on the epitope specificity of the antibody. As observed with anti-human GH MAbs, no MAb specificity was inhibitory when employed singularly (Aston *et al.*, 1986).



Figure 10. Schematic representation of the three dimensional structure of GH, based on Abel-Meguid et al. (1987). Location and amino acid sequences of peptides 120-140 and 134-154 are shown on the loop which links helices 3 and 4. A further peptide (35-53), identified previously to elicit growth enhancing antibodies (Bomford and Aston, 1989), is partially shown on the loop between helices 1 and 2. The structural relationship between the protease sensitive region (KQTYDKFDTN; dark-shaded) and the sequences that elicit growth-enhancing antibodies (diagonal lines) is shown. Cleavage at this site has been shown previously to result in enhancement in the growth promoting activity of hormone. The grey-shaded loop regions, 53-73 and 167-191, represent synthetic peptides which failed to elicit growth-enhancing antibodies. The general region which may be involved in the enhancement phenomenon is indicated.

Table 1. Plasma insulin-like growth factor (IGF-I) concentrations (ng/ml) of lactating ewes before and during treatment with monoclonal antibody (OA11) (Mab), bGH and Mab-bGH complexes

Treatment (N)	MAb (4)	bGH (5)	MAb-bGH (5)	SD <sup>1</sup>
Pre-treatment (d -3)	81.5	93.8	113.6	35.3
Treatment (d 16)	128.5ª	193.5⁵	254.5°	31.8

<sup>1</sup>Values with different superscripts differ (P<0.05).

# Enhancement of diabetogenic activity

In order to determine whether enhancing MAb OA11 could increase the diabetogenic activity of circulating GH, ewe lambs were treated with the hormone in the presence or absence of MAb, or with MAb only. Changes in diabetogenic activity were measured by the decrease in plasma glucose following insulin administration to the animals (Figure 11). The ability of MAb only to enhance endogenous GH activity

is reflected by the corresponding increase in insulin tolerance; it is noteworthy that neither GH nor MAb were as diabetogenic as the complex. In the latter case, the animals were virtually resistant to the insulin (Pell *et al.*, 1989b).



Figure 11. Insulin (80 mU/kg) tolerance tests on ewe lambs before treatment (solid lines) and during treatment (dashed lines) with (a) phosphate-buffered saline, (b) 0.15 mg/kg bGH, (c) 5.0 mg monoclonal antibody (MAb), and (d) MAb-bGH complex (0.15 mg/kg bGH + 5.0 mg OA11). Values are means  $\pm$  SE (n=5).

# Enhancement of GH galactopoietic activity

The ability of passive antibody administration to enhance either endogenous hormone or exogenously administered GH has been studied in two lactation trials (Pell *et al.*, 1989a,b,1990). In the former it was shown that enhancing MAb OA11 (to bGH and oGH) could increase the milk production induced by injected bGH by over 30%. More recently, it has been shown that ovine antiserum to sequence region 134-154 of bGH could significantly enhance endogenous GH activity in recipient ewes (Figure 12). The effect of exogenous antiserum was more apparent in terms of prolonging the production of milk in animals as compared to the control Ig treated group.

### Enhancement of GH activity by active vaccination

The observations in the passive antibody administration trials (described above) formed the basis to proceed to an active vaccination trial in growing lambs. It is noteworthy, however that during the course of a passive antibody trial only low circulating antibody titres can be achieved. This limition is set by the small volume of Ig that can be physically administered to an animal. In contrast, during active vaccination animals can produce constant and high levels of circulating antibodies. The results of our first active vaccination trial are shown in Figure 13 and are compared with a large dose of passively administered anti-134-154 Ig derived from immunized sheep (Pell et al., submitted). In the passive aspect of this experiment, lambs were treated with purified Ig from sheep immunized against peptide sequence 134-154 of GH (identical to the corresponding sequence of pGH). Such antisera crossreact with both bovine and porcine GHs with equal efficacy. Animals receiving either GH or anti-peptide antiserum had significant increases in total carcass protein; a combination of both exogenous GH and anti-peptide antiserum gave 'a further improvement in total protein although this was not significant over GH or anti-peptide antibody only treated groups. Within the same study, actively vaccinated animals with peptide 134-154 linked to ovalbumin (four injections of antigen in FCA), gave a significantly improved total carcass protein over control vaccinated animals (ovalbumin only). Similarly, GH treated animals had corresponding increases in total protein; combination treatment of GH and active vaccination failed to give significant improvement over GH only or active vaccination only treated groups.



Figure 12. Effect of daily injections of GH and/or anti-peptide hormones on milk production in ewes (treatments; Control O, GH  $\Box$ , Anti-peptide antibody  $\bullet$ , GH + anti-peptide antibody  $\blacksquare$ ).



Figure 13. Total carcass protein in lambs treated with Ig to ovine GH by immunizing sheep with peptide #30 (134-154) (passive) or vaccinated directly with peptide #30 (134-154) linked to ovalbumin (active). (Treatments; C: control Ig or control vaccine (ovalbumin only), GH: growth hormone (0.1 mg/kg), AP: anti-peptide Ig, AV: active vaccine of sequence 134-154).

# Discussion

The development of a growth promoting vaccine, based on the observation that MAbs can enhance injected GH activity, has required the demonstration that: (i) autoimmunization can be achieved and (ii) that endogenous GH activity can be enhanced. In the studies of Pell *et al.*, (submitted) it has been shown that animals vaccinated against the 134-154 region of GH have significantly increased production parameters. The antigenic overlap of this sequence region with that from the porcine GH sequence has now also enabled the undertaking of a pig growth trial using this approach. Preliminary data (Agergaard *et al.*, in preparation) have yielded highly encouraging results in pig growth rates and food efficiency. Success in the vaccine approach to improving animal production may provide a viable alternative to hormone treatment; such a product would be analogous to currently available vaccines for controlling animal fertility.

# SYMPOSIUM CONCLUSION

### F.R. Dunshea

We do indeed live in exciting times. Biotechnology has provided us with some novel means to manipulate growth as well as providing us with tools to utilize in our attempts to understand the regulation of growth and development. While the commercial dollar has been geared towards developing strategies to manipulate the GH/IGF-I axis, much basic science has emerged from these efforts. For example, if nothing comes from commercializing the development of GH, we would have at least obtained invaluable data on the mode of action of endogenous GH and established new production thresholds which may be ultimately achieved through conventional techniques.

What is more likely, is that one or more of these technologies will result in commercial application with the ultimate winners being the producers (through improved efficiency and premium prices), the consumers (through a more healthy, leaner product) and of course the commercial backer of the successful product(s). One aspect which is particularly pleasing, and is clearly evident from this symposium, is the prominance of Australian research in the practical application of biotechnology to the pig industry. This can only be of benefit for our industry and the PRDC are to be congratulated on their foresight and active participation in these areas.

Importantly, Steele et al. (1991) high-lighted the need for a proactive scientific community in the education of members of the regulatory bodies and the general community. In addition, as scientists we should remain objective and realistic in our evaluation of the role of biotechnology in the pig industry.

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# PLASMA INSULIN-LIKE GROWTH FACTOR-I (IGF-I) CORRELATES WITH GROWTH RATE OF BOARS

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IGF-I and -II are polypeptides present in pig blood, and both can inhibit protein breakdown and stimulate protein synthesis in cultured rat muscle cells (Francis *et al.*, 1989). Anabolic responses to treatment with growth hormone (GH) are associated with increased levels of IGF-I and reduced levels of IGF-II in blood from "finisher" pigs (Owens *et al.*, 1990), indicating that in pigs, as in other species, IGF-I mediates anabolic actions of GH. It is very likely that IGF-I itself is anabolic in pigs since *human* IGF-I, which is structurally identical to *porcine* IGF-I (Francis *et al.*, 1989), improves nitrogen balance in rats (Tomas *et al.*, 1991). The aim of the present study was to determine whether plasma IGF-I concentrations are related to the growth rates of boars.

Large white boars (n=76) were fed *ad libitum* from weaning until age 22 weeks (weight, mean  $\pm$ SEM; 97.9  $\pm$  1.4 kg). Blood was collected on four occasions from each boar and IGF-I was measured in plasma by radioimmunoassay (Francis *et al.*, 1990). Plasma was extracted with acid-ethanol before IGF-I assay to precipitate IGF-binding proteins that can produce artefacts in IGF assays by competing with anti-IGF-I immuno-globulins for association with the ligand. To assess this technique, originally designed for human plasma, IGF-I measurements of acid-ethanol plasma supernatants were compared with those from assay of fractions obtained by size exclusion high performance liquid chromatography of plasma in acid. The latter completely separates IGF-I from binding proteins and eliminates their interference from the IGF-I assay.

In boars aged 9 (20.3  $\pm$  0.5 kg) to 15 weeks (51.6  $\pm$  1.0 kg), acid-ethanol IGF-I estimates correlated positively with definitive measurements obtained by IGF-I assay after acid chromatography (r= 0.76 to 0.88; P<0.0005). These acid-ethanol values under-estimated true IGF-I levels, as previously reported for 90 kg pigs (Owens *et al.*, 1990). In younger boars however, IGF-I assay of acid-ethanol plasma supernatants failed completely to estimate IGF-I levels in plasma. Relationships between growth and IGF-I levels were assessed by linear regression analysis using only IGF-I measurements with acid column extracts of plasma. The interassay coefficient of variation was 7.4%.

Plasma IGF-I increased from  $61 \pm 4$  ng/ml at 5 weeks (7.1  $\pm$  0.2 kg) to  $295 \pm 9$  ng/ml at 15 weeks of age. IGF-I levels in young boars correlated with liveweight measurements at all ages studied (IGF-I at 5 weeks with weight at 5, 7, 9, 11, 15, 19 and 22 weeks; r= 0.46 to 0.72; P<0.001). Also at 5, 7 and 9 weeks of age, IGF-I correlated strongly with rate of liveweight gain at those ages (r= 0.71, 0.68 and 0.66; P<0.0005).

We conclude that the blood plasma level of the anabolic peptide IGF-I in young boars is a major correlate of postweaning growth rate and suggest it may have a significant influence over liveweight at slaughter.

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# EFFECTS OF PORCINE GROWTH HORMONE ON MUSCLE FIBRE CHARACTERISTICS IN PIGS

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Administration of porcine growth hormone (pGH) induces skeletal muscle hypertrophy in pigs. Muscle fibre characteristics may influence post-mortem metabolism in skeletal muscles and eventual meat quality. This study investigated the effect of pGH on skeletal muscle fibres in male and female finishing pigs fed varying amounts of digestible energy. The muscle samples were from the experiment reported by Warner and King (1989). Samples of the *m. longissimus* were taken at the last rib 24 h after slaughter and frozen. Fibre types were distinguished by staining for myofibrillar adenosine triphosphatase (ATPase) after incubation at pH 4.2. Muscle fibre sizes and proportions were analysed by computerised image analysis. About 800 fibres were traced for each sample.

	Males		Females			Statistics <sup>1</sup>			
	Cont	pGH	Cont	pGH	SED	Sex	pGH	SexxpGH	
Type I, µm²	3717	3691	3644	3590	239	NS	NS	NS	
Type II, µm²	4896	4938	5070	5774	309	*	NS	NS	
Giant, µm²	11508	12005	11370	13246	911	NS	NS	NS	
% type I	12.6	11.7	11.0	12.1	1.03	NS	NS	NS	
% type II	86.5	87.2	88.5	87.0	1.05	NS	NS	NS	
% Ĝiant	0.83	0.98	0.55	0.83	0.153	*	*	, ŃS	

# Table 1. Muscle fibre histology in pGH-treated boars and gilts

<sup>1</sup>NS, non significant, P>0.05; \* P<0.05.

Since feeding level did not alter the effects of sex and pGH on muscle fibre characteristics or meat quality, data were pooled across feeding levels within sex and pGH groups. Type II fibres were larger in females than in males, and pGH tended to increase type II fibre sizes (P=0.09) at the same slaughter weight. In contrast Solomon *et al.* (1988) reported an increase of muscle fibre size with treatment of pGH; this discrepancy may be due to their use of younger pigs (25-55 kg live weight). In contrast to Solomon *et al.* (1988), giant fibres were observed in both control and pGH-treated muscles. The occurrence of giant fibres was significantly greater in males than in females, and was increased further by pGH. Since pGH did not affect pH, colour and tenderness in longissimus muscle (Warner and King, 1989), the small population of giant fibres does not appear to be associated with meat quality.

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# TEMPORAL RESPONSE OF METABOLITES TO RACTOPAMINE TREATMENT IN THE GROWING PIG

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Ractopamine (RAC) is a  $\beta$ -adrenergic agonist which increases lean tissue deposition in pigs, without necessarily affecting fat deposition (Dunshea *et al.*, 1990). The aim of this study was to determine the responses of intermediate metabolites to dietary RAC. Eight gilts (73 kg live weight) prepared with jugular vein catheters were fed 3.0 kg/d (6 x 0.5 kg every 4 h) a wheat-based diet (14.5 MJ DE/kg, 0.65 g available lysine/MJ DE) containing either 0 or 20 ppm RAC. Blood samples, for plasma non-esterified fatty acids (NEFA), glucose and urea nitrogen (N) analyses, were taken every 2 h on d 1, 2, 8 and 22 of treatment. RAC was withdrawn on d 24 and pigs were again bled on d 29.

				•						
	Da	y 1	Da	y 2	Da	iy 8	Da	y 22		
Ractopamine (ppm)	0	20	0	20	0	20	0	20	CV (%)	Sign <sup>1,2</sup>
NEFA (µmol/l) Glucose (mg/dl) Urea N(mg/dl)	57 96 18.1	73 94 18.2	57 99 18.3	69 94 17.0	70 95 17.4	64 90 16.2	63 91 18.3	68 86 17.3	14.2 3.1 4.6	D*** R*,D*

# Table 1. Effect of ractopamine on daily mean plasma metabolite concentrations

<sup>1</sup>R, ractopamine treatment; D, day. <sup>2\*</sup> P<0.05; <sup>\*\*\*</sup> P<0.001.

Average daily gain (ADG) upto d 24 was increased by RAC (754±30 vs 1030±41 g/d, mean±SE for control and ractopamine treatment). For the week following withdrawal of RAC there was no difference in ADG ( $674\pm43$  vs  $736\pm38$  g/d). Plasma NEFA concentrations were not significantly affected by RAC (P=0.45) suggesting no increase in apparent fat mobilisation. The marginally higher mean plasma NEFA observed in the RAC group would correspond to an increase in fat mobilisation of only 8 g/d (Dunshea et al., 1991). No acute effects of RAC were evident from the plasma NEFA profile over the first 2 d. In contrast, Hancock and Anderson (1990) observed acute but not chronic increases in plasma NEFA during RAC treatment. Differences in acute responses may be due to more rapid uptake of RAC in their more restrictively-fed pigs  $(2 \times 1 \text{ kg/d})$ . There was no effect of RAC on plasma glucose concentrations, although plasma glucose did decrease with time. Plasma urea N concentrations remained at basal levels for the first day of RAC treatment, commencing to decline on the second day. Thereafter, plasma urea N remained lower for the duration of RAC treatment. Withdrawal of RAC resulted in a significant increase in plasma urea N ( $17.5\pm0.5$  vs  $21.0\pm0.7$  mg/dl, P<0.01) but no change in either plasma NEFA or glucose. The temporal responses of metabolites to RAC are consistent with increased lean tissue deposition with little change in fat deposition (Dunshea et al., 1990).

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# SKELETAL MUSCLE FIBRE TYPE CHARACTERISTICS IN RACTOPAMINE-FED PIGS

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This study was conducted to investigate the effects of ractopamine on skeletal muscle fibre characteristics in finishing pigs (16 males, 16 females and 16 castrates). The muscle samples were from the experiment reported by Dunshea *et al.* (1990). Samples of the *m. longissimus* were taken at the last rib 24 h after slaughter and frozen. Fibre types were distinguished by staining for myofibrillar adenosine triphosphatase (ATPase) after incubation at pH 4.2. Muscle fibre sizes and proportions were analysed by computerised image analysis. About 800 fibres were traced for each sample.

Sex	Ma	les	Fem	ales	C	Castrates			Statistics	1
Treatment <sup>2</sup>	CON	RAC	CON	RAC	CON	RAC	SED	Sex	RAC	SxR
Type I, µm²	2072	2314	1951	1869	1779	1993	192	NS	NS	NS
Type II, µm²	2190	2281	1889	2040	1829	1978	230	NS	NS	NS
Giant, µm²	6560	6636	5674	6191	5230	5955	452	*	NS	NS
% Type I	9.3	9.5	9.1	8.8	11.4	9.8	1.4	NS	NS	NS
% Type II	90.2	90.1	90.5	90.9	88.4	89.8	1.4	NS	NS	NS
% Giant	0.51	0.40	0.37	0.25	0.25	0.46	0.14	NS	NS	NS

# Table 1. Effects of sex and ractopamine on muscle fibre histology in pigs

 $^{1}$ NS, non significant, P>0.05; \* P<0.05.  $^{2}$ CON, controls; RAC, ractopamine (20 mg/kg diet).

Giant fibres were found in muscles of both control and ractopamine-fed pigs. Type I and giant fibres were larger in males than in females or castrates. Ractopamine did not affect the size or proportion of muscle fibre types. Muscle fibre histology was unrelated to meat quality, except for a low correlation between the size of type II fibres and colour (r = -0.53, -0.56 and -0.63 for L\*, a\* and b\* respectively). The absence of major effects of

ractopamine on fibre characteristics is consistent with the results of Dunshea *et al.* (1990) who found no effects of ractopamine on loin eye area. The poor relationships among fibre characteristics and meat quality preclude any firm conclusions about cytological effects of growth promotants and pig meat quality.

#### References

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# GENETIC PROGRAMMES TO IMPROVE LITTER SIZE IN PIGS

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### Introduction

Until the mid-1980's, European genetic programmes had concentrated mainly on growth and carcase traits. The reasons were the lower heritability and economic value of litter size, the inability to measure litter size in males, and the already high reproductive performance of European crosses.

As carcase fat levels decline, genetic improvement of feed efficiency can be expected to slow down. Reproductive efficiency will therefore become more important as a selection objective (Ollivier *et al.*, 1990). At the same time, new mixed model statistical techniques offer the opportunity for faster genetic improvement of reproduction.

Today the emphasis on litter size in selection programmes is increasing. Prolific breeds from China may offer even greater potential for improvement in the near future. This paper reviews the genetic options for improvement of litter size, together with their application in a large international breeding company.

# Genetics of litter size

#### Additive variation

The genetic influence is readily visible as differences among breeds. Within breeds, litter size at birth consistently shows a heritability of around 10%, with a repeatability between parities of around 0.15 (see review by Haley *et al.*, 1988). The coefficient of variation is high at 25%. Litter sizes at birth and weaning appear to be influenced by the same genes. They show a high genetic correlation and have similar heritabilities.

Within breeds, litter size is largely determined by the genotype of the dam, with little influence of the sire genotype via the foetus. Litter sizes at different parities appear largely due to the same genes, with high genetic correlations between adjacent parities. There is no clear evidence that heritability increases with parity.

### Maternal effects

Cross-fostering studies have shown that the litter size of gilts reared in artificially large litters can be depressed by around 0.1 piglet for every extra littermate (Van der Steen, 1985). This negative maternal effect does not appear to be present when the variation in litter size occurs naturally. It is also unclear whether the effect persists beyond the first litter.

A negative effect of this size would theoretically only reduce the selection response in litter size by 5 to 10%. A practical safeguard will be to avoid extremes of litter size by fostering at birth.

There have been reports of cytoplasmic inheritance, mediated via mitochondrial DNA, in dairy cattle. However, REML (restricted maximum likelihood) models capable of separating maternal additive and cytoplasmic effects have only recently become available (Southwood *et al.*, 1989). These have yet to be applied to pigs.

## Association with other traits

Genetic correlations of litter size with other reproductive traits are generally poorly estimated. As expected, a genetic increase in litter size appears to be associated with a decrease in piglet birth weight and survival (Haley *et al.*, 1988), although the extent of the correlation is low and variable. Genetically increased litter size may also lead to later puberty through greater mature size, and possibly to an extended

# farrowing interval.

Genetic correlations of litter size with growth and carcase traits are low. More recently there is an indication of an unfavourable association with backfat (Johannson and Kennedy, 1983). One may speculate only that very low backfat could generate an adverse correlation by depleting body reserves. The litter size in which a pig is reared may exert a maternal environmental effect on growth performance, but this can easily be removed by regression (Haley, 1989).

### Selection studies

The few experiments in pigs attempting longterm direct selection for litter size have been unsuccessful mainly due to inadequacies of population size and/or selection intensity (eg Bolet *et al.*, 1989). By contrast, more well controlled experiments in laboratory mice have successfully demonstrated genetic improvements in litter size (Table 1).

 Table 1. Review of selection responses for increased litter size in five experiments

 with mice (Morris et al., 1990)

Parameter	Mean	Range
Number of generations	14	10 to 20
Realised heritability (%)	13	4 to 20
Realised response - total mice - % of control - mice per generation	2.0 21.7 0.15	0 to 3.8 0 to 35 0 to 0.26

In pigs, single generation attempts to identify a small proportion of genetically superior sows from very large populations have met with greater success (Bichard and David, 1985; Le Roy *et al.*, 1987). Observed responses accord with a heritability of 10% for litter size, and give confidence that inclusion of litter size in genetic improvement programmes is worthwhile.

### Heterosis

The low heritability of litter size is compensated by its high levels of heterosis (Table 2). This occurs in the genotypes of both dam and progeny, and when both are fully crossbred can raise litter size weaned by 17% or around 1.5 piglets per litter.

Trait	Dam	Progeny
Litter size bone alive	8	3
Litter size weaned	11	6
Litter weight weaned	10	12
Post-weaning growth rate	0	6
Food conversion ratio	0	3
Carcase composition	0	0

### Table 2. Heterosis levels (% of parental average) (Sellier, 1976)

 $F_1$  crossbred boars show heterosis of around 20% in semen quality and mating performance (Buchanan, 1988). While they are easier to manage and may improve conception rate by up to 15% in their early working life, little benefit has been observed in litter size from heterosis in the genotype of the boar *per se*.

In a UK trial, meat-type sires showed advantages of 0.22 piglet per litter born alive and 0.30 piglet weaned over Large White sires (MLC, 1989). This presumably arose from extra heterosis in the genotype of foetuses containing Pietrain-type genes from the meaty sires, rather than from heterosis in the sire genotype.

# Improvement programmes

### Choice of methods

Design of the optimum genetic improvement programme involves three steps :

- 1. Select the best lines
- 2. Maximise heterosis by crossing
- 3. Select within the best lines

Correct choice of the best foundation populations may "save" many generations of selection. At any stage, immigration or line substitution may lead to faster improvement than selection.

Maximum heterosis results when the sire, dam and offspring are all crosses of unrelated strains (Table 3). However, additional lines must be of sufficiently high performance that the extra heterosis more than compensates for lower additive merit.

Crossing system	Genoty	Genotype showing heterosis			
		Maternal	Paternal	Individual	
Discontinuous ·				· · · · · · · · · · · · · · · · · · ·	
F <sub>1</sub>	A x B	0	0	100	
F <sub>2</sub>	(A x B) x (A x B)	100	100	50	
Backcross	A x (A x B)	100	0	50	
3-breed cross	C x (A x B)	100	0	100	
4-breed cross	$(C \times D) \times (A \times B)$	100	100	100	
Continuous <sup>1</sup>					
2-breed rotation	(B x (A x (B x (A x B)	67	0	67	
3-breed rotation	(B x (A x (C x (A x B)	86	0	86	
4-breed rotation	(A x (D x (C x (A x B)	94	0	94	
Purebred male and	d 2 breed rotation female				
	C x ((A x (B x (A x B)	67	0	100	

Table 3. Expected levels of heterosis in genotypes of parents and offspring from different crossing systems (expressed as a percentage of heterosis in  $F_1$  (from Hill and Webb, 1982)

<sup>1</sup>Heterosis levels shown are those attained at equilibrium, roughly five generations after starting the rotation crosses.

Theoretically, reciprocal recurrent selection within pure lines on the basis of crossing ability could give greater longterm response in litter size due to its high heterosis (Wei and Van der Steen, 1991). Against this, short-term response may be lower, and the choice of lines to be crossed may change over time.

#### Selection objectives

The strategy to improve litter size depends on its importance relative to other

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objectives. Past European programmes have concentrated selection on finishing performance, and relied solely on heterosis to boost litter size.

Much of the past genetic improvement in feed efficiency is the result of substituting lean for fat. As backfat levels decline and selection emphasis moves elsewhere, improvement in feed efficiency is therefore expected to slow down. At the optimum fat level feed efficiency can only be improved by either faster growth (using less total food for maintenance), or increased efficiency of nutrient retention. For finishing performance, the main longterm goal must therefore be lean tissue growth rate (Webb and Bampton, 1988).

As genetic improvement in feed efficiency slows down, the relative importance of improvement in reproductive efficiency will increase. Improvements in husbandry practice can be expected to reduce mortality from birth to weaning, so that the limiting factor will be **litter size born alive**. This new breeding objective must be achieved without any penalties in birthweight, farrowing interval or sow longevity.

### Sire and dam lines

Where the selection objectives are litter size and lean growth, maximum improvement will be obtained by dividing the populations into sire and dam lines. Dam lines are selected both for litter size and lean growth. Sire lines are selected only for lean growth, since litter size is genetically determined largely by the dam.

In Europe during the 1970-80s the relative economic importance of litter size was low, and the genetic association with lean growth apparently small. Theoretically, the benefit of selection in sire and dam lines from a common foundation was therefore small at around 4% of the annual response (Smith, 1964).

In practice selection of sire and dam lines was carried out between rather than within breed types. Large-hammed Pietrain types with high lean yield and very poor litter size were adopted as terminal sires giving an immediate benefit. Their disadvantages included the halothane gene and small population size, making further improvement difficult.

Parameter	1964	1987	1994
$a_R/a_L$	2.00	2.00	4.00
r <sub>ti(R)</sub> /r <sub>ti(L)</sub>	0.29	0.35	0.62
s <sub>T(R)</sub> /s <sub>T(L)</sub>	0.58	0.53	0.64
Relative improvement <sup>1</sup>			
$r_{\rm G} = +0.2$	103	104	111
$r_{G} = 0.0$	104	105	115
$r_{\rm G} = -0.2$	105	106	122

Table 4. Relative improvement from selection in specialised sire and dam lines on separate indices for reproduction (R) and lean growth (L), with changing economic values (a), accuracy  $(r_{Tl})$  and genetic variation  $(s_T)$  (Webb and Bampton, 1987)

<sup>1</sup>Improvement from selection in sire and dam lines relative to single line (100);  $r_{G}$  = genetic correlation of R with L.

At ideal backfat levels, the greater relative importance of litter size coupled with a small negative genetic correlation (-0.2) with lean growth could increase the benefit of specialised lines to around 20% of the annual selection response in financial terms (Table 4). As an example, the Cotswold populations are divided into two principal dam lines selected for litter size and lean growth, and two principal sire lines selected for lean growth. The resulting four-line cross makes maximum use of heterosis.

### Selection techniques

#### Selection response

The major genetic handicaps for litter size are its low heritability and the inability to measure males directly. Although this is compensated for to some extent by a high phenotypic standard deviation (ca. 25% coefficient of variation), simple selection of replacement breeding stock from the largest litters produces very slow improvement.

The annual response (R) to selection can be predicted as:

 $R = ir_{TI}s_T/L$ 

where i is the selection intensity,  $r_{TI}$  the accuracy of prediction of genetic merit (T) using an index (I),  $s_T$  the genetic standard deviation, and L the generation interval. With  $s_T$  largely fixed, courses of action to improve R would be:

- 1. Increase population size to maximise i
- 2. Predict merit of males as well as females to increase i
- 3. Increase the accuracy r<sub>TI</sub>
- 4. reduce the generation interval L

Three different approaches are compared below.

### Hyperprolific lines

This approach involves the identification of a very small proportion of highly prolific sows from multiplier herds on the basis of three or more litters. Using AI or hysterectomy to preserve health status, their genes are returned to the nucleus at the top of the breeding pyramid.

Since as few as 20 sows may be selected out of several thousand, the method gives a very high selection intensity (i). Even selection on phenotype gives an expected genetic superiority for these "hyperprolific" sows of around one piglet per litter, and this has been borne out in practice (eg Haley, 1988). Selection on predicted genotype rather than phenotypic would of course increase the selection differential.

However, there are two drawbacks. First, the hyperprolific genes from multiplier level lag behind the nucleus in genetic merit for lean growth. Second, a new cycle of hyperprolific selection cannot begin until the genes from the first have filled the multiplication tier. This can lead to a delay of up to seven years (Bichard and David, 1985), and obviously invites a high rate of inbreeding.

### Progeny testing

A national scheme with widespread use of AI and litter recording offers the possibility of improving litter size by progeny testing AI boars. A proportion of boars can be preselected for progeny testing on the basis of their dam's family litter size (Avalos and Smith, 1987). Very high accuracy  $(r_{TI})$  from the progeny test makes up for lower selection intensity (i) and a much longer generation interval (L).

A serious drawback in practice is that many schemes make no clear distinction between sire and dam lines, with many breeds treated as dual purpose. Also, tens of thousands of sows would need to be mated by the AI boars, so the scheme would need to be very well co-ordinated.

# Family index

It is now clear that worthwhile rates of improvement in litter size can be obtained within the nucleus population itself. Genetic merit for litter size can be estimated from the litter records of large numbers of female relatives going back several generations. This permits more accurate selection of both boars and gilts at the end of performance test. A theoretical study using a traditional selection index approach has shown that the Family Index can give roughly 50% faster improvement in litter size than the Hyperprolific method (Table 5). With single-trait selection for litter size, theoretical rates of improvement with the Family Index can reach 0.5 piglet per litter per year. An AI Progeny Testing Scheme with 50,000 AI-mated sows could also approach this rate.

Table 5. Examples of possible annual genetic improvements in litter size (from Avalos and Smith, 1987)

Method	Accuracy r <sub>TI</sub>	Piglets per litter
Dam's litter record	0.21	0.12
Hyperprolific lines	0.55	0.30
AI boar progeny test	0.75	0.40
Family index	0.31	0.47

In practice, roughly half the selection emphasis in a dam line must go to lean growth (Smith, 1964). Again theoretically, selection on a family index of litter size and lean growth using UK economic weightings in a nucleus of only 100 sows and 10 boars could improve litter size by 0.33 piglet per litter per year (Toro *et al.*, 1988). The penalty, as expected, would be a high rate of inbreeding (3% per year), which could eventually depress reproductive performance.

# Practical implementation

# Mixed models (BLUP)

Mixed model methods such as BLUP (best linear unbiased prediction) maximise the accuracy  $(r_{TI})$  with which genetic merit can be predicted, and widen the range of options for the design of breeding programmes (Webb and Bampton, 1988):

- 1. Use of performance records on all relatives in the population to predict genetic merit of the individual.
- Comparisons of genetic merit of pigs performing in different environments, allowing for example genetic comparisons between herds and between generations.
- 3. Comparisons of genetic merit among individuals subjected to different amounts of prior selection, and with different numbers of measurements.
- 4. A running estimate of the rate of genetic change in the population, providing a check on progress without the need for either a control population or repeat matings.

The power of BLUP lies in its ability to simultaneously estimate, and therefore "separate", genetic and non-genetic effects on performance. It takes into account genetic relationships among all individuals in the population, and also removes any influence of selection and overlapping generations.

For litter size with its low (10%) heritability, BLUP selection in a herd of 156 sows and 26 boars would increase annual genetic improvement by some 11% over the traditional selection index (Wray, 1989). Sequential culling, the replacement of sows as soon as a young gilt of higher genetic merit is available, would add a further 5%. However, the rate of inbreeding would be more than doubled compared with the traditional index.

Superimposing BLUP selection on the theoretical 100-sow herd of Toro *et al.* (1988) would increase expected response in litter size to around 0.38 piglet per litter per year, and raise annual inbreeding above 6%. Clearly very much larger nucleus populations will be required to ensure consistent improvement of litter size at the new high rates.

### Population structure

In breeding company pyramids, nucleus herds of 200 sows per line have been sufficient to improve lean growth at a competitive rate. However, a nucleus of at least 500 sows is required to safely implement family index selection for litter size. The ability of BLUP to compare merit across herds now offers a cost-effective means to achieve this.

As an example, in 1986 Cotswold introduced BLUP selection for litter size and lean growth in 1200-sow nucleus populations for each of its two dam lines. Each nucleus consists of five distinct herds linked together by AI in a new structure known as a "Group Nucleus" (Figure 1).



Figure 1. Structure of the Cotswold Group Nucleus.

The two Group Nucleus populations were created by "promoting" purebred multipliers to nucleus level (Figure 2). Decreasing the number of tiers in the breeding pyramid from four to three has an important spinoff in reducing genetic lag.



Figure 2. Transformation of breeding pyramid to create Group Nucleus populations for dam lines A and B.
AI boars are mated to females in all herds to provide a high degree of connectedness for across-herd comparisons. A full animal model BLUP evaluation is run weekly for litter size and lean growth. Progeny are pre-selected for performance testing for lean growth on their unit of birth. Sequential culling is practiced on females.

Table 6.	Average	AI	boar	utilisation	per	line	in	the	two	Cotswold	Group	Nucleus
populatio	ons during	z 19	90		-						•	

Total AI boars	90
Litters per boar	29
Percentage AI matings	82
Weeks at service	10.6
Percentage boars selection	1.1
Generation interval: months <sup>1</sup>	13
Annual inbreading (%)	0.15

# <sup>1</sup>Boars only.

Selection differentials are closely monitored, together with generation intervals and family size. In 1990 an average of 90 AI boars were used per line, with 82% matings by AI, and less than 11 weeks at service per boar (Table 6). Selection intensity on boars is around 1% (see also Webb *et al.*, 1991).

## Central testing stations

Past national improvement schemes have involved many small privately-owned nucleus herds. Selection among herds has been achieved by providing a standard environment in the form of a central testing station. Although giving an accurate evaluation for lean growth, drawbacks of the stations are health control and small numbers tested.

For dam lines at least, a shift of emphasis towards litter size, together with the advent of BLUP, would in principle make these stations redundant. Provided there are adequate connections via AI, BLUP should allow direct comparisons of "on-farm" test performance among the member herds.

It could therefore be argued that the investment in central stations would now be better switched to AI centres, providing genetic links across herds. The large across-herd selection differentials so created for lean growth as well as litter size, together with large numbers of relatives, would more than compensate for a less accurate performance test on each individual pig. In Denmark for example, it has been estimated that AI, BLUP and on-farm testing would raise annual genetic improvement by 20% compared with station testing (Christensen *et al.*, 1986).

# Indirect selection

## Component traits

Litter size is the product of ovulation rate and prenatal survival. Although ovulation rate has a high heritability (40%), selection for this component has resulted in very little change in litter size (Johnson *et al.*, 1984). This implies a strong negative genetic correlation between ovulation rate and prenatal survival, which also has a lower heritability (15%).

If practical, selection on an index of ovulation rate and embryo survival prior to first farrowing might be expected to lead to faster genetic improvement. This would involve mating followed by laparotomy on all candidate gilts. Unfortunately, a high proportion of embryo loss may occur after the optimum time for measurement at day 50 of pregnancy (Johnson and Neal, 1988).

With the high rates of improvement now possible using BLUP and the family index, it seems unlikely that the costs of laparotomy would justify the extra selection response. Any form of surgery would be unpopular with the welfare lobby.

## Correlated traits

Genetically correlated traits which can be measured prior to puberty and/or in males offer the prospect of shortening the generation interval or increasing selection intensity. Testis size for example is moderately heritable (ca. 25%) and positively correlated with litter size (Young *et al.*, 1986). The association is complicated by the age and weight at measurement, and selection can easily change age at puberty or mature weight rather than litter size (Lee and Land, 1984).

A further possibility in the male is testosterone response to GnRH challenge, which has successfully improved litter size in one selection study (Robison, 1986). Another is selection for uterine capacity following unilateral hysterectomy and ovariectomy of gilts (Leymaster and Bennett, 1990). The remaining ovary compensates to place selection pressure on overcrowding in the remaining uterine horn. In theory, the optimum index of ovulation rate and uterine capacity could improve litter size 39% faster than direct selection involving the same number of measured individuals.

## Single genes

Single genes with economically important effects are rare. An exception is the recessive halothane gene which, at least in homozygous form within a breed, appears to reduce both litter size and weight (Simpson *et al.*, 1986).

Halothane screening may be worthwhile to reduce the frequency in any prospective dam line. However, BLUP selection for litter size and lean growth, placing a relatively high emphasis on growth rate, would be expected to favour large mature size and "automatically" discriminate against the gene.

Genes of the swine lymphocyte antigen complex (SLA) show an association with litter size (Rothschild, 1989). The degree of association is likely to vary between populations, but the test is relatively cheap and can be performed at a young age.

Indirect selection carries the risks of low correlation with litter size itself as well as undesirable and unpredictable changes in other traits. Since they are expensive to measure, most correlated traits will be confined to few individuals. By making use of BLUP, less accurate measurements on a much larger number of individuals may offer faster improvement. Direct selection on litter size in a larger population is therefore an increasingly attractive option.

# Chinese meishan

## Characteristics

The Meishan is just one of the highly prolific Taihu strains originating from a small area around Shanghai. The breed has an advantage of 3-4 piglets born alive, reaches puberty at three months of age, and has extra teats (Table 7). It is a docile careful mother with improved suckling behaviour (Meunier-Salaun *et al.*, 1991). Its great disadvantage is slow growth, excessive fat, and poor carcase conformation (Table 8).

Early French research suggested that the Meishan advantage stems entirely from higher embryo survival, but later British studies accounting for age and weight at measurement indicate improvements in both ovulation and embryo survival (Figure 3). Similarly, British studies show no evidence for either a faster rate of embryo development or lower embryonic variation for the Meishan. The enhanced prolificacy does not appear to originate from any single factor, ruling out the possibility of a single "Booroola-like" gene which might be transferred to other breeds.

	C	Implied		
Trait	LW MS I		LW x MS	heterosis (%)
Number of females	56	63	73	-
Born alive	9.8	13.2	13.8	20
Ovulation rate	14.9	18.9	17.7	5
Prenatal survival	66	71	78	14
Piglet birthweight (kg)	1.28	0.93	1.20	9
Number of teats	14.2	17.3	16.3	3

Table 7. Litter performance of Meishan (MS), Large White (LW) and  $F_1$  crossbred dams in Edinburgh experiments (Haley *et al.*, 1990)

Table 8. Growth and carcase performance of Meishan (MS), Large White (LW) and  $F_1$  crossbred slaughter pigs in Edinburgh and Newcastle experiments (D'Agaro *et al.*, (1990)

Trait	LW	MS	LW x MS
Number of pigs	72	57	144
Growth rate (g/day)	801	500	711
Food conversion ratio	2.49	3.37	2.76
P <sub>2</sub> fat (mm)	8.5	22.5	15.3
Killing out (%)	75.7	75.9	75.0
Carcase length (mm)	732	701	721
Eye-muscle area (cm <sup>2</sup> )	35.6	20.2	28.0



Figure 3. Relationship between prenatal survival and ovulation rate in Large White (LW), Meishan (MS) and  $F_1$  crosses (Haley et al., 1990).

UK Meishan research is partly funded by a consortium of five breeding companies together with the Department of Trade and Industry (Cotswold Pig Development Co. Ltd., Masterbreeders (Livestock Development) Ltd., National Pig Development Co. Ltd., Pig Improvement Co. Ltd., UPB-Porcofram Plc.). Similar groupings of local organisations exist in the USA and the Netherlands.

## Crossbreeding

Crossbreeding studies suggest that the high prolificacy is largely a maternal breed effect. Large quantities of heterosis in litter productivity are observed for Meishan x Western crosses (Table 7). However the crosses are intermediate in lean growth (Table 8), and show little advantage in eating quality (Table 9).

Trait	LW	MS	LW x MS
Tenderness score <sup>1</sup>	3.7	3.4	3.6
Juiciness score	3.7	3.3	3.7
Flavour score	4.0	3.0	3.8
Overall acceptability score	3.8	3.8	3.8
Cooking loss (%)	22	16	19

Table 9. Eating quality of Meishan (MS), Large White (LW) and  $F_1$  crossbreds in Newcastle experiments (Ellis *et al.*, 1990)

<sup>1</sup>Scores 1-8 : low values are desirable.

A parent gilt containing one quarter Meishan would be predicted to have a litter size advantage of 1-2 piglets reared. The resulting one eighth slaughter generation would have a disadvantage of  $1.5 - 2.5 \text{ mm P}_2$  at 95 kg on *ad libitum* feeding. In the short term this extra fat may be at least in part corrected by the use of ultra-lean terminal sires and/or lower density diets.

## Genetic improvement

Clearly the Meishan would offer great promise for improving litter size if its lean growth could be corrected by selection. In the longterm, greatest improvement would result from selection within the purebred Meishan itself as a maternal grandparent (Figure 4). However, this would be expensive due to the low carcase value, and there is difficulty in obtaining reliable ultrasonic fat measurements due to the thick skin, coarse hair and variable fat depot anatomy.



Figure 4. Relative economic value of slaughter generation containing no Meishan (base 100) or maternal grandparents selected for lean growth as either pure Meishan (MS) or a Meishan Landrace (MS-LR) synthetic line (from Bidanel et al., 1990).

The next best option is to select for lean growth and litter size in a synthetic line containing both Meishan and Western genes. Cotswold has developed such a line which is now in the third generation of selection. The coefficient of variation of fat depths is double that of Western breeds. The risk is the possibility of an unfavourable genetic correlation between fatness and litter size within the Meishan.

## New technology

## Manipulation of reproduction

Reproductive biology can be expected to yield a variety of techniques which may impact on genetic improvement (eg First, 1990):

- 1. Nonsurgical embryo transfer
- 2. Embryo freezing
- 3. Sex determination
- 4. In vitro fertilisation and maturation of occytes
- 5. Cloning

Limited success has been reported for both nonsurgical embryo transfer and embryo freezing in pigs. However, multiple ovulation and embryo transfer (MOET) would have little impact on genetic improvement of litter size in pigs, which already have large full- and half-sib families. The Cotswold Group Nucleus is similar in concept to cattle MOET schemes (eg Woolliams, 1989), but with the advantage of larger population size.

Manipulation of the sex ratio could optimise selection intensity within a nucleus. Sorting of semen by flow cytometry into male and female sperm now appears to be possible (Johnson and Clarke, 1988). However, the sorting process is presently very slow, and either smaller numbers of sperm will need to be introduced higher up the reproductive tract or the sort rate drastically increased before the technique is a practical proposition.

An alternative could be genetic manipulation of the SRY gene which has recently been shown to determine maleness in mice (Koopman *et al.*, 1991). Transfer onto one of the autosomes could change the sex ratio, but the presence of two X chromosomes still appears to confer infertility.

Cloning and in vitro production of embryos will be useful mainly to service genetic manipulation. Assuming that nonsurgical embryo transfer becomes possible, cloning might in principle be used to produce a uniform slaughter generation. Rapid cloning of the best nucleus genotypes could result in the slaughter generation performing at a higher level than the nucleus, creating negative genetic lag (Teepker and Smith, 1989).

## Genetic manipulation

Genetic manipulation could allow transfer of genes across species and alteration of the timing and expression of existing genes. Even with embryo stem cell techniques, cost effective methods for gene transfer appear a long way off. The problems still remain of which genes to manipulate, and how to control their expression.

In Europe, sixteen research centres are collaborating to produce a genetic map of the pig (Haley and Archibald, 1990). Known as the European Pig Gene Mapping Project (PiGMaP), this will show the location of any major genes. More important it will increase understanding of the control of expression, for example allowing the timing of traits such as puberty or fat deposition to be altered.

The possibility of a major gene for litter size in the Meishan appears to be receding. Instead the genetic map may throw up RFLP (restriction fragment length polymorphism) marker genes which are associated with litter size. Marker-assisted selection could then speed up the improvement of litter size. However, to be cost-

effective the RFLP markers would need to be both cheap and well-correlated with litter size.

## Future systems

How might litter size, and overall production efficiency, be maximised using all available technology already on the horizon? One possibility would be to use Meishan crosses as "surrogate mothers", who by nonsurgical embryo transfer produce pure sire line offspring (Figure 5).



Figure 5. Hypothetical scheme to exploit the prolificacy of Chinese Taihu dam lines by embryo transfer.

Two Taihu dam lines might be selected exclusively for litter size using the Group Nucleus system, to improve their already high litter size at around 0.4 piglet per litter per year. Sire lines would be selected exclusively for lean growth. Their embryos would be cloned and nonsurgically transferred to the Taihu surrogate mothers. Such a system simply illustrates the biological opportunity to attain 30 pigs per sow per year and 2 kg per day growth rates. It is likely to encounter ethical as well as technical barriers.

## Research priorities

Future research priorities lie with both statistics and physiology. Further effort is needed to optimise BLUP statistical models to avoid confounding of effects, particularly for the estimation of genetic trends over time. Similarly, the genetic structure of Group Nucleus schemes needs to be optimised to give the correct balance between inbreeding and selection. Examples are the proportion of each sex to be performance tested, the proportion of AI matings, the number of matings per sire and the value of restrictions on the number of sons per sire.

Research on the physiological basis for the Meishan prolificacy has so far been disappointing. Further effort must surely yield ways of improving Western breeds. A limiting factor on genetic improvement must be the negative association of ovulation rate with embryo survival. Extremes of lean growth in dam as well as sire lines may also invoke negative genetic associations which will need to be understood. Of the opportunities to artificially manipulate reproduction, nonsurgical embryo transfer and sex determination may offer the greatest prize.

## Conclusions

As the genetic reduction of backfat proceeds towards an economic optimum, litter size is increasing in importance as a selection objective. Litter size born alive is a clear limiting factor, uncomplicated by environmental effects on preweaning mortality.

BLUP methodology together with AI now permits more accurate selection for

litter size in larger multi-herd populations, creating higher selection differentials with lower inbreeding. In dam lines selected for lean growth and litter size together, annual genetic improvements of 0.2 to 0.3 piglet per litter are a practical reality.

The use of larger populations and relatives' records makes indirect selection for litter size using correlated traits such as SLA-type or testis size less attractive. Maternal effects are unlikely to seriously prejudice genetic progress in litter size. In Europe the Meishan will almost certainly have a role in improving litter size, as a constituent of a crossbred dam.

Priority areas for future research include the physiological basis for the Meishan advantage, and the genetics of prenatal survival. Statistical priorities include the refinement of mixed models, and the impact of population structure on the balance between inbreeding and selection.

The obvious danger of rapid genetic improvement of litter size is the inability to rear the additional piglets. Clearly adverse correlated changes in birthweight, teat number and milk yield must be avoided. In future, husbandry and veterinary practice may have to adapt to deal with one extra piglet per litter every 4 to 5 years.

Future production systems will require lines which are designed and selected for specialised roles. Yet in many countries there is increasing uncertainty over the future of animal production, with strong animal welfare, environmental and health lobbies. To guard against this uncertainty, a policy of genetic flexibility is required. Cotswold for example maintains a "library" of twelve different lines which can be quickly crossed together in any combination to meet changing circumstances.

In summary, litter size is now a major breeding objective, and the new statistical technology together with Chinese genes offers the potential for rapid change. It is likely that this challenge will be met by increasing the scale of breeding operations rather than by increasing the sophistication of measures of reproduction.

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# ESTIMATION OF GENETIC PARAMETERS FOR PRODUCTION AND REPRODUCTIVE TRAITS USING AUSTRALIAN PIG FIELD DATA

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Klassen *et al.* (1988) reported on a study to estimate heritabilities  $(h^2)$  for backfat in millimetres (BF), average daily gain in grams/day (ADG) and litter size born alive (PBA) from a large sample of Australian pig field data. This study used a univariate animal model so additional random effects, such as the genetic covariance between BF and ADG or permanent environmental effects for PBA, were not estimated. Estimates of these effects are important when using a multiple trait animal model for genetic evaluation as is done in PIGBLUP. The purpose of the present study was to use a multiple trait animal model to estimate heritabilities, genetic correlations and common litter ( $c^2$ ) effects for BF and ADG and heritability and repeatability for PBA.

The data used in this study were described by Klassen *et al.* (1988). These data included 24,501 production records and 13,688 litter records. An additional data set was obtained for estimation of parameters for PBA that contained 35,656 litter records. All analyses were carried out using a simulated Restricted Maximum Likelihood procedure described by Klassen and Smith (1990). With this iterative procedure, standard errors of estimates are not produced. It was assumed that correlations between production traits (BF and ADG) and PBA were zero so production and reproduction analyses were carried out separately. Results were pooled across data sets and are presented in Table 1.

		Variances		Covariances (ADG, BF)			
	Additive genetic	Common litter	Residual	Additive genetic	Common litter	Residual	
ADG	672	291	1852	4.41	0.0 <sup>1</sup>	20.57	
BF	2.06	0.43	2.10	-	-	-	
PBA	0.69	0.37 <sup>2</sup>	5.38				

Table 1. Estimates of variances and covariances for ADG, BF and PBA

<sup>1</sup>Litter covariance was not estimated in any analyses and was assumed to be 0. <sup>2</sup>Permanent environmental variance.

These values give heritabilities of 0.24 and 0.45 for ADG and BF, respectively, with a genetic correlation between ADG and BF of 0.12. Common litter effects ( $c^2$ ) were 0.10 for both ADG and BF. Heritability and repeatability for PBA were 0.11 and 0.165, respectively. These values are the default values for genetic parameters being used in the PIGBLUP genetic evaluation system.

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# MATE SELECTION: MAXIMISING RESPONSE WHILE MINIMISING INBREEDING - IS IT POSSIBLE?

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Simulation studies have shown increased response to selection using BLUP technology (Belonsky and Kennedy, 1988). Further selection response could be provided by positive assortative mating systems and optimally arranging pedigree information (Smith and Hammond, 1987). Unfortunately these approaches to selection and mating decisions may result in elevated rates of inbreeding above other alternatives. The purpose of this study was to investigate the application of mate selection (selection of mating pairs or groups) for the control of inbreeding while maximising expected progeny breeding value (PBV).

A closed 260 sow herd was simulated to evaluate selection response and inbreeding over 16 years. The trait considered had a heritability of 0.1 and a phenotypic standard deviation of 100 units. Selection policies included selection on individual performance (I), or the use of best linear unbiased predictions (B), where a single record was available for both sexes. Mating systems were random (R), assortative (A), or one of five mate selection alternatives: MS1) mating on PBV, ignoring inbreeding, MS2-4) mating on PBV with increasing penalities on inbreeding, and MS5) mating to minimise inbreeding and ignoring PBV. Mate selection alternatives used linear programming procedures to maximise an objective function containing information on PBVs and inbreeding for all possible matings.

Results showed that where information on expected PBVs was ignored (MS5) or inbreeding coefficients were excluded from the objective function (MS1), suboptimal responses were obtained. However, MS5 substantially reduced average percentage inbreeding compared to random mating following BLUP selection (BR). For the intermediate objective functions (MS2-4) small differences in response did not reflect marked differences in average percentage inbreeding. These preliminary results suggest that an intermediate approach, eg. (BMS4) can achieve a compromise between selection response and inbreeding. More research is required to investigate other potential mate selection alternatives, particularly for the control of inbreeding and in the multiple trait situation.

Yr	IR	IA	BR	BA	BMS1	BMS2	BMS3	BMS4	BMS5
0	1(1) <sup>1</sup>	-1(1)	1(1)	0(1)	-1(1)	1(1)	0(1)	2(1)	0(1)
	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	<b>0(0)</b>	0(0)	<b>0(0)</b>
8	101(2)	99(2)	178(3)	198(3)	202(3)	204(5)	209(3)	198(3)	167(3)
	<b>4(0)</b>	4(0)	<b>19(1)</b>	<b>28(1)</b>	<b>29(1)</b>	27(1)	<b>22(1)</b>	<b>15(0)</b>	<b>9(0)</b>
16	198(4)	197(2)	325(4)	349(5)	353(4)	359(5)	362(4)	362(3)	310(4)
	8(0)	8(0)	35(1)	51(1)	50(1)	50(1)	<b>41(1)</b>	<b>31(1)</b>	20(0)

Table 1. Cumulative genetic response and average percentage inbreeding (bolded) over 16 years for alternative selection and mating schemes

<sup>1</sup>Standard errors in brackets.

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# INDEX WEIGHTINGS AND EXPECTED GAINS FOR DIFFERENT BREED/LINE TYPES IN PIGS

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It is important to specify clear breeding objectives with respect to a range of parameters. This paper reports on analyses using PIGBLUP software to estimate the effect of breed/line type specification of the \$Index - EBV. This index combines the EBVs for average daily gain (ADG), backfat (BF) and number born alive (NBA) of an animal into a single \$-value using a bioeconomic profit function (Stewart *et al.*, 1990).

The assumed economic and production inputs resulted in economic values as shown in Table 1.

Table 1. Economic values of the traits per unit

Subobjective	ADG (g)	BF (mm)	NBA (pig)
Growth finishing	0.26	-1.60	-
Sow herd	1.05	-6.36	31.58

Holding these inputs constant the objective for the breed/line was varied. The relative weightings of the EBVs are presented as regressions of the \$Index on the EBVs in Table 2 for three different situations.

Line type	Variance	Inde	x weigh	tings	Expected gain per sd		
specification	of \$Index	ADG	BF	NBA	ADG	BF	NBA
Terminal line	31.9	0.502	-3.03	9.47	8.39	-0.113	0.115
Maternal line	126.2	0.896	-5.41	25.26	7.54	-0.102	0.155
Slaughter	70.7	0.699	-4.22	17.37	7.85	-0.105	0.142

# Table 2. Index weightings and expected gains

The difference in the variance of the Index was caused by the different importance of the traits in the \$Index. This reflects that the line specifications define a different product.

The expected gains are derived by using an approximation as described by . Schneeberger *et al.* (1991). They represent the changes in the selection criteria (EBV's) per standard deviation of the \$Index and illustrate the direction of the selection for the different traits if it is based on the \$Index. If, for example, a maternal index is used instead of a terminal one the genetic improvement is 35% higher for NBA and about 10% less for ADG and BF.

These results underline the importance of a clear breeding objective differentiating between breed/line type.

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# PIG GENES: AN OVERVIEW OF PRESENT KNOWLEDGE

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A concerted effort is now underway in Europe, the USA and Australia to create a genetic map of the pig. For reasons explained by Le Tissier *et al.* (these proceedings), most of the markers in this map will be from non-coding regions. However, considerable effort is also being devoted to mapping functional genes. In order to facilitate this latter task, we have established a database which provides upto-the-minute information on all known pig genes.

No-one knows how many functional genes there are in the pig, or in any other higher organism; but the number is likely to be at least 50,000, and could be greater than 200,000. In contrast, the number of known pig genes is miniscule. In the last decade, however, our knowledge of pig genes has begun to grow rapidly, mainly because of the power of molecular genetics as a tool for identification and mapping of functional genes.

An indication of the recent explosion in knowledge is provided by the following figures. When Ollivier and Sellier's comprehensive review was published in 1982, the total number of identified pig genes was approximately 100. Given that knowledge had been gradually accumulated since the turn of the century, this corresponds to an average annual rate of about 1.3 genes identified per year. In contrast, during the next eight years, the total number of identified pig genes increased to 226 (Nicholas, 1990), corresponding to more than a ten-fold increase in the rate of identification.

At the time of writing (September, 1991), a total of 332 pig genes have been identified. Of these, 121 have been cloned, and 41 have been mapped to a chromosome or chromosomal region. For each identified gene, our database records all relevant information, including symbols and names, and linkage and cloning information. So as to maximise the benefits in relation to comparative gene mapping, the whole database is modelled on McKusick's (1988) catalogue of human genes: the main identifier of each pig gene is the relevant McKusick number (if the pig gene is homologous to a human gene) or a McKusick-type number (if there is no known human homologue).

With pig genes now being identified at the rate of almost one per week, and with many of these being cloned, our knowledge of the pig genome will be greatly expanded in the next few years. In providing up-to-date information not only on genes that have been mapped, but also on those that have been identified but not yet mapped, our database highlights those genes that deserve the attention of research workers involved in gene mapping programmes.

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# GENE MAPPING AND THE IDENTIFICATION OF QUANTITATIVE TRAIT LOCI IN PIGS

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A linkage map of the porcine genome with a resolution of 20 cM would be a valuable asset, allowing the positioning of quantitative trait loci (QTL) and marker assisted selection (MAS) (Beckmann and Soller, 1983). To accomplish this, polymorphic DNA markers are required, both to construct the linkage map and to follow the inheritance of superior alleles once they have been identified.

To generate a linkage map, we are using a cross between Large White and Chinese Meishan pigs. There should be a high degree of genetic divergence (and thus F2 polymorphism) between these breeds and also large differences in a number of economically important traits (litter size, growth rate, age at onset of puberty and lean meat content). Quantitative data from F2 progeny will be collected which will allow the loci controlling these traits to be mapped.

Anonymous cosmid clones, selected at random from a porcine cosmid library, cDNA clones of known function, and heterologous probes are being used to detect variation in restriction fragment length by Southern analysis of DNA cut with nine restriction enzymes. The ten parents used in the cross are being screened to determine which enzyme/probe combination reveals a variant. Having established the best probe/enzyme combinations, the F2 progeny from the Large White/Meishan cross will be typed. Several individuals of each of the "European" breeds of pigs in Australia are also being screened with those probes found to reveal polymorphisms in the Large White/Meishan cross. This will give an indication of which probes will be of use in breeding programmes which only involve those breeds already present in Australia.

To date, several probes have been produced which detect restriction fragments specific to Meishan and Large White pigs, while others detect allelic variants common to these two populations. However, both types will be useful for mapping.

By using a diverse cross, not only will a linkage map in the pig be constructed, but information on the position of loci which control a number of economically important traits will be gained. This may be of great benefit in increasing the genetic potential of pigs more effectively than was previously possible.

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# IDENTIFICATION OF THE HALOTHANE GENOTYPE OF AUSTRALIAN PIGS

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Pigs of halothane (Hal) genotype nn are readily identified using the halothane test. They have a high incidence of Porcine Stress Syndrome (PSS) and pale soft exudative meat (PSE). The effects of PSS in pigs of Hal genotype Nn have not yet been investigated in Australian breeds. A project was initiated to establish methods for detecting Nn pigs and then to compare the performance and meat quality of these pigs with the nn and NN Hal genotypes. This communication describes the method for detecting heterozygotes.

Genetic markers provide a means of identifying the Hal genotype of pigs (Gahne and Juneja, 1985). The process, known as Marker Assisted Selection, relies upon the identification of three blood proteins produced by marker loci closely linked to the Hal locus.

Over a two year period, blood was collected from 2,400 pigs at a research facility. Agarose and polyacrylamide gel electrophoresis techniques were used to separate and identify alleles of the proteins phosphohexose isomerase (Phi), 6-phosphogluconate dehydrogenase (Pgd) and postalbumin-2 (Po2). The marker haplotypes of complete families were predicted from the blood typing results and compared with the halothane test results of progeny. The Hal genotypes were then predicted. The haplotype sequences associated with the Hal gene (n) and the normal gene (N) and the frequencies of these haplotypes are described in Table 1. Both nsbf and nfbf can be assigned once a halothane reactor has been identified in the family.

Haplotype	LW x LR	Haplotype	LW x LR
Hal, Po2, Phi, Pgd		Hal, Po2, Phi, Pgd	
Nfaf	37.0	nfaf	0
Nfbf	16.0	nfbf	4.4
Nfas	3.8	nfas	0
Nfbs	7.1	nfbs	0
Nsaf	0.7	nsaf	0
Nsbf	0	nsbf	31.0
Nsas	0	nsas	0
Nsbs	0	nsbs	0

## Table 1. Haplotype frequencies (%) in Large White Landrace (LW x LR) pigs

The blood typing technique provided an accurate prediction (92% accuracy) of the halothane genotype in LW x LR pigs. Further sampling is being undertaken to enable a similar evaluation of the technique in Landrace and Large White pigs.

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# PORCINE STRESS SYNDROME AND THE RYANODINE RECEPTOR

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Porcine Stress Syndrome (PSS) is an economically important genetic disease of pigs characterized by muscular hypermetabolism that leads to inferior meat quality and, frequently, death in susceptible individuals following exposure to a stress such as transportation.

In recent years much physiological and genetic evidence has strongly suggested that an abnormality of a specific muscle protein known as the ryanodine receptor (RR) is the causative lesion in PSS (see Davies, 1990). If this is the case, cloning the RR gene in the pig will lead to a definitive diagnostic test for both PSS susceptible and carrier pigs. This paper details work towards this goal being undertaken in this laboratory.

Attempts to clone the porcine RR gene have been greatly aided by the fact that both the rabbit (Takeshima *et al.*, 1989) and human (Zorzato *et al.*, 1990) cDNA sequences have been published (i.e. DNA transcribed from the processed mRNA of the gene). Oligonucleotide primers for use in the polymerase chain reaction (PCR) were designed to be identical to sequences in both rabbit and human. This strategy was employed to increase the probability that the primers would also be identical to homologous sequences in the pig. PCR with genomic DNA from human, rabbit, and pig, proved unsuccessful, probably due to the presence of an intron in the region. To circumvent this possibility cDNA was synthesized from rabbit muscle mRNA and used as the PCR template. The resultant product closely matched the expected fragment size of 662 bp and its identity was confirmed with restriction digests. A similar approach is now being followed with pig cDNA.

To develop a definitive test it is important to locate the specific causative mutation. Work continues toward this goal with the isolation of genomic clones, and the expected use of denaturing gradient-electrophoresis. In the meantime, all RR fragments cloned will be tested as genetic markers in families segregating for PSS.

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# HALOTHANE HETEROZYGOTES AND PERFORMANCE IN A FAT AND A LEAN PIG LINE

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From Halothane testing of boars at testing stations it is estimated that about 30% of Large White and Landrace pigs in Australia are heterozygous for the halothane gene (Nn), the remaining 70% being normal (NN). This study compares the performance, carcass quality and survival of NN and Nn genotypes from two lines of pigs in an environment subject to high temperatures (Central Qld.) and pre-slaughter transport stress (>600km). The lines, of common Large White x Landrace origin, are a lean line which had undergone five generations of selection for rapid lean growth, and a fat line which had been maintained as an unselected control (McPhee *et al.*, 1988). The frequency of the halothane gene has been increased to 65% in the fat line and 26% in the lean line by selection using the halothane test and blood typing (Gahne and Juneja, 1985). Table 1 gives growth and carcass measurements on 58 pigs fed *ad libitum* from 25kg to 90kg, and transit death rates in 1315 pigs comprising the NN and Nn genotypes from both lines.

Table 1.	Performance,	carcass,	meat	quality	and	transit	deaths	of	two	halothane
genotypes	in the lean ar	n <mark>d fat li</mark> n	es; lea	st squar	e me	ans ±SI	Ξ			

Line (L)	Le	an	F	Sign.	
Halothane (H)	NN	Nn	NN	Nn	(P<0.05)
Growth rate (kg/d)	0.95±0.02	0.93±0.02	0.79±0.02	0.78±0.02	L
Food conversion ratio	2.73±0.08	$2.60 \pm 0.07$	2.91±0.07	3.08±0.07	L
Fat at P2 (mm)	13.7±1.3	14.1±1.2	21.7±1.1	22.1±1.1	L
Carcass lean (%)	54.3±1.1	54.8±1.0	49.9±0.9	48.5±0.9	L
Eye muscle area (cm <sup>2</sup> )	42.7±1.4	38.1±1.2	37.5±1.1	35.7±1.1	L
pH (45 min)	6.50±0.08	6.28±0.08	6.31±0.08	6.17±0.08	L,H
Optical probe (units)	21.1±2.6	25.0±2.6	24.0±2.4	26.9±2.4	L,H
Transit death (%)	, 1.21±0.6	$5.17 \pm 2.1$	$0.96 \pm 0.48$	3.27±1.1	Н

Compared with the fat line, the lean line grew faster, was more efficient and had more carcass lean, which was less acid and had a darker colour. The halothane gene made no contribution to these differences between the lean and fat lines but within both lines it increased the acidity and paleness of lean and transit death rate.

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# IN VIVO MEASUREMENT OF PROTEIN IN PIGS BY IVNAA

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The *In Vivo* Neutron Activation Analysis (IVNAA) technique is used at the Monash Medical Centre for measuring nitrogen (N) in human patients (Rayner *et al.*, 1991). This technique employs nuclear reactions induced in the subject's body by neutrons from a <sup>252</sup>Cf radioactive source. For example, hydrogen can capture a neutron to form deuterium and <sup>14</sup>N can be changed to <sup>15</sup>N. The excess energy is immediately released as gamma rays with energies characteristic of these nuclei and are counted by a pair of large NaI gamma ray detectors. With a radiation dose of 0.1 mSv to the patient (compare natural background of 3 mSv per year) hydrogen and nitrogen can be easily observed and N can be measured with a precision of  $\pm 4\%$  in a measurement that takes 15 minutes. The more common method for determining N is by chemical analysis of the homogenised animal carcass.

Two experiments have been conducted to compare the IVNAA technique with chemical analysis and to demonstrate that the technique is suitable for pigs. Firstly, N was measured in two pig carcasses by IVNAA prior to homogenisation and chemical analysis. In the second experiment, a pile of minced, lean beef patties were analysed by both methods. The patties were stacked on the IVNAA equipment to form a cube approximately 300 mm on each side. Results are shown in Table 1. Estimates of the total errors are shown for the IVNAA method. When chemically analysing the patties, care was taken to thoroughly mix the mince before taking samples; twenty samples were taken and the error shown is the SD of the 20 measurements. The same percent error has been assumed for the pig analyses.

	Ni	Nitrogen	
	IVNAA	Chemical analysis	IVNAA/CA <sup>1</sup>
Pig 1	2.30±0.10	2.48±0.08	0.93±0.05
Pig 2	2.53±0.10	2.38±0.08	1.06±0.05
Patties	3.22±0.20	3.23±0.09	$1.00 \pm 0.07$

Table 1. Comparison of measurements of nitrogen (%)

<sup>1</sup>CA Chemical analysis.

The ratio of the two results is shown in the final column; the fractional error of the ratio squared equals the sum of the other two squared. From these data it can be concluded that the two methods of measuring N are consistent within the estimated errors.

It is concluded that the IVNAA technique is suitable for measuring N in pigs or pig carcasses with an accuracy of  $\pm 4\%$ . The two significant advantages of the IVNAA technique when compared to chemical analysis are that the measurement can be repeated at frequent intervals while an animal is being reared and the test gives a non-invasive average over the whole carcass. Commercial applications of the IVNAA technique should now be investigated.

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# ESTIMATION OF GENETIC PARAMETERS FOR NUMBER OF PIGS WEANED

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Treacy (1989) estimated the value of one extra pig weaned/litter to be worth between \$75 and \$150/sow unit, depending on utilisation of grower shed space. This poses the question of whether it is useful to include pigs weaned/litter (NPW) as a selection criterion in a genetic improvement program. The objective of the current study was to estimate genetic parameters for NPW.

An Australian data set was obtained for this study which contained 17,895 weaning records. A derivative-free approach for estimating variance components by Restricted Maximum Likelihood, DFREML (Meyer, 1988), was used to analyse these data. An animal model was used which included year-season and parity of sow as fixed effects and the animal's additive genetic effect and repeated records as random effects. Number of pigs after transfer (up to 3 days post farrowing) was also included in the model as a covariable, accounting for both linear and quadratic effects. With these data crossfostering was minimal after 3 days post-farrowing. Estimates of variance components, heritability and repeatability for NPW are presented in Table 1.

Additive genetic	Permanent environmental	Residual	Heritability	Repeatability
0.021	0.003	1.121	0.018	0.021

Table 1. Estimates of variance components, heritability and repeatability for NPW

These results indicate low heritability and repeatability for NPW. This agrees with results from Johansson and Kennedy (1985), who found lower heritabilities for NPW than for number of pigs born alive (NBA), but disagrees with Haley *et al.* (1988), who found similar heritabilities for NBA and NPW. Heritability for NBA using these data was 0.11.

The solutions for the linear and quadratic effects of number after transfer on NPW were: linear,  $0.686 \pm 0.006$  and quadratic,  $-0.074 \pm 0.002$ . Not surprisingly, number of pigs after transfer had a positive linear effect on NPW. The negative quadratic effect indicated the non-linear nature of the relationship, suggesting that large numbers of pigs after transfer could have a deleterious effect on NPW. With very little crossfostering 3 days post-farrowing, these results indicate that crossfostering affects NPW but does not explain the low heritability for NPW.

Results in Table 1 indicate little genetic variation for NPW, but these are preliminary results from a single trait animal model and further work is needed to ascertain whether other models or inclusion of other traits (e.g. litter weight weaned) would enhance genetic analysis of reproduction.

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# NEUROTRANSMITTER CONCENTRATIONS IN BRAIN REGIONS OF HALOTHANE-NEGATIVE AND HALOTHANE-POSITIVE PIGS AND EFFECTS OF DIETARY TRYPTOPHAN AND TYROSINE

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Pale, soft and exudative (PSE) pork results in financial losses to the pork industry (cf. Tarrant *et al.*, 1987). Stress, an unavoidable problem during the marketing and slaughter of pigs, often results in PSE pork. Pigs which carry the halothane gene are much more susceptible to stress than normal pigs and result in a higher incidence of PSE pork. The concentrations of brain neurotransmitters are related to stress susceptibility in many species. Therefore, experiments were conducted to determine if brain neurotransmitters in halothane-positive and halothane-negative pigs were different and whether feeding tryptophan, a precursor of serotonin, or tyrosine, a precursor of catecholamines, could alter brain metabolites.

Concentrations of neurotransmitters were measured in brain regions associated with neural regulation of stress response (hypothalamus, hippocampus, and caudate nucleus) of halothane-positive and halothane-negative pigs. Pigs were anaesthetised with ketamine then exsanguinated, following which brain regions were sampled. Pigs receiving supplementary dietary tryptophan or tyrosine were slaughtered under commercial conditions and only the hypothalamus was removed. Tissues were analysed for catecholamines and indoleamines by high performance liquid chromatography.

In one experiment (n=10) halothane-positive pigs had one-half the concentrations (nmol/g) of hypothalamic serotonin and 5-hydroxy tryptophan compared to halothane-negative pigs (6.25 vs 13.67 and 0.72 vs 1.41), respectively) (P<0.05). Analysis of brain regions from pigs heterozygous for the halothane gene (n=6) showed that concentrations of serotonin and 5-hydroxy tryptophan tended (P<0.1) to be lower than the halothane-negative pigs and higher than the halothane-positive pigs.

A control, tryptophan supplemented (5 g/kg) or tyrosine supplemented (10 g/kg) diet was fed to halothane-negative pigs (n=24) for five days in another experiment. Concentration of serotonin in the hypothalamus was increased by dietary tryptophan vs. control (4.02 vs 2.79 nmol/g, P<0.05) but not significantly affected by added tyrosine. Administration of tyrosine had no significant effect on epinephrine or norepinephrine concentrations but increased the concentrations (P<0.05) of several catecholamine metabolites (dopamine, homovanillic acid and dihydroxyphenyl acetic acid).

These data support the hypothesis that stress susceptibility in normal and stresssusceptible pigs may be related to concentrations of brain neurotransmitters. Administration of amino acid precursors for neurotransmitters may be a possible method for altering brain metabolism and thus reducing the response to stress and the subsequent incidence or severity of PSE pork.

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