

# **MANIPULATING PIG PRODUCTION IX**

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AUSTRALASIAN PIG SCIENCE ASSOCIATION (INC.)  
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## ACKNOWLEDGEMENTS

*Manipulating Pig Production IX* represents a substantial body of work, effort and support from all facets of the Australasian pig science community. The Australasian Pig Science Association (Inc.) thanks everybody involved in the preparation, organisation and conduct of this year's conference, and especially to all delegates for attending and contributing. Thanks go also to the international delegates that have supported APSA (Inc.) by contributing papers or simply attending the conference. Your support is warmly appreciated.

The scientific program of an APSA conference has always pivoted on invited reviews, symposia and 1-page papers. In this regard, appreciation is expressed to Egbert Kanis, who presented the Dunkin Memorial Lecture, to reviewers Mark Nottle, Frank Dunshea and Peter Collignon, and to symposium presenters Andrew Fisher, Paul Hemsworth and John Barnett. Thanks are also expressed to the organisers of the 'Alternative Housing' symposium, held in conjunction with the Producers' Day. APSA (Inc.) also thanks the various chairpersons who ensured that the program ran to time, and to Dr J-K Kim for collating all the PowerPoint presentations before the conference.

Conference organisation is all about people. Accordingly, I would like to thank the organising committee for their support and efforts in the last two years, namely Pat Spicer (Vice President), Hugh Payne (Secretary), Darryl D'Souza (Treasurer), Ian Williams (Past President), and committee members Ian Barugh, Frank Dunshea, Susanne Hermes, Ray King and Geogy Philp. It was great having a few 'seasoned hands' on the committee, and I believe our meetings and discussions went well. I also wish to thank the conference Secretariat, Margaret Hector and Johanna Pluske, for their patience, support and organisational skills in handling the many and varied tasks required of them. Kim Nairn and Barb Frey, from Portec Pty Ltd, are also thanked for their help and assistance with the Producer Day, as are all the other contributors. Finally, I would like to reiterate my thanks to the referees who volunteered their time, which is becoming scarcer, to 'do their bit' for the conference. Without you, *Manipulating Pig Production IX* would not have been possible.

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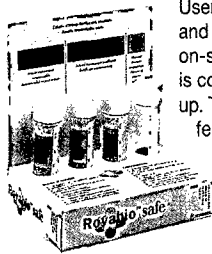
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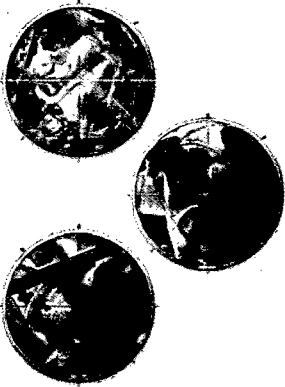
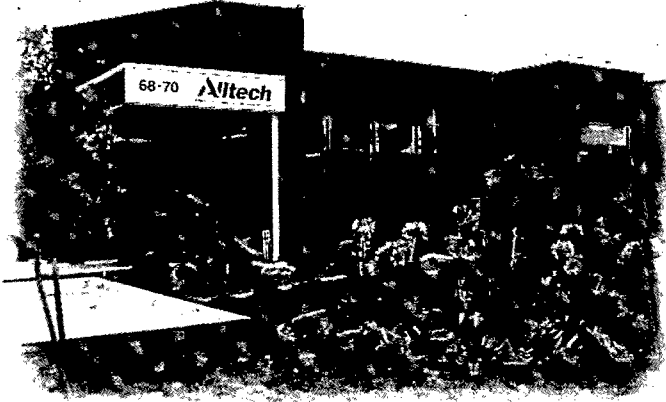
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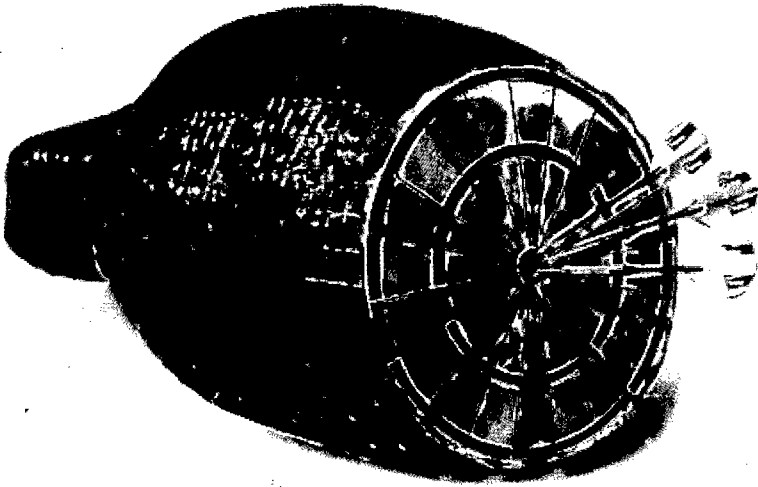
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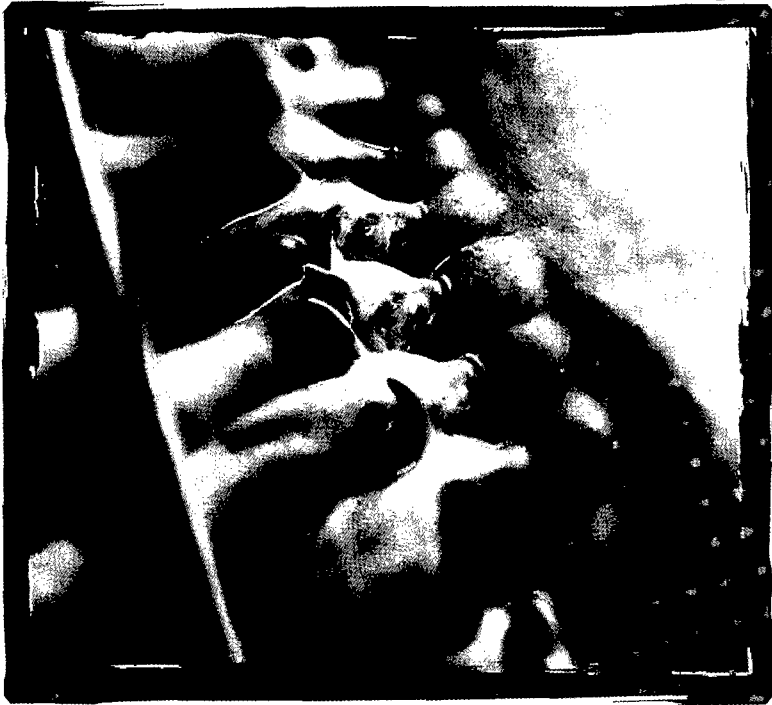
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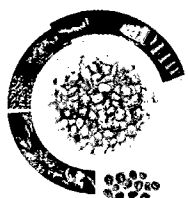
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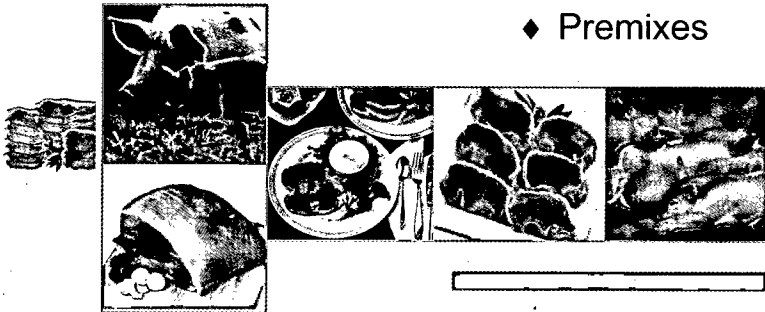
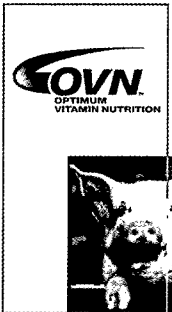
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The Proceedings 'Manipulating Pig Production IX' contains 81 one-page papers, four Reviews and one Symposium. As is the policy of the Association, all one-page papers, Reviews and Symposia were reviewed by external referees. The committee of APSA and the Editor gratefully acknowledge the assistance generously given during 2003 by the following referees and those who may have been inadvertently omitted from the list.

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Wynn, P.

## PREFACE

*Manipulating Pig Production IX* documents the proceedings of the IX<sup>th</sup> Biennial Conference of the Australasian Pig Science Association (Inc.). The reviews, symposia and contributed one-page papers in the 2003 proceedings are of a very high standard and reinforce the excellent reputation that the conference, and indeed the proceedings, holds in both the Australasian and international pig science community. This reputation has been earned because APSA (Inc.) has intentionally promoted high-quality science that 'stands up' in the national and international science community.

The running of the IX<sup>th</sup> Biennial Conference saw several changes, namely a new Editor, the appointment of a dedicated Sales and Marketing Manager, and a change of venue. Peter Cranwell, who edited the APSA proceedings (volumes V-VIII) in 1995 (conjointly with David Hennessy), and as sole editor in 1997, 1999 and 2001, did not tender to edit this year's proceedings. Janet Paterson, from SciScribe Scientific Copywriting, successfully tendered for the position. Janet has done an excellent job in continuing the very high standard of editing and presentation expected of the proceedings. Peter Cranwell, never one to completely relinquish his association with APSA (Inc.), has assisted Janet with many aspects of the editing process. I thank Peter for his efforts in this regard. Peter was also responsible for publication of the proceedings and will continue his links with APSA (Inc.) by promoting and marketing the proceedings throughout Australasia and, indeed, the world. And finally, the APSA conference has come to Western Australia for the first time!

APSA has continued the tradition of providing every person who has a one-page paper with the opportunity to speak, and hence be seen and heard. Time constraints mean that we cannot offer an 'oral' presentation to everybody with a one-page paper, resulting in some contributors being asked to prepare a poster. My experience at many conferences is that posters are generally given the 'cold shoulder', with delegates often questioning their efforts to bring a poster in a long white tube halfway across the world (or the continent) in the first place. Our meeting is unique in providing a person presenting a poster with a 3-minute opportunity to speak, which reinforces the value of a poster to the conference. We have further enhanced the value of posters this year by holding a 'Happy Hour' in the poster room, to promote discussion and interaction among presenters and delegates in more convivial circumstances.

The Batterham Memorial Award remains a feature of any APSA conference. One of my endearing memories of Ted Batterham was in the bar at the 1989 APSA conference in Albury, where Ted was asleep standing up – and holding a beer. A strong list of candidates has again trotted through the gate for this year's award. Continued thanks to the sponsors of this award are expressed, as is their recognition of the importance of the award to the advancement of pig science in Australasia.

I wish to comment on the decline in the number of one-page papers being published in the APSA proceedings. Figure 1 on the next page shows the number of submitted and accepted one-page papers in APSA proceedings between 1993 and 2003. The graph is characterised by a steady decline since the 1997 meeting in the number of submitted and accepted papers, going from a high of 117 one-page papers in 1997 to approximately 80 in this meeting. I believe these data highlight the changing atmosphere of pig research in Australia, and pose a number of questions that the Association, and the pig science community in general, should possibly address. Interesting times ahead, I believe.

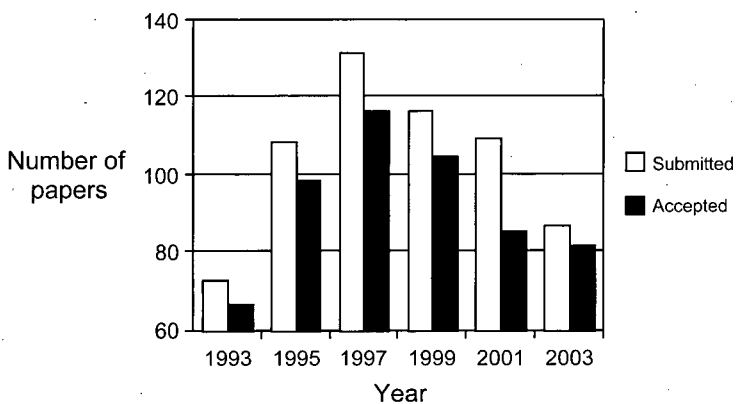
Due, in part, to the declining number of contributions to the conference, the APSA Committee decided to instigate a dedicated 'Producer's Day' for this year's conference. This idea has been toyed with previously, but this year the Committee has promoted the concept more vigorously and, we hope, a day such as this will mesh scientists with producers and reinforce the

value of pig science to the wider pig community. Conferences are not becoming any cheaper to run, and fully-engaged pig scientists are becoming fewer on the ground, so for the long-term viability and direction of the Association, I believe that further innovations such as these will be required in the future. Greater consultation, participation and stimulation from the wider pig industry community will be required to ensure that APSA (Inc.) continues its pivotal role in the pig industry.

Successful conferences need sponsorship, and this year we have again been fortunate to have Australian Pork Limited as the principal sponsor. Special thanks go to Dr Ian Johnson for his efforts in securing the support of APL for this conference. This year's conference is noteworthy for the record level of sponsorship secured. In this respect I, and I'm sure I speak for all delegates, thank the inspired efforts of Dr Pat Spicer for securing and coordinating this most important aspect of the conference. In tough times for the Australasian pig industry, I think the level of support APSA (Inc.) has received only reinforces the tremendous importance and value the industry as a whole places on this conference.

On this note I will sign off, and trust that you all have a terrific time at the conference and come away just that little bit wiser.

John Pluske  
President,  
Australasian Pig Science Association (Inc.)



**Figure 1.** The number of submitted and accepted one-page papers in APSA proceedings between 1993 (Volume IV) and 2003 (Volume IX).

## **A REVIEW – CONCERNS ABOUT PORK PRODUCTION IN WESTERN EUROPE – EFFORTS TO GET THE LIGHTS ON GREEN**

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### **Abstract**

Pork production in Western Europe and particularly in The Netherlands increasingly encounters a variety of societal concerns. The greatest concerns relate to food safety and healthiness, animal welfare and health, environmental impacts, sensory quality and pork price. Most of these concerns have their basis in the large increase during past decades in the number of pigs kept at high densities. A number of painful incidents in food production during the past seven years, and the extensive media attention that they attracted, have certainly contributed to these concerns. In general, the concerns can be dealt with by legislative measures, participation in (alternative) production schemes and the utilisation of opportunities. With respect to pork safety, animal welfare and environmental impacts, legislation by the Dutch government and the European Union (EU) is having a large impact on the Dutch pork production sector. In order to meet these legislative requirements high investment costs are often needed, with many pig farmers deciding to instead cease production in the face of static pork prices. Because of this, one of the aims of the legislation is being met by default – to reduce the total number of pigs. The other aim is to provide the remaining pork production sector with a ‘license to produce’. Real progress has already been made during the past decade to guarantee pork safety, to improve pig welfare and to diminish the environmental burden of pork production. However, with the exception of certain niche markets, there is little prospect of financial compensation for these efforts and it is unrealistic to expect all pork that enters the market to fulfill the same production and animal welfare standards. For the Dutch pig production sector the lights are not yet on ‘green’ because it has to compromise between its corporate social responsibility and its survival in the economic competition of the EU and world markets. Within the strict legislative framework that has been built to meet societal concerns, only the joint efforts of consumers, citizens, governments and producers can result in a sustainable pig production sector.

### **Introduction**

Like other agricultural activities in developed countries, the production of pork is currently subject to increasing societal criticism and concern regarding its acceptability. That many people earn their living directly or indirectly from pig and pork production and that this branch of the agricultural industry substantially contributes to the human need for high quality protein, is no longer a sufficient justification for its existence. Consumer criticism of pork production systems is only partly related to real changes in these production systems. Consumers now have an increasing knowledge and awareness of pork production because of a more active media role in the agricultural industries. In addition, a number of large-scale incidents followed by various interventions by authorities, have strongly supported this societal criticism.

To consolidate or strengthen its ‘license to produce’, the modern pork production sector needs ‘green lights’ from society on the various intersections it has to cross. Such green lights can only be obtained by taking a number of drastic measures. This paper provides an overview of recent developments in pork production and public opinions and concerns in Western Europe, and in particular in The Netherlands. In addition, a number of measures will be discussed that could improve the sustainability of pork production in Western Europe. Lastly, the dilemma of balancing societal concerns with price competition in the world market will be discussed.

### **Size and trends in pig farming**

The dimensions of pig production in Australia are smaller than in The Netherlands, despite Australia having a bigger population (19.8 M) than The Netherlands (16.1 M) and more than 150 times the area of arable land and pasture (Table 1). Consumption of pork per capita in Australia is less than half the Dutch consumption. In both countries, the number of pig farms shows a similar, decreasing trend. Due to an increasing number of pigs per farm and an increasing average

slaughter weight, the total number of pigs and the amount of pork produced have been relatively stable in both countries throughout the decade 1990-1999. In The Netherlands, however, the total number of pigs has clearly decreased since its peak in 1997 (over 15 million) to 12.8 million in December 2000 and 10.7 million in April 2003. This strong downward trend was mainly due to activities of the Dutch government that encouraged pig farmers to cease pig production, and to imposed national and European Union (EU) regulations related to the manure production and housing of pigs. Most of these regulations are the result of increasing societal concerns about environmental pollution and animal welfare which may be reflected in the decreasing pork consumption trend in The Netherlands (Table 1).

**Table 1. Characteristics of Australian and Dutch pig production.**

	Australia			The Netherlands		
	1990	1999	2001	1990	1999	2001
Number of sows (x 1000)	335	305	305	1,497	1,373	1,259
Number of pig farms	6,847	3,018	2,507	29,211	16,426	12,822
Pigs slaughtered (x 10 <sup>6</sup> )	± 4.9 <sup>a</sup>	± 5.2 <sup>a</sup>	± 5.0 <sup>a</sup>	19.9 <sup>b</sup>	19.6 <sup>b</sup>	15.7 <sup>b</sup>
Average carcass weight (kg)	± 62.5 <sup>a</sup>	± 71.0 <sup>a</sup>	± 72.5 <sup>a</sup>	84.3	87.5	91.2
Consumption (kg cwt/cap.)	± 19.0	19.0	19.0	45.2	43.6	42.4
Export (tons cwt x 1000)	± 7 <sup>a</sup>	37	55	1,067	1,107	892

Sources: Australian Pork Limited, Statistics Netherlands and USDA Foreign Agricultural Service.

<sup>a</sup>Estimated from trend lines.

<sup>b</sup>In addition, 4 to 5 million pigs born in The Netherlands were exported for slaughter annually.

Various societal concerns have been strengthened by a number of incidents in animal production in The Netherlands that have also had negative consequences for the export of pork. About 10% of The Netherlands' pork is exported to markets outside the EU. Part of the lost, Dutch-pork export market in Asia may have been taken over by Australia - supported by the strong growth of the Australian pork export to Japan and Singapore. Australian pork is perceived as fresh and healthy and is an attractive product to Asia's demanding markets (Ronan *et al.* 2001; Anonymous, 2003).

#### Recent incidents in animal production

The response of authorities and industry to a number of pig-industry incidents during the past decade (and the extensive media attention that ensued) have certainly contributed to increased public concern about pig production. Although the intensity of these concerns fluctuates, there is a definite increasing trend. Recent major incidents in animal production in The Netherlands or neighbouring countries include:

- February 1997, Classical Swine Fever (CSF). The start of a disastrous outbreak involving a total of 429 contaminated farms. From another 1,286 farms all pigs were preventively removed and destroyed. Due to the associated restrictions in transporting pigs, huge welfare problems arose on most other pig farms in The Netherlands. In consequence, more than eight million pigs were purchased by the national government. In total, almost 10 million pigs were destroyed because of CSF.
- March 1997, Bovine Spongiform Encephalopathy (BSE). Since the first BSE cow was detected until the time of writing (September 2003), 63 cows on 63 different farms in The Netherlands have been diagnosed with BSE. There is a ban on the use of animal protein in animal feed for all domestic animals. All slaughtered cattle older than 30 months of age are now routinely tested for BSE. To date, more than 7,000 cattle have been destroyed because of BSE detected in a herd mate or a relative.
- May 1999, Dioxin. Animal feeds containing unacceptable concentrations of dioxin may have been imported to The Netherlands from Belgium. The source was fat from one fat processor which was used in animal feed. Many products have been removed from retail shelves. Farms that may have used contaminated feed were quarantined until the feed was tested and the animal products did not exceed maximum dioxin norms.
- March 2001, Foot and Mouth Disease (FMD). One month after the start of the large outbreak in England, goats on a Dutch farm were diagnosed with FMD. In the next few months, the disease spread to 26 farms. On about 2,500 farms (one large region and a few smaller regions),



all susceptible animals were (compulsorily) removed to prevent further spread, and over 280,000 animals were destroyed because of FMD. The EU non-vaccination policy has resulted in extensive societal discussions on its acceptability.

- May 2002, Nitrofen. Organically grown German wheat was found to be contaminated with Nitrofen, a carcinogenic herbicide prohibited in the EU. The wheat was used in poultry and pig feeds. In Germany, batches of organic eggs and poultry meat were detected to have unacceptable concentrations of Nitrofen.
- July 2002, Medroxy Progesteron Acetate (MPA). After finding sows with reproductive problems, the hormone MPA was detected in pig feed. The source was glucose from a pharmaceutical company that was processed in pig feed in Belgium and exported to at least 26 pig farms in The Netherlands. Although MPA was not found in pig meat from those farms, all 50,000 pigs were destroyed.
- March 2003, Avian Influenza (AI). Feral water birds probably infected outdoor-kept chickens in an area with a high concentration of poultry. The disease spread out over other regions in The Netherlands, including Belgium and Germany. By June 2003, 255 Dutch poultry farms were found to be contaminated with AI (including 22 farms with just hobby animals) and 28 million birds were (preventively) killed.

As a result of many of these food safety and/or disease incidents, recall of food, feed or animals occurs and transport of animals and animal products (including exports) is severely restricted. Farms and food companies may be blocked and many animals are destroyed. Often, relatively few animals are culled because they are affected, with many more healthy animals killed to prevent further distribution of a disease, or to maintain a production image and prevent the close of (export) markets. These sorts of incidences often lead to media 'hypes'. In cases where large numbers of healthy animals have to be killed to prevent further disease outbreaks, the question always arises as to why such destruction could not be prevented by large-scale vaccinations. Citizens do not often understand or accept that economic reasons (export) are usually the basis for such decisions. Animals that have to be killed are often collected and removed mechanically and in large numbers. Due to the detailed attention to food scandals by the media, the public is generally well informed about such incidents in the food chain and about the views and actions of the various parties involved. Although quick and effective action to prevent further damage certainly contributes positively to the public confidence in food production, the fact that such incidents repeatedly happen, along with the associated images and discussions in the media, is usually considered as substantially negative for the animal (food) production sector.

### **Concerns about pork and pork production**

Pork producers in Western Europe encounter a variety of societal concerns about pork and pork production. The term 'societal concerns' is used here to indicate that the concerns are not only economic concerns related to production costs and retail prices. Kanis *et al.* (2003a) presented an overview of possible concerns and put them in a general framework based on the whole pork-production system. Concerns can arise amongst each of three groups of 'stakeholders' - consumers (who want to buy and eat pork (or not)), citizens (who want to improve society (or not)) and producers (who want to earn a good and sustainable living from their enterprise). Governments can also be considered one of the 'stakeholders' that, through legislation, exert influence on pork production. However, in a democracy, the actions of the government should also reflect the concerns of its citizens, who may in turn be consumers and/or producers. The pork production system can be divided into three components - the production chain (the consecutive activities and their scales), the chain input (quality of feed, medicines, type of animal transport, etc.) and the chain output (pork, manure, but also animal welfare, landscape, human working conditions, etc.). Each of these three components can cause concerns with each of the three groups described above. Examples of possible concerns, excluding global concerns like the world food security and the world trade issue are given in Table 2. Many concerns are not limited to just one component of the production system. For example, some citizens may be concerned, for ethical reasons, about (1) the 'genetic modification of feed and animals' (production chain), or (2) about certain kinds of genetic modification (chain input) for example, the application of recombinant porcine somatotropin (rPST) because they believe it is bad for the welfare of pigs, or (3) about pig welfare, no matter what the cause is (chain output).

**Table 2. Examples of possible societal concerns regarding pork production within components of the production system.**

Components	Stakeholders		
	Consumers	Citizens	Producers
Production chain	Limited availability of some (e.g. regional) pork products. Low transparency of the chain and little information about production methods.	Pork production unnatural, too intensive and industrialised (large farms, use of growth promoters or GMO feed, ethics, unemployment). Low transparency of the chain, little information.	High costs of production, transport, quality control, etc. Marketing problems. Competition.
Chain input	Use of low-quality feed (resulting in unsafe or unhealthy pork). Bad veterinary care (e.g. resulting in medicine residues in pork). Amounts of fat, salt or water added to processed products.	Unbalanced breeding goal (e.g. only market traits). Type of molecular genetics and modern reproduction techniques used. Bad handling or housing of animals (castration, tail docking, little day light or long-distance transport).	Availability of raw material at low costs (piglets, feed, veterinary care, expertise, etc.) Way of presenting products with butchers and in supermarkets.
Chain output	Unsafe pork (residues of hormones, medicines and herbicides, pork decay). Unhealthy pork (fat, cholesterol, allergens) Bad sensory quality (colour, taste, boar taint). Pork quality too variable, insufficiently guaranteed, product not well labelled, etc. Pork too expensive.	Pigs are unhealthy, have leg problems or other physical problems. Pigs have low welfare, are stressed, etc. Bad effects on quality of air, water and land. Exhaustion of natural resources (food, energy, biodiversity) and soils. Production of odours and noise. Decreasing attractiveness of landscape.	Low product prices. Costs and possibilities to get rid of manure surplus and offal. Pigs too stressed (meat not suitable for some kinds of products). Bad working conditions (heavy labour, dust and noise).

### Importance of concerns

The following concerns about pork and pork production are deemed the most important in Western Europe (Verbeke *et al.* 1999; Brom, 2000; Harper and Makatouni, 2002):

- Pork safety and healthiness for the consumer
- Welfare and health of pigs
- Environmental impact of pork production
- Sensory quality of pork
- Price of pork

It is difficult, however, to draw general conclusions about the (relative) importance of these concerns for the following reasons:

- Concerns are difficult to measure and are often indirectly derived by analysing purchase data or perceptions about products instead of directly surveying the respective groups of stakeholders for their concerns or measuring concerns in an experimental setting (Verbeke, 2000).
- Apart from the research methods used, the number and kind of possible concerns that are simultaneously investigated are often different between studies, and (ranking) results may therefore be difficult to compare. Also, research is often aimed at the whole meat production sector (not just pork), which makes it difficult to draw conclusions specific to pork production.
- As discussed above, concerns may be different for different stakeholders and are often time-dependent because they are strongly influenced by the occurrence of incidents and the kind of information provided.
- Concerns may have different origins. For example, Harper and Henson (2001) concluded that consumers seemed to be concerned about animal welfare because they believed that consumption of products from animals with a low welfare status had a negative impact on their own health.
- Concerns are often interwoven and may depend on many circumstances that vary with time. For example, during an economic crisis a high pork-price might be a much more important concern for consumers than low animal welfare. With increasing prosperity, animal welfare issues are likely to become more important.

To investigate the (relative) importance of concerns, the number of times respondents mention a particular concern can be analysed. An alternative method is to ask respondents to mark

or rank their concerns. A third approach is to ask respondents for their willingness to pay more for a certain improvement in pork quality or in the pork production system. However, this may not be a reliable estimate of importance because willingness to pay depends on socio-demographic characteristics like income and level of education. Also, many respondents may not want to pay anything extra because they don't consider it their responsibility to ensure acceptable production and product standards (Harper and Henson, 2001).

A summary of literature that quantifies concerns, partly taken from Kanis *et al.* (2003a), is presented below. In a study of European consumers in 1999 (PVE, 2001), in which they were asked to mention one or more concerns, 28% said they had no concerns about meat production in general (not only pork), 21% mentioned BSE as a concern and 11% mentioned growth promoters and additives. Other concerns were mentioned by less than 10% of respondents, but with a large variation among countries. However, when the surveyor asked for potential concerns, 89% of European consumers expressed concerns about residues, 85% about hormones, and 77% about animal welfare. From a series of studies (PVE, 1996; Schifferstein *et al.* 1998; PVE, 1999) covering 1991, 1995, and 1997 and with over 800 Dutch consumers per year, it was concluded that the overall image of fresh meat depended on four dimensions. These were (1) 'sensory characteristics' (including taste and tenderness) which ranked highest, followed by (2) 'ease of use,' (3) 'special' (including price and healthiness), and (4) 'production system' (containing the items - hormones or additives used, animal friendly, environment friendly, and hygienic production). Pork had an overall image score similar to veal, but lower than chicken, beef, and fish. Compared to other meats, pork was rated as a widely available cheap product, but not as a premium product. Pork was considered easy to prepare and able to be used for many dishes. The main disadvantages were that pork production was not considered as animal- or 'environmentally-friendly', that pork might contain hormones, and that pork rated low for healthiness and leanness. In the study by Schifferstein *et al.* (1998), the image of 'production system' was more important in 1997 than in 1995 or 1991, suggesting that consumers had become increasingly concerned about the 'naturalness' of pork production, although this was not measured.

Meuwissen *et al.* (2003) concluded from a survey of 1000 French and 1320 Dutch pork eaters, that in France, the safety of pork was the number one concern, while in The Netherlands both food safety and animal welfare were perceived as very important. Other possible concerns investigated were environmental aspects of pork production, sensory quality of pork and naturalness of pork production. Willingness to pay more for improved pork seems to be slightly higher in The Netherlands than in France.

Results by Becker *et al.* (2000) suggest that German pork consumers, with respect to safety of pork, were most concerned about antibiotics and hormones in pork, followed by Salmonella and other bacteria, and then by fat or cholesterol. Fat or cholesterol in pork was perceived a little more important than in beef. For beef, BSE contamination appeared the most concerning and for chicken meat this was Salmonella.

In Belgium, a survey (Verbeke and Viaene, 2000) revealed that meat consumers attached importance to the following meat attributes in decreasing order: healthiness, leanness, free of harmful substances, animal friendly production, compatible with a range of dishes and widely available. Pork was perceived as a fatty kind of meat and less healthy than poultry, but containing less harmful substances than beef. Verbeke and Viaene (2000) concluded that consumers were most concerned about meat safety, which could be explained by recent safety crises (hormones, BSE, antibiotics, tranquillisers, and dioxin). With Issanchou (1996) and Schifferstein *et al.* (1998), Verbeke and Viaene (2000) concluded that animal welfare in general ranked low among fresh pork attributes, but that it would gain importance.

However, opinions or concerns that people, as citizens, express about production systems are often not expressed in their purchasing behaviour as consumers. Even respondents' answers to questions about their willingness to pay more for products that are produced in an animal friendly way are poor predictors of their actual purchasing behaviour (Burrell and Vrieze, 2000; Harper and Henson, 2001). This indicates that relative to other possible concerns, even in wealthy societies a high pork price is an important concern for consumers. The study by Harper and Henson (2001) also revealed that consumers were not well informed about the production of beef, lamb, pork, and veal, and that they were concerned about the quality of the information they received. There was a

general distrust of the main sources of information, notably the government and the food industry, whereas campaign organisations were trusted more.

#### **How to deal with concerns?**

Kanis *et al.* (2003a) argue that the development of a stakeholder concern is the result of:

- the reality regarding pork and the pork production system,
- the information that reaches the stakeholder and,
- the stakeholder's norms and values.

The stakeholder's norms and values depend largely on his/her cultural background, education, ethical and societal consciousness, etc. and are difficult to influence in the short term. The information provided by producers to consumers and citizens increasingly deserves attention (see Powell, 2000), because people want to know about what they eat, or at least to have the information made available to them. A butcher can usually directly communicate with his customers about the background and quality of the pork he sells. In the supermarket, labelling of pork can partly take over this role. Classic communication media, like newspapers or television, or modern means like the Internet (e.g. by the use of web cameras in pig houses, which are already used by a Dutch veal chain) can provide more general information about the pork production system for citizens. However, combined with a good identification and registration system, the Internet can also provide specific information for the consumer about a particular batch or package of pork, in addition to the information already present on the label attached to the product. Because information can be affected or modified by, for example, journalists during its way to the consumer or citizen, it is important that producers get involved in the information provision and quality control. Labelling of pork is a good opportunity for producers to inform consumers about various aspects of pork quality and the pork production system.

Since most concerns are related to aspects of the production system, the most important way to deal with them is to adapt the relevant aspects of pork production, and subsequently to inform the stakeholders about these modifications as well as possible. If, for example, consumers are concerned about the fatness of pork, then producers can adapt the breeding goal and the feeding level to meet these concerns. Because these adaptations are relatively simple and, moreover, profitable for the producer, they will easily be implemented. However, producers will not be able to deal so easily with concerns relating to the welfare of pigs or to the ecological impact of pork production, because of the complexity and high implementation costs involved, which are unlikely to be compensated for by higher product prices. If the concerns are wide spread in society they can often only be met by legislative measures.

Legislation only guarantees that the production system fulfils certain minimum standards. Such minimum standards can then be raised stepwise by additional legal provisions. However, legislation can usually not comply with all the concerns of all stakeholders. Therefore, in many countries several national production schemes have been developed that, in addition to the legislative requirements, pay extra attention to a few or several concerns. In this paper reference is made to three production schemes. EU-Organic farming (EU-wide and based on regulations recorded in EU Regulation EEC/2092/91) and Freedom Food (initiated by the British Royal Society for the Prevention of Cruelty to Animals (RSPCA)) are some of the well known and wide spread production schemes. In The Netherlands the Integrated Quality Control system (IKB) was introduced in 1992 - see Kanis *et al.* (2003a) for a comparison of these schemes. Producers can voluntarily commit themselves to such production schemes, although more and more retailers require pork to be produced according to a particular production scheme. Participating producers have to produce according to certain standards for the benefit of, for example, a guaranteed market or higher product prices. The production process is controlled and certified by independent agencies. The products from production schemes are usually labelled and sold to consumers at a higher price. Due to the higher price and the availability of regularly produced pork that fulfils the gradually increasing legal requirements, the market for pork from production schemes that substantially differ from regular production (e.g. organic pork), in the EU-member states is expected to be limited.

Along with the imposition of legislation and the participation in (alternative) production schemes, producers can seize several other opportunities to meet societal concerns. These include – placing an extra emphasis on the sensory quality of pork in breeding programs, selling dried and

granulised manure, showing their pig houses to citizens and explaining the production system to them, etc.

In the following sections the most promising and accepted ways to meet important concerns regarding pork and pork production are discussed with regard to their legislation, production scheme and opportunities. Although there are several biotechnologies that could help to meet concerns (Bonneau and Laarveld, 1999), these techniques also raise concerns and the EU is therefore reserved about them. An example of these techniques is the genetic modification of animals and plants and the use of products from genetically modified organisms. Despite the increasing worldwide-use of genetically modified crops in pig feeds, it is reassuring that even with highly sensitive detective methods, no fragments of the transgenes nor of the proteins coded for by those transgenes, have been detected in samples of loin muscle from pigs fed with Roundup Ready soybean meal (Jennings *et al.* 2003).

#### **Pork safety and healthiness for the consumer**

Pork safety refers to the absence of (1) chemical residues from veterinary medicines and/or feed additives and (2) micro-organisms on or in the pork that are harmful for public health. Pork healthiness is related to the natural composition of pork and its healthiness to the consumer. Pork is often perceived as relatively fatty and therefore relatively unhealthy. However, the intramuscular fat content in pork is on average lower than in beef and lamb (Verbeke *et al.* 1999). Compared to some decades ago, the proportion of fat in pig carcasses has decreased tremendously due to improved feed (e.g. addition of free essential amino acids) and feeding systems (e.g. phase feeding) and due to effective genetic selection for leanness and feed efficiency (Gibson *et al.* 2001; Knap, 2002). Studies on consumer behaviour regarding preference for pork, consistently show, however, that most consumers still prefer pork with little fat cover (Grunert, 2002; Kanis, 2003, unpublished data).

#### *Legislation*

Pig feed is one of the major risk factors with respect to residues, as shown by the recent dioxin, nitrofin and MPA crises in the EU. Because pig feed is usually a mixture of raw feedstuffs from various origins, it is extremely important that all feed components are fully safe and contain no banned substances. There are detailed EU regulations on banned substances and maximum levels allowed in pig feed. The new European Food Authority, which covers the whole food chain, now governs food safety in Europe. For example, most antibiotics, growth promoters, tranquillisers and products from genetically modified crops are forbidden. For medicines and feed additives a so-called positive list of allowed products is used in the EU. Further, strict regulations apply to the use of slaughter offal and catering waste in feed. In addition, EU-legislation must be complied with regarding the application of medicines for slaughter procedures, processing, transport and storage of pork. Hygiene and cooling are the key words to prevent contamination and growth of harmful microbes on pig carcasses and meat cuts.

#### *Production schemes*

The EU-Organic farming scheme requires that pigs are fed at least 80% of organically produced feed. This is feed produced without the use of herbicides or artificial fertilisers and with no additives. EU-Organic farming rejects the use of chemical medicines and the preventive use of medicines. Only sick animals may be treated with, preferably, natural or homeopathic medicines. In addition, organic breeding programs aim for robust and healthy animals.

The Dutch IKB scheme originally placed much emphasis on pork safety by developing lists of medicines and additives that could be used and by improving tracing and tracking possibilities of pigs and pork by requiring good identification and registration of pigs. Many of these activities have now got a legislative basis.

#### *Opportunities*

Almost all Dutch feed companies participate in several quality systems like GMP+ (Good Manufacturing/Management Practices) which includes HACCP (Hazard Analysis and Critical Control Points) guaranteeing that all components of pig feed and its production process fulfil the highest safety requirements.

As with poultry, Salmonella (Swanenburg *et al.* 2001) and Campylobacter (Nesbakken *et al.* 2003) in pigs are now the most threatening microbes for public health since the classical pork-born disease trichinellosis (Van Knapen, 2000) has virtually disappeared in Western Europe. Programs

to reduce the risk of contamination of pork by *Salmonella* and *Campylobacter* have been developed by the industry. It is important also to educate the consumer with these programs, because contamination can easily occur in the home refrigerator or at the kitchen bench, but can also be easily prevented by applying adequate kitchen procedures.

Fat content is an important issue with respect to the healthiness of pork. The use of entire boars as slaughter pigs instead of castrated boars could further diminish the deposition of inter- and intramuscular fat. The risk of boar taint in pork, however, would work against this option particularly if slaughter weights increase. The use of the welfare-friendly immunocastration technique, through the active immunisation against gonadotrophin releasing hormone (Bonneau, 1998; Oonk *et al.* 1998), could provide an alternative to the surgical castration of boars. This would enable the combination of the positive effects of entire boars (little fat, high feed efficiency) with the absence of boar taint. However, this procedure is not currently permitted in the EU.

There is increasing interest to manipulate the fatty acid composition of pork (as well as in meat from other species) towards a more healthy ratio (higher than 0.4) of polyunsaturated to saturated fatty acids. Recently, nutritionists have focussed on the ratio between different types of polyunsaturated fatty acids. In particular the n-6:n-3 ratio, which is 7.22 in pork, but 2.11 and 1.32 in beef and lamb (Enser *et al.* 1996 cited by Wood *et al.* 2004), should be less than four to reduce the risk of cancers and coronary heart disease. (Wood *et al.* 2004) found genetic and nutritional effects on the n-6:n-3 ratio but Cameron *et al.* (2000) concluded that nutritional approaches are the most effective at reducing the n-6:n-3 ratio.

Genetic selection for robustness and disease resistance, although complicated (see Simianer and König, 2002), could play a significant role in reducing the use of medicines (Henryon *et al.* 2001) and thereby reducing the risk of residues in pork.

#### **Welfare and health of pigs**

As indicated previously, in most Western Europe societies, many people are concerned about the welfare of farm animals and particularly animals, like pigs, that are kept in intensive production systems. In response to these welfare concerns, several pig husbandry measures have recently been implemented or announced by the Dutch government and the EU-council to improve the welfare of pigs.

#### *Legislation*

Since 1886, there has been official legislation regarding the protection of animals in The Netherlands. In 1961, a national law was accepted concerning animal health and mistreatment. Since 1992, legislation about animal welfare has been included in the new 'Health and Welfare Law for Animals'. This is a so-called 'framework law' consisting of many Acts that can be changed independently. Part of this law is an act for the health and welfare of pigs. The general principle is that it is no longer permitted to keep animals or to handle them unless it is explicitly legally accepted.

More recently, EU-legislation has been developed in the field of animal welfare and health that should also harmonise the legislation in the member states. This means that for many aspects of pig production in The Netherlands the EU-legislation is now the minimum standard but for some aspects this may be overruled by the stricter national legislation. In Table 3 we give a short overview of the main rules on welfare of pigs regarding their housing, based on the Council Directive 2001/88/EC (EU, 2001) and Ministeries van Justitie en van LNV (1998, 2003).

Owners of existing pig houses have until 2013 to implement the necessary alterations to their buildings. New pig houses or pig houses that will be renovated are required to fulfil the new requirements immediately. For the future, the EU is considering further improvements to the welfare of pigs, for example, the abolishment of surgical castration, teeth clipping and tail-docking.

Welfare legislation is also being considered with respect to pig transport and the slaughtering of pigs.

**Table 3. Overview of the main rules on welfare of pigs in the EU and The Netherlands.**

Item	EU-Legislation	Dutch Pig Act <sup>a</sup>
Housing system	Group housing for gilts and sows from four weeks after service to one week before farrowing by 1st January 2006.	From five days after service to one week before farrowing.
Formation of stable groups	Within one week after weaning. Mixing of groups as little as possible. Escaping and hiding from other pigs should be possible.	
Minimum un-obstructed floor area	0.15 m <sup>2</sup> for weaners or rearing pigs of 10 kg up to 1.00 m <sup>2</sup> for pigs over 110 kg kept in groups. 1.64 m <sup>2</sup> for gilts after service and 2.25 m <sup>2</sup> for sows kept in groups.	About 30% more than EU. Gilts also 2.25 m <sup>2</sup> , ≥ 1.3 m <sup>2</sup> solid floor.
Concrete slats with group housing	Minimum slat width: 50 mm for piglets and up to 80 mm for gilts and sows. Maximum width of openings: 11 mm for piglets and up to 20 mm for gilts and sows.	Piglets: 10 mm for concrete slats and 12 mm for other slats.
Nesting material	To be provided to sows in the week before farrowing.	
Access to water	Permanent access from two weeks of age.	
Age at weaning	28 days, or 21 days with specialised housing.	
Tail docking, teeth clipping of piglets	Not routinely, only in case of evidence of injuries, not later than seven days old.	
Access to distraction material	Straw, hay, wood, sawdust, mushroom compost, etc. available in sufficient quantities.	
Access to bulky or high-fibre feed	Required for pregnant sows and gilts.	
Light and noise	Minimum of 40 lux during eight hours per day, avoid noise levels of 85 dBA.	

<sup>a</sup>Only indicated where the Dutch Pig Act is stricter than the EU-legislation.

### Production schemes

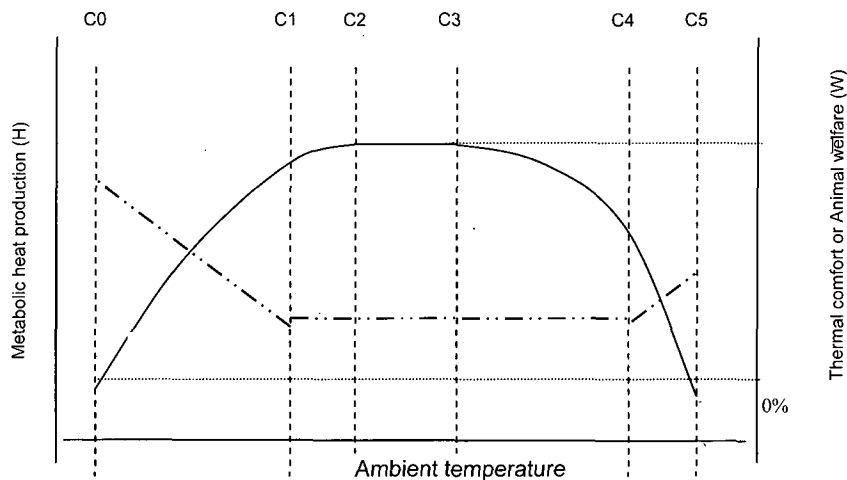
Most production schemes are attentive to animal welfare and health. In EU-Organic production, pigs are housed in groups with open-air runs and minimum standards for floor surface area, which are higher than legally prescribed. For example, sows with piglets must have 7.5 m<sup>2</sup> inside and 2.5 m<sup>2</sup> outside – considerably more than the minimum of 1.3 m<sup>2</sup> (plus 0.6 m<sup>2</sup> for the piglets) required in conventional systems. Teeth clipping and tail docking are not allowed and minimum weaning age of piglets is 40 days. The Freedom Food Scheme is aimed particularly at improving animal welfare by adhering to the five freedoms – freedom from (1) hunger and thirst, (2) discomfort, (3) pain, injury, or disease, (4) fear and distress, and (5) freedom to express normal behaviour (RSPCA, 2000). In addition to space and management requirements, attention is paid to training stockmen to minimise the stress and injuries experienced by their animals. The IKB scheme includes the welfare rules laid down in the Dutch Pig Act (see Table 3) and makes extra demands of pig transport methods and lairage in slaughter houses.

### Opportunities

The general opinion has always been that animal welfare should primarily be improved by implementing better husbandry methods (housing, feeding, management). This is reflected in the large number of legislative measures regarding welfare and the interest in production schemes with welfare standards higher than the minimum level required by legislation. However, there is also a genetic component involved in the improvement of animal welfare and health. During the process of domestication pig populations have gradually adapted to the conditions man has offered them. It is plausible that pigs that are better adapted to, or that can better cope with a certain husbandry or production system, have a better welfare. This can be deduced from the lower incidences of aberrant behaviour (e.g. aggressive and stereotypic behaviour), a better health and probably also a better production level. Several studies have demonstrated that behaviour shows genetic variation (e.g. Hemsworth *et al.* 1990; Kjaer *et al.* 2001). Based on animal responses to varying ambient temperatures, Kanis *et al.* (2003b) developed a theoretical framework to describe the welfare of pigs in terms of the parameters of the environment in which it is kept (e.g. ambient temperature, number of pen mates) and its coping abilities (Figure 1). Between C2 and C3 in Figure 1, the ambient temperature is such that body temperature can be kept constant by vasoconstriction and vasodilatation. Beyond this comfort zone, some conscious adaptive behaviour is needed to maintain body temperature, for example wetting of the skin above C3 and huddling below C2. It is assumed that between C2 and C3, thermal comfort is optimal and from C1 to C2 as well as from C3 to C4 it is still acceptable because normal adaptive behaviour is sufficient to maintain body temperature. Beyond the zone C1 to C4, adaptive behaviour that should be considered as

abnormal, is necessary to maintain body temperature, e.g. panting above C4 and shivering below C1. The C-values thus quantify adaptive capacity and the environmental tolerance of pigs.

Kanis *et al.* (2003b) argue that this framework in Figure 1 can be used to describe welfare for several other environmental characteristics and that it can be used to find criteria for genetic selection. If the environment is worsened gradually, there comes a time when a pig cannot sufficiently cope with the situation and begins to change its behaviour or physiology. Pigs that show no change in behaviour (or only in very bad circumstances) are apparently better able to cope with the situation and are likely to have a better welfare under circumstances that are not optimal. By selecting these animals as parents of the next generation, we can probably extend the zone C1-C4 and, therefore, welfare parameters with respect to particular environmental circumstances.



**Figure 1.** Diagrammatic representation (after Mount, 1979) of the relationship between ambient temperature and metabolic heat production ( $H$ ) and their assumed relationship with animal welfare ( $W$ ). C0 to C5 are critical ambient temperatures associated with different thermo-regulatory mechanisms (see text for further explanation).

### Environmental effects of pork production

The impact of pork production on the environment can be divided into its impact on surrounding ecosystems, its effect on 'nature values' such as the quality of the landscape and biodiversity, and other impacts such as the production of odour, dust and noise. In The Netherlands and Brittany in France, the ecological effects of pig production cause most concern in regions with a high pig density. There are three kinds of ecological problems – (1) the surplus of minerals accumulating in the soil, water and air, (2) the use of fossil energy and its contribution to greenhouse gases and (3) the accumulation in the environment of heavy metals and residues from medicines. Lowering the amount of fossil energy used can be achieved by insulating animal buildings, using bedding for pigs, using photo-voltaic cells on pig-house roofs, and fermenting manure for methane production. Surplus minerals entering ecosystems from pig production systems is considered the most important environmental issue and is discussed below.

Pig manure is usually appreciated as a fertiliser on agricultural land. However, in particular regions with high numbers of pigs fed with feed imported from abroad, manure production may exceed the amount required for fertiliser. This has sometimes resulted in the over-fertilisation, and even dumping, of manure on certain fields, causing excessive concentrations of N, P and K, (and possibly heavy metals) in the soil. If the concentration of minerals in the soil exceeds the mineral-binding capacity of the soil, and if the crops cannot take up this mineral surplus, then minerals will flow to the groundwater and ultimately to the surface water. This results in an oxygen shortage in canals and lakes and an increased growth of undesired algae and weeds (eutrophication) leading to the death of fish and other aquatic animals.



Another problem is the emission into the air of ammonia (NH<sub>3</sub>), methane (CH<sub>4</sub>) and odours from pig slurry in pig houses and storage facilities as well as during the spreading of manure on land. Emissions may contribute to acid rain (NH<sub>3</sub>) and the greenhouse effect (CH<sub>4</sub>).

#### *Legislation*

The so-called 'EU Nitrate Directive' has been implemented in the EU to control the quality of surface waters and requires that ground water does not contain more than 50 mg nitrate per litre. Since January 1 2003, the 'EU Nitrate Directive' allows for a yearly application of 170 kg N per hectare from animal manure (reduced from 210 kg N per hectare pre-January 2003). The ultimate aim is to control the quality of surface water directly. In principle, the amount of 170 kg N is independent of the soil type or type of crops grown. EU-member states can get permission to apply higher quantities of N from animal manure if they present good arguments. To cover all types of application of manure on to land, The Netherlands has developed a detailed proposal for N-application based on (1) the type and humidity of soils, (2) crop production, (3) amount of artificial fertiliser and (4) amount of air-born deposition of N. For example, the proposal suggests an application of 250, 170 and 80 kg N/ha on highly productive grasslands, arable land and nature reserves, respectively.

In 1984, the Dutch government started to limit the production of manure through legislation. Since then, various 'manure laws' have been passed. Currently, the Dutch pig farmer must have so called 'pig rights' which are based on historical numbers of pigs on his farm and which allow him to keep a certain maximum number of (standard) pigs (derived from the P production per pig category). Pig rights can be 'skimmed' by the government if the farm is to be sold. Furthermore, each Dutch animal farmer must have a 'manure disposal contract' by which he proves that he has sustainable methods to get rid of the manure that he cannot apply to his own land. These are usually contracts with arable farmers or manure processors and often greatly increase his production costs. All Dutch agricultural farms must also use a 'minerals book-keeping system' through which all N and P that enters the farm in feed, animals or fertiliser must balance with the N and P that leaves in crops, animals, animal products and manure. A levy is paid on manure that cannot be accounted for or that has been applied on the farmer's own land above the enforced limit. This manure system is controlled by legislation that governs exceptions, controls and sanctions.

To reduce emissions from manure, the covering of outdoor manure stores and the application of manure into the soil by injection is obligatory in The Netherlands. Furthermore, applying manure between 1 September and 31 January is prohibited due to the reduced uptake of minerals by plants during this period.

#### *Production schemes*

The EU-Organic production scheme requires that farmers have enough land of their own or in conjunction with neighbouring farmers, to apply the manure their farm produces. The number of pigs that can be kept is linked to the number of hectares available. A maximum of 170 kg N can be applied per hectare per year in organic farming. Further, no artificial fertiliser or herbicides can be used. These regulations ensure that the risk of accumulated residues in the soil is very low. The Freedom Food and IKB production schemes pay no explicit attention to the environmental friendliness of pork production.

#### *Opportunities*

Pig farmers who do not want to reduce production to manage manure levels have implemented ways to reduce the mineral surplus on their farms. These include (1) transport of (dried) manure from surplus areas to deficient areas (including export), and (2) processing of manure to dry manure granules that can be used in gardens. Excretion of minerals per unit of pork produced can be reduced by increasing production efficiency and in particular feed efficiency (see Jongbloed and Lenis, 1998). Historically, pig breeders have focussed on increasing production efficiency and reducing maintenance requirements by genetic selection for feed efficiency, growth rate, carcass lean percentage and number of piglets produced per sow per year. Such selection for efficiency will continue, but the conditions in which pigs achieve this higher efficiency will be better defined. Interestingly, Crocker and Robison (2002) suggest that there is genetic variation in the excretion of nutrients and that a faster daily gain may be associated with a higher total excretion until slaughter weight.

Enhancing phosphorus digestibility by adding microbial phytase to pig feed has been very successful in reducing P-excretion of pigs by reducing their P-input from feed. Another effective way to increase N-use is to feed pigs via a multi-phase system with a diet reduced in protein content but supplemented with limiting essential amino acids (lysine, methionine, threonine and tryptophan). Multi-phase feeding results in reduced N and P excretion by 10 to 20% (Van der Peet-Schwering *et al.* 1999). For the time being, the Europeans will not accept genetically modified pigs with phytase genes (Golovan *et al.* 2001). Transgenic pigs possess a gene construct, based on an *E. coli* phytase gene and a mouse promoter gene, that stimulates phytase production by the salivary glands, apparently without any undesired side effects.

Van der Peet-Schwering *et al.* (1999) showed that reducing the excretion of N also reduces the emission of NH<sub>3</sub>. Methods to prevent emission from pig houses include lowering the pH of manure, cooling the manure and the use of filters in air outlets. Reducing the emitting surface in pig stables by using manure pits with a smaller area and preventing floor and animal contamination with manure through the use of partly slatted floors are also effective ways to diminish emissions. Keeping pigs on straw or sawdust bedding is another option available to reduce NH<sub>3</sub> and odour emission and to produce valuable compost. Between 1990 and 2000, many 'Green Label' pig houses were built with the aid of government subsidies that aimed to reduce emission to the air significantly. Subsequently, the Green Label concept has been extended to pig welfare and health as well as the use of fossil energy.

### **Sensory quality of pork**

The most important sensory characteristics of pork are (1) appearance in the supermarket or at the butcher, (2) shrinkage during cooking, and (3) eating quality (taste, tenderness, structure, juiciness, flavour). Appearance traits are the proportions of visible fat and bone, muscle and fat colour and the amount of drip.

#### *Legislation*

There is no legislation addressing sensory aspects of pork in the pig production phases of the pork chain. In the processing and marketing phases there are (safety) regulations concerning, for example, the addition of substances that improve pork colour, but these are beyond the scope of this paper.

#### *Production schemes*

Sensory aspects are not usually directly addressed in production schemes, although some suggest that better production conditions and pig welfare result in a better pork taste (e.g. RSPCA, 2000). Kanis *et al.* (2003a), concluded that there is currently no consensus on the most desired sensory quality attributes (and no economic trigger to improve them) and that sensory quality can only be improved through breeding which is not a core activity of most production schemes.

Nevertheless in some countries, famous pork products are produced such as Parma hams in Italy and Serrano hams in Spain, that rate very highly for perceived sensory quality. Such products are usually based on a complete chain concept with particular breeds and feeds, deviant slaughter weights and a special way of processing. However, in contrast to the production schemes mentioned before, often only certain parts of the pig carcass (e.g. the hams) can be marketed with the special label.

#### *Opportunities*

Some sensory aspects of pork can be influenced by breed or line of the slaughter pigs, sex and the presence of particular DNA alleles like the Halothane gene and the Rendement Napole gene, (for a recent review see Rosenvold and Andersen, 2003). Usually, pork from halothane-positive animals (homozygous) or carriers (heterozygous) shows a faster pH decline right after slaughter than pork from non-carriers and this is associated with a lower water holding capacity, a paler colour and a reduced tenderness (Channon *et al.* 2000; Brewer *et al.* 2002; Rosenvold and Andersen, 2003). Pork from carriers of the low Rendement Napole allele (RN<sup>-</sup>) usually shows a lower pH at 24 hours after slaughter than pork from non-carriers. The pork is also associated with a lower technological yield, a somewhat lower water holding capacity and a less pink colour (Brewer *et al.* 2002; Rosenvold and Andersen, 2003). However, eating quality (juiciness, tenderness) is often better in RN<sup>-</sup> carriers (Josell *et al.* 2003).

Regarding breed effects, Berkshire and Duroc are well known because of their higher intramuscular fat percentage and marbling. Pietrain has a high percentage of lean in the pork chop (e.g. Wood *et al.* 1996; Brewer *et al.* 2002).

Small and variable differences in sensory pork attributes have been measured between pigs kept in different housing systems (Gentry *et al.* 2002b). A higher feeding level may result in fatter pigs with more intramuscular fat and, therefore, a somewhat better taste and tenderness (Wood *et al.* 1996). Rosenvold and Andersen (2003) state that a holistic approach at the production-system level is needed to understand the effects of single factors. However, differences in sensory quality between production systems also seem to be small. Van der Wal *et al.* (1993) found no difference in tenderness and juiciness between loins from free-range pigs and conventionally finished pigs. Van der Wal (1993) suggested that the better sensory properties of pork from free-range pigs found earlier are due to labelling effects (perception bias from the consumers). Gentry *et al.* (2002a) found no differences in pork quality of loins between indoor housing on slatted floors and three alternative housing systems (indoor deep-bedded buildings, outdoor on dirt, outdoor on alfalfa pasture). Sundrum *et al.* (2000) found higher amounts of intramuscular fat in organic pork. In a study by Jönsall *et al.* (2002), consumers showed no difference in preference for organic pork compared to conventionally reared pigs, although a trained taste-panel scored organically-produced loins lower for juiciness and higher for crumbliness. However, in a study by Maw *et al.* (2001), bacon from pigs housed on straw tended to be darker, more tender and with more intense positive flavours than bacon from pigs kept on slatted or concrete floored houses.

#### **Consequences for the costs of pork**

For most Dutch pig producers, the cost associated with implementing legislative measures is their largest concern followed by the low prices they receive for their piglets or slaughter pigs. During the past few years, many pig farmers have ceased farming because of decreasing margins and because the Dutch government offered favourable buy-out agreements. The declining trend in the number of pig farms is expected to continue for the coming years. The remaining pig farms will either produce very efficiently and on a larger scale, or they will produce for niche markets.

To satisfy all legislative welfare measures, the cost to the pig farmer largely depends on his present housing system. If pig buildings are rebuilt to incorporate group housing on partly slatted floors, or should larger farrowing pens be required, it may be expensive. Providing pigs with distraction or bedding material and removing it later will also cost extra labour. According to Praktijkonderzoek Veehouderij (2002), the building costs of a new sow house that fulfils all present legal requirements are about €2500 per sow place (375 sows present on average). The construction cost for a new building for growing and finishing pigs is around €525 per place (2160 places). These costs include tax but do not include the costs for feeding equipment, feed storage, climate control, manure removal, reduction of NH<sub>3</sub> emission, etc. There are no clear figures available about the welfare and environmental components in these building costs. There is no substantial increase in cost to group-house pigs compared to housing of sows individually. Any extra costs are due mainly to the increased surface area required per animal and the inclusion of automatic feeders and equipment to minimise NH<sub>3</sub> emission. Farmers who install equipment to reduce NH<sub>3</sub> emissions can apply for financial support from the Dutch government for 25 to 30% of the investment costs, as long as the environmental component of the total investment cost is at least 30%.

Costs in relation to the disposal of manure depend almost entirely on the area of land the pig farmer has available. To transport manure to a crop producer usually requires payment to the crop farmer for taking the manure plus the cost of transport. On average, the costs for transport and the disposal contract are €6.75 and €11.25 per metric ton, respectively, corresponding with annual extra costs per animal present of about €20 for growing pigs and €90 for sows if the pig farmer doesn't own land (Praktijkonderzoek Veehouderij (2002)). Furthermore, farmers who don't remove enough minerals from their farm have to pay a levy of about €9.0 and €3.0 per surplus kg of P and N, respectively. In 2000, about 50% of Dutch pig farmers had to pay a manure levy of an average of €4500 per farmer (resulting in a total of about 26.5-million euro).

Using computer simulation, Krieter (2002) evaluated different pig production systems for their economic, welfare and environmental aspects. Compared to standard pork production (slatted floors, individual housing of sows, small groups of fattening pigs and production costs of €132 per

slaughter pig), group housing for gestating sows (slatted floor) and keeping fattening pigs in large groups (40 pigs per group) reduced costs per slaughter pig by 3.5%. However, group housing of sows in straw during lactation, mating and gestation (with prolonged lactation length of five weeks) raised production costs by 24.6% (+€ 32 per slaughter pig). In the latter situation, the welfare score was very high, but N and P excretion increased by 12.7% and 10.5% respectively.

Organic production is more expensive due to higher feed costs, lower feed efficiency and a lower number of piglets weaned per sow per year. In the UK, Bornett *et al.* (2003) estimated organic pork production to be 44% more expensive per kg carcass weight than free-range production (143.2 vs. 99.3 pence/kg) while costs for free-range production were 8% higher than for a conventional partly slatted system (92.0 pence/kg). In The Netherlands in 2001 and 2002, the organic production costs were calculated to be € 90 and € 94 per piglet, and € 2.51 and € 2.63 per kg carcass weight, respectively (Hoste, 2002 and Hoste, 2003, pers. comm.). This is about double the costs of conventional production. However, due to higher prices for organic piglets and pork, balances of organic piglet and pork production (excluding labour costs) were estimated to be 66% and 44% higher, respectively, than conventional production (Platform Biologica, 2002). In 1999, to stimulate transition to organic pork production, 22 Dutch parties in the whole pork chain agreed on price guarantees for a number of years.

Production costs for pork produced according to the Freedom Food scheme in the UK are about 7% higher than for a conventional partly-slatted production system (Bornett *et al.* 2003). These higher costs can be partly explained by the increased labour costs associated with the time required to clean out and replenish bedding (straw). Increasing space allowance by about 60% in accordance with new EU legislation, results in 1.5 and 2.1 pence/kg higher production costs for the partly slatted and Freedom Food system, respectively. Pork produced according to the IKB production scheme costs somewhat more than common production although accurate figures are not available. Although the impact of mainly welfare measures on total production costs seems to be modest, their effects on farm income are much larger and may well represent the difference between a viable and a non-viable business (Bornett *et al.* 2003).

### Discussion

The Netherlands is probably the country in the world where most of the concerns about pork and pork production originated. In that respect it might serve as a lighthouse for other countries or regions and as a stimulus to prevent similar problems. Although, in the past, profits for pig farmers have shown strong yearly fluctuations, in general terms pig production has been profitable, especially if production per farm was increased gradually. The number of pigs in The Netherlands increased dramatically in a relatively short time, from 5.5 million in 1970 to 10 million in 1980, to almost 14 million in 1990 and to a peak of over 15 million in 1997. Paralleling this increase in pig numbers, but with some delay, societal concerns also rose, which again with some delay, resulted in the legislation described above. A combination of incidents, legislation and a worsening economic perspective resulted in a rapid decline in the number of pigs to less than 11 million in 2003. The expectation is that the number will decrease further. As well as the increasing number of pigs, there has also been a concomitant increase in the number of Dutch chickens (laying hens and broilers) to a peak of almost 105 million, followed recently by a strong decline. Considering also there are about 4 million cattle, 0.7 million veal calves, 0.5 million horses and ponies, 1.4 million sheep and goats, 1.5 million turkeys and 16 million people, it is not surprising that a country as small as The Netherlands has enormous space and environmental problems. Also, the recent animal disease problems and the related animal welfare problems are, according to public opinion, mainly due to the high density of animals, and in particular pigs and poultry. Further, because many people think that sensory quality of pork depends to some extent on pig welfare, the whole complex of societal concerns is perceived to be largely related to the high numbers of pigs kept in intensive production systems on small land areas.

The policy of the Dutch government, now supported by the EU, is clearly aimed at reducing the number of pigs and providing the remaining Dutch pig production sector with a 'license to produce'. With respect to this second goal, the Dutch policy is predominantly aimed at meeting the concerns of citizens by legislatively-affecting the production process and by providing subsidies to producers to stimulate participation in alternative production schemes and to use various opportunities. This far-reaching legislation obviously results in strong concerns among pig farmers

because it affects their production 'freedom' and, more importantly, it costs them considerable money. Nevertheless, the efforts of the Dutch government seem to be yielding success. After years with little visible effect of legislation on the number of pigs, since 1998 a strong decrease in the number of pigs (and pig farmers) has been measured. Nevertheless, there is still a considerable production volume. This may be explained by the strong endurance of family farms that are willing to spend much of their own labour with low compensation, combined with high levels of technology and production efficiency. The final size of the Dutch pig industry is still difficult to predict and will depend largely on market developments. There is a chance, however, that part of the lost production in The Netherlands will be taken over by countries outside the EU. If these countries have pork safety and animal welfare rules that are less strict than in the EU, the net effect of legislation might be negative, at least in the short term, and that is certainly not what has been intended.

With respect to the remaining pig production sector, the efforts to meet the societal concerns seem to be succeeding. Currently there is a strong emphasis on safety of pork by legislation and by means of various safety-guaranteed programs. With respect to pig welfare, a number of legislative measures are already in effect (explorative material for all pigs, bulky feed for pregnant sows, minimum weaning age) and for others (group housing, minimum floor area) there is a transition period. Regarding environmental pollution, according to the Dutch RIVM institute, the emission of N and P to the soil has been reduced from 1980 to 2000 by 29% and 42%, respectively (RIVM, 2002). During the same period, NH<sub>3</sub> emission to the air has been reduced by 37%. In 2001 about 15% of the Dutch pigs were kept in emission-poor housing systems (RIVM, 2002).

Despite these successes in meeting societal concerns, there is no guarantee that the effects on public opinion will be sufficient to give approval for the remaining pig production sector. First, there is an information lag between the citizens and the pig industry and second, public opinion will always remain critical about keeping animals for production purposes. This (increasingly) critical attitude in Western Europe may be related to a combination of wealth and culture. If food availability and purchasing power were no longer major concerns, people may become more interested in other aspects of food, like the quality of food and food production. Moreover, in Western Europe there is an increasing consciousness of animal rights and integrity of animals. This means that animals should be kept under animal friendly ('humane') conditions and that humans cannot do everything they want to do with animals. In such a social climate, keeping and treating animals for production purposes, and in particular killing and slaughtering them, becomes questionable. Similar trends with respect to animal welfare are also apparent in other developed countries (e.g. Blandford and Fulponi, 1999).

In practice, a 'license to produce' and 'green lights' for the pig production sector would mean that the pork production system is found to be acceptable to society and that the sector receives full reward for its efforts. However, in the interim, the sector has to compromise between its corporate social responsibility and its survival in the economic competition of EU and world markets. Within the EU, national governments are not allowed to compensate farmers financially for higher levels of legislation. Developments on the world market also suggest that higher production costs are not likely to be compensated. The obvious solution is that local markets give the green light and reward the better production circumstances, resulting in a higher market price. However, the lack of substantial willingness to pay shows the ineffectiveness of the market to support societal demands. Another solution, often suggested by producers to promote a fair competition, is that all pork on the relevant markets should fulfil the same requirements, at least with respect to animal welfare. This option also seems to be a utopia, at least in the short term. In the long term, hopefully all pork producers will realise that societal concerns should be taken seriously.

Lack of financial compensation for the various efforts to meet societal concerns means that pork producers cannot do more than the minimum required. This bottom line is usually formed by legislation. Only a few pork producers can produce for niche markets and receive a higher price for their higher efforts. For the majority of future pork producers, the challenge is therefore to acquire a market share in a highly competitive world market within the legislative framework. Joint efforts of all sectors are needed to get a sustainable pig production sector. Consumers should show more considered purchasing behaviour, citizens must realise that too much pressure on the

pork production sector could have undesirable effects, governments should facilitate innovations as much as possible and the producers themselves should use all available opportunities to increase efficiency and quality of pork production.

### Conclusion

Pork production in Western Europe and in particular in The Netherlands is increasingly confronted with various societal concerns, mainly because of the high density of pigs. The most important concerns are related to pork safety and healthiness, animal welfare and health, environmental effects, sensory quality of pork, and pork price. In fact, these concerns have resulted in a red light for the Dutch pork production sector. Currently, these concerns are dealt with through legislation, promoting participation in alternative production systems and using various opportunities. Legislation has been effective in guaranteeing safer pork, a better welfare of pigs and less environmental pollution. However, the costs to the farmer have been high. In consequence, many farmers have ceased production and the number of pigs has decreased, which by default has reduced environmental problems. Unfortunately, because the remaining producers receive minimal reward for their efforts, in order to survive the economic competition of EU and world markets they cannot afford to do more than the minimum required. This means that animal-welfare concerns will not be fully met and the lights will not turn green for the pork industry. Solutions to this dilemma seem to be a higher consumer price for pork that satisfies the societal concerns or imposing the condition that all pork on the relevant markets fulfils the same standards, at least with respect to animal welfare. However, both options are not realistic in the short term. Within the legislative framework, only the joint efforts of consumers, citizens, governments and producers can result in a sustainable pig production sector.

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## PORK EATING QUALITY VARIES BETWEEN BUTCHERS

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Ageing pork for seven days post-slaughter improves sensory perceptions of tenderness and other eating quality attributes (Channon *et al.* 2001). In this experiment we evaluated the eating quality of commercially available pork purchased from three retail butchers using different ageing periods before sale. This experiment formed part of a larger marketing study through which consumers were supplied with quality-assured pork (Channon *et al.* 2003). It was postulated that pork aged at the store either as carcasses or in vacuum packaging for four days or more would have superior eating qualities to pork eaten after the two-days of ageing that often occurs in many retail situations.

Five caudal sections of deboned and denuded pork loins (*M. longissimus lumborum*) were obtained on four separate occasions from three independent retail butchers in Melbourne who were all supplied from the same processor/wholesaler. A total of 60 carcasses (n=20 per retailer) were represented. All pigs were of similar genetics and slaughtered at the same abattoir with carcasses distributed through the same channels. Loins from Retailer A were aged for 4 days on the carcass, Retailer B aged pork loins in vacuum packs for at least 7 days and Retailer C provided loins aged for only 2 days post-slaughter (control). Four 2.5 cm thick steaks were prepared from each loin before being frozen. Steaks were thawed before being grilled at 190°C for 5 min and rested for 2 min before presentation to consumers. A total of 80 consumers were involved in four taste panels, with each panellist assessing six half-steaks for tenderness, juiciness, flavour and overall quality (all 0-100 continuous line scales) and quality grade (1-5, ranging from unsatisfactory to premium quality), with higher scores indicating increased liking. Each loin was assessed by a total of eight consumers (8 half-steaks/loin).

**Table 1. Effect of ageing treatment (Retailer A:  $\geq 4$  days as carcass; Retailer B:  $\geq 7$  days in vacuum; Retailer C: 2 days ageing) on sensory attributes of pork loin steaks.**

	Retailer A	Retailer B	Retailer C	SED	P value
Tenderness	52.3	54.7	43.9	2.52	< 0.001
Overall Liking	55.9	57.1	49.6	2.36	0.003
Quality Category	3.21	3.30	2.91	0.12	0.003

Pork from Retailers A and B was more tender ( $P < 0.001$ ) and scored higher ( $P = 0.003$ ) for both overall liking and quality category compared with pork purchased from Retailer C (Table 1). No differences ( $P > 0.05$ ) in juiciness and flavour were found between retailers (data not presented).

These findings indicate that pork eating-quality can vary between butchers depending on the retailing systems they have in place. It is most probable that ageing of pork for at least 4 days post-slaughter resulted in superior eating quality compared with pork aged for two days. Since pork eating-quality was similar between retailers A and B, ageing either as a carcass or as a vacuum-packaged primal could be implemented at the retail level to improve consistency of pork eating quality.

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## COMMERCIAL VALIDATION OF ELECTRICAL STIMULATION OF PIG CARCASSES ON PORK QUALITY

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Channon *et al.* (2003) found that constant current electrical stimulation (CCES) of pig carcasses with 150 mA for 30 s at 5 min post-stunning improved eating quality, particularly tenderness and overall liking, without detrimentally affecting drip loss and pork colour. Given that constant voltage ES systems can increase drip loss from pork (Rees *et al.* 2003), the following experiment was done after the installation of CCES equipment at a commercial domestic pig abattoir. The purpose of the experiment was to demonstrate, in a commercial environment, the impact of electrical stimulation of pigs on pork quality.

A total of 36 entire male (Large White x Landrace) pigs from one producer (liveweight 75-85 kg) was processed according to normal commercial practices following three CCES treatments (n=12 per treatment) applied at 5 min post-stunning: (i) non-stimulated (control), (ii) stimulated with 150 mA for 20 s and (iii) stimulated with 300 mA for 20 s. Muscle pH of the loin *M. longissimus thoracis* (LT) was measured at the P2 site (located at the last thoracic rib 65 mm from the carcass midline) at 45 min, 3 h, 6 h and 24 h post-slaughter. Drip loss and muscle lightness (CIE L\*) were measured at 24 h post-slaughter on the LT muscle.

**Table 1. Effect of stimulation treatment (control, 150 mA or 300 mA) on muscle pH at 45 min and 3, 6 and 24 h post-slaughter, muscle lightness (L\* value) and drip loss in the loin (*M. longissimus thoracis*).**

	Control	150 mA	300 mA	SED	P value
pH 45 min	6.25	6.11	6.14	0.111	0.41
pH 3 h	6.07	5.94	5.79	0.135	0.14
pH 6 h	5.94	5.77	5.69	0.104	0.07
pH 24 h	5.59	5.50	5.48	0.055	0.12
Muscle lightness	44.08	46.07	48.30	1.823	0.08
% Drip loss	2.19	2.57	3.53	0.676	0.14

No effect ( $P>0.05$ ) of CCES was found for loin muscle pH measured at 45 min, 3 h and 24 h post-slaughter (Table 1). However at 6 h post-slaughter, muscles from control pigs had a higher pH ( $P=0.07$ ) compared with those stimulated with 150 mA and 300 mA. Pork from pigs stimulated with 300 mA tended to be paler in colour ( $P=0.08$ ) compared with control pigs and those stimulated with 150 mA. Although not significant ( $P>0.05$ ), average drip loss of pork from pigs stimulated with 300 mA was 0.96% higher than meat from carcasses stimulated with 150 mA.

These results suggest that CCES of pig carcasses with 300 mA is not ideal due to the higher drip losses observed from the loin muscle. As no differences in muscle lightness and drip loss were found between carcasses stimulated with 150 mA and those non-stimulated, CCES using 150 mA applied for 20 s at 5 min post-stunning was approved for commercial application to pig carcasses destined for a major supermarket chain.

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## ELECTRICAL STIMULATION OF PIG CARCASSES IMPROVES PORK EATING QUALITY

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Electrical stimulation of pigs with 150 mA for 30 s using a constant current system can improve the eating quality of pork (Channon *et al.* 2003). Following the commercial installation of a constant current electrical stimulation (CCES) unit, an experiment was done to determine if the measured increases in pork eating quality, resulting from electrical stimulation, were discernible to consumers.

Caudal sections of de-boned and denuded pork loins (*M. longissimus lumborum*) from the left side of 20 carcasses (electrically stimulated with 150 mA for 20 s at 5 min post-stunning) and 20 non-stimulated (control) carcasses from the same producer were obtained from the collaborating processor at 24 h post-slaughter. Four 2.5 cm thick steaks were prepared from each loin. All steaks were individually coded, vacuum packaged and frozen at 48 h post-slaughter. Steaks were thawed at 2°C for 24 h before being cooked on a Silex flat-plate grill at 190°C for 5 min and rested for 2 min before presentation to consumers. A total of 80 consumers were involved and each panellist was presented with four, pork-loin half-steaks. Each loin was assessed by a total of eight consumers for tenderness, juiciness, flavour and overall quality, with two consumers assessing each individual steak. Consumers used a continuous line scale to assess the quality of the steaks. The scales ranged from 0 to 100 with high scores indicating a positive feature.

**Table 1. Effect of electrical stimulation treatment on sensory attributes of pork loin steaks.**

	Control	Stimulation	SED	P value
Tenderness	48.3	53.9	2.39	0.020
Juiciness	59.2	62.0	1.90	0.14
Flavour	58.8	60.5	1.85	0.37
Overall Liking	53.4	58.1	2.19	0.032

Pork steaks from stimulated carcasses were more tender and obtained higher scores for overall liking compared with pork from control carcasses (Table 1). Although scores for juiciness and flavour were higher for loin steaks from stimulated carcasses, these differences were not significant ( $P>0.05$ ).

These findings indicate that the commercial application of constant current electrical stimulation to pig carcasses results in improvements in tenderness and overall liking of pork. The results also support previous work on CCES of pigs using 150 mA applied for 30 s at 5 min post-stunning that was done in a pilot abattoir (Channon *et al.* 2003). The adoption of this technology would therefore provide pig processors with a cost-effective means of enhancing the eating quality of pork.

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## BELLY FAT DETERMINATION USING DIGITAL ANALYSIS

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Belly fat content is a critical characteristic in the Singapore and domestic markets, where excessive fat is considered a product defect. In this work a method is described whereby the fat content of the belly can be predicted by computation based on the P2 backfat and analysis of a digital image of the belly.

We used 95 pigs of mixed sex between 78 to 147 kg liveweight and a P2 backfat ranging from 5 to 17 mm. Standard measurements including hot standard carcass weight and P2 backfat were taken. An image was taken of the anterior of the belly at the cross section level with the third thoracic vertebrae using a Fujifilm 4800Z digital camera set at 4 mega pixels. Digital image analysis was used to measure the intermuscular fat area (IMFA), subcutaneous fat area (SFA) and bone and muscle area (BMA). Analysis of each digital image was performed using Image Pro Plus v4.1.

Bellies were string-boned, partially frozen, minced and homogenised. A 1000 g sub-sample was mixed with 23 g sodium chloride and 300 g ice to produce the fine emulsion necessary to allow fat percentage to be measured using near infra-red (NIR) technology (Technicon 450 Infralyzer). The correlations between NIR measurements over the range of fat values were based on chemical analysis by means of soxhlet extraction using petroleum ether (40-60°C).

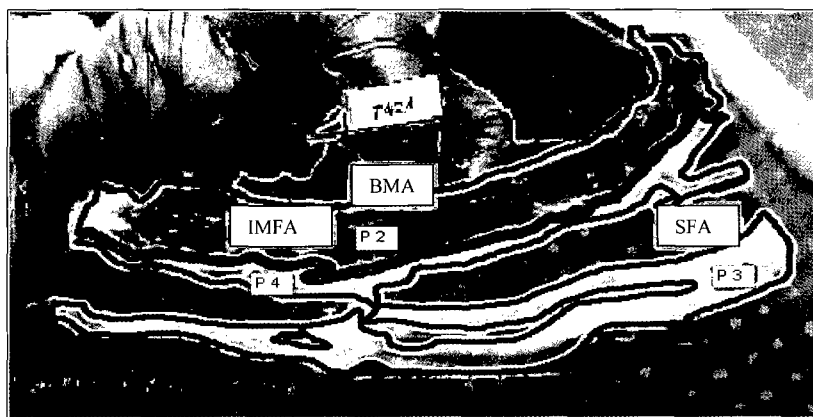


Figure 1. Definition of area measurements used for digital analysis.

Stepwise regression analyses were performed using listwise and pairwise exclusion of missing values to predict the belly fat percentage from the variables. Multiple regression accounted for 76.2% of the variation in belly fat.

$$\text{Belly Fat Percentage} = 13.689 + (0.484 * \text{IMFA}) + (0.549 * \text{P2}) - (0.226 * \text{BMA}) + (0.271 * \text{SFA}).$$

This procedure permits an accurate assessment of belly fat content that can be incorporated as a selection criterion of breeding stock when targeting the Singapore and domestic markets.

## PORK QUALITY DEFECTS IN 16 AUSTRALIAN ABATTOIRS

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Pale, soft, exudative (PSE) pork costs the Australian pork industry in excess of \$22M per year (Whan, 1993). Dark, firm and dry (DFD) pork is also unappealing and has recently become an issue for the export market. In this experiment we surveyed 16 Australian abattoirs across all states, covering about 80% of the Australian slaughter, to quantify the incidence of PSE and DFD. We then made recommendations to each abattoir on how to reduce PSE and DFD pork and re-surveyed the same abattoirs for the incidence of PSE and DFD pork. Surveys were done in autumn and spring.

Surveys were done over two consecutive days at each abattoir. About 20% of the daily slaughter was randomly assessed for meat quality. Pig handling, lairage management, the stunning process, temperature and time in scald-tank, dehairing, processing times along the chain, deep butt temperatures and chiller management were assessed at each abattoir. Muscle pH was measured at 6-8 h post-slaughter in the loin (*M. longissimus thoracis*, at the P2 site between the third- and fourth-last rib, 65 mm from midline) and the ham (*M. semimembranosus* next to the *tuber ischii*). Pork quality was described as PSE if the pH was  $\leq 5.6$ , normal if the pH was  $> 5.6$  and  $< 6.0$  and DFD when  $\text{pH} \geq 6.0$  (Joo *et al.* 1995). Carcasses were described as having an extensive (Ext) quality defect if the condition was found in both the loin and ham, or localised (Loc) if the condition was found in only one of the two sites. Analysis was performed using ANOVA.

**Table 1. Incidences (%) of pork quality categories across 16 Australian abattoirs.**

	Survey 1	Survey 2	sed	P value
No of pigs	4501	5128		
Ext PSE	14.3	6.5	3.10	0.027
Loc PSE	18.1	13.0	2.30	0.044
Normal	38.5	46.8	5.47	0.125
Loc DFD	16.1	18.3	1.47	0.290
Ext DFD	13.0	15.4	1.73	0.225

These data indicate a significant reduction in the level of PSE in response to management interventions. In general, there was a shift from PSE into the normal category. However, the increase (29.1 vs 33.7%,  $P=0.14$ ) in DFD is of growing concern to the export market. The management changes made following Survey 1 included improved handling of pigs, judicious use of electric goads, improved stunning procedure and improved management of the carcass in the chiller including spacing and rate of chill. Improvements in management can therefore be made at minimal cost that impact positively on pork quality. Abattoirs that implement positive changes, at pre- and post-slaughter, can expect to increase the production of high quality pork, resulting in economic benefits to the company and to the Australian pork industry as a whole.

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## TIME OF BONING AND TEMPERATURE CONDITIONING INFLUENCE TENDERNESS AND COLOUR OF PORK LOIN

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Pork carcasses are usually boned or distributed after chilling overnight. Hot boning involves processing the carcass before the body temperature has been substantially reduced. In this experiment we examined the impact of time of boning and temperature conditioning of carcasses on the quality of the pork loin. We hypothesised that hot-boning and fast chilling would cause cold-shortening and make the pork tough (Rees *et al.* 2002) but that this could be overcome by chilling the meat more slowly during the pre-rigor period.

Forty-five pigs were slaughtered over three days (15 pigs per slaughter) and the two carcass sides were randomly allocated to treatments in a 3 x 3+1 (control) design. The 3 x 3 treatments were: 1) Time of boning of the loin *M. longissimus et lumborum* (LTL), 3 h (WB3), 6 h (WB6) or 24 h (CB) and 2) Chill temperature, -20°C for 2 h then 2°C (T-20), 2°C (T2) or 14°C (T14). The +1 treatment was hot-boning at 45 min post-slaughter (HB). Before boning, the LTL was placed in a vacuum bag and stored at 2°C. The pH and temperature of the LTL were measured at 3 h post-slaughter. At 24 h post-slaughter, the Warner-Bratzler peak shear force (WBSF), surface colour (L\*) and drip loss (D. loss) were measured and samples collected for measurement of sarcomere length and then snap frozen.

At 3 h post-slaughter, the LTL temperature was different between treatments (15.7, 12.6, 18.2 and 23.4°C for HB, T-20, T2 and T14 respectively,  $P<0.05$ ) and the pH was lower for T14 (6.42, 6.42, 6.36, 6.28;  $P<0.05$ ). HB produced the shortest sarcomeres of all the treatments (1.72, 1.81  $\mu\text{m}$  respectively;  $P<0.05$ ). The LTL from carcasses that were cold-boned and chilled at 2°C or 14°C were generally the most tender (lowest WBSF) whereas the LTL from carcasses chilled at -20°C and boned at 6 h or hot-boned, were the toughest (highest WBSF) (Table 1). The LTL of carcasses chilled at 14°C and warm-boned at 6 h or cold-boned were lighter in surface colour (L\*) as was the LTL of carcasses cold-boned and chilled at 2°C. Surface lightness was detrimentally affected by 14°C treatment and even LTL from a 2°C chilling/cold-boning process was unacceptably pale. Tenderness was detrimentally affected by hot or warm boning and by -20°C or 2°C chilling and this was only overcome by 14°C chilling pre-rigor and warm-boning at 6 h, supporting the hypothesis. These results highlight the trade-off in the ideal temperature decline to achieve optimum meat quality in terms of colour and tenderness.

**Table 1. The effect of chilling temperature and time of boning on meat quality traits.**

	Chill at -20°C (T-20)				Chill at 2°C (T2)			Chill at 14°C (T14)		
	HB	WB3	WB6	CB	WB3	WB6	CB	WB3	WB6	CB
D.Loss (%)	2.9 <sup>a</sup>	3.1 <sup>a</sup>	4.4 <sup>b</sup>	2.9 <sup>a</sup>	2.7 <sup>a</sup>	3.5 <sup>a</sup>	3.0 <sup>a</sup>	3.3 <sup>a</sup>	2.7 <sup>a</sup>	3.2 <sup>a</sup>
WBSF (kg)	10.8 <sup>ab</sup>	9.5 <sup>b</sup>	11.8 <sup>a</sup>	9.5 <sup>b</sup>	9.6 <sup>b</sup>	10.0 <sup>b</sup>	7.1 <sup>c</sup>	10.3 <sup>ab</sup>	8.8 <sup>b</sup>	7.5 <sup>c</sup>
Surface L*	45.6 <sup>a</sup>	46.5 <sup>a</sup>	45.3 <sup>a</sup>	46.8 <sup>a</sup>	46.6 <sup>a</sup>	46.0 <sup>a</sup>	50.0 <sup>b</sup>	47.1 <sup>a</sup>	48.2 <sup>b</sup>	49.3 <sup>b</sup>

<sup>abc</sup> Different letters across columns show differences between treatments ( $P<0.05$ ).

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## BENCHMARKING THE EATING QUALITY OF BRANDED PORK IN WESTERN AUSTRALIA

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Two consumer-focused alliances have been set up in Western Australia to offer the consumer a high eating-quality 'branded' pork product. The 'Select Pork' brand (supermarket alliance) involves quality interventions at the producer (crossbred Duroc bloodlines, Halothane free genetics, and immunocastration), processor (aitch bone carcass hanging), and retailer (ageing protocol) stages. The processor and retailer stage interventions for Select Pork will be implemented in mid-2003. The 'Linley Valley Fresh' brand (food service alliance) involves quality interventions at the producer (genetics, breed, and sex) and the processor (brine enhancement) stages. The aim of this experiment was to compare the eating quality of 'branded' pork with non-branded 'generic' pork.

Pork samples from the *Longissimus thoracis* (loin) muscle of male pigs were selected 24 h post-slaughter from five Select Pork producers, one Linley Valley Fresh producer and two 'generic' pork producers. Four loins per producer were randomly selected and five steaks per loin were used in a consumer taste panel. One hundred and sixty boneless *Longissimus thoracis* steaks (2 cm thick) were cooked using a Silex flat-plate grill and were cooked for 5 min at 190°C (internal temperature 75°C; medium/well done). Each steak was halved after cooking and tasted by two consumers. Sixty-four consumers tasted a total of five half-steaks each, over one session. Consumers assessed the steaks for aroma, tenderness, juiciness, flavour and overall acceptance. All data were analysed by ANOVA.

**Table 1. The eating quality of the *Longissimus* muscle from generic pork (G), Select Pork (SP) and Linley Valley Fresh (LVF) pork.**

Brand	G	SP	LVF	l.s.d	Significance
Aroma <sup>1</sup>	55	63	57	6.54	0.002
Flavour <sup>1</sup>	54	66	76	6.11	<0.001
Juiciness <sup>1</sup>	43	58	75	6.85	<0.001
Tenderness <sup>1</sup>	41	59	75	7.40	<0.001
Overall acceptability <sup>1</sup>	48	64	76	6.67	<0.001

<sup>1</sup>Acceptability score (line scale): 0 = dislike extremely and 100 = like extremely.

The consumer taste panel results indicate that steaks from Select Pork were considered to have the better aroma when compared to steaks from generic and Linley Valley Fresh pork (Table 1). Pork from Linley Valley Fresh was considered to have the best flavour, juiciness, tenderness, and overall acceptability scores followed by Select Pork and then generic pork. Branded pork from Linley Valley Fresh and Select Pork were considered by consumers to have significantly better eating quality when compared to generic pork while Linley Valley Fresh pork was considered to have the best eating quality when compared to Select Pork and generic pork.

## A CAPSAICIN ANALOGUE IMPROVES GROWTH AND DRESSING RATE IN PIGS, PARTICULARLY GILTS

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Capsaicin (8-methyl-N-vanillyl-6-nonenamide), a constituent of capsicum, is known to react with capsaicin receptors in many tissues in the body and there is evidence that feeding red peppers to humans can increase fat oxidation via a stimulation of the  $\beta$ -adrenergic system (Yoshioka *et al.* 1998). The aim of this experiment was to determine if a dietary capsaicin analogue could increase growth and decrease fat deposition in the finisher pig.

Thirty-six Large White x Landrace pigs (18 boars and 18 gilts) were allocated to a 6 x 2 factorial design with the respective factors being dietary capsaicin analogue N-vanillylnonamide (0, 5, 15, 40, 80 and 120 mg/kg feed) and sex. Pigs were kept in individual pens and from 56 kg liveweight (LW) were fed their respective diets containing 14.4 MJ DE/kg and 0.5 g available lysine/MJ DE for five weeks. Body composition was determined by DXA at the start of the experiment and again just before slaughter to determine tissue deposition rates. Dressing rate was determined by dividing head-on carcass weight by liveweight. Data were analysed by ANOVA.

**Table 1. Effect of sex (S) and dietary vanillylnonamide (V) in finisher pigs.**

	Sex	Vanillylnonamide (mg/kg)						SED	Significance <sup>A</sup>
		0	5	15	40	80	120		
Daily gain (g/d)	Gilt	829	1217	1121	941	1156	998	100.2	S*** V+
	Boar	1333	1354	1269	1276	1261	1406		SxV*
Lean gain (g/d)	Gilt	450	523	495	480	547	483	54.9	S***
	Boar	668	661	602	650	639	711		
Fat gain (g/d)	Gilt	247	384	372	282	391	332	51.5	S*** SxV+
	Boar	435	424	424	393	396	513		
Dressing rate (g/kg)	Gilt	817	824	814	824	826	835	10.3	S* V*
	Boar	798	810	824	804	813	820		Linear V*

<sup>A</sup> Significance levels as follows: \*P<0.10; \*\*P<0.05; \*\*\*P<0.01; \*\*\*\*P<0.001.

Pigs fed diets containing vanillylnonamide tended to grow faster than control pigs (1221 vs 1327 g/d, P=0.059, Table 1). However, there was a significant interaction between sex and vanillylnonamide (P=0.029) such that vanillylnonamide increased daily gain in gilts (829 vs 1084 g/d) but not boars (1333 vs 1313 g/d). Effects of vanillylnonamide on tissue deposition rates were not as apparent as the effects on growth performance. While there was no significant effect of level of vanillylnonamide on lean tissue or fat deposition, bone mineral deposition tended (P=0.067, data not shown) to increase linearly with increasing level of dietary vanillylnonamide. There was also a linear increase (P=0.034) in dressing-out rate with increasing level of dietary vanillylnonamide. Pigs fed diets containing vanillylnonamide had a 12 g/kg increase in dressing-out rate compared to control pigs (808 vs 820 g/kg, P=0.047). In conclusion, capsaicin, or at least the analogue vanillylnonamide, is biologically active in finisher pigs and can improve growth rate and dressing rate, particularly in gilts.

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## CAPSAICIN TREATMENT OF NEONATAL PIGS DECREASES SUBSEQUENT LEAN AND FAT DEPOSITION

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Capsaicin-treatment (8-methyl-N-vanillyl-6-nonenamide) of neonate rats causes deafferentation of capsaicin-sensitive sensory nerves with long-term effects on feed intake, metabolism and fat mass (Melnik and Himms-Hagen, 1995). A major constraint to the Australian pig industry is the excessive deposition of fat and associated poor feed-conversion efficiency of pigs. The aim of this experiment was to determine whether capsaicin treatment of neonatal pigs reduced subsequent feed intake and fat deposition.

Twelve, mixed-parity Large White x Landrace sows (average litter size, 10) were used to nurse pigs. On day one of lactation, the median four male pigs from each litter were randomly allocated to one of four doses of capsaicin (0, 20, 40 and 80 mg/kg) administered subcutaneously on days one and four of life. Pigs were weaned at 21 days and offered feed *ad libitum* until slaughter at 134 days of age. Body composition was measured using dual energy X-ray absorptiometry at 21, 49, 77, 105 and 133 days of age (Suster *et al.* 2003). Data were analysed by ANOVA.

**Table 1. Effect of capsaicin on growth and tissue deposition rate of pigs between 105 and 133 days of age.**

	Dose of capsaicin (mg/kg)				sed	Significance	
	0	20	40	80		Linear	Quadratic
Daily gain (g/d)	1137	1098	1092	1034	52.6	0.062	0.99
Feed intake (g/d)	3162	3078	3082	2957	116.0	0.097	0.96
FCR (g/g)	2.78	2.85	2.84	2.90	0.102	0.30	0.85
Lean gain (g/d)	731	707	674	669	31.5	0.049	0.38
Fat gain (g/d)	264	298	271	250	24.3	0.29	0.24

Capsaicin did not increase growth performance during the nursing, weaner and grower phases however, over the finisher period there were linear decreases in daily gain, feed intake and lean tissue gain but not fat gain, with increasing dose of neonatal capsaicin treatment (Table 1). There were linear decreases in lifetime fat (130, 131, 126 and 116 g/d for pigs treated with 0, 20, 40 and 80 mg/kg capsaicin,  $P=0.046$ ) and lean deposition (561, 567, 536 and 526 g/d,  $P=0.029$ ) with increasing dose of neonatal capsaicin. As lean tissue deposition is decreased with neonatal capsaicin treatment, capsaicin does not appear to be a viable means of reducing feed intake and fat deposition.

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## RISK FACTORS ASSOCIATED WITH *LAWSONIA INTRACELLULARIS* INFECTION

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In this experiment we sought to identify (1) the production phase during which pigs become infected with *L. intracellularis* within a range of piggery management systems and (2) the management factors most associated with an increased risk of *L. intracellularis* infection.

Management and disease information from 12 piggeries in NSW and Victoria was collected through a questionnaire and serological testing of about 5% of pigs at the end of each stage of production (about 10, 14, 17 and 22 weeks of age). Sera were diluted 1:30 and tested for IgG antibodies to *L. intracellularis* using a method specific for pigs exposed to *L. intracellularis* and with a sensitivity of 0.9 and a specificity of 0.99 (Knittel *et al.* 1998). The questionnaire variables were analysed by logistic regression using GenStat.

Discrete variables included the age (weeks) when pigs were bled and the number of pigs sold per week. The proportion of pigs with antibodies to *L. intracellularis* was entered as a continuous variable. Categorical variables included: the frequency of diarrhoea (described by producers as present in no batches, 10-20% of batches, or more than 50% of batches); production flow (all-in-all-out *vs* continuous); flooring type (slatted *vs* solid); mixing of pigs within and between production phases; the stocking rate relative to the code of practice and APL recommendations (Payne *et al.* 2000); the cleaning and/or disinfection of pens between batches, and housing type (ecosheds or conventional). Antibiotic medication was recorded for the production period preceding the age when pigs were bled and categorised on whether it prevented *L. intracellularis* infection or not (Collins *et al.* 2001).

Initially all 51 production-groups were used in a stepwise regression to determine the subset of variables that explained most of the variation in the proportion of pigs with antibodies to *L. intracellularis*. However, risk factors may be obscured in pigs medicated with antibiotics that prevent *L. intracellularis* infection and the regression was therefore repeated omitting the 10 sample groups that used preventative antibiotics. The strength of association between management factors and the proportion of pigs infected with *L. intracellularis* was measured by odds ratio analysis.

Continuous flow production systems led to a 5.87-fold increase in the odds of *L. intracellularis* infection compared with all-in-all-out batch production ( $P=0.003$ ). The odds of *L. intracellularis* infection also increased significantly for pigs where diarrhoea was observed in more than 50% of batches, or in 10-20% of batches compared with pigs where diarrhoea was never observed (31.94-fold and 6.70-fold respectively). Diarrhoea was predominantly observed in weaner pigs and is not a specific clinical sign of *L. intracellularis* infection.

The serological assay identified when pigs became infected with *L. intracellularis* and that the antibiotic changes and the movement and mixing of pigs within and between production phases were the principal management factors associated with *L. intracellularis* infection. The logistic regression model identified continuous production systems and the increased incidence of diarrhoea as significant risk factors for *L. intracellularis* infection.

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## HAEMOLYSIS IN BACTERIAL POPULATIONS PRESENT IN RECTAL SWABS FROM HEALTHY AND SCOURING WEANERS

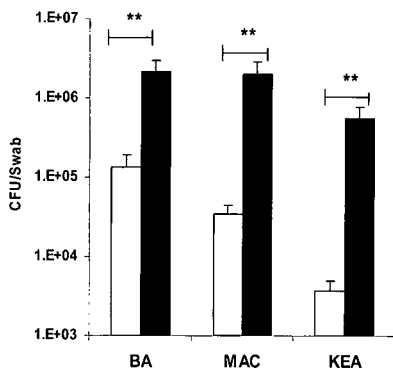
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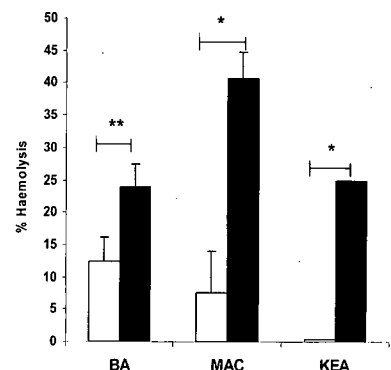
In Australia, post-weaning diarrhoea is associated with haemolytic enterotoxigenic *E. coli* (ETEC) and is predominantly caused by one of a number of serotypes: O8, O139, O141 and O149. Bacteriological confirmation of colibacillosis is carried out routinely by streaking faeces onto nutrient Blood Agar (BA) or selective MacConkey Agar (MAC). One or two haemolytic and lactose-fermenting colonies are selected and then serotyped. This protocol assumes that all colonies in the scouring samples are identical ETECs belonging to a single clonal serotype. The aim of this experiment was to confirm the clonal status of ETECs, as well as other species in the faecal population, and to assess differences in frequency of haemolysis between samples from scouring and healthy weaner piglets.

Rectal swabs were collected from weaner piglets (eleven healthy – five male and six female, and eleven scouring – only one female) aged between five and eight weeks. Individual swabs were expressed in BHIB containing 20% glycerol. Aliquots were spread directly onto hydrophobic grid membrane filters (HGMFs) which were then incubated (37°C overnight) on Blood (BA), MacConkeys (MAC), and Kanamycin-Esculin-Azide (KEA) agars respectively. Each filter was then replicated using sterile 1600-point inoculators. These were then enumerated for haemolysis by inverted placement onto BA.

Bacteria (colony forming units or CFU) from swabs of scouring weaners showed a consistently higher recovery rate on BA, MAC and KEA compared to healthy controls. On a population basis (representing between 200-300 CFU per filter), there was a consistently higher incidence of haemolytic bacteria on BA in scourers compared to healthy controls (Figure 2). Interestingly, the extent of haemolytic bacteria from the MAC grid population was only 40% in scourers and less than 10% in healthy weaners. This indicates that the *E. coli* clones in the scouring population are most likely not clonal and cannot be identical as more than half of the population are not haemolytic. Similar analysis with the KEA grid population also showed a significant presence of haemolytic *Streptococci* in the bacterial population from scourers compared to healthy weaners. Analysis of a bacterial population on selective media can provide a more realistic picture of the role of both pathogenic and non-pathogenic bacterial communities in health and disease.



**Figure 1.** Enumeration of bacteria in swabs collected from healthy (□) and scouring (■) pigs on Blood (BA), MacConkeys (MAC) and Kanamycin-Esculin-Azide (KEA) Agar. \*\* $P < 0.01$  (t-Test)



**Figure 2.** Frequency of haemolytic colonies collected from healthy (□) and scouring (■) pigs in representative grid-replicated populations of bacteria cultivated on Blood (BA), MacConkeys (MAC) and Kanamycin-Esculin-Azide (KEA) Agar. \* $P < 0.05$ , \*\* $P < 0.01$  (t-Test)

## PLASMA IGF-I AS A POTENTIAL METABOLIC INDICATOR OF STRESS AND WELFARE IN GROWING PIGS

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Given the multi-factorial nature of its regulation, it is highly likely that circulating IGF-I may serve as an index of metabolic well being, thereby acting as a measure of the stress and welfare status of an animal. We have shown that plasma IGF-I concentrations of pigs decrease in response to the psychological stress of grouping unfamiliar pigs, and also when growing pigs are exposed to high ambient temperatures for 7 days (Kerr *et al.* 2002). As IGF-I is a circulating anabolic growth factor influenced by many of the key metabolic regulators, it is not surprising that IGF-I status reflects lean tissue growth and growth rate in growing pigs. This is the converse of the negative relationship between these factors in the newborn pig, an observation that is used currently as a growth selection tool. These relationships are complicated further through the paracrine and autocrine modes of action of IGF-I in the musculature and other metabolically important tissues. In this experiment we evaluated correlations between plasma IGF-I concentrations with measures of growth performance, plasma cortisol status and metabolic indices in response to a pathological, psychological and environmental challenge.

Plasma IGF-I concentrations were measured by ELISA in response to: 1) a subclinical dose of *Actinobacillus pleuropneumoniae* (App) and heat stress (Kerr *et al.* 1999) and 2) in response to grouping of unfamiliar pigs and heat stress (Kerr *et al.* 2002). Feed intake, weight gain and plasma IGF-I were significantly depressed ( $P < 0.05$ ) in response to the treatments in both experiments and cortisol increased in response to only App in Experiment 1. We evaluated all possible correlation coefficients (Pearson's) between plasma IGF-I concentrations and growth performance indicators, plasma cortisol and plasma metabolic indicators. These calculations are reported here when the effects of the stress challenges were maximal for weight gain and feed intake: 48 hours post App infection and after 24 h at 30°C for Experiment 1 and 7 days after grouping and/or 30°C for Experiment 2.

**Table 1. Summary of significant ( $P < 0.05$ ) plasma IGF-I concentration correlations.**

Expt.	Feed Intake	Weight Gain	Plasma Cortisol	Skin Temp.	Respiration Rate	Plasma Glucose	Plasma Urea Nitrogen	Plasma Insulin
1 <sub>App</sub>	0.67	-	ns	ns	ns	-	-	-
1 <sub>Heat</sub>	0.69	0.61	0.44	-0.39	ns	-	-	-
2	0.31	0.57	0.22	-	-	ns	0.22	ns

Plasma IGF-I was positively correlated with feed intake and weight gain in both experiments. Plasma IGF-I was positively correlated with plasma cortisol for two of the time points where plasma cortisol was not significantly altered. These two experiments provide strong evidence that plasma IGF-I concentrations may be a useful metabolic measure of the impact of stress and welfare status in growing pigs.

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## BIRTH WEIGHT AND WEANING WEIGHT AS PREDICTORS OF PIG WEIGHT AT SLAUGHTER

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Pig performance during suckling and immediately post-weaning, must be optimised to minimise ill health and mortality. Weaning weight and growth during the week after weaning are significant predictors of subsequent piglet performance (Miller *et al.* 1999). The aim of this experiment was to determine whether weaning weight is a better predictor of lifetime performance than birth weight. We also examined the relationship between pre-and post-weaning growth and growth performance to slaughter.

One hundred and ninety-four hybrid pigs (62.5% Large White, 25% Landrace, 12.5% Duroc) from 44 litters were tagged and weighed within 12 h of birth. Pigs were weighed at weaning (25.7 ± 0.2 days) and on days 7, 20 and 118 (slaughter) after weaning. Pigs were housed under normal commercial conditions. Linear and multiple regression analyses were performed with Minitab 12.2 to identify relationships between pig weight on day 118 post weaning (d118) and pre- and post-weaning performance parameters.

**Table 1. Pig weights between birth and slaughter (mean ± sem), average daily gain (ADG) between birth and weaning and between weaning and day 20 post-weaning. Relationship between pig weight on day 118 and birth weight (wt), wean wt, day 20 post-weaning wt, ADG (average daily gain) between birth and weaning, ADG between weaning and day 20 post-weaning (ADGw-20), and wean age.**

Measured Variables	Linear Regression analyses (relationship with day 118 wt)				
	Predictor	R-Sq %	Regression equation	P value	
Birth weight	1.69 ± 0.02	Birth wt	7.2	y = 74.6 + 0.009x	<0.001
Wean weight	8.69 ± 0.12	Wean wt	20.9	y = 64.4 + 2.92x	<0.001
Day 20 wt	15.2 ± 0.2	Wean age	16.8	y = 58.6 + 1.21x	<0.001
Day 118 wt	89.8 ± 0.7	ADG birth-wean	4.1	y = 77.9 + 0.044x	<0.05
ADG birth-wean	272 ± 3.5	ADG wean-d20	25.0	y = 71.1 + 0.058x	<0.001
ADG wean-d 20	325 ± 6.4				

Weaning weight and growth during the initial post-weaning period were more important than birth weight and growth during the suckling period for predicting pig performance to slaughter (Table 1). Multiple regression analysis showed that weaning weight and ADGw-20 were additive predictors of d118 wt; d118 wt = 57.2 (SEM = 3.51) + 2.07 (SEM = 0.40) wean wt + 0.05 (SEM = 0.01) ADGw-20 (R-sq = 34.3%, P<0.001). This highlights the significance of the immediate post-weaning period in determining the lifetime performance of the pig. Thus, improving piglet performance by increasing feed intake and food conversion efficiency during this period should result in pigs reaching the desired slaughter weight more rapidly. Surprisingly, birth weight and growth during the suckling period made little contribution to pig weight on d118, despite being good predictors of wean weight (wean wt = 4.76 + 0.002 birth wt, R-sq=19.5%, P<0.001; wean wt = 1.65 + 0.026 ADG birth-wean, R-sq= 58.58%, P<0.001). However, maximising growth during the suckling period will produce a heavier, more viable piglet at weaning, and lead to higher growth rates to slaughter.

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## ONIONS DECREASE TRIGLYCERIDES IN PIGS WITH A HISTORY OF HIGH DIETARY FAT CONSUMPTION

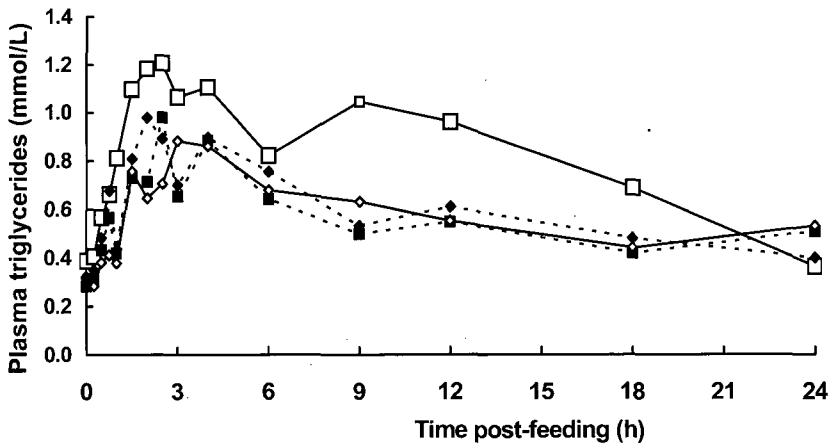
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Consumption of raw onion (*Allium cepa*) has been shown to reduce circulating triglycerides (TG) in pigs in some studies (Ostrowska *et al.* 2001) but not in others (Gabler *et al.* 2003). The different findings may have been because the onions varied in their concentration of the active compounds, cysteine sulfoxides and flavonols, or because of historical differences in fat intake or the time of sampling for TG. In this experiment, we examined the effect of fat intake and sampling time on plasma TG.

Eighteen boars and 18 gilts (Large White x Landrace) were individually-penned and pre-fed either a low (5%) or high (25%) fat diet for 21 days at 1.67 MJ DE/kg<sup>0.75</sup>. The fat content of both diets consisted of 58% tallow and 33% canola oil. After 21 days, all pigs were fed the high-fat diet either alone or supplemented with raw brown onion (25 g/MJ DE) for a further 28 days. After 21 days the pigs were surgically prepared with venous catheters. On day 28 frequent blood samples were obtained post-feeding and plasma analysed for TG.



**Figure 1.** Effect of high and low historical fat consumption and dietary onion on mean plasma triglycerides. Data are pooled across sexes (SED=0.168). (□ high fat, ■ high fat onion, ◇ low fat, ◆ low fat onion).

Consuming brown onion tended to reduce mean plasma TG (0.71 vs 0.54 mmol/L,  $P=0.053$ ) with the response most pronounced between 6 h and 18 h post-feeding ( $P=0.012$ ) (Figure 1). However, this occurred only in pigs previously fed a high-fat diet, as indicated by the dietary fat x onion interaction ( $P=0.020$ ). Over the 6-18 h interval, dietary onion decreased plasma TG in pigs that had been fed a high-fat (0.93 vs 0.48 mmol/L) but not a low-fat (0.48 vs 0.54 mmol/L) diet during the pre-feeding period. In conclusion, TG concentration is affected by time of sampling and raw onion consumption may aid in reducing postprandial TG in subjects with a history of high fat intake.

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## COMPARISON OF DIAGNOSTIC PROCEDURES FOR THE IDENTIFICATION OF *MYCOPLASMA HYPONEUMONIAE*

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Definitive diagnosis of *Mycoplasma hyopneumoniae* (Mhp) infections can often be difficult. Routine diagnostic culture is not practical for Mhp detection, as the organism is fastidious and extremely slow growing by nature. The National Pig Health Monitoring Scheme (PHMS) (Pointon *et al.* 1999) has been in place for some time and is widely used to identify disease problems present in finisher pigs at the time of slaughter. Although PHMS is effective in identifying the presence of pneumonia, it cannot clearly determine the causative agent responsible for the disease.

This experiment involved a survey of 10 pig herds (five known to be Mhp positive and five known to be Mhp negative) and the use of four diagnostic procedures to detect Mhp from samples collected at slaughter. A piece of the apical lobe of the lung and a corresponding serum sample were taken from 30 animals from each herd and tested. A single round Polymerase Chain Reaction (PCR), with primers specific to Mhp (Baumeister *et al.* 1998) was performed on DNA extracted from lung tissue. Serum was analysed in a commercial Mhp-specific Enzyme Linked Immunosorbent Assay (ELISA) (DAKO K0043). Culture of Mhp was attempted using a variation of the Friis media (Friis, 1975) and scoring of the lung was performed using the PHMS technique. The results of these tests (Table 1) were analysed for association using the *kappa test*.

**Table 1. Percentage of positive pigs under each test for herds 1 through 10.**

Test/Herd	1	2	3	4	5	6	7	8	9	10
PCR	16.6	50.0	83.3	80	90.0	0	0	0	0	0
ELISA	60.0	100.0	60.0	86.6	86.6	0	0	0	0	0
Culture	0	0	0	0	0	0	0	0	0	0
Lung score	36.6	70.0	33.3	23.3	36.6	3.3	0	0	10.0	0

All herds were negative for culture, indicating the difficulty of culturing Mhp, while lung scoring was an effective method of monitoring the disease status of a herd. The ELISA and PCR tests had a good association ( $\kappa=0.62$ ,  $p<0.001$ ) to each other. Both PCR and ELISA were also moderately associated with the presence of pneumonia (acute and chronic lesions). The PCR and ELISA both appeared to be sensitive and specific for detecting the presence of Mhp in finishers from infected herds. No false positive results were returned for the five negative herds for either PCR or ELISA. Variations in correlation between the two tests are likely to be due to differences in sensitivities of the tests and the delay between infection and seroconversion. Both PCR and ELISA appear to be useful tools for confirming the Mhp status of a herd.

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## INDUCED PROLIFERATIVE HAEMORRHAGIC ENTEROPATHY AND ITS EFFECT ON PERFORMANCE OF FINISHER PIGS

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Proliferative Haemorrhagic Enteropathy (PHE) is the most severe form of the Porcine Proliferative Enteropathies (PPE) caused by *Lawsonia intracellularis*. The disease occurs more frequently in adult pigs and causes bloody diarrhoea and high mortality. Experimentally, PHE has been reproduced by oral challenge of adult pigs with fresh faeces from PPE-affected pigs (Collins, 2000). The aim of this experiment was to investigate if PHE could be reproduced by challenge of finisher pigs using mucosal homogenate from PHE-affected intestine and to assess the effect of the disease on the performance of these challenged pigs.

Twelve 17-month old Landrace x Large White pigs were housed in two pens with automatic feeders and orally dosed with mucosal homogenate containing approximately  $10^8$  *L. intracellularis*. Faeces and blood were collected weekly for detection of *L. intracellularis* DNA by polymerase chain reaction (PCR) and specific IgG antibodies against *L. intracellularis* by immunofluorescent antibody test (IFAT). Clinical signs, weight gain (G) and feed (F) consumption were recorded daily for a period of four weeks. Pigs that developed signs of PHE were slaughtered and examined for gross and histological lesions of PHE.

Faecal shedding of *L. intracellularis* was detected in all pigs 7-14 days post inoculation (pi). All pigs developed IgG antibodies as detected by the IFAT from day 21 pi. Two pigs developed watery, dark diarrhoea and anorexia on day 20 pi. Post-mortem and histological examination revealed gross lesions and histopathological changes of PHE. Severe diarrhoea and weight loss were observed in the other four pigs. Five out of six pigs with diarrhoea developed specific IgG antibodies that were detectable by IFAT at 1:240 dilution while a lower level of IgG (less than 1:120 dilution) was detected in the other infected pigs. On average, the finisher pigs gained  $0.7 \pm 0.32$  kg and consumed  $1.9 \pm 0.28$  kg feed/d. Average daily feed to gain ratio (F/G) was 3.95 for the whole group for four weeks. Analysis of variance showed that significantly reduced daily weight gains and increased F/G ratios occurred in the fourth week of the experiment (Table 1).

**Table 1. Average daily weight gain, feed consumption and feed to gain ratio of challenged pigs over four weeks (n=12).**

Week pi	Average daily G	Average daily F	Average daily F/G
1	0.88 <sup>a</sup>	1.77	2.11
2	0.87 <sup>a</sup>	2.13	2.71
3	0.60	1.90	3.91
4	0.44 <sup>b</sup>	1.79	7.06
Mean	0.68	1.90	3.95
SEM	0.11	0.08	1.10
P value	0.01	0.06	0.08

<sup>ab</sup>Data in columns with different superscripts differ significantly  $P < 0.05$ .

Oral challenge with mucosal homogenate from pig intestine affected by PHE reproduced PHE in a portion of exposed finisher pigs. Pigs with more severe clinical signs of the disease developed a more intense level of IgG antibodies against *L. intracellularis*. Four weeks after initial infection, daily weight gain was significantly reduced and the feed to gain ratio was increased in finisher pigs challenged with *L. intracellularis*.

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## HAEMOLYSIS IN BACTERIAL POPULATIONS FROM RECTAL SWABS OF HEALTHY AND SCOURING NEONATAL PIGLETS

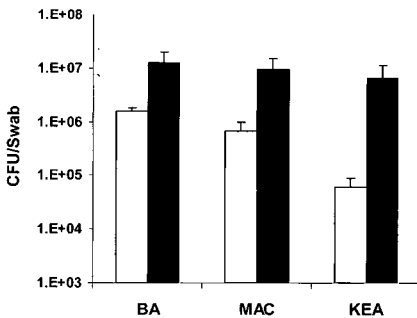
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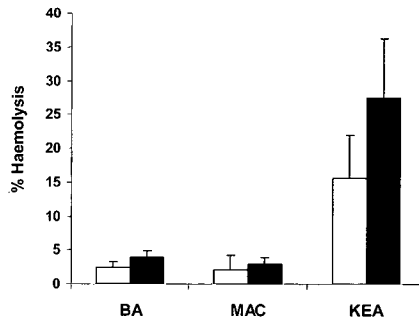
Neonatal diarrhoea associated with *E. coli* belonging to serotypes O8, O9, O20, O64, O101, O138, O141, O149 and O157 occurs in piglets in the first 96 h of life. Diagnosis is confirmed by plating faecal samples onto MacConkeys Agar (MAC) followed by serotyping of one or two lactose-fermenting colonies. Such restrictive sampling for *E. coli* does not provide information about other types of bacteria that may also be associated with neonatal diarrhoea. The aim of this experiment was to investigate the role of other bacterial species in neonatal diarrhoea by comparing haemolytic bacterial populations in swabs taken from healthy and affected piglets.

Rectal swabs were collected from piglets (six healthy - three male and three female, and eight scouring - two male and six female) up to 96 h old. Individual swabs were expressed in BHIB containing 20% glycerol. Aliquots were spread directly onto hydrophobic grid membrane filters (HGMFs) which were then incubated (37°C overnight) on blood (BA), MacConkeys (MAC) and Kanamycin-Esculin-Azide (KEA) agars respectively. Each filter was then replicated using sterile 1600-point inoculators. These were then enumerated for haemolysis by inverted placement onto BA.

There was a consistently higher recovery of bacteria from swabs of scouring piglets (Figure 1). This could be due to the higher absorption rate of wet faecal material from the scourers into the swabs. Although the level of haemolysis was relatively low, there was a significant ( $P < 0.05$ ) increase in the haemolytic bacteria in the MAC population of scourers compared to healthy neonates (Figure 2).



**Figure 1.** Enumeration of bacteria in swabs collected from healthy (□) and scouring (■) piglets. Bacteria were cultivated on Blood (BA), MacConkeys (MAC) and Kanamycin-Esculin-Azide (KEA) Agars.



**Figure 2.** Frequency of haemolytic colonies in representative grid-replicated populations of bacteria cultivated on blood (BA), MacConkeys (MAC) and Kanamycin-Esculin-Azide (KEA) Agar.

Interestingly, there was a trend towards higher levels of haemolysis in the *Streptococci* population from scouring piglets which, while not statistically significant, warrants further analysis to determine the role of *Streptococci* in neonatal scours.

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## RAW AND COOKED ONION CONSUMPTION ALTERS LIPID METABOLISM AND LIPOGENIC ENZYMES IN THE PIG

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Consuming raw onion (*Allium cepa*) reduces blood lipid and cholesterol levels (Ostrowska *et al.* 2001). The compounds responsible for this bio-activity of onion, cysteine sulfoxide (CSO) and quercetin, are affected by food processing (Gabler *et al.* 2003). The aim of this experiment was to determine if raw and cooked onion affects blood lipids and *de novo* fatty acid synthesis in pigs.

Forty individually-penned Large White x Landrace pigs (gilts) were pre-fed a high fat (25%) diet for 21 days at 1.67 MJ DE/kg<sup>0.75</sup>. After 21 days, all pigs were fed the same diet either alone or supplemented with raw, steamed or fried brown onion (25 g/MJ DE) for a further 42 days. Three-hour postprandial blood samples were collected at 28 and 42 days and fat biopsies collected at 42 days from control-pigs and pigs supplemented with raw onion. Blood was analysed for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides (TG) and lipase activity. Fat biopsies were assayed for fatty acid synthase (FAS), glycerol-3-phosphate dehydrogenase (G3PDH) and glucose-6-phosphate dehydrogenase (G6PDH). Data were analysed by ANOVA using Genstat v5.

**Table 1. Effects of dietary raw onion on adipose tissue and plasma enzymes.**

Variable	Treatment		SED	P-value
	Control	Raw		
FAS (mU/g adipose tissue)	20.1	19.7	1.28	0.800
G3PDH (mU/g adipose tissue)	749	693	26.2	0.053
G6PDH (mU/g adipose tissue)	132	208	33.7	0.041
Plasma lipase U/L	34.9	27.6	2.94	0.026

G6PDH-activity increased in pigs fed onion suggesting that onion increased the production of NADPH within adipose tissue. However, the activity of FAS, which uses NADPH for fatty acid synthesis, was unchanged. Feeding raw onion suppressed G3PDH and plasma lipase activities compared to control pigs, indicating that *de novo* TG metabolism was altered. Additionally, raw, fried and steamed onion increased mean postprandial plasma TG (P=0.02) but not cholesterol. This contrasts with our previous studies and may be related to differences in the CSO and quercetin content between onion varieties or the timing of the blood sampling, since decreases in TG are most apparent between 6 and 18 h post-feeding (Ostrowska *et al.* 2003).

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## RAW AND COOKED ONION CONSUMPTION ALTERS PLASMA OXIDATION STATUS IN THE PIG

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Consumption of *Allium* plants such as garlic (*Allium sativum*), onion (*Allium cepa*) and their derivatives such as organosulfur compounds and quercetin, inhibit lipoprotein oxidation and reduce the risk of cardiovascular disease (Griffiths *et al.* 2002). In this experiment we assessed the ability of raw and cooked brown onion to reduce serum lipoprotein oxidation in the pig.

Forty Large White x Landrace gilts (initial liveweight  $22.5 \pm 1.7$  kg) were allocated to individual pens and pre-fed with a high fat diet for 21 days. The diet contained 19.5 MJ DE/kg and 25.8% (w/w) total fat, similar to that of a typical 'western' diet. Following pre-feeding, pigs were allocated to one of four high-fat diets either without onion or supplemented with raw, fried or steamed onion at 25 g onion/MJ DE for 42 days. Pigs were fed about 90-95% of *ad libitum* intake (1.67 MJ DE/kg<sup>0.75</sup>). Blood samples were collected 3 h postprandial at 28 and 42 days of onion feeding. A modified *in vitro* assay that measures oxidative modifications of lipoprotein (Kontush and Beisiegel, 1999) was used to assess serum antioxidant status. The effective time (ET in minutes) taken to reach 50% of maximum absorption (Rmax) at 234 nm was determined.

**Table 1. Effect of dietary onion on lipoprotein oxidation.**

Variable	Treatment				SED Treatment <sup>1</sup>	P-value	
	Control	Raw	Fried	Steamed		Treatment <sup>2</sup>	Control vs Onion <sup>3</sup>
Rmax (abs)	0.282	0.313	0.292	0.292	0.0214	0.529	0.353
ET50 (min)	280	655	553	491	84.9	0.159	<0.001

<sup>1</sup>Standard error of the difference for Control vs Raw vs Fried vs Steamed. For standard error for comparison of control vs pooled onion multiply by 0.816. <sup>2</sup>P-value for overall comparison of Control vs Raw vs Fried vs Steamed. <sup>3</sup>P-value for comparison of Control vs pooled onion.

Cooking the onion reduced quercetin concentrations by up to 42% in both the steamed (0.275 mg/g fresh weight) and fried (0.183 mg/g fresh weight) preparations compared to raw onion (0.315 mg/g fresh weight). Cooking also reduced the cysteine sulfoxides (CSO) by up to 44% compared to raw onion (0.055 vs 0.098 mg/g fresh weight). However, all onion treatments significantly ( $P < 0.001$ ) inhibited copper-induced lipoprotein oxidation. Raw, fried and steamed onion increased ET50 by 134%, 98% and 75% compared to the control pigs, while Rmax was unaffected. These data confirm that cooking onions reduces their bio-active compounds, especially the volatile CSO (Kubec *et al.* 1999). Despite this, consumption of raw, fried or steamed brown onions offers cardiovascular health benefits. The compound most likely conferring these benefits is quercetin.

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## PIGLET AGE AT CASTRATION INFLUENCES SERUM CORTISOL PROFILES BUT NOT ACUTE GROWTH PERFORMANCE

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The age at which male pigs should be castrated remains an area of debate. Heart rate and vocalisation data reported by White *et al.* (1995) indicated that castration-induced stress was greater for pigs castrated at eight days of age or older. However, Taylor *et al.* (2001) reported that pig age had no effect on behavioural responses to castration at 3, 10 or 17 days of age. In the present experiment we tested two hypotheses. First, that the stress response associated with castration, as indicated by serum cortisol, would increase as the piglet aged. Second, that piglet age at castration would not affect the acute growth performance during the pre-weaning period.

Ninety Landrace x Large White male piglets (n=9-13 pigs/group) were assigned to treatment groups castrated (C) or non-castrated (N) based on age (3, 6, 9 or 12 days) and bodyweight. A jugular catheter was placed in all piglets using a non-surgical method (Carroll *et al.* 1999) 3 h before treatment. Pigs in the C group were held upside down, sprayed with Betadine® solution, and then castrated using a sterile scalpel blade. Pigs in the N group were held upside down, sprayed with Betadine® solution, and held for 45-60 seconds. Blood samples were collected at pre-castration (0 h), and 0.5, 1, 1.5, 2, 24 and 48 h after treatment. Bodyweights were recorded at 0, 24 and 48 h. All data were analysed using analysis of variance. The statistical model included the effects of time, age, treatment and interactions.

**Table 1. Effect of piglet age on serum cortisol (ng/ml) in non-castrated (N) and castrated (C) male piglets during the first 48h post-castration. Values represent the mean  $\pm$  SEM.**

Time (h)	Piglet Age (days)							
	3		6		9		12	
	N	C	N	C	N	C	N	C
0	44.1 + 9.9	23.9 + 3.4	22.2 + 5.1	25.3 + 4.2	50.1 + 5.9	39.0 + 8.0	29.6 + 3.0	33.0 + 3.9
0.5	57.8 + 7.4 <sup>a</sup>	112.1 + 16.8 <sup>b</sup>	48.3 + 9.1 <sup>a</sup>	100.1 + 11.4 <sup>b</sup>	71.2 + 10.3 <sup>a</sup>	118.7 + 17.7 <sup>b</sup>	47.1 + 7.7 <sup>a</sup>	97.3 + 11.9 <sup>b</sup>
1	32.3 + 6.4 <sup>a</sup>	73.3 + 9.0 <sup>b</sup>	42.9 + 8.4 <sup>a</sup>	104.1 + 10.3 <sup>b</sup>	51.9 + 9.9	89.6 + 16.4	51.4 + 12.6 <sup>a</sup>	99.2 + 18.0 <sup>b</sup>
1.5	35.4 + 8.5	51.0 + 8.6	36.5 + 5.3 <sup>a</sup>	67.1 + 10.1 <sup>b</sup>	49.6 + 4.4	65.6 + 10.0	50.6 + 11.7 <sup>a</sup>	95.5 + 17.0 <sup>b</sup>
2.0	30.4 + 5.3	48.9 + 11.0	34.7 + 5.7	59.9 + 12.5	34.6 + 6.3	47.7 + 5.5	32.2 + 3.6 <sup>a</sup>	68.5 + 16.3 <sup>b</sup>
24	22.0 + 4.0	24.5 + 6.3	26.0 + 6.9	22.6 + 4.3	39.0 + 11.5	34.2 + 6.5	36.7 + 11.4	32.4 + 8.7
48	29.5 + 6.8	19.2 + 3.6	70.9 + 8.5	52.8 + 15.3	87.3 + 16.5	63.3 + 20.3	76.5 + 19.8	96.7 + 17.2

<sup>ab</sup>Means within a row and within the same age group with different superscripts differ (P<0.05).

Acute growth performance during the first 48 h following castration was not affected by piglet age. During the first 24 h, serum cortisol was elevated in both groups (P<0.0001). There was an overall treatment effect (P<0.0001) such that serum cortisol was greater in C vs N males. At 48 h, there was no effect of treatment (P>0.28) on serum cortisol however serum cortisol was lower in 3-day old pigs compared to 6, 9 or 12-day old pigs. The cortisol data suggest that after three days of age, pigs have a greater stress response to being handled.

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## TREATMENT OF NEONATES WITH PORCINE SOMATOTROPIN INCREASES LIFETIME GROWTH BUT THE COMPOSITION OF GROWTH VARIES WITH THE SEX OF THE PIGS

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Treatment of neonate male pigs with high doses of porcine somatotropin (pST) reduced lifetime fat but did not affect lean deposition (Dunshea *et al.* 2003). The failure to stimulate lean tissue growth may have been due to down-regulation of endogenous pST-production as lean tissue deposition was decreased in the period immediately post-weaning. Administration of growth hormone releasing hormone (GHRH) may ensure endogenous pituitary pST-production. The aim of this experiment was to determine the effects of sex and neonatal pST and GHRH treatment on subsequent tissue growth.

Seven Large White × Landrace sows with an average litter of 10 were used to nurse pigs for this experiment. Forty-two pigs (six/sow) were allocated to a 2 × 3 factorial design with the respective factors being sex (boar vs gilt) and administration of saline (control) or pST (1 mg pST/kg LW.d until 21 days) or pST (1 mg pST/kg LW.d until 16 days) followed by GHRH (0.50 nmol GHRH (D-Ala<sup>2</sup> hGHRH (1-29) NH<sub>2</sub>)/kg LW.d from day 17-21). Pigs were weaned at 21 days and commercially reared. Pig body composition was measured by dual energy X-ray absorptiometry at 1, 21, 49, 77, 105 and 133 days of age (Suster *et al.* 2003).

**Table 1. Effect of sex (S) and pre-weaning treatment (T) with daily pST (P) or pST followed by GHRH (P+G) on growth, tissue deposition and body composition.**

S	Boar			Gilt			SED <sup>a</sup>	Significance		
	Cont	P	P+G	Cont	P	P+G		S	T	S × T
Lifetime rate of gain (g/d)										
Weight	649	706	672	590	637	621	30.7	0.002	0.076	0.90
Lean	435	467	449	396	395	398	18.1	<0.001	0.413	0.49
Fat	114	126	117	99	125	132	9.6	0.92	0.009	0.21
Empty body composition at day 133										
Fat (kg)	15.3	16.9	15.7	13.3	16.7	17.7	1.28	0.91	0.009	0.21
Fat %	19.6	20.0	19.5	18.8	22.4	23.5	1.08	0.006	0.009	0.018
Lean (kg)	60.2	64.6	62.1	55.0	55.3	54.9	2.40	<0.001	0.39	0.49
Lean %	77.2	76.8	77.3	77.9	74.3	73.4	1.08	0.005	0.010	0.020

<sup>a</sup>SED for interaction between sex and treatment.

Neonatal treatment with pST for 21 days increased lifetime daily gain (619 vs 672 g/d, P=0.046) while in pigs treated with pST for 17 days followed by GHRH, daily gain was intermediate (647 g/d). However, the composition of the increased gain in the pST-treated pigs depended on sex, as indicated by significant treatment × sex interactions (Table 1). In boars, the composition of the increased gain was similar to the controls while gilts treated with pST as neonates deposited more fat than controls. The reason for this sexual dimorphism remains unclear. In conclusion, neonatal pST-treatment may provide a means of improving growth and maintaining carcass quality in boars but not in gilts since the increased growth in gilts was predominantly as fat.

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## THE INTERACTION BETWEEN STEEPING TIME AND EFFICACY OF A XYLANASE IN WHEAT-BASED DIETS FOR WEANER PIGS

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Grains contain endogenous enzymes that may be activated when temperature and moisture conditions become favourable. Providing diets in a liquid form enhances the action of endogenous enzymes on dietary substrates (Brooks *et al.* 1996). The endogenous enzymes act on the substrates in a similar manner to supplemental enzymes and have been shown to improve growth performance in chickens (Choct and Hughes, 1997). The aim of this experiment was to determine the effect of steeping diets with or without a supplemental xylanase on growth performance in weaner pigs.

Seventy-two male, Large White x Landrace pigs, weaned at 28 days, were fed a wheat-based meal diet containing 14.5 MJ DE/kg DM and 0.85g available lysine/MJ DE (as-fed basis). The wheat was pre-characterised as medium intake, based on the non-starch available carbohydrates it contained. This was verified by a 'growth assay' (Cadogan *et al.* 2001). The experimental design was a 3 x 2 factorial with steeping time (0 dry, 1 h, 24 h) and xylanase supplementation (0, 300ppm Biofeed Wheat<sup>®</sup> Novozymes, Denmark). Liquid diets contained 2.5 l water/kg of feed and were soaked for 15 h before feeding. Data were analysed using a multi-factor ANOVA and Fisher's LSD.

**Table 1. Effects of steeping time and xylanase supplementation on the performance of male weaner pigs from 5–26 days post-weaning (100% DM basis; n=72).**

Diet	Steeping time (h)	Enzyme	5 d Wt. (kg)	26 d Wt. (kg)	ADG (g/d)	FCR (g:g)	ADI (g/d)
Dry	0	-	7.29 <sup>ab</sup>	16.55 <sup>a</sup>	426 <sup>a</sup>	1.18 <sup>a</sup>	514 <sup>a</sup>
Dry	0	+	7.33 <sup>a</sup>	16.72 <sup>a</sup>	438 <sup>a</sup>	1.19 <sup>a</sup>	532 <sup>a</sup>
Liquid	1	-	7.40 <sup>b</sup>	17.81 <sup>ab</sup>	494 <sup>ab</sup>	1.15 <sup>a</sup>	549 <sup>ab</sup>
Liquid	1	+	7.68 <sup>ab</sup>	17.37 <sup>ab</sup>	470 <sup>ab</sup>	1.15 <sup>a</sup>	527 <sup>ab</sup>
Liquid	24	-	7.74 <sup>a</sup>	18.71 <sup>b</sup>	516 <sup>b</sup>	1.13 <sup>ab</sup>	579 <sup>b</sup>
Liquid	24	+	8.03 <sup>a</sup>	17.93 <sup>ab</sup>	492 <sup>ab</sup>	1.02 <sup>b</sup>	502 <sup>a</sup>
P value	Steeping (S)		*	*	*	*	NS
	SE <sub>D</sub>		0.09	0.47	17.2	0.03	17.2
	Enzyme (E)		NS	NS	NS	NS	NS
	SE <sub>D</sub>		0.07	0.38	14.0	0.02	14.1

\*P<0.05; NS=not significant. <sup>ab</sup>values within column with different superscripts differ significantly (P<0.05). ADG=average daily gain; FCR=feed conversion ratio; ADI=average daily intake.

Pigs fed steeped diets were heavier and had higher daily gain than pigs fed dry diets (Table 1). While steeping did not significantly influence feed intake, feed conversion ratios were superior for steeped diets and improved as steeping time increased from 1 to 24 h. There were no significant effects of enzyme or steeping by enzyme interactions.

Steeping for 24 h negated the effect of supplemental xylanase. Thus, the use of carbohydrate-degrading enzymes in liquid feed requires a different approach compared with traditional application in dry diets. The efficacy of enzymes depends on the length of steeping and the diet composition. In addition, diets steeped for more than 12 h allow the endogenous enzymes sufficient time to act on substrates, negating the need for exogenous enzymes.

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## NUTRIENT DIGESTIBILITY OF WHEAT FOR WEANER PIGS DEPENDS ON STARCH STRUCTURE, PARTICLE SIZE AND ENZYME

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Black, (2001) hypothesised that the structure of endosperm starch may influence nutrient digestibility and animal performance because the branching structure of amylopectin disturbs the close packing between the polymer chains of amylose, making a cereal with high amylopectin ('waxy') starch more digestible than a less-branched ('normal') cereal.

However, it has also been reported that waxy barley contains more cell wall non-starch polysaccharides (NSP) than normal barley, which could limit access to starch by digestive enzymes (Xue *et al.* 1991). Fine grinding and supplementation of a 'waxy' grain with enzymes that degrade NSP might increase starch digestion by increasing contact with amylolytic enzymes. In this experiment, we defined how the type of starch in the endosperm, particle size and supplementation with exogenous enzymes changes digestibility of wheat-based diets fed to weaner pigs.

The experimental design was a 2 x 2 x 2 factorial with the factors being starch structure (waxy vs normal, variety Janz), particle size [560 (fine) vs 930 (coarse)  $\mu\text{m}$ ] and enzyme addition ( $\pm$ Grindazyme™ 5000, minimum activity 5000 U/g endo-1,4- $\beta$ -glucanase and 12000 U/g endo-1,4- $\beta$ -xylanase, Danisco Animal Nutrition, UK). Forty-eight male pigs (Landrace x Large White) weaned at 21 days of age ( $6.0 \pm 0.08$  kg) were used. The diet contained 650 g/kg wheat and 1 g/kg  $\text{TiO}_2$  added as an inert marker for calculating faecal digestibility. All diets contained similar concentrations of calculated digestible energy (14.4 MJ/kg) and available lysine (0.80 g/MJ DE). The pigs were fed for 10 days after weaning, and faecal 'grab' samples were collected at 0800, 1000, 1200, 1400 and 1600 h for three consecutive days from day seven. The GLM procedure of Minitab (Minitab Inc., PA, USA) was used for statistical analyses.

**Table 1. Effects of waxy or normal starch of wheat (W), particle size (P) and enzyme addition (E) on faecal digestibility coefficients (DC) determined with male weaner pigs (n=6).**

Wheat	Waxy Janz				Normal Janz				SEM	Significance <sup>1</sup>			
	Coarse		Fine		Coarse		Fine			W	P	E	PxE
Particle size	-	+	-	+	-	+	-	+					
DC <sub>Starch</sub>	97.1	98.8	98.2	99.0	97.8	99.0	99.3	99.4	0.17	†	**	**	†
DC <sub>GE</sub>	75.1	76.2	76.3	72.6	73.2	76.9	76.9	76.3	0.62	NS	NS	NS	†
DC <sub>CP</sub>	69.0	73.1	72.9	65.1	71.8	75.1	76.2	70.7	0.96	†	NS	NS	**

NS non-significant, †P<0.10, \*\*P<0.01; GE=gross energy; CP=crude protein.

Amylopectin content had no effect on nutrient digestibility, although the waxy wheat tended to have a higher starch (98.9 vs 98.2%, P=0.06) and protein (73.5 vs 69.7, P=0.06) digestibility after weaning than the normal wheat. Fine grinding (99.0 vs 98.1%, P<0.01) and the enzyme (99.0 vs 98.1%, P<0.01) increased starch digestibility at the faecal level. The DCCP was influenced (P<0.01) by an interaction between particle size and enzyme supplementation, such that finely-ground wheat plus the enzyme caused a decrease in faecal digestibility. These data suggest that the faecal digestibility of wheat for weaner pigs depends of the structure of starch, particle size and enzyme, but the response differs according to the nutrient in question.

Grains were provided through the 'Premium Grains for Livestock Program' coordinated by the Grains Research and Development Corporation.

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## INFLUENCE OF LYISOFORTE™ ON NUTRIENT DIGESTION IN THE SMALL INTESTINE OF GROWING PIGS

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Lysoforte™ is a mixture of lyso-phospholipids designed to promote emulsification of fats and micelle formation in the gut. In diets deriving a significant proportion of energy from added fats, adding Lysoforte™ should not only improve energy yield, but also enhance the digestion of other nutrients such as amino acids by ensuring the dietary fat does not impede enzymic action. The aim of this experiment was to assess the influence of Lysoforte™ addition to commercial growing-pig diets varying in tallow content, on the digestion of nutrients in the small intestine.

Wheat-based and barley-based diets (13.9 MJ/kg digestible energy (DE), 0.7 g available lysine/MJ DE) were formulated for this experiment – the wheat-based diet contained wheat (417 g/kg), barley (100 g/kg), animal proteins (120 g/kg), legumes (300 g/kg) and tallow (10 g/kg) while the barley-based diet contained barley (450 g/kg), animal proteins (80 g/kg), legumes (400 g/kg) and tallow (25 g/kg). Diets were fed with and without added Lysoforte™ (0.75 g/kg). Celite® was added to the diets as an acid-insoluble ash marker. Large White male pigs (40-45 kg bodyweight) fitted with simple T-piece ileal cannulas were provided diets based on a 4 x 4 Latin square design. Diets were fed for 7 days (three-times maintenance before 8 h digesta collections over two consecutive days. Data were analysed using an analysis of variance.

**Table 1. Apparent ileal energy, nitrogen and lysine digestibility (proportion of total) and ileal diet digestible energy content (MJ/kg) of wheat and barley-based diets fed to growing pigs fitted with simple T-piece ileal cannulas.**

	Diet				Statistics	
	Wheat <sup>1</sup>	Wheat+Lyso <sup>2</sup>	Barley <sup>3</sup>	Barley+Lyso <sup>4</sup>	P	SEM <sup>6</sup>
Nitrogen	0.82 <sup>a</sup>	0.82 <sup>a</sup>	0.70 <sup>b</sup>	0.80 <sup>a</sup>	0.024	0.021
Energy	0.67	0.70	0.65	0.73	0.098	0.020
Lysine	0.84 <sup>a</sup>	0.84 <sup>a</sup>	0.75 <sup>b</sup>	0.83 <sup>a</sup>	0.014	0.015
Ileal diet DE <sup>5</sup> (MJ/kg)	12.39	12.78	11.75	13.49	0.069	0.364

<sup>1</sup>Commercial wheat-based diet. <sup>2</sup>Commercial wheat-based diet plus Lysoforte™. <sup>3</sup>Commercial barley-based diet.

<sup>4</sup>Commercial barley-based diet plus Lysoforte™. <sup>5</sup>Digestible energy. <sup>6</sup>Standard error of mean. <sup>ab</sup>Means in a row with different superscripts differ significantly (P<0.05).

Addition of Lysoforte™ significantly improved (P<0.05) the digestion of nitrogen and lysine in the commercial barley-based diet which had a higher fat content than the wheat-based diet (Table 1). Similarly, ileal diet DE content increased from 11.75 to 13.49 MJ/kg (P=0.069), but lacked significance. The results suggest that the action of Lysoforte™ improves the capacity of the growing pig to digest protein and amino acids in the small intestine, possibly through enhanced enzymic access to protein substrates. The improved nutrient digestibility observed in growing pigs is supported by positive growth responses observed in weaner pigs by Carter and Henman (2003) with Lysoforte™ addition to diets containing tallow. In the present experiment, the tallow content of diets was only 1% (wheat base) and 2.5% (barley base). Higher fat contents may provide additional substrate for the action of Lysoforte™ to further increase energy yield and more research is required to assess the influence of Lysoforte™ addition on whole-tract DE yield in growing pigs.

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## **A REVIEW - NEW REPRODUCTIVE TECHNOLOGIES FOR PRODUCTION AND RESEARCH IN THE PIG**

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### **Abstract**

During the past two decades there has been considerable interest in the improvement of existing reproductive technologies as well as the development of new ones for the pig industry. This has been brought about by a consolidation in the pig industry worldwide and an increased interest in using pigs for various biomedical applications. During the past 10 years we have witnessed the production of piglets using sexed semen from frozen, thawed embryos and more recently cloned pigs using somatic cell nuclear transfer. The rate at which these and other reproductive technologies are adopted will be increased by the recent development of non-surgical methods for deep uterine insemination and embryo transfer. In particular, the development of cloning technology is advancing at a rapid rate. The power of this technology lies not only in its ability to disseminate an elite animal's genetics at a much greater rate than is currently possible; using AI for example, but also in its ability to allow the porcine genome to be manipulated precisely for the first time. This, together with the development of mammalian artificial chromosomes as vectors for introducing large transgenes, gene clusters and chromosomal segments will allow us to explore the full potential of this equally powerful technology. These technologies, together with the ability to freeze these genetics either as cells or embryos, will change the way pig genetics are disseminated in the future.

### **Introduction**

During the past two decades there has been considerable interest in the improvement of existing reproductive technologies as well as the development of new ones for the pig industry. This has been brought about by a consolidation in the pig industry in many developed countries, including Australia, and an increased interest in using pigs for various biomedical applications such as xenotransplantation. As the pig industry consolidates, breeding companies in particular are becoming increasingly receptive to the development of existing technologies such as artificial insemination (AI), as well as the development of new ones as a means of increasing production efficiency through increases in genetic improvement, reduction in labour costs, etc. These changes together with recent advances in related areas such as transgenesis and functional genomics will change the way pig genetics are disseminated in the future. The aim of this review is two-fold. First, existing reproductive technologies such as AI, their limitations and the potential to improve these, are discussed. Where relevant, recent data regarding these have been tabulated so that the reader can gain an appreciation of their overall efficiency. The second part of the review focuses on the newer technologies such as cloning and takes a more speculative approach to examining how these alone, and together with the new genetic technologies, might impact on production and research.

### **Artificial insemination.**

Artificial insemination was first reported in pigs by Chris Polge in 1956 (Polge, 1956) and its use has increased dramatically over the past 20 years in countries such as The Netherlands, where over 85% of herds are inseminated artificially (Weitze *et al.* 2000). In contrast, less than 30% of Australian herds are inseminated artificially (P. Hughes, pers. comm.). In terms of genetic improvement, current AI technology provides an efficient method for disseminating a boar's genetics, as well decreasing the risk of disease importation, greater worker safety and reduced costs (Flowers and Alhusen, 1992).

#### *Fresh Semen*

While numerous studies have been undertaken on the development of new extenders, methods for evaluating semen fertility, etc., AI has remained relatively unchanged since Polge's first report. Relatively large numbers of spermatozoa ( $2.5-5 \times 10^9$  sperm) are deposited in relatively large volumes (50-100 ml) intra-cervically, usually twice during standing oestrus. This

limits the number of doses that can be prepared from one semen sample to about 20. This number can be reduced 100-fold if sperm are deposited surgically at the uterotubal junction (Krueger *et al.* 1999). However, the convoluted nature of the pig cervix and uterine horns has prevented non-surgical approaches similar to those used extensively in the cattle industry, from being developed for pigs.

Recently, a new method for non-surgical deep uterine insemination of pigs without anaesthesia has been reported by Martinez *et al.* (2002). Insemination was performed by firstly inserting a commercial AI spirette through the vagina into the cervix, which was then used to manipulate a specially designed flexible catheter (working length 1.8 m, outer diameter 4 mm, diameter of inner tubing 1.8 m) as far as possible into one uterine horn. In this experiment, farrowing rates and litter sizes following deep intrauterine insemination with  $15 \times 10^7$  and  $5 \times 10^7$  sperm, 36 hours after hCG injection, did not differ from that compared with standard AI with  $3 \times 10^9$  spermatozoa (Table 1). On the basis of these results Martinez *et al.* (2002) have suggested that a 20- to 60-fold reduction in the number of spermatozoa inseminated and an 8- to 10-fold reduction in the volume used can be obtained with deep uterine insemination.

#### *Frozen Semen*

The first piglets born from insemination with frozen semen by surgical insemination into the oviducts (Polge *et al.* 1970) and by intracervical insemination with thawed spermatozoa (Crabo and Einarsson, 1971; Graham *et al.* 1971) were reported over 30 years ago. Recent improvements using 5 ml flat plastic packages (Eriksson and Rodriguez-Martinez, 2000), or 0.5 ml straws (Bussiere *et al.* 2000) have seen fertility rates increase from around 30% to more than twice this rate. However, this is still lower than that expected from fresh semen. It is well established that boar spermatozoa are particularly sensitive to freezing and thawing compared with other species (Watson, 1996). As a consequence it is usual to double the concentration of spermatozoa when using frozen thawed semen.

The use of deep uterine insemination may improve these efficiencies by allowing a reduced number of sperm to be deposited near the site of fertilisation. In preliminary experiments, deep uterine insemination using  $1 \times 10^9$  frozen thawed spermatozoa resulted in a 78% farrowing rate and average litter size of 9.3 piglets, which was not different from that obtained with cervical insemination using  $6 \times 10^9$  frozen thawed spermatozoa. Although lower than the results obtained with fresh semen in a subsequent experiment (70 vs 84% farrowing rate, no difference in litter size), these results suggest that deep uterine insemination can result in acceptable fertility using a relatively small number of frozen spermatozoa (Roca *et al.* 2003).

#### *Sexed semen*

The ability to sex semen into X and Y bearing fractions has long been an objective for a number of animal industries, including the pig industry. However, the ability to offer sexed semen on a commercial scale is beyond the capability of existing technology that results in relatively few sperm whose fertility is compromised. For example, the current method of high speed and high-pressure flow cytometry produces  $5\text{-}6 \times 10^6$  sperm per hour (Johnson and Welch, 1999). However, sexed semen can be combined with deep uterine insemination, *in vitro* fertilisation or intracytoplasmic sperm injection to overcome the need for large numbers of sperm. For example, using deep uterine insemination, farrowing rates of up to 50% and litter sizes of nine have been obtained with  $14 \times 10^7$  sperm in preliminary experiments by Vazquez *et al.* (2003). Sexed semen can also be used for *in vitro* fertilisation to produce large numbers of embryos using oocytes from elite females (or from the ovaries of commercial animals slaughtered at the abattoir) and matured *in vitro*. This may offer an advantage compared with AI, in that both the male and female genomes can be disseminated. In the case of elite females, the oocytes could only be obtained by a limited number of aspirations of follicles from the surface of the ovary done surgically under anaesthesia. In contrast, the same procedure can be performed repeatedly in cattle non-surgically. Using intracytoplasmic injection (ICSI) relatively few sexed sperm would be required because these are injected directly into the oocyte. The birth of piglets using this approach has been reported recently by Probst and Rath (2003).

**Table 1. Artificial insemination using deep uterine insemination (DUI) with fresh, frozen and sex sorted spermatozoa.**

Semen	Method of insemination	Spermatozoa concentration (x number of inseminations)	Farrowing rate (%)	Litter size	Reference
Fresh semen	Intracervical	$3 \times 10^9$ (x 2)	83	10.0	Martinez <i>et al.</i> (2002)
	DUI	$15 \times 10^7$ (x 1)	83	9.7	
Frozen semen	Intracervical	$6 \times 10^9$ (x 1)	83	10.0	Roca <i>et al.</i> (2003)
	DUI	$1 \times 10^6$ (x 1)	78	9.3	
Fresh sexed semen	Non sorted DUI	$14 \times 10^7$ (x 1)	86	9.9	Vazquez <i>et al.</i> (2003)
	Sorted DUI	$14 \times 10^7$ (x 1)	54	9.2	

### ***In vitro* production of embryos and *in vitro* follicle growth systems**

Systems for the *in vitro* maturation of immature oocytes obtained from abattoir-derived ovaries have been developed for many livestock species and are available commercially for beef and dairy cattle (Thibier, 2002). In the pig, *in vitro* maturation, fertilisation and culture of embryos have been used extensively to produce mature oocytes and embryos for research purposes. However, pigs have been produced using IVM with frozen thawed spermatozoa (Abeydeera *et al.* 1998b) and sexed spermatozoa (Abeydeera *et al.* 1998a), demonstrating the potential of this approach as a method for accessing both male and female genomes (Table 2). In the future it may even be possible to harvest the majority of the 200 000 eggs found in the pig ovary and mature these in their follicles *in vitro* (so-called *in vitro* growth systems). This is an intriguing concept because by the time the female animal reaches maturity, about 75% of the oocytes in the ovaries are already lost which represents an enormous wastage of genetic material (Gosdon and Telfer, 1987). Furthermore once puberty is reached, only relatively few of the remaining oocytes ovulate, while the majority become atretic. *In vitro* growth systems that use the primordial and pre-antral follicles which contain nearly all the oocytes, have already been developed for mice and offspring (Eppig and Schroeder, 1989). Similar systems are being developed for the pig. Although no offspring have been produced, porcine pre-antral follicles have been cultured for up to 20 days and meiotically competent oocytes produced (Telfer *et al.* 2000).

**Table 2. Production of piglets using *in vitro* maturation, fertilisation and culture of embryos.**

Source of oocytes	Source of spermatozoa	Embryonic stage at which transferred	Piglets /recipients	Reference
IVM	Frozen thawed	8 cell-morula	82/12	Abeydeera <i>et al.</i> (2000)
IVM	Frozen thawed	Blastocyst	19/3	Kikuchi <i>et al.</i> (2002)
IVM	X-sperm	8 cell-morula	4/5	Abeydeera <i>et al.</i> (1998a)
	Y-sperm	8 cell-morula	9/3	
IVM	ICSI using Y-sperm	One cell	13/4	Probst and Rath (2003)

### **Embryo transfer**

Embryo transfer is used extensively in the cattle industry with over half a million embryos transferred world-wide each year. In contrast, embryo transfer in the pig industry is limited (Thibier, 2002). While many factors are involved in an industry's decision to adopt a particular technology such as embryo transfer, one of the major factors in the beef and dairy industries has been the ease with which the embryo transfer catheter can be passed through the cervix. This has allowed embryo transfer as well as other technologies to be performed non-surgically on-farm by veterinarians, trained technicians or producers themselves. In contrast, the convoluted nature of the pig cervix and uterine horns means that the same procedures have, until recently, been performed surgically under anaesthesia in the pig. This limits the number of times embryos can be collected from donor animals for embryo transfer, for example, because of the build-up of adhesions and scar tissue. Procedures which minimise the amount of trauma associated with surgery are being developed which, if successful, will enable embryos to be collected and transferred repeatedly. Examples of such techniques include, mini-laparotomy (Huang *et al.* 2002), semi-endoscopic (Wallenhorst and Holtz, 2002) and non-surgical collection of embryos based on the flexible catheter used for deep uterine insemination (Martinez *et al.* 2001).

In addition, the development of a number of underpinning technologies has tended to lag behind that developed for other livestock species, notably cattle. For example, FSH is widely used to super-ovulate cattle because it has been shown to be superior in terms of the number of transferable embryos produced compared with equine chorionic gonadotrophin (eCG or PMSG). In contrast, eCG is still used exclusively for the induction of ovulation and super-ovulation in pigs, despite similar results to that in cattle being obtained in preliminary experiments with pigs (Takahagi *et al.* 1999). Similarly, the development of culture systems for the *in vitro* maturation, fertilisation and culture of embryos have only been developed relatively recently; all of which still have limitations compared with the *in vivo* situation. For example, current *in vitro* culture systems delay the development of embryos to the blastocyst stage by about 24 hours and result in embryos with fewer numbers of inner cell mass cells; both of which affect their ability to develop to term (Machaty *et al.* 1998).

#### Cryopreservation of embryos

Embryo freezing is an important tool for maximising genetic improvement in the livestock species. It also provides a low cost method for importing and exporting genetic material with a minimum risk of disease transmission, and is used widely in the cattle industry for these reasons (Thibier, 2002). In contrast, embryo freezing in pigs has only recently been developed. Nagashima *et al.* (1995) showed that the high lipid content found in early stage embryos prevented them from being frozen using conventional slow cooling methods. To demonstrate this, they centrifuged embryos to polarise the lipid in the embryo and then removed this by aspirating it through a micropipette. Following the removal of the majority of the lipid, these embryos survived freezing and thawing using slow cooling, whereas embryos which still contained the lipid did not (Nagashima *et al.* 1995). Technology has now been developed where the embryo is simply centrifuged to polarise the lipid and then frozen within seconds using vitrification, before moving back into the embryo proper (Cameron *et al.* 2000). This, together with other improvements (Beebe *et al.* 2002; Dobrinsky, 2001), now means that embryo freezing will soon be available commercially (Table 3).

**Table 3. Production of piglets from vitrified frozen/thawed embryos.**

Embryonic stage	Embryos transferred per recipient	Piglets born/ recipients	Reference
Blastocyst	36-38	5/4	Cameron <i>et al.</i> (2000)
Blastocyst	20	38/20	Berthelot <i>et al.</i> (2001)
Morulae-early blastocyst	25-35	61/11	Dobrinsky (2001)
Morulae	20	26/10	Berthelot <i>et al.</i> (2001)
Morulae-early blastocyst	30-36 (Experiment 2, Group 1.)	17/5	Misumi <i>et al.</i> (2003)

#### Cloning

Of all the reproductive technologies that have been developed, somatic cell nuclear transfer or cloning, as it is more commonly known, offers the most potential in terms of the rate at which genetics can be disseminated. Cloning has the potential to replace many of the existing reproductive technologies described previously, including AI, sexed sperm and embryo transfer. Cloning, in theory at least, allows an elite animal's genetics to be replicated indefinitely. By simply taking ear notch tissue from such an animal it is possible to produce millions of cells, which can then be frozen relatively simply and stored in liquid nitrogen. This is valuable in itself, in that it provides a relatively inexpensive way of conserving genetic material (so-called gene banking, within and between breeds). These cells can then be thawed and used for cloning to produce virtually identical animals. However, in practice, current cloning efficiencies are still relatively low with less than 1% of cloned embryos developing to term. Further losses also occur around the time of birth (reviewed in Wilmut *et al.* 2002). Cloning is still in its infancy and tremendous progress has been made since the birth of Dolly (Wilmut *et al.* 1997) and the first cloned pigs less than three years ago (Onishi *et al.* 2000). For example, Walker *et al.* (2002) recently reported that transferring between 34-74 embryos to five recipients resulted in four recipients farrowing with an average litter size of seven. This represents an overall efficiency of around 12% of embryos transferred resulting in live-born (Table 4). Furthermore all except one piglet, which had an anal

atresia, were normal and consistent with the emerging view that pigs do not experience the same sorts of problems seen in cattle and sheep around the time of birth (Cibelli *et al.* 2002). This experiment was done using cells harvested from fetuses however, if similar results can be obtained on a larger scale using adult fibroblasts obtained from an ear notch for example, it is possible that cloning could be used as a breeding tool in the near future.

The value of cloning technology to the pig industry can be defined in two ways. First, in the better use of superior genetics at the commercial level as currently, genetics companies sell more than half of all the animals they produce which only obviously differ in their genetic merit. A cost-effective cloning technology would allow breeders to identify the top 1% of their boars for example, and use these as commercial sires or their semen. The second advantage of this technology comes from the production of a more uniform product. Currently, the majority of pigs are marketed on their percentage lean content. A significant problem is the large natural variation in this trait with up to 50% of this variation due to individual genetic difference. Cloning has the potential to reduce this variation substantially and this would improve both the cost of production and the value of the carcass.

**Table 4. Production of piglets using somatic cell cloning.**

Cell donor source	Oocyte maturation	Piglets born/recipients	Reference
Fetal	In vivo	1/4	Onishi <i>et al.</i> (2000)
Adult	In vivo	5/5	Polejaeva <i>et al.</i> (2000)
Fetal	In vitro	28/5	Walker <i>et al.</i> (2002)

### Transgenesis

While the potential benefits of cloning technology are enormous, perhaps even more powerful is the ability to use this technology in the pig and other livestock species to modify the genome precisely. Currently, transgenic livestock are produced using pro-nuclear micro-injection that involves injecting hundreds of copies of a particular transgene into one of the pronuclei of a recently fertilised embryo. This method is relatively inefficient and suffers from a number of inherent limitations that prevent the full potential of transgenesis from being explored in these species. Most of these limitations stem from the fact that it is impossible to control where the transgene becomes inserted, resulting in no expression in around half of the transgenic animals produced and variable levels of expression in the remainder due to the influence of surrounding genes. Furthermore, it is not possible to use this method to 'knock out' existing genes. In contrast, a single copy of a transgene can be inserted at a predetermined site in the mouse genome using embryonic stem cell and gene targeting technologies avoiding interference with expression. This method has also been used to knock out existing genes and has revolutionised our understanding of mammalian gene function. However, despite considerable effort for more than twenty years, embryonic stem cells are yet to be isolated for any of the livestock species. The advent of cloning technology now means that we can also make these same precise changes using somatic cells instead of ES cells in the pig as evident by the recent production of 'knock out' pigs (Lai *et al.* 2002).

Transgenic technology holds considerable promise for the pig industry, from improvements in animal productivity to the conferring of disease resistance. Furthermore, as our knowledge of the genetic basis for productive traits increases as a result of the sequencing of the human genome, studies in functional genomics and proteomics, further uses for this technology will become apparent. Many of these traits are likely to be controlled by a number of genes acting together. As such, the ability to isolate gene clusters, chromosomal segments and combine these to form mammalian artificial chromosomes (MACs) which can then be inserted into the genome using cloning technology offers enormous potential for the livestock industries. This will add a new dimension to transgenic technology that is currently limited to the insertion of relatively small transgenes (<100 kb of DNA). Transgenic cattle inserted with a 10 Mb human artificial chromosome (HAC) containing the entire un-rearranged sequences of the human immunoglobulin heavy chain and lambda light chain loci (1-1.5Mb for each locus) have already been produced (Kuroiwa *et al.* 2002). Furthermore transmission of MACs to the next generation has been demonstrated in mice (Tomizuka *et al.* 1997). It is possible therefore, that MACs may become the

preferred method for performing transgenesis in the future, not only for inserting single transgenes but also allowing gene clusters and chromosomal segments to be incorporated into the genome.

### Summary

In conclusion, as the pig industry consolidates, we will witness an increase in the development of existing as well as new reproductive technologies. During the past ten years we have witnessed the production of piglets using sexed semen, from frozen thawed embryos, as well as the production of gene 'knock out' pigs using cloning technology. The rate at which these and other reproductive technologies are adopted will be increased by the recent development of non-surgical methods for deep uterine insemination and embryo transfer. The development of cloning technology in particular is advancing at a rapid rate. The power of this technology lies not only in the ability to disseminate an animal's genetics, male or female, at a greater rate than is currently possible, but also in its ability to allow for the first time, a method whereby the porcine genome can be manipulated precisely. This, together with the advent of mammalian artificial chromosomes as vectors for gene clusters and chromosomal segments, will allow us to capitalise on the information gained from gene mapping, functional genomics and proteomic studies, and explore the full potential of transgenic technology. These technologies, together with ability to freeze these genetics either as cells or embryos, will change the way pig genetics are disseminated in the future.

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## INTERRELATIONSHIP BETWEEN WHEAT TYPE AND XYLANASE ADDITION ON DIGESTA TRANSIT RATE IN PIGS

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Supplementing wheat of sub-optimal quality with xylanase increases feed intake in pigs (Choct *et al.* 1999). Ivan and Farrell (1976) reported that pigs fed poor quality wheats had lower rates of digesta flow than pigs fed higher quality wheat. In this experiment we hypothesised that the digesta transit rate would vary in pigs fed two types of wheat with and without xylanase supplementation.

Forty male pigs (liveweight  $7.0 \pm 0.4$  kg) were placed in individual crates and randomly allocated to a 2 x 2 factorial design. The respective factors were two wheat types (low and high quality wheats, *viz.* Diets 1 and 2) with or without a xylanase (derived from *Thermomyces lanuginosus*). The diets were formulated to contain 14.5 MJ DE/kg, 0.85 g/MJ DE available lysine and were pelleted at 80°C. Pigs were offered the treatment diets and water *ad libitum* for 11 days. On day 12, the diets were withheld from pigs for 12 h before offering the same diets for 30 min but which now contained insoluble (800 g/kg alkane; C<sub>36</sub>H<sub>72</sub>) and soluble (50 ppm Cr from Cr-EDTA) markers. The intake of the diets containing the marker was recorded during the 30 min feeding period. The pigs were then given their original treatment diets *ad libitum* for 6.5 h, after which they were slaughtered and digesta samples collected from the stomach, duodenum (Duo), jejunum (Jej), ileum, terminal ileum (TI), caecum, middle colon (MC) and rectum for analysis of marker concentrations. The data were analysed using multi-ANOVA and means compared using LSD. The concentrations of alkane and Cr-EDTA were corrected according to the actual marker intakes (Table 1).

**Table 1. Influence of wheat type and xylanase on alkane and Cr concentrations along the gut of young male pigs.**

Diet	Enzyme	Stomach	Duo	Jej	Ileum	TI	Caecum	MC	Rectum
Alkane concentration (mg/kg digesta)									
1	-	271	235	470	629	694	1200	749 <sup>ab</sup>	121
1	+	240	248	371	535	769	1248	511 <sup>b</sup>	202
2	-	227	164	468	665	663	907	923 <sup>a</sup>	80
2	+	258	307	515	620	783	1179	780 <sup>ab</sup>	294
SEM		25.7	43.5	70.1	72.8	108.9	105.8	82.3	80.5
Cr concentration (mg/kg digesta)									
1	-	10 <sup>ab</sup>	10	21	37 <sup>ab</sup>	86	132	98	12
1	+	8 <sup>b</sup>	8	13	26 <sup>b</sup>	95	127	81	20
2	-	12 <sup>a</sup>	13	23	47 <sup>a</sup>	58	107	99	8
2	+	9 <sup>ab</sup>	11	34	28 <sup>b</sup>	97	125	96	15
SEM		1.1	1.6	2.5	4.0	12.4	8.3	6.8	4.3

<sup>ab</sup>Means for a marker in a column with same superscripts are not different ( $P < 0.05$ ).

Both marker levels peaked in the caecum after 6.5 h. Wheat type influenced alkane level in the MC ( $P=0.047$ ), and xylanase reduced Cr-EDTA levels in the stomach ( $P=0.022$ ), ileum ( $P=0.050$ ) and tended to decrease alkane level in the MC ( $P=0.077$ ). This demonstrates that wheat type and xylanase can influence digesta transit rates.

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## THE RESPONSE OF WEANLING PIGLETS TO ENZYME SUPPLEMENTATION WHEN FED DIETS DIFFERING IN NSP COMPOSITION - A: EFFECTS ON ENERGY DIGESTIBILITY

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The composition of NSP in cereals varies greatly and enzyme supplementation is often targeted only to the predominant NSP. This has led to a large number of enzyme preparations offered by the industry. The aim of this experiment was to determine if a single enzyme preparation containing multi-enzyme activities was able to increase energy digestibility in diets based on cereals differing markedly in their NSP composition.

Two experiments were done with weanling piglets to determine the faecal apparent energy digestibility of cereal diets with and without added enzymes. In Experiment 1, 16 crossbred piglets at 8.0 kg LW were divided into two groups and fed for 35 days either a marker (celite 545, 5 g/kg) supplemented barley-based basal diet (barley 691.3 g/kg, soybean meal 245.7 g/kg, soybean oil 26.2 g/kg, premix 36.8 g/kg Lys 11 g/kg, ME 12.9 MJ/kg) or the same basal diet supplemented with the enzyme preparation Rovabio™ Excel (Adisseo, France). In Experiment 2, 16 PIC piglets at 7.5 kg LW were divided into two groups and fed for 28 days either a corn/soy-based basal diet (corn 670 g/kg, soybean meal 240 g/kg, molasses 40 g/kg, premix 40 g/kg Lys 11 g/kg, ME 13.4 MJ/kg) or the basal diet supplemented with the enzyme preparation. The animals were housed individually, fed *ad libitum* and distributed according to a completely randomised block design. In the last five days of the experiment, faeces were collected as grab samples (Experiment 1) or quantitatively (Experiment 2). Data were analysed for each experiment using one-way ANOVA.

**Table 1. Influence of the broad-spectrum enzyme on energy digestibility (mean ± SD).**

	Barley (Experiment 1)		Corn-Soy (Experiment 2)	
	Control	+ Excel	Control	+ Excel
Energy Digestibility (%)	80.6 <sup>a</sup> ± 2.6	82.5 <sup>b</sup> ± 1.5	85.3 <sup>a</sup> ± 3.8	87.7 <sup>b</sup> ± 2.4
Energy Improvement (ME)		+ 0.31 MJ/kg		+ 0.37 MJ/kg

<sup>ab</sup>Means within an experiment with different superscripts differ ( $P < 0.05$ ).

In this experiment, treatment diets were based on different cereals resulting in differing compositions of NSP. Corn consists of 2% cellulose and 5.1% insoluble pentosans while barley contains 3.9% cellulose, 7.1% insoluble pentosans and 3.6% soluble  $\beta$ -glucans (Choct *et al.* 1997). Although the diets were different in NSP composition, adding the enzyme preparation increased ( $P < 0.05$ ) energy digestibility of the diets because of the broad spectrum of enzyme activities in the enzyme product. In an in-vitro experiment, Mathlouthi *et al.* (2002) found that xylanase reduced viscosity caused by wheat-NSP by 90%, but only by 15% in corn and 68% in barley. In the same experiment glucanase had a good effect on barley (84%), but almost no effect on corn and wheat. When xylanase and glucanase were combined, the viscosity deriving from corn NSP was reduced by only 6%. When a multi-enzyme preparation was used, viscosity of wheat, barley and corn was reduced by 94-100%. Versatile enzyme preparations, containing a range of activities, can increase energy availability in diets based on different cereals.

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## THE RESPONSE OF WEANED PIGLETS TO ENZYME SUPPLEMENTATION WHEN FED DIETS DIFFERING IN NSP COMPOSITION - B: EFFECTS ON PERFORMANCE

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Adding enzymes to hydrolyse non-starch polysaccharides (NSP) has been shown to increase the nutritional value of cereal-based feeds, but the practical application of this is often complicated by many marketed enzyme preparations being adapted only to the specific NSP content of the cereal used. Because of this, nutritionists must purchase a number of enzyme preparations and change these according to the feed produced. To increase the flexibility of diet formulation, a more versatile enzyme preparation is required. The aim of this experiment was to determine if a single enzyme preparation, containing a broad range of enzyme activities, was able to increase the performance of weaned piglets fed diets differing in NSP-composition.

Two experiments were done. In Experiment 1, 48 crossbred piglets (8.0 kg LW) were divided into two groups, housed individually, and randomly allocated (for 35 days) to a barley-based basal diet (barley 691.3 g/kg, soybean meal 245.7 g/kg, soybean oil 26.2 g/kg, premix 36.8 g/kg, Lys 11 g/kg, ME 12.9 MJ/kg) with or without supplementation with the enzyme preparation Rovabio™ Excel (Adisseo, France). In Experiment 2, 232 crossbred piglets (10.0 kg LW) were allocated to a completely randomised design using four replicates of 28 or 30 animals and fed for 42 days either a wheat-based basal diet (wheat 500 g/kg, barley 120 g/kg, rye 100 g/kg, soybean meal 210 g/kg, soybean oil 30 g/kg, premix 40 g/kg, Lys 13 g/kg, ME 13.9 MJ/kg) or the basal diet supplemented with the enzyme preparation. Growth (individually in both experiments) and feed intake (individually in Experiment 1 and per pen in Experiment 2) were recorded. Average daily gain (ADG), daily feed intake (DFI) and feed conversion ratio (FCR) were calculated and statistically analysed for each experiment using one-way ANOVA.

**Table 1. Influence of enzyme on piglet performance (mean ± SD)**

	Barley (Experiment 1)		Wheat (Experiment 2)	
	Control	+ Excel	Control	+ Excel
DFI (g/d)	658 ± 95	691 ± 106	803 ± 29	839 ± 36
ADG (g/d)	427 ± 56	458 ± 73	458 <sup>a</sup> ± 71	486 <sup>b</sup> ± 54
FCR	1.54 ± 0.10	1.51 ± 0.07	1.75 ± 0.04	1.73 ± 0.01

<sup>ab</sup>Means within an experiment with different superscripts differ (P < 0.01).

Supplementing the barley- and wheat-based diets with the enzyme preparation increased pig performance. Enzyme supplementation improved ADG [in tendency for barley (P < 0.08), significantly for wheat (P < 0.01)] and the pigs gained 7.3% (barley) and 6.1% (wheat) more per day than the control animals. Interestingly the variability of the FCR was reduced by the enzyme supplementation in both experiments, indicating a more homogeneous herd in terms of the transfer of dietary energy into growth.

## AVAILA<sup>®</sup>CU FOR GROWING PIGS

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Copper sulphate (CuSO<sub>4</sub>, 200 to 250 ppm Cu) is routinely added to pig diets to improve growth and feed conversion. However, increasing regulatory pressure regarding the impact of animal waste on the environment has meant that alternatives to CuSO<sub>4</sub> are required. Ward *et al.* (1997) recommended that complexed Cu could be used at lower levels (100 ppm Cu) to replace the high levels of CuSO<sub>4</sub> that are currently used in many swine diets. Addition of Cu from CuPLEX<sup>®</sup> copper lysine complex improved pig performance compared with CuSO<sub>4</sub> (Coffey *et al.* 1994).

This experiment was designed to evaluate the ability of a copper amino acid complex (Availa<sup>®</sup>Cu) to improve growth performance of pigs compared to CuSO<sub>4</sub>. A total of 480 pigs (240 males and 240 females) were used in a 2 x 3 factorial experiment with main effects of gender and Cu treatment. Dietary treatments were 1) no added Cu (Control); 2) Control plus 200 ppm Cu from CuSO<sub>4</sub> (CuSO<sub>4</sub>); 3) Control plus 100 ppm Cu from Availa<sup>®</sup>Cu. Four pens of 20 male pigs and four pens of 20 female pigs were allotted to each treatment at weaning (24 days). Pig bodyweights and average daily feed intakes were determined on day 21, 42, 63 and 91. The effect of treatment on faecal colour was measured from grab samples taken at weaning, day 42 and 84.

**Table 1. Growth of pigs fed diets containing CuSO<sub>4</sub> or Availa<sup>®</sup>Cu.**

Treatment	Start Weight (kg)	Final Weight (kg)	Rate of Gain (g/d)	FCR	Feed Intake (g/d)
Control (No added Cu)	8.07	61.4	601	2.06 <sup>ab</sup>	1.24
CuSO <sub>4</sub> (200 ppm Cu)	8.06	61.1	598	2.15 <sup>a</sup>	1.29
Availa-Cu (100 ppm Cu)	8.15	62.3	612	1.97 <sup>b</sup>	1.20
SEM	0.112	0.542	0.006	0.032	0.021
Treatment (T)	0.933	0.663	0.618	0.046	0.250
Sex (S)	0.232	0.279	0.398	0.024	0.016
T x S	0.967	0.709	0.653	0.170	0.531

<sup>ab</sup>Means with different subscripts differ significantly (P<0.05).

Dietary treatments had no effect (P>0.05) on growth performance, although pigs fed Availa<sup>®</sup>Cu were 1.2 kg heavier than pigs fed CuSO<sub>4</sub>. Adding Availa<sup>®</sup>Cu to the diets of the pigs improved feed conversion (P=0.046). Pigs fed Availa<sup>®</sup>Cu had lighter coloured (P=0.052) faeces compared to pigs fed the other two treatments. Using these data and an average feed cost of \$250/t, a break-even cost of \$17/kg for the addition of 100 ppm Cu from Availa<sup>®</sup>Cu to grower pig diets is required.

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## BETAINE AND A XYLANASE ENZYME HAVE SYNERGISTIC AND ADDITIVE EFFECTS IN RESTRICTIVELY-FED GILTS

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Dietary betaine can improve growth by reducing maintenance requirements, with the best responses generally occurring when dietary energy is limiting. For example, betaine increases lean growth in restrictively-fed boars but not in boars fed *ad libitum* (Suster *et al.* 2002). Many cereals contain complex carbohydrates, some of which are resistant to digestion in the small intestine of pigs. Addition of xylanase can increase the digestibility of these complex carbohydrates. The aim of this experiment was to determine the interactions and additivity of betaine and xylanase in restrictively-fed finisher gilts.

Twenty-four individually penned gilts (initial weight 59 kg) were allocated to a 2 x 2 factorial experiment with the respective factors being dietary betaine (0 and 1.25 g/kg Betafin) and xylanase (0 and 1.0 g/kg Porzyme). Feed intake was restricted to just below that which maximises lean deposition in this class of gilts (ca. 75% *ad libitum*). Pigs were weighed weekly and feed intake adjusted according to a sliding scale related to liveweight. Diets contained 50% wheat, 20% barley and 0.65 g available lysine/MJ DE. Pigs were slaughtered after five weeks and the carcasses dissected to a retail level.

**Table 1. Effect of xylanase (Porzyme, Por) and betaine (B) on growth and carcass characteristics in restrictively-fed gilts.**

Porzyme (Por, g/kg)	0		1.0		LSD <sub>05</sub>	Significance		
	Betaine (B, g/kg)	0	1.25	0		1.25	B	Por
Daily gain 0-14 d (g/d)	771	840	800	898	103.7	0.028	0.24	0.69
Daily gain 0-35 d (g/d)	799	844	832	878	72.5	0.080	0.18	0.99
Feed conversion ratio 0-14 d	2.86	2.63	2.82	2.50	0.29	0.01	0.39	0.64
Feed conversion ratio 0-35 d	2.87	2.77	2.85	2.69	0.18	0.045	0.41	0.59
Cold carcass weight (kg)	63.2	63.4	65.1	65.4	2.51	0.74	0.034	0.95
Carcass lean (kg)	38.3	38.1	37.6	40.0	3.33	0.34	0.57	0.26
Carcass fat (kg)	6.41	7.02	9.53	6.39	2.44	0.14	0.15	0.034
P2 (mm)	11.7	11.5	12.3	11.0	1.14	0.009	0.99	0.19
Loin eye area (cm <sup>2</sup> )	38.4	41.2	39.2	43.1	5.04	0.067	0.44	0.75

Over the first 14 days, daily gain was increased in pigs fed betaine (786 vs 869 g/d, P=0.028) but was not altered by xylanase (805 vs 849 g/d, P=0.24) (Table 1). However, the effects were additive with gilts receiving the combined treatment growing 16% (P<0.05) faster than controls. Responses diminished over the subsequent three weeks, perhaps because feed intake was then beyond that which maximised lean deposition. Feed intake averaged 2.21 and 2.44 kg/d over the periods from 0 to 14 days and 14 to 35 days, respectively. There was no effect of betaine on carcass weight (64.2 vs 64.4 kg, P=0.74) whereas xylanase increased carcass weight (63.3 vs 65.3 kg, P=0.034). P2 was reduced by dietary betaine (12.0 vs 11.2 mm, P=0.009) but not by xylanase (11.6 vs 11.6, P=0.99). There was an interaction (P=0.034) between the dietary treatments such that xylanase alone increased fat mass (6.41 vs 9.53 kg, P<0.05) whereas the combination had no effect on fat mass (6.41 vs 6.39 kg, P=0.91). In conclusion, betaine increased growth in gilts that were restrictively fed but not when their intake increased above that required to maximise lean deposition. The mechanisms responsible for the synergy between betaine and xylanase are unclear.

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## WEANMOR<sup>®</sup> INCREASES SOW PRODUCTIVITY BY REDUCING STILLBIRTHS

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Sows kept under confinement conditions become inactive and this may reduce muscular tone and increase the risk of uterine infections. Calcium is involved in muscular contractions and diet acidification can modify gastric pH, reducing bacterial infection. The objective of this experiment was to determine if WEANMOR<sup>®</sup> (an encapsulated blend of mixed organic acids and calcium chloride) could reduce stillbirths and pre-weaning mortality. (Loughmiller *et al.* 2002)

Two commercial farms in the USA were used in this experiment. On Farm 1, a total of 442 KPA sows and average parity of 5.1 was used (control 232 sows, treatment 210 sows). On Farm 2, a total of 90 PIC sows and average parity of 3.3 was used (control 43 sows, treatment 47 sows). All parities were equally represented in control and treatment groups. Sows were allocated to treatments in an unbalanced, completely randomised experimental design. All sows received a lactation diet that met or exceeded NRC requirements (1998) and had a minimum DE 12.8 MJ/kg, crude protein 180 g/kg and total lysine 9.4 g/kg. The treated sows received 60 g/d of WEANMOR<sup>®</sup> top-dressed from day four prepartum to day seven postpartum. Each sow was fed 1.8 kg/day of feed before farrowing and *ad libitum* after farrowing. Piglets were weaned at about 19 days of age. Data were analysed using the model SAS that included treatment and parity.

**Table 1. Reproductive performance of sows fed WEANMOR<sup>®</sup>**

	Farm 1					Farm 2				
	Control	S.E.	Weanmor	S.E.	P value	Control	S.E.	Weanmor	S.E.	P value
Total born	11.88		12.11			11.79		11.27		
Born alive	10.89	±0.19	11.37	±0.20	0.092	11.01	±0.43	10.99	±0.48	0.960
Stillborn (% of total)	8.33	±0.70	6.11	±0.73	0.485	6.11	±1.10	2.30	±1.22	0.010
Weaned	9.90	±0.11	10.42	±0.12	0.002	9.97	±0.10	9.69	±0.11	0.030

Feeding WEANMOR<sup>®</sup> at 60 g/sow/d from day four prepartum to day seven postpartum reduced stillborn pigs by 27-62%. Sows fed WEANMOR<sup>®</sup> at Farm 1 had 2.22% less stillborn pigs than control sows and in Farm 2 there were 3.81% less stillborn pigs (Table 1). An extra 0.52 pigs were weaned relative to control sows in Farm 1. Unfortunately, Farm 2 used cross fostering during the trial period and no improvement in number of weaned pigs or in pre-weaning mortality could be recorded. No parity effect was detected for the treatment response on either farm.

The mode of action of WEANMOR<sup>®</sup> is hypothesised to be that calcium chloride elevates blood calcium concentrations aiding uterine muscle contractions and improving farrowing rate. The protected acids alter pH in the gastrointestinal tract and lower the pH of urine and this may reduce bacterial load in the urinogenital tract and improve the hygienic environment of the birth canal.

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## THE EFFECTS OF DIETARY SUPPLEMENTATION WITH PLANT EXTRACTS ON MILK COMPOSITION, FEED INTAKE AND BACKFAT LOSS IN LACTATING SOWS

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Extracts of capsicum, carvacrol and cinnamaldehyde improve diet digestibility and suckling piglet performance when supplemented to lactating sows (Ilesley *et al.* 2002). The purpose of this experiment was to determine whether these extracts could improve voluntary food intake, backfat loss and milk composition of lactating sows.

Forty-four hybrid sows (mean parity  $2.2 \pm 0.3$ ) were allocated to one of two dietary treatments on day 107 of gestation. Treatments were 'C' - standard sow lactation diet (14 MJ/kg DE, 10 g/kg lysine), and 'P' - diet C supplemented with 100 mg/kg plant extracts (containing 5% carvacrol, 3% cinnamaldehyde, 2% capsicum). Sows received 2.5 kg/d of the appropriate diet until farrowing. Sows were fed 1 kg/day on day 0, increasing by 1 kg/d up to day 7, after which food was offered *ad libitum*. Sow P2 backfat was measured by ultrasonics at farrowing and weaning. Ten sows per treatment were sampled for colostrum (day 0) and again for milk on day 21 of lactation. Data were analysed using the General Linear Model procedure of Minitab 12.2.

**Table 1. Effect of sow dietary treatment on percentage fat, protein and lactose, fat to lactose ratio, and immunoglobulin concentrations in colostrum and milk.**

	Colostrum (day 0)				Milk (day 21)			
	C	P	SEM	P value	C	P	SEM	P value
Fat %	4.57	4.14	0.24	ns	7.83	6.48	0.66	ns
Protein %	14.46	14.25	0.61	ns	4.88	4.36	0.23	ns
Lactose %	2.42	2.80	0.16	P<0.1	4.87	5.37	0.05	P<0.01
Fat: Lactose	1.929	1.511	0.179	P<0.1	1.619	1.230	0.137	ns
IgG mg/ml	106.2	93.5	5.9	P<0.1	0.72	0.65	0.02	P<0.1
IgA mg/ml	28.0	24.6	1.7	P<0.1	3.41	3.24	0.12	ns

**Table 2. Effects of dietary treatment on sow P2 backfat at farrowing, backfat loss between farrowing and weaning and average daily feed intake (ADFI) during lactation (21 days).**

	Control	Xtract	SEM	P value
Start P2 (mm)	18.2	19.0	0.9	ns
P2 loss (mm)	2.10	0.98	0.57	P<0.1
ADFI kg/d	7.63	7.32	0.22	ns

Despite similar feed intakes during lactation, loss of P2 backfat tended to be lower in supplemented sows (P<0.1). This suggests an improved efficiency of feed utilisation. The increased lactose levels in the milk and colostrum of sows fed the extract suggests either an effect on nutrient partitioning, increased substrate availability or a stimulation of the lactose synthesis pathway. The tendency towards reduced IgG and IgA concentrations may indicate lower immune stimulation of the sow, perhaps a result of antimicrobial activity by the plant extracts. The higher fat to lactose ratio in control sow colostrum may point to greater fat reserve mobilisation. In conclusion, plant extracts may reduce sow backfat loss during lactation and influence milk composition.

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## POTENTIAL DAMAGE TO THE UTERINE LINING AFTER NON-SURGICAL DEEP INTRAUTERINE IMSEMINATION OF SOWS

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Deep intrauterine (DIU) insemination allows for a 20-fold reduction in the required dose of fresh sperm from traditional insemination (Martinez *et al.* 2002). However, litter size may be reduced (Bathgate *et al.* 2003; Day *et al.* 2003), possibly due to trauma caused to the reproductive tract during insemination. In this experiment we investigated if there was a correlation between bleeding from the reproductive-tract and fertility when DIU was carried out with 272 crossbred sows using the Firflex<sup>®</sup> catheter (Magapor, Spain).

Fertility data from the experiment have been reported previously (Bathgate *et al.* 2003). In summary, conception rates at the same piggery and over the same period were lower using DIU than contemporary conventional cervical inseminations. We observed the incidence of blood on the DIU catheter on withdrawal (cervical bleeding) and emanating from the vagina 12 or more hours after AI. A significant proportion of sows (15%) displayed bleeding 12 or more hours post-insemination. This bleeding appeared to originate in the uterus (uterine bleeding), while another 15% of sows showed blood during or immediately after the insemination that appeared to originate in the cervix or vagina (cervical bleeding). Yet another 4% apparently bled from both sites. To gauge the impact of this blood on the non-return rate (NRR), farrowing rate and litter sizes of the sows, these factors were compared between the groups. Results from commercial matings over the same period were also compared (cervical bleeding occurred in <1% of these cases). All sows inseminated using DIU showed lower NRR and farrowing rates than sows inseminated using conventional AI (ANOVA  $P < 0.05$ ). The groups of sows that displayed uterine bleeding, or no blood, had a significantly lower litter size than the conventional AI group (Chi-squared,  $P < 0.05$ ) (Table 1).

**Table 1. Results of DIU trial when considering bleeding caused by the insemination.**

	Number of sows	Number conceived (%)	Number farrowed (%)	Total born mean $\pm$ SEM
Conventional AI	65	54 (83) <sup>a</sup>	51 (78) <sup>a</sup>	11.0 $\pm$ 0.6 <sup>a</sup>
No blood (DIU)	202	103 (51) <sup>b</sup>	69 (34) <sup>b</sup>	8.2 $\pm$ 0.1 <sup>b</sup>
Cervical Bleeding	30	18 (60) <sup>b</sup>	14 (47) <sup>b</sup>	9.1 $\pm$ 0.3 <sup>ab</sup>
Uterine Bleeding	31	12 (39) <sup>b</sup>	7 (23) <sup>b</sup>	5.4 $\pm$ 0.5 <sup>b</sup>
Both bleeding types	9	6 (67) <sup>b</sup>	3 (33) <sup>b</sup>	9.0 $\pm$ 0.7 <sup>ab</sup>

<sup>a,b</sup> Different superscripts in same column indicate significant differences ( $P < 0.05$ ).

These data do not indicate that visible cervical or uterine bleeding was the major cause of the reduced fertility of DIU sows, although the reduced litter size measured after visible uterine bleeding may be worthy of further research.

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## THE RELATIONSHIPS BETWEEN SOW BODY CONDITION, REPRODUCTIVE PERFORMANCE AND CULLING

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Australian pig producers have been aware for some time that breeding-herd performance is static or reducing and annual rates of sow replacement are exceptionally high at 60-70% (Pigstats, 2001). Inadequate gilt management and in particular poor nutrition of the gilt before herd entry and throughout her breeding life have been suggested as the primary causes of these changes in breeding herd performance (Hughes and Varley, 2002). In this experiment the bodyweight and body condition (P2 backfat depth and visual appraisal) of 2184 gilts and sows being culled and 491 dying gilts and sows were compared with the same parameters in a balanced cross-section (5323 gilts and sows) of the whole breeding herd.

The recorded reasons for culling sows agreed well with previous reports (see Hughes and Varley, 2002): 50% due to reproductive inefficiency or failure, 12% due to locomotor problems, and only 20% due to the age/parity of the sow. Culled and dying/euthanased sows were significantly lower in body condition than sows remaining in the herd (Table 1). Low body condition, causing anoestrus or skeletal/locomotor problems, was a particularly significant pre disposing factor for culling (Table 2).

**Table 1. Mean bodyweight ( $\pm$  SEM) and P2 backfat ( $\pm$ SEM) for a cross-section of the herd, culled gilts/sows and dead gilts/sows.**

	Herd cross-section	Culled gilts/sows	Dead gilts/sows
Mean bodyweight (kg)	218.5 <sup>a</sup> $\pm$ 0.54	205.9 <sup>b</sup> $\pm$ 1.05	206.1 <sup>b</sup> $\pm$ 2.04
Mean P2 backfat depth (mm)	15.5 <sup>a</sup> $\pm$ 0.06	14.3 <sup>b</sup> $\pm$ 0.09	14.6 <sup>b</sup> $\pm$ 0.19

<sup>ab</sup> Within rows means with different superscripts are significantly different ( $P < 0.001$ ).

**Table 2. The relative body condition of culled gilts/sows and the herd mean.**

Reason for culling	Parameter	Condition relative to mean*
Reproductive failure: anoestrus	Liveweight	-26.8 kg
	P2 backfat	-1.3 mm
Locomotor problems	Liveweight	-39.6 kg
	P2 backfat	-2.6 mm

\*Mean calculated for each culling reason on the basis of parity profile of the culled sows.

The results indicate that improving sow body condition may alleviate, but not resolve, the problem of high rates of sow culling and sow death.

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## MOLECULAR VIRULENCE GENE TYPING OF CLINICAL *E. COLI* ISOLATES FROM PIGS WITH POST-WEANING DIARRHOEA

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Post-weaning diarrhoea (PWD) is associated with the isolation of haemolytic lactose-fermenting bacilli - *Escherichia coli* (*E. coli*). Possession of certain virulence genes is a key requirement for pathogenicity in enterotoxigenic *E. coli* (ETEC). However, some human ETECs also carry virulence genes (Dezfulian *et al.* 2003) which, until recently, were believed to be exclusive to the extra-intestinal pathotypes (ExPECs) that cause urinary tract infections (UTI) or septicaemia.

The aim of this experiment was to determine whether PWD ETECs, like their human counterparts, also possess ExPEC virulence genes. Fifty-one *E. coli* isolates recovered from PWD piglets were obtained from NSW (19), Qld. (23) and Vietnam (9). Three serogroups represented by O149 (38), O141 (8) and O8G7 (4) were found to predominate. The distribution of these genes as a function of geographical origin is depicted in Table 1. Following PCR analysis, only eight out of 32 ExPEC virulence genes were found to be present in PWD *E. coli* isolates; *fimH* (type 1 fimbriae), *hlyA* (-hemolysin), *traT* (serum resistant protein gene), *iha* (iron-regulated gene), *fyuA* (siderophore receptor), *ompT* (outer membrane protease T), *k5* (K5 capsule) and *kpsMTII* (Group II capsule). The association of *fimH* with serogroup O141 and O8G7; *traT* with O149 and O8G7, and *iha* with O149 and O141 demonstrate that porcine PWD ETECs also harbour extra-intestinal virulence genes. Furthermore, the pattern of ownership of these ExPEC genes with certain serotypes suggest that it will be possible to identify and epidemiologically map the prevalence of PWD *E. coli* serogroups using a simple faecal PCR test.

PWD ETEC isolates could also be discriminated using different combinations of enteric virulence genes (Table 1). For instance, O141 isolates were *stx2*<sup>+</sup> (Shiga toxin II), *LT* (heat-labile toxin) and *east-1* (EaggEC heat-stable enterotoxin) while O149 isolates were *cdt* (cytolethal distending toxin) and *stx2*. Virulence gene exclusivity could not be used to type O8G7 isolates.

**Table 1. Prevalence of virulence factor genes detected in PWD *E. coli* isolates.**

	O149 (NSW) n=7	O149(Qld.) n=23	O149 (Vietnam) n=8	O141(NSW) n=8	O8G7 n=4
ExPEC VF gene					
<i>fimH</i>	0	0	0	100%	100%
<i>hlyA</i>	100%	74% (17/23)	88% (7/8)	88% (7/8)	50% (2/4)
<i>traT</i>	100%	61% (14/23)	100%	12% (1/8)	100%
<i>iha</i>	100%	100%	100%	88% (7/8)	25% (1/4)
Enteric VF gene					
<i>LT</i>	100%	100%	100%	0	75% (3/4)
<i>STa</i>	100%	83% (19/23)	100%	88% (7/8)	100%
<i>STb</i>	100%	100%	100%	88% (7/8)	100%
<i>F4</i>	100%	100%	100%	0	50% (2/4)
<i>F18</i>	0	0	0	88% (7/8)	50% (2/4)
<i>east-1</i>	100%	100%	100%	0	100%
<i>cdt</i>	0	0	0	0	50% (2/4)
<i>stx2</i>	0	0	0	88% (7/8)	0

Note: *STa* and *STb*: heat-stable enterotoxin a and b; *F4*: F4 fimbriae (k88); *F18*: F18 fimbriae

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## VARIATION IN ESTIMATED BREEDING VALUES (EBVs) OF YOUNG BOARS

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Estimated Breeding Values (EBVs) predict differences in genetic merit between animals. On average, half of the genetic superiority of an animal is observed in the performance of its progeny (Macbeth and McPhee, 1999), since a parent passes half of its genes to its offspring. Hence, selecting genetically superior replacement stock increases the profitability of pig production. In this experiment we evaluated the amount of variation in EBVs of young boars and derived the economic benefits of using genetically superior boars.

Across-herd EBVs from eight herds were available from the National Pig Improvement Program (NPIP) (<http://npip.une.edu.au>) for 1287 Large White young boars, all less than 200 days of age at the time of analysis (May 7, 2003). The PIGBLUP software (Crump, 2003) is used in the NPIP to predict EBVs. A profit function is used to derive a Terminal Sire (TSI) and Maternal (MI) index (basis: \$/litter). Partial regression coefficients were 0.53 (TSI) and 0.57 (MI) for average daily gain (ADG), -25.0 (TSI) and -5.8 (MI) for backfat (BF) and 4.55 (TSI) and 27.0 (MI) for number born alive (NBA). In comparison with the base year of 1991, mean EBVs were 38.5 g/d for ADG, -2.78 mm for BF and 0.53 piglets/litter for NBA, showing the genetic superiority of this group of young boars (Table 1). The mean TSI and MI indexes were \$96.5 and \$51.3 per litter.

Ranking boars according to their TSI (top 10%) shows the emphasis this index places on BF and ADG. Mean EBVs of the top 10% group were 1.41 mm below the mean for BF and 9.5 g/d above the mean for ADG. In contrast, the top 10% of boars based on the MI had mean EBVs for NBA of 1.03 piglets/litter above the mean and 12 g/d above the mean for ADG. The total variation in EBVs was further partitioned into within-herd and between-herd variation. The between-herd variation comprised 19% of the variation in TSI. In contrast, more variation existed between herds (64%) for the MI, because of a larger between-herd variance for NBA.

**Table 1. Across-herd EBVs for average daily gain (ADG), backfat (BF) and number born alive (NBA) along with the Terminal Sire index (TSI) and Maternal index (MI).**

	ADG	BF	NBA	TSI	MI
	(g/d)	(mm)	(piglets/litter)	\$/litter	\$/litter
Mean	38.5	-2.78	0.53	96.5	51.3
Standard deviation	18.6	1.02	0.69	23.7	21.6
Mean EBVs - top 10% for TSI	48.0	-4.19	0.30	133.7	57.9
Mean EBVs - top 10% for MI	59.3	-2.50	1.56	105.4	89.4

The economic difference between choosing a young boar from the top 10% over an average boar was \$37.2 per litter for the TSI. On average, half of this difference will be realised by his progeny. In this example, profitability for a 200-sow herd with 2.2 litters/sow/year increased by \$8184 per year. Producers and breeders alike can achieve a similar increase in profitability by simply choosing boars based on their genetic merit.

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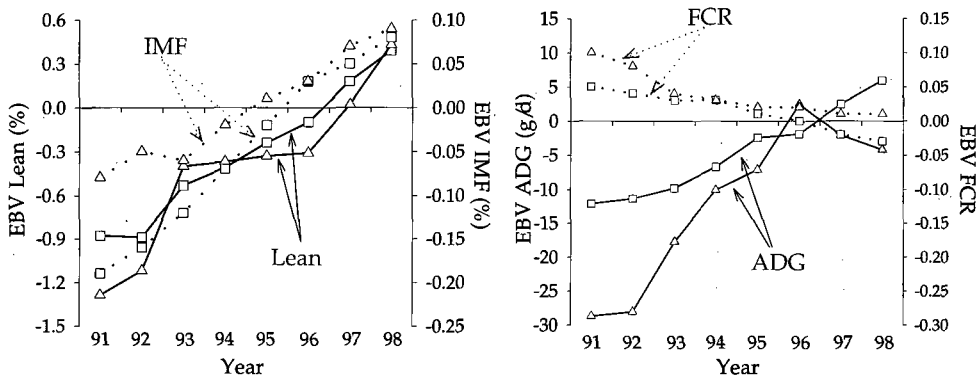
## SELECTION FOR INTRAMUSCULAR FAT IN THE LARGE WHITE AND LANDRACE PIG BREEDS IN SWITZERLAND

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The level of intramuscular fat (IMF) in the *M. longissimus dorsi* in Australian pork is now below the recommended optimal value for eating quality of 2.5% (Hermesh *et al.* 1997). This also occurred in Switzerland in the mid-1980s. Since 1985, the IMF levels of all full sibs performance-tested at the Testing Station between 30-103 kg liveweight, have been routinely measured by Near Infrared Spectroscopy. In 1989, a new selection index combining average daily gain during test (ADG), feed conversion ratio (FCR), percentage lean cuts (% lean), IMF, and a meat-quality score based on pH and light reflectance measurements (MQ; 1=normal to 4=PSE or DFD) was introduced (Morel *et al.* 1988). The index was derived to achieve a genetic gain of 0.03% for IMF and 0.005 point for MQ per generation (Niebel, 1979). The aim of this experiment was to show that classical index selection could be successfully used to improve IMF level in pure breeds.

Estimated Breeding Values (EBV) of 21 340 LW and 5712 LR pigs born between 1991 and 1998 were calculated using BLUP (Schwörer *et al.* 2000). These EBVs are given in Figure 1 to illustrate the genetic trends in these two breeds.



**Figure 1.** Estimated Breeding Value (EBV) for Intramuscular Fat (IMF), % Lean, Feed Conversion Ratio (FCR), Average Daily Gain (ADG) for Large White (□) and Landrace (Δ) pigs.

Between 1991 and 1998, a total genetic gain in IMF of 0.27% in LW and 0.17% in LR was achieved. No changes in MQ parameters were measured. Genetic improvement in IMF may have reduced, but did not compromise, the genetic gain in the others traits: % Lean (LW +1.27% and LR +1.7%), FCR (LW -0.08 and LR -0.09), or ADG (LW +18 g/d and LR +24.5 g/d). Eating quality and growth performance traits can therefore be improved genetically by index selection.

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## THE EFFECT OF XYLANASES ON THE NSP DIGESTIBILITY OF PIG DIETS HIGH IN WHEAT BY-PRODUCTS.

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Millrun is a mixture of wheat bran and wheat pollard used as a low-cost ingredient in weaner-pig diets in Australia. Millrun is added to feed at relatively low levels, between 50-100 g/kg DM, and although low in DE (11.0 to 11.5 MJ/kg) it contains around 140-160 g/kg protein. However, because millrun is also rich in dietary fibre (350-450 g/kg DM), young pigs find it difficult to digest. The fibre is largely insoluble and the 30-40 g/kg of soluble fibre is not as viscous as that of intact wheat (Choct *et al.* unpublished data) enabling the digestibility of millrun to be possibly increased by liquid feeding. Supplementation with exogenous enzyme targeting the NSP (arabinoxylans) may also improve the nutritive value of millrun.

In this experiment we measured the ability of two xylanases to increase the digestibility of millrun offered in either a dry or liquid form. The aim was to determine if depolymerisation of soluble NSP, through enzyme addition, increased millrun digestibility more than releasing the easily fermentable carbohydrates (also through enzyme addition) from insoluble NSP. One of the xylanases used has an affinity for soluble and insoluble arabinoxylans while the other xylanase only targets insoluble arabinoxylans.

One hundred and twelve male, Large White x Landrace pigs (weaned at 28 days) were fed a diet containing 500g/kg DM millrun, formulated to contain a marginal DE content of 13.80 MJ DE/kg DM and 0.80 g available lysine/MJ DE, in a 2 x 4 factorial design. The factors were (1) enzyme (0, 300 ppm Biofeed Wheat<sup>®</sup>, 400 ppm Biofeed Plus<sup>®</sup>, and a combination of 300 ppm Biofeed Wheat<sup>®</sup> + 400ppm Biofeed Plus<sup>®</sup>) and (2) feed form (dry vs liquid). Liquid diets contained 2.5 L water/kg feed and were soaked for 24 h before feeding. Statistical analyses included a multi-factor Analysis of Variance and Fisher's LSD. Digesta samples from 40 pigs (five pigs per treatment) were taken after 26 days from three sections of the gut (duodenum, ileum and colon) to determine nutrient digestibility (protein, starch, and NSP) through the GIT.

Liquid feeding improved ( $P<0.05$ ) the FCR of weaners offered diets high in millrun (liquid=1.11 vs dry=1.17). Liquid feeding also decreased digesta viscosity and reduced VFA production in the colon ( $P<0.05$ ), suggesting that liquid feeding altered the site of millrun fermentation.

Liquid feeding improved ( $P<0.05$ ) ileal gross energy digestibility, but not when the xylanase capable of solubilising insoluble NSP was added to the diet. Adding the enzymes to the high-fibre diet had no significant effect on weaner growth performance. Starch and energy digestibility in the ileum, were depressed (in both feed forms) when the xylanase capable of solubilising insoluble NSP was used, highlighting the detrimental effect of releasing soluble NSP in the small intestine. This reduction in the efficiency of enzymatic digestion has been measured in poultry with similar effects on production (Bedford and Classen, 1992; Choct and Anison, 1992). These results suggest that soluble NSP may be a significant anti-nutrient in weaner pig diets. Caution must be applied when using enzymes with affinity for only the insoluble NSP, especially when diets contain a high level of fibre.

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## INCREASING THE LEVEL OF MILLRUN SUBSTITUTION (FIBRE) IN WHEAT-BASED DIETS FOR WEANER PIGS

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Increasing fibre in the diets of weaner pigs may negatively affect performance due to the effects of fibre on nutrient digestion and absorption (Bach Knudsen and Hansen, 1991; Jorgensen *et al.* 1996). However, it is not clear whether increasing fibre is detrimental to weaner-pig performance when diets are balanced in energy and amino acids. Millrun is one of the most commonly used fibre-rich by-products in Australia and is made up of wheat bran and wheat pollard. The hypothesis tested in this experiment was that feed intake and growth performance of weaner pigs would be reduced as millrun substitution was increased.

Forty-eight male, Large White x Landrace pigs, weaned at 28 days, were fed wheat- and barley-based diets with 0-500 g/kg millrun substitution. Diets were formulated to contain a marginal digestible energy of 13.8 – 13.9 MJ DE/kg and 0.85 g available lysine/MJ DE. Animals were fed dry meal diets *ad libitum* for 21 days. The experimental treatments were five graded levels of millrun inclusion: 0, 125, 250, 375 and 500 g/kg millrun. Statistical analyses included a multi-factor Analysis of Variance and Fisher's LSD.

**Table 1. Effect of increasing millrun up to 500 g/kg in weaner diets on performance of male weaner pigs from 5-26 days post weaning.**

Millrun Inclusion (g/kg)	5d Wt.(kg)	26d Wt.(kg)	Daily gain (g/d)	FCR (g:g)	Feed intake (g/d)	DE Intake (MJ DE/day)	DE (MJ/ kg DM)
0	7.97	17.61	459	1.27	583	10.00	14.01 <sup>a</sup>
125	8.10	17.96	469	1.36	640	10.95	14.64 <sup>ab</sup>
250	7.99	17.64	459	1.36	625	10.68	15.35 <sup>bc</sup>
375	7.97	17.71	464	1.41	654	11.70	15.51 <sup>bc</sup>
500	7.97	16.99	429	1.37	590	10.74	15.97 <sup>c</sup>
Millrun	NS	NS	NS	NS	NS	NS	*
SE <sub>D</sub>	0.13	0.70	30.1	0.05	34.4	0.60	0.38

\* P<0.05; NS = not significant. <sup>abcd</sup> within a column different superscripts differ significantly (P<0.05)

There was no significant effect of millrun on pig performance. However, feed intake and growth rate tended (P<0.07) to decrease when millrun was included at 500 g/kg. Daily DE intake also tended to be higher as millrun substitution increased (P<0.08). This suggests that millrun does not significantly decrease growth performance in weaner pigs when the diet is adequate in energy and nutrients. The DE measured in the diets was higher than expected with no difference in growth observed, so diets high in millrun were in fact impeding performance as the nutrient supply from these diets was superior. The tendency for feed intake and daily gain to decrease as millrun increased in the diet indicates that the physical bulk and rate of digestion may limit intake through increased gut fill associated with the millrun.

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## ACTIVE TEMPERAMENT INCREASES EYE MUSCLE DEPTH IN MALE PIGS

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Hessing *et al.* (1994) found that female and castrate-male pigs grew faster when housed in groups containing pigs with active and passive temperaments. In contrast, van Erp-van der Kooij *et al.* (2000) found no association between growth and temperament, but a significant positive relationship between lean meat content at slaughter and activity assessed using the backtest at weaning. The present experiment was designed to test two hypotheses: (1) male pigs housed in groups containing both active and passive animals will grow faster when maintained at a stocking density of either 0.75 or 1.5 m<sup>2</sup>/pig; and (2) active male pigs will have a higher body lean content.

Seventy-two hybrid male pigs (Large White x Landrace) were selected at weaning (28 days of age) as having either an active (more than six escapes) or passive (zero escapes) temperament when held in the supine position for one-minute. The pigs were allocated at 16 ± 0.83 kg (mean ± SE) to three temperament treatments (active, mixed and passive) and two stocking rates (0.75 or 1.5 m<sup>2</sup>/pig) and allocated to pens (n=6 pigs per pen). The experiment continued until the pigs reached 80.6 ± 1.70 kg liveweight. Pigs were offered a commercial mash diet and water *ad libitum*. Backfat thickness and eye-muscle depth at the P2 position measured by real-time ultrasound were recorded together with liveweight weekly throughout the experiment. The pen was used as the statistical unit.

**Table 1. The effect of temperament<sup>1</sup> (T) and stocking rate (SR) on mean average daily liveweight gain (ADG), P2 backfat thickness and eye-muscle depth (EMD) measured at 80 kg liveweight on 66 male pigs grown from 16 to 80 kg liveweight.**

Temperament	Active		Mixed		Passive		SE	Significance <sup>2</sup>		
	0.75	1.5	0.75	1.5	0.75 <sup>3</sup>	1.5		T	SR	T x SR
ADG (g)	936	981	940	960	932	952	8	NS	*	NS
P2 (mm)	11.9	11.9	10.8	12.1	10.5	12.6	0.7	NS	NS	NS
EMD (mm)	54.5	52.2	51.2	52.7	49.8	52.5	0.7	*	NS	*

<sup>1</sup>Active (more than six escapes) or passive (zero escapes) temperament at weaning when held in the supine position for one minute. <sup>2</sup>NS, not significant, \*P<0.05. <sup>3</sup>One pen was removed from the analysis due to suspected pneumonia infection.

The results did not support the hypothesis that male pigs grow faster when housed in group-pens of mixed temperament (half active, half passive). Daily liveweight gain was not significantly different in group-pens containing either active pigs (958 g), mixed (950 g) or passive (942 g) animals. Pigs with active temperament, however, displayed a significant (P<0.05) increase in eye muscle depth (54.5 mm) compared to passive animals (49.8 mm) when housed at 0.75 m<sup>2</sup>/pig. In contrast, when housed at 1.5 m<sup>2</sup>/pig, eye-muscle depth was similar for active (52.3 mm), mixed (52.7 mm) and passive (52.5 mm) treatments. These results suggest that increased struggling behaviour in male piglets during the backtest is associated with increased eye-muscle depth at 80-kg liveweight when housed in group-pens at the higher stocking rate.

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## TEAT NUMBER AND ITS RELATIONSHIP TO FEMALE REPRODUCTION TRAITS

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Teat number (TN) is one of the traits used for pre-selection of pigs at Thailand government farms. Pigs with higher phenotypic TN are selected to guarantee that sufficient functional teats are available to nurse the litter. The aim of this experiment was to investigate the genetic relationships between TN and reproduction traits that are commonly recorded in sows in Thai pig populations on government farms.

Combined data from Duroc, Large White and Landrace breeds were used for genetic analysis. These data included TN from 5099 pigs and reproduction traits from 994 sows (3309 litters) recorded between 1991 and 2001. The reproductive traits used were: total number born (TNB), number of pigs born alive (NBA), litter size at 21 and 28 days after farrowing (LS21 and LS28), litter weight at birth, 21 and 28 days after farrowing (LWB, LW21 and LW28). Bivariate animal model analyses were performed using ASREML (Gilmour *et al.* 2002) to estimate genetic parameters for and between TN and reproduction traits. For TN, fixed effects of breed-line and herd-year-season (four month intervals of birth dates) and the random additive genetic effect were included in the model. For reproduction traits, the model included breed-line, herd-year-season (four month intervals of farrowing dates) and age class of sows as fixed effects and random effects of additive genetic and permanent environment of the sow. Litter weight traits were analysed with and without linear and quadratic adjustments for litter size. Results of the genetic analyses are given in Table 1.

**Table 1. Heritability estimates ( $h^2$ ) ( $\times 100$ ) for reproduction traits, and genetic ( $r_g$ ) and phenotypic ( $r_p$ ) correlations ( $\times 100$ ) between teat number and reproduction traits.**

	TNB	NBA	LS21	LS28	LWB	LW21	LW28	LWB <sup>1</sup>	LW21 <sup>1</sup>	LW28 <sup>1</sup>
$h^2$	10	14	7	5	9	7	8	9	16	20
$r_g$	-1	7	-1	-5	21	17	11	22	20	13
$r_p$	1	2	4	3	2	4	2	1	1	-2

<sup>1</sup>Weights adjusted for litter size.

The mean TN was  $14.2 \pm 1.3$ . The heritability estimate for TN was  $0.37 \pm 0.03$  and the heritability estimates for reproduction traits were low with standard errors (SE) ranging from 0.03 to 0.07. Genetic correlations of TN with litter-size traits were effectively zero whereas those of TN with litter-weight traits tended to be slightly positive. However, due to the high SE (0.12-0.21) they were not significantly different from zero. Phenotypic correlations were close to zero with SE ranging from 0.03 to 0.04. Our results were similar to studies of Ligonésche *et al.* (1995) who reported genetic correlations of TN and reproductive traits close to zero and Lu and Lian (2000) who reported positive genetic correlations between TN and litter weight and negative correlations between TN and litter size with large SE. Selection to increase TN is therefore unlikely to affect litter size but could have a favourable effect on litter weight.

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## THEORETICAL CONSIDERATIONS ON GENETIC SELECTION

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Pigs are traditionally selected on phenotypic traits such as feed conversion ratio (FCR), backfat thickness (BF) or average daily gain (ADG) using classical population genetic methods. After several generations of breeding, a plateau is reached and no further genetic gain is achieved. In this experiment, we used a simulation model to investigate what effect different environments have on the number of generations of breeding it takes to reach the plateau.

A simulation model has been developed to link individual gene actions, pig growth modelling and non-linear optimisation. In this model, 39 loci each with six alleles ( $3.686 \times 10^{51}$  genomic combinations) control five traits, and these are used to characterise the genotype in a pig growth model (De Lange, 1995). The growth model then simulates FCR, BF and ADG for this given genotype and a given diet. Using the model, we investigated the effect of dietary nutrient density (High: 14.5 MJDE/kg and 17.4 g available lysine/kg and Low: 12.8 and 7.1 available lysine/kg) during growth on theoretical genetic gain ( $\Delta G$  with  $i\text{-bar}=1$ ). A search of the genome for the best value for FCR, BF or ADG was made using a genetic algorithm. To get estimates of the theoretical genetic gain, genomes for 50 000 sire/dam pairs were generated at random as the base population, with each pair bred to produce one offspring. FCR, BF and ADG were simulated with the model for each diet and genetic parameters estimated. The theoretical number of generations (N), assuming no Bulmer effect (Bulmer, 1971), between the base population and the best value genome was then calculated for each trait and diet combination (Table 1).

**Table 1. Population mean, phenotypic standard deviation (sp), heritabilities ( $h^2$ ), genetic gain ( $\Delta G$ ), best genome performance (BG), theoretical number of generations (N) for FCR, BF and ADG with High and Low diets (SE in bracket).**

Trait	Diet	Mean	sp	$h^2$	$\Delta G$	BG	N
FCR	High	2.18	0.207	0.389	0.0806	1.39 (0.008)	9.8
	Low	2.42	0.173	0.393	0.0680	1.89 (0.005)	7.8
BF (mm)	High	12.3	2.56	0.332	0.850	3.51 (0.151)	10.3
	Low	14.7	2.31	0.321	0.741	5.34 (0.161)	12.1
ADG (kg/d)	High	0.924	0.119	0.317	0.0379	1.62 (0.014)	18.4
	Low	0.924	0.103	0.299	0.0307	1.47 (0.009)	17.8

Nutrient density in the diet had only a small effect on heritability, but a more significant effect on  $\Delta G$  values. Based on  $\Delta G$ , it would take about 9.8, 10.3 and 18.4 generations for FCR, BF and ADG for the base population to move from its starting performance to the best possible genome when the high diet is fed. When the low diet is fed, the number of generations is 7.8, 12.1 and 17.8 for FCR, BF, and ADG. This indicates that diet can play an important role in how far traits are from their biological limit and demonstrates that genetic improvement for a given trait is achieved through different pathways in different environments.

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## MICROARRAY EXPRESSION PROFILING OF PERIPHERAL BLOOD LEUCOCYTES FROM PIGS CHALLENGED WITH *ACTINOBACILLUS PLEUROPNEUMONIAE*

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Individual pigs display degrees of severity after being challenged with *Actinobacillus pleuropneumoniae* (App) at a subclinical dose (Kerr *et al.* 1999). Pigs displaying extreme phenotypes during App infections are obvious targets for gene expression profiling using microarrays to reveal the genes controlling the degree of susceptibility to the disease. In this experiment, 18 pigs were ranked according to their susceptibility to App based on a combination of performance measurements and clinical data incorporating principal component analysis. The two most distant pigs (top and bottom rank) in the resulting ranking order were selected for microarray expression profiling.

We constructed a 6.5 K complementary DNA (cDNA) microarray comprising anonymous clones that were picked from 10 subtracted libraries generated from porcine immune tissues and cells. This microarray chip was used to compare gene expression in peripheral blood leucocytes from the two selected phenotypically-extreme pigs at 0 h and 24 h post-infection with App. The data obtained from 16 microarray hybridisations were analysed using a mixed analysis of variance model and Bayesian model-based clustering approach. A list of 307 differentially expressed (DE) elements of which 179 were up-regulated in the least susceptible (R) and non- or down-regulated in the susceptible pig (S) (*Rup-Sdown*) was generated. The remaining 128 genes showed the reverse trend by being up-regulated in S and non- or down-regulated in R (*Rdown-Sup*). The majority of DE elements with the *Rup-Sdown* profile originated from a blood leukocyte library, while the majority of the DE elements with the *Rdown-Sup* profile originated from an immune tissue library. Overall, 278 sequences were retrieved and 44 single genes or contigs were identified by searching the gene database using BLAST (basic local alignment search tool) (Table 1).

**Table 1. Overview of sequencing results and BLAST search summary.**

Expression profile	DE Elements	Sequences retrieved	BLAST summary		
			Known function +good match	Unknown function +good match	Known function +poor match
Rup-Sdown	179	166	28	2	8
Rdown-Sup	128	112	5	-	1

Based on the putative BLAST gene identity, the 44 genes were assigned to seven functional groups: 1) Cell signalling/Inflammatory response, 2) Cellular physiology/ Energy metabolism, 3) Endothelial function/Cytoskeletal structure/Cell adhesion, 4) Secretory pathway, 5) Immunoglobulins, 6) Translation/Other and 7) Unknown function. Most of the 44 DE candidates have been implicated in immune response and several candidates were identified as being part of the innate component of the immune system. Therefore, it is assumed that the diverse set of identified genes is potentially related to disease resistance in App infected pigs.

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## COMPARISON OF PIG ENTEROTOXIGENIC *E. COLI* ISOLATES BY PULSED-FIELD GEL ELECTROPHORESIS (PFGE)

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Enterotoxigenic *E. coli* (ETEC) is a common cause of post-weaning diarrhoea (PWD) in pigs and characteristically belongs to only a limited number of serotypes. Osek (2000) has used virulence gene typing and PFGE with European ETEC isolates and classified these as one genetically-related pathogenic group.

The aim of this experiment was to determine the molecular relationship between endemic PWD ETEC isolates from serogroups O141 and O149:K88 by PFGE genomic fingerprinting. Forty-five ETEC isolates from NSW (7 O149:K88; 8 O141), Qld. (23 O149:K88) and Vietnam (7 O149:K88) were assembled. Each of these clinical isolates possessed at least one enteric virulence gene (F4, F18, LT, STa, STb and EAST-1). PFGE was performed by digestion of embedded genomic DNA with *NotI* or *XbaI* endonucleases. The resulting fragments were separated in a GeneNavigator PFGE system (Pharmacia). Clustering relationships were established using GelCompar software (Figure 1).

As expected, the two serogroups were distributed in two main clusters. Digestion with *NotI* and *XbaI* generated identical clustering results. All O149:K88-isolates could be subdivided into three further groupings, primarily based on country of geographical origin. The Queensland isolates fell into two clusters (A & B) that were the furthest apart in relatedness. The Vietnam isolates fell into two tight but distinctively separate clusters that were closer to the Queensland-A group. The NSW isolates fell into a single cluster as did the isolates in the Queensland-B grouping. These tight genetic clusters support the theory that O149:K88 ETEC isolates from different geographical origins are highly clonal and argue against a horizontal mode of gene transfer.

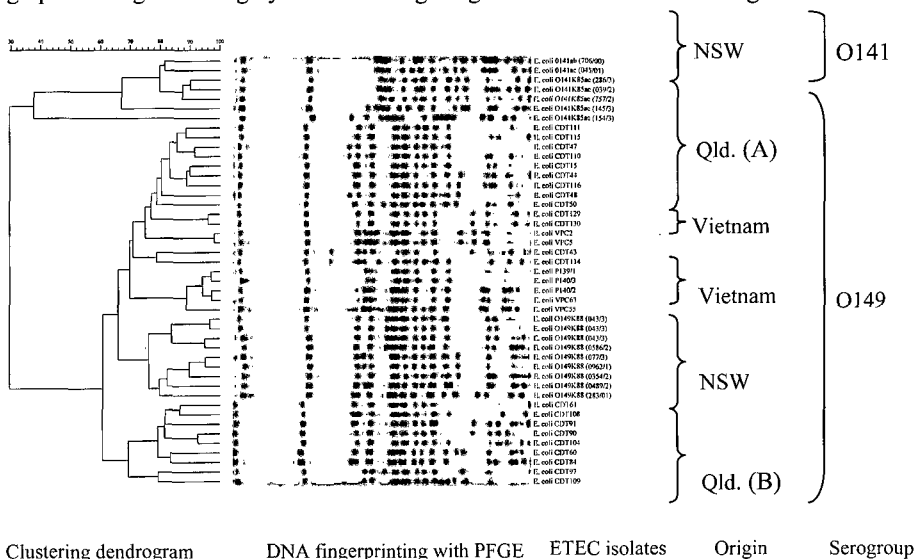


Figure 1. PFGE relationship analysis of 46 ETEC isolates from NSW, Qld. and Vietnam.

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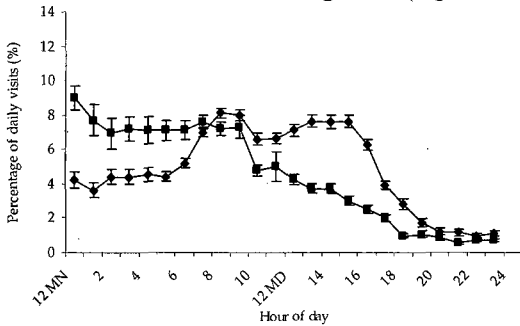
## EFFECTS OF FEEDING REGIME ON FEEDING PATTERNS OF GROUP-HOUSED PIGS

J.M. McSweeney, S. Hermesch\*, R.E. Crump\* and B.G. Luxford\*\*

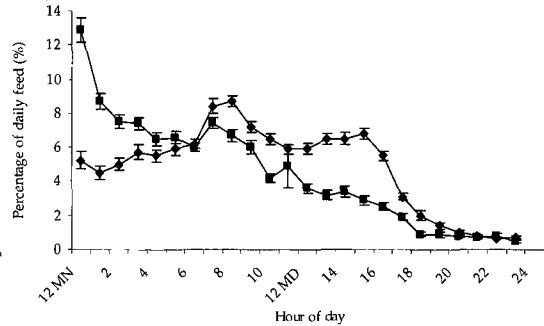
Genetic Solutions, PO Box 145, Albion, Qld. 4010. \*Animal Genetics and Breeding Unit, Armidale, NSW 2351. \*\*QAF Meat Industries, PO Box 78, Corowa, NSW 2646.

Diurnal feeding patterns have been described for wild foraging pigs (Signoret *et al.* 1975) as well as commercial *ad libitum* fed pigs (Hall, 1997). Restricted feeding is used in some breeding programs for selection of lean meat growth. In this experiment, we investigated whether feeding regime affects the feeding patterns of pigs.

Pigs ( $n=278$ ) from terminal sire lines were fed over seven weeks using electronic feeders and randomly allocated to one of three feeding levels 115%, 100% ( $\mu=2.37\text{kg}$ ) and 85% (McSweeney *et al.* 2001). Group size was about 30, with all feeding regimes represented in all groups. Daily feed allowances were allocated at midnight each day. The average feed eaten and average number of visits per hour, as a percentage of the total feed eaten and total number of visits per day, are shown for the 115% and 85% feeding levels (Figures 1 and 2).



**Figure 1.** Feed eaten/h as a % of daily feed for 115% (♦) and 85% (■) feeding levels



**Figure 2.** Visits/h as a % of daily visits for 115% (♦) and 85% (■) feeding levels

The feeding patterns exhibited a diurnal pattern for the 115% feeding-level group. Pigs allocated less feed ate a larger proportion of their feed shortly after midnight. Pigs responded rapidly to the daily feed allocation, as evidenced by the increased number of visits in the hour following midnight for all feeding levels. These changes in feeding patterns are specific to this feeding system. The development of any alternative feeding system should consider changes in feeding patterns, competition and other measures of group dynamics to maximise the well being of pigs. Data on feeding patterns could be of value in genetic improvement in nucleus herds, providing information on the feed requirements of individual animals and indirectly on other traits. Data were collected by QAF Meat Industries.

AGBU is a joint venture of NSW Agriculture and The University of New England.

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## GENETIC RELATIONSHIP BETWEEN A NEW FEEDING PATTERN TRAIT AND PERFORMANCE TRAITS

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Pigs adapt their feeding patterns to the level of feed restriction by modifying their feed intake in the early hours of the day (McSweeney *et al.* 2003). This observation led to the investigation of a new feeding-pattern trait, defined as the percentage of the total daily feed intake eaten between midnight and 0600 h (P6AM). This trait ( $\mu=35\%$ ) is specific to the feeding system studied and may provide additional information for genetic improvement of performance traits. The aim of this experiment was to estimate genetic correlations between P6AM and economically important traits.

Data on individual feed-intake were collected from group-housed boars ( $n=1459$ , 821 litters, group size=30, average start liveweight=71kg, seven week test period) from three different lines between February and October 2001. Feed was dispensed continuously at a set rate using QAF electronic feeders (McSweeney, 2002) and daily feed allowances were based on starting weight and increased each week for extra maintenance requirements.

The performance traits measured were daily feed intake (DFI), backfat measured at the P2 site (BF), lifetime and test average daily gain (IADG and ADG) and feed conversion ratio (FCR). Appropriate fixed effect models were developed for each trait (McSweeney, 2002). An individual animal model was applied to estimate the genetic parameters for P6AM and the performance traits using ASREML (Gilmour *et al.* 1999).

**Table 1. Heritability and genetic correlation estimates (x100) between percentage of daily feed eaten before 0600 h (P6AM), daily feed intake (DFI), backfat (BF), lifetime and test average daily gain (IADG and ADG) and feed conversion ratio (FCR).**

	P6AM	DFI	BF	IADG	ADG	FCR
Heritability	25 ± 7	29 ± 7	57 ± 8	43 ± 8	33 ± 8	31 ± 7
Genetic correlation		69 ± 12	20 ± 16	57 ± 12	32 ± 17	16 ± 19

A heritability of 0.25 (Table 1) and a phenotypic variance of 179<sup>2</sup>% for P6AM indicate that this trait would respond to selection. The trait P6AM had genetic correlations of 0.69 (DFI) and 0.32 (ADG). The information on P6AM was evaluated through selection index calculations (McSweeney, 2002). The basic index included IADG, BF, FCR (*ad libitum*) and litter size (LS) as objective traits and used information on IADG, BF, LS, ADG and DFI (restricted). Adding information on P6AM (restricted) to this index increased economic gain by 4.84%. Electronic feeders can automatically record information on feeding pattern traits. Relevant feeding pattern traits should be specified for a given feeding system and this extra information should be used in breeding programs if electronic feeders are already in place.

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## SUPPLEMENTATION WITH SAPONIN-RICH PLANT EXTRACTS REDUCES PIGLET STILLBIRTHS AND IMPROVES DIET DIGESTIBILITY IN LACTATING SOWS

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Dietary saponin has been shown to improve growth, immunity and rumen fermentation (Cheeke, 2000). *Yucca shidigera* and *Quillaja saponaria* are both plants rich in saponin. Cline *et al.* (1996) found that a *Yucca* plant extract reduced piglet stillbirths when fed to sows during late gestation. In this experiment we aimed to further establish the influence of saponin on piglet stillbirth and survival, and its effect on diet digestibility.

Sixty hybrid sows of mixed parity and fatness were allocated to one of three dietary treatments on day 107 of gestation. Treatments were C (control) sow lactation diet, Y supplemented with 200 mg/kg *Yucca shidigera* extract, and Q supplemented with 250 mg/kg *Quillaja saponaria* extract. Sows received 2.5 kg/d of the appropriate diet until farrowing. During lactation, allocated diets were offered *ad libitum*. At farrowing, piglets were weighed and the number of stillbirths within each litter recorded. Farrowings were attended but no intervention occurred. Deaths during the suckling period were also recorded. Faecal samples were collected daily for the first week of lactation for digestibility analyses. Data were analysed using linear regression analysis and the General Linear Model procedure of Minitab 12.2.

**Table 1. Effect of sow dietary treatment on piglet stillbirth incidence, pre-weaning mortality and sow diet digestibility coefficients in terms of dry matter (DM), organic matter (OM) and crude protein (CP), (OM and CP on a DM basis).**

	Litter size	Birth wt (g)	% Stillborn	% Mortality	Digestibility		
					DM	OM	CP
C	11.1	1599	8.9	12.8	0.820	0.846	0.862 <sup>b</sup>
Y	11.2	1598	4.2	12.7	0.848	0.873	0.878 <sup>ab</sup>
Q	12.6	1529	3.8	11.3	0.859	0.886	0.907 <sup>a</sup>
SEM	0.87	61.8	1.9	3.4	0.011	0.010	0.010
P-value	ns	ns	P<0.1	ns	P<0.1	P<0.1	P<0.05

<sup>ab</sup>Means in the same column without a common superscript differ significantly (P<0.05).

The tendency for the *Yucca* (and *Quillaja*) extracts to reduce stillbirths supports the results of Cline *et al.* (1996). Pre-weaning mortality was unaffected suggesting there was no improvement in piglet vigour. Duration of farrowing was not affected by treatment and was not linked to stillbirth incidence or pre-weaning mortality (P>0.1; % stillborn = 1.9+0.03 farrowing time (minutes), R<sup>2</sup>=7.8; % mortality = 0.5+0.001 farrowing time (minutes), R<sup>2</sup>= 0.8). The mechanism underlying the reduced incidence of stillbirth remains unclear. It is possible that oxygen supply to the foetuses was improved before and during farrowing, and that this reduced asphyxia. The improved diet digestibility in supplemented sows is most likely due to the saponin component of the extracts, which has a strong surfactant activity and is able to increase membrane permeability. Such an effect in the small intestine of the sow would lead to increased nutrient uptake during the first week of lactation.

This work was funded by AXISS France SAS.

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## REPRODUCTIVE PERFORMANCE INCREASES WITH AGE IN INDUCED PRE-PUBERTAL GILTS

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By using exogenous hormones to induce oestrous in gilts, strategies can be developed to maintain a constant volume of weaners throughout the year. We reported that the pregnancy rates of induced gilts increased linearly with age between 18-24 weeks of age (Smits *et al.* 2001). In this experiment we tested the hypothesis that pregnancy rates and embryo number would also increase in induced gilts between 24-29 weeks of age.

One hundred and forty-seven, female finisher progeny from Large White x Landrace F1 cross sows were selected at 24, 27 and 29 weeks of age in August. A further 99 gilts were selected at 29 weeks of age as a control group for which oestrus was not induced artificially. Gilts were housed in groups of 25 (1.25 m<sup>2</sup>/gilt) and those on the induced treatment were injected with 1000 iu PMSG (Folligon™) followed, after 72 h, by 500 iu hCG (Chorulon™). Non-induced gilts were stimulated once a day from 24 weeks of age until standing heat was observed between 29-31 weeks of age. All gilts were inseminated ( $3 \times 10^9$  cells) in stalls in the presence of a mature boar. Induced gilts were inseminated 30 h and 48 h after hCG. Non-induced gilts were inseminated when they exhibited a standing heat response. At 30 days post-mating, gilts were slaughtered and embryos were recovered. One-way analysis of variance and Chi-square were used to evaluate statistical differences between treatments.

**Table 1. Pregnancy rate and mean  $\pm$  SD embryo recovery of induced gilts at 24, 27 or 29 weeks of age compared to non-induced gilts.**

	Size of gilt pool at start	No. mated of gilt pool	Pregnant rate		Embryos recovered
			% gilts mated	% gilt pool	
24 weeks induced	50	50 <sup>a</sup>	46.0	46.0 <sup>a</sup>	7.6 $\pm$ 2.7 <sup>a</sup>
27 weeks induced	50	50 <sup>a</sup>	62.0	62.0 <sup>a</sup>	9.3 $\pm$ 4.0 <sup>ab</sup>
29 weeks induced	47	47 <sup>a</sup>	48.9	48.9 <sup>a</sup>	10.7 $\pm$ 5.4 <sup>bc</sup>
Un-induced	99	24 <sup>b</sup>	62.5	15.2 <sup>b</sup>	12.7 $\pm$ 2.8 <sup>c</sup>
Significance		***	NS	***	**

\*\*P $\leq$ 0.01, \*\*\*P $\leq$ 0.001. NS, treatment-effect not significantly different. <sup>abc</sup>Mean values with different superscripts differ significantly (P<0.05).

The mating age of the non-induced gilts was 31.0  $\pm$  0.7 (mean  $\pm$  SD) weeks of age. As hypothesised, there was a significant increase (P<0.01) in the number of embryos recovered per pregnant gilt with an increase in age from 24-29 weeks in the induced treatments. At 29 weeks of age, there were a similar number of embryos in induced and non-induced gilts. However, there were fewer gilts mated and a lower pregnancy rate in the non-induced treatment from the available gilt pool (P<0.001). Swine dysentery was observed during the experiment and may have disrupted the normal oestrous cycle of gilts in the non-induced group. In this experiment, the use of hormone induction in gilts up to 29 weeks of age successfully increased the productivity from the gilt pool.

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## **A REVIEW - THE USE OF ANTIBIOTICS IN FOOD PRODUCTION ANIMALS - DOES THIS CAUSE PROBLEMS IN HUMAN HEALTH?**

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### **Abstract**

One of the frequent consequences of antibiotic use is the development and spread of resistant bacteria in people and animals. If animals carry resistant bacteria, then food produced from these animals will often be colonised with these bacteria. After ingesting these foods, people can then carry these resistant bacteria and in some cases develop infections from them.

Some of this resistance can be to antibiotics that are 'last-line' agents needed to treat life-threatening infections in people. The development and spread of these multi-resistant bacteria can follow the use of 'last-line' (or similar) antibiotics in food production animals. Examples include ciprofloxacin-resistant strains of *Salmonella spp.*, *Campylobacter spp.* and *E. coli*, vancomycin-resistant strains of enterococcus (VRE) and third generation cephalosporin-resistance in Gram-negative bacteria.

In Denmark, pig and poultry producers voluntarily ceased the routine use of in-feed antibiotic for growth promotion and prophylaxis purposes in 1998. Poultry production was unaffected other than a one percent increase in feed intake (there were no effects on weight gain or mortality). In finisher pigs there were also no important detrimental effects. In weaners there was a 0.5% increase in mortality and a small decrease in daily weight gain. However there were no detrimental effects on overall pork production or exports which both continued to rise.

If three basic principles of antibiotic use were adopted in the agriculture sector, most of the driving factors for unnecessary antibiotic resistance would be substantially reduced or eliminated. This can be done without compromising the therapy of sick animals or the economic production of food animals. These principles are:

- Antibiotics that are 'critical' or 'last-line' for serious human infections should not be used in food production animals or agriculture.
- The use of antibiotics for prophylactic purposes in animals should be kept to a minimum. The use of methods (other than antibiotics) to prevent infections should be expanded and developed.
- Antibiotics should not be used as growth promoters.

### **Introduction**

Antibiotics are used extensively in both humans and animals. The majority of the problems arising from antibiotic resistance in humans is due to the over-use of antibiotics in people and (frequently) the sub optimal infection control and hygiene practices that enable these bacteria to then spread easily from person to person. There are, however, also antibiotic resistant bacteria that can be ingested by people via foods and concerns about this have generated many international reports and recommendations (JETACAR 1999; WHO 1997; WHO 1998; WHO 2000; WHO 2001). Some of these resistant bacteria are 'super-bugs' - multi-resistant bacteria for which there may be few or no therapeutic options available. In Australia, the amount of antibiotic used in animals is much greater than in humans (JETACAR 1999). The three main uses of antibiotics in livestock are for growth promotion, prophylaxis and to treat sick animals (Collignon 1999b; JETACAR 1999; WHO 2001).

Antibiotic use in animals, however, is a potential problem for human medicine because antibiotic resistant bacteria can pass through the food chain to people (JETACAR 1999, WHO 1997). In the past, the main bacterial concerns we were aware of were those involving food-borne bacteria that produced either frequent or severe disease in people (eg gastroenteritis with *Salmonella spp.* or *Campylobacter spp.*). However more recently there have been growing concerns about bacteria that only infrequently cause disease in people but which are transferred more frequently via the food chain (eg *Escherichia coli* and *enterococci*). These latter bacteria

frequently carry genes encoding for antibiotic resistance (as do *Salmonella* species and *Campylobacter* species) (JETACAR 1999; Collignon 1999b; WHO 2000; Witte 2000).

#### **Vancomycin resistance is linked to antibiotic use in animals**

In Europe there is strong evidence (Collignon 1999a, Witte 2000) that one type of the vancomycin-resistant enterococci (VRE - vanA) developed in animals fed an antibiotic called avoparcin (a glycopeptide or vancomycin-like antibiotic). VRE carrying the vanA gene-cassette remained on the carcasses of animals after slaughter. VRE was also frequently found on foods that were sold at the retail level (for example, in the Netherlands in one experiment over 70% of chickens tested at the retail level had VRE present (Collignon 1999a). In studies of the European population, between 2 - 17% of people had these multi-antibiotic resistant bacteria present in their bowel (Collignon 1999a, Witte 2000). The conclusion from these data was that VRE was widespread in the general population in Europe and that avoparcin use in animals was a major cause of this and the consequent spread of vanA VRE via the food chain. Vancomycin resistance is a concern in human medicine in Australia because it is a 'last-line' antibiotic for some hospital-acquired infections of enterococci and staphylococci that have become resistant to the more commonly used antibiotics for these infections. Thus, should bacterial resistance develop to vancomycin we will have no or few alternate antibiotics available to treat people if they develop such infections. Another major concern regarding this type of bacterial resistance is that the vancomycin-resistance genes can spread from VRE to bacteria that are much more common and aggressive such as the multi-resistant strains of *Staphylococcus aureus* (MRSA). Experimentally, this has occurred *in vitro* and has now also occurred with patients in the USA (Sievert 2002). However, currently the most common form of vancomycin resistance in *S. aureus* is caused by a completely different genetic mechanism and is related to the extensive use of vancomycin in hospitals. The amount of vancomycin (or other antibiotics that co-select for VRE) used in hospitals is the main driving force behind how many vancomycin-resistant bacteria amplify and spread. One of the most common and aggressive bacteria causing human infections (*Staphylococcus aureus*) may be untreatable with currently-available antibiotics if vanA spreads from VRE in hospitals to staphylococci and if these MRSA isolates pick up vancomycin-resistance genes and genetic material that allows them to spread more easily. In Australia we have much less data than Europe on the spread of VRE through the food chain. There is, however, suggestive evidence that spread of VRE via foods has occurred (especially with VRE carrying the vanA gene). Recently in Australia, Choice magazine found that 11-14% of chicken meat sold at the retail level in Sydney and Brisbane contained VRE (Australian Consumers Association 2002). We also know that VRE in Australia in hospitalised patients is geographically widespread and has been found in small community hospitals as well as large hospitals (Collignon 1999a, JETACAR 1999). Van A VRE has been isolated in food production animals and foods in Australia (mainly chickens but not from pigs; Barton 1999; Barton and Wilkins 2001, JETACAR 1999, Australian Consumers Association 2002). The most logical explanation of this diverse spread in Australia is that many strains of VRE have been spread through the food chain.

Wherever antibiotics are used, we know that one of the consequences of their use is that resistance can develop. The amount of resistance that eventuates is related to the total amount of antibiotic used. In 1992 over 120,000 kilograms of avoparcin (10% active ingredient by weight) was used in animals in Australia (predominantly as a growth promoter), while only 68 kilograms of vancomycin was used in people (JETACAR 1999). There is debate as to whether antibiotics used as growth promoters still lead to any significant economic benefits (eg weight gains and improved feed efficiency) and in many recent studies, no or minimal benefits were measured (Engster *et al.* 2002; Emborg *et al.* 2001; WHO 2003). In Denmark there has been no decrease in weight gains of poultry or pigs at slaughter time since the use of antibiotic growth promoter (AGP) ceased (Emborg *et al.*, 2001; WHO 2003). In the USA one of the largest poultry producers found the weight gain from the use of AGPs was, at most, only 0.4% (Engster *et al.* 2002). This is much lower than the general belief and expectation in industry that weight gains with the use of AGPs are between 5 to 10%. At best, with good farming methods, (and relying on figures produced by the pharmaceutical companies themselves) this economic gain is only a few percent in weight gain (JETACAR 1999).

It is also important to note that in much of the promotional material from pharmaceutical companies about their own AGPs, figures are presented which shows their competitors' AGPs



often have a very poor weight gain associated with their use! However, these claimed optimistic benefits usually translate to no more than three cents per chicken or a few cents per kilogram in pork (JETACAR 1999). When it was available, the large amounts of avoparcin used (which is in the class of antibiotics that are 'last line' or 'critical' to humans) appear to have been a waste of a precious resource. Avoparcin has not been registered for use in Australia since 2000. There have been no reports of major losses in production or increases in disease in food animals since the withdrawal of avoparcin. It appears that the use of avoparcin was therefore a waste as it was not essential for the agriculture sector and is now associated with the development, spread and persistence of VRE in food animals and on foods.

Avoparcin was banned in the EU in 1995 and is no longer registered for use in Australia. It is, however, an important antibiotic to study because even after its ban there is continued evidence of the development of avoparcin resistance and the subsequent spread of these resistant bacteria to people along with co-selection of VRE by the use of other antibiotics (eg tylosin) (Aarestrup 2001). There is no reason to believe that resistance in other bacteria following the use of antibiotics different to avoparcin, will develop and spread via similar mechanisms (Witte 2000).

From a medical perspective, any small economic benefits that may have flowed to the agricultural sector from the use of antibiotics as growth promoters appears to have been more than outweighed by the potentially wide-spread circulation of these multi-resistant bacteria throughout the food chain. Antibiotic use in animal production has also resulted in the public perceiving food as a possible source of 'super-bugs'. From a livestock perspective, however, the value of these antibiotics in prevention and control of serious diseases must be factored in. Clearly, it would be much better to remove any ambiguity or misunderstanding about their use and register them as therapeutic agents and not as growth promoters. Failure to do so could potentially compromise their use to treat sick animals if it induces inappropriate changes in laws or regulations in the future.

#### **Ciprofloxacin resistance is linked to antibiotic use in animals**

Throughout most of the world, a fluoroquinolone similar to ciprofloxacin (enrofloxacin) has been associated with the spread to humans (through the food chain) of ciprofloxacin-resistant *Salmonella* and *Campylobacter* species and resistant *E. coli* (Molbak *et al.* 1999; Glynn *et al.* 1999; Smith *et al.* 1999). This has resulted in infections of *Salmonella spp.* in humans that are multi-resistant and in some cases for which there are no available antibiotics (ciprofloxacin is also a 'last-line' human antibiotic). In Australia, fluoroquinolones are not approved for use in food production animals and as a consequence, Australia appears to be one of the few countries in the world where there is not a major problem with fluoroquinolone resistant *Salmonella* and *Campylobacter* species (JETACAR 1999).

#### *The Danish Experience*

In May 1995 Denmark banned the anti-microbial growth promoter avoparcin (a glycopeptide) because of concerns that its use contributed to the creation of an animal reservoir of VRE, which then posed a potential risk to public health. In December 1997 the EU banned avoparcin in all member states. In January 1998 Denmark also banned the anti-microbial growth promoter, virginiamycin, (a streptogramin) because of concerns that its use contributed to creation of an animal reservoir of streptogramin-resistant *Enterococcus faecium* that posed a potential risk to public health. In February 1998 the Danish cattle and broiler chicken industries voluntarily stopped the use of all anti-microbial growth promoters in response to consumer concerns that the use of anti-microbial growth promoters posed a potential risk to public health. At that time, the Danish swine industry also voluntarily stopped the use of all anti-microbial growth promoters in pigs over 35 kg (finishers). In July 1999 the EU banned another four anti-microbial growth promoters (tylosin, spiramycin, bacitracin and virginiamycin), because they belonged to classes of anti-microbial drugs also used in human medicine. In December 1999, the Danish swine industry voluntarily stopped the use of all remaining anti-microbial growth promoters in pigs under 35kg (weaners). The effects of the terminations of all antibiotics as growth promoters have recently been reviewed (WHO 2003).

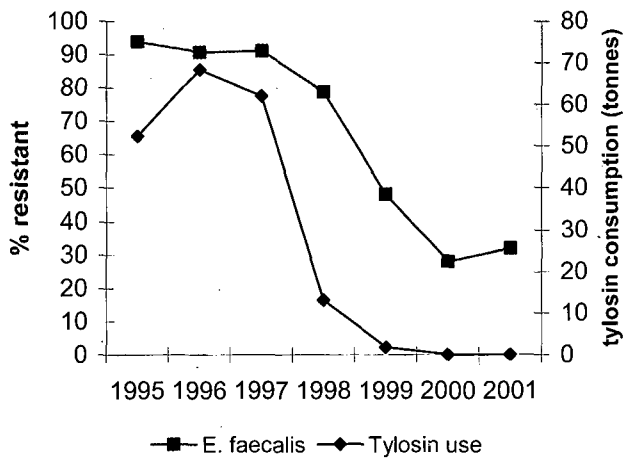
In 2001, Denmark had a population of 5.35 million people. It remains a net exporter of both poultry and pork (about 50% of poultry production and 85% of pork production). Broilers and pigs are raised intensively and more than 130-million broilers are produced annually. Typically,

broilers are raised using 'all-in-all-out' (AIAO) management and barns that are cleaned and disinfected between flocks. About 13,500 pig producers raise 22.5 million pigs annually and 95% are slaughtered in two farmer-owned cooperative slaughterhouses. Most new pig facilities use AIAO management.

There has been a substantial reduction in the total amounts of antibiotic used in food production animals in Denmark since the cessation of all AGPs in 1998-99 (eg 99,650 kg of AGPs were used in 1992, 115,786 in 1994, 105,548 kg in 1996, 49,294 kg in 1998 and none in 2000; (DANMAP 2001). The therapeutic use of antibiotics has been variable from year to year, but it appears that similar levels were used before and after the termination of AGPs (range 48,000 – 89,900 kg between 1986–96 to 57,300 – 80,600 kg between 1998-2000; (DANMAP 2000). Despite arguments that the therapeutic use of antibiotics would replace the discontinued use of antibiotics as growth promoters and routine in-feed prophylaxis, the therapeutic use of antibiotics in Denmark remains much lower per kilogram of meat produced than in nearly all other countries in the EU. Danish use of antibiotics is also much lower than in other EU countries that have continued to use some in-feed antibiotics (EMEA 1999). The total use of antibiotics (that is both as AGPs and therapeutically) fell from a peak of 205,686 kg in 1994 to 80,600 kg in 2000 (DANMAP 2001).

*Impact of the ban on anti-microbial growth promoters on anti-microbial resistance in Denmark*

In Denmark there has been a substantial reduction in the levels of antibiotic resistant bacteria carried by food production animals since the cessation of AGP use (Aarestrup *et al.* 2001, Aarestrup *et al.* 2002). This reduction occurred for most antibiotics relatively soon after the removal of the in-feed antibiotics. With virginiamycin, resistance in isolates of enterococcus from pigs dropped from 60% in 1998 to 5% in 2000 (DANMAP 2001). There were some exceptions, however. Relatively high levels of VRE persisted in pigs at a level of 20% even after avoparcin use was ceased in 1995. It was not until the cessation of another antibiotic in a different class in 1998 (which was co-selecting for the resistant strains – tylosin and a macrolide) that rates dropped to only a few percent in 2000 (Aarestrup *et al.* 2001, DANMAP 2001).



**Figure 1.** Trends in tylosin use for growth promotion and erythromycin resistance among *Enterococcus faecalis* isolated from pigs at slaughter from 1995 to 2001 (WHO 2003; Aarestrup *et al.* 2001).

Despite concerns that ceasing AGP use would result in an increased carriage of *Salmonella* in food production animals and/or an increase in human disease caused by *Salmonella*, this was not seen in Denmark. In fact, human cases of *Salmonella* infection have decreased since the termination of AGP-use from 100 cases per 100,000 population in 1997, to 55 cases per 100,000 in 2001. The carriage of *Salmonella* in food animals has shown a steady decrease since 1990 and this downward trend appears to have been unaffected by the termination of AGP use (Evans *et al.* 2003; WHO 2003).

The termination of anti-microbial growth promoters in Denmark has had no major impact on animal production. The amount of pig meat produced in Denmark has continued to rise since 1960 with no obvious effects from the termination of AGPs in swine production in 1998-99 (Figure 2). There were also no effects on pork exports, which have continued to increase over the last 10 years. The number of pig producers continued to decline in Denmark from 1972-2001 but there were no obvious effects following the termination of AGPs in 1998-99 (WHO 2003).

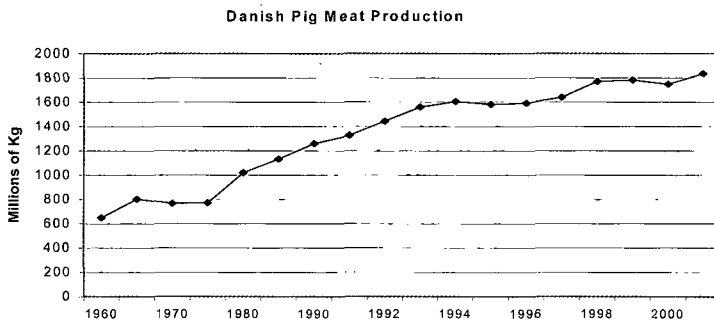


Figure 2. Total production of pork in Denmark between 1960 and 2001

The termination of AGPs in swine production in 1998-99 had no effect on production parameters in finisher pigs. Feed efficiency improved between 1995 and 2001 (from 2.95 to 2.89 feed units/weight gain). In weaners however, there were effects with an increase in mortality of about 0.5% and a decrease in daily weight gain (WHO 2003). Feed efficiency also decreased over the periods 1995 to 2001 (97.9 to 99.3 feed units per produced pig). Some of these effects in weaners may have been due to the ban on the use of olaquinox and carbadox, rather than the termination of other antibiotic growth promoters. Before the ban on quinoxaline-based anti-microbial growth promoters (e.g., carbadox and olaquinox), olaquinox was the most commonly used anti-microbial growth promoter in weaners, in terms of total kilograms of anti-microbial growth promoters used (WHO 2003). One of the major reasons for the increased morbidity in weaners appeared to be an increase in diarrhoea due possibly to *E. coli* and/or *Lawsonia intracellularis* infections (Jensen 2002; WHO 2003).

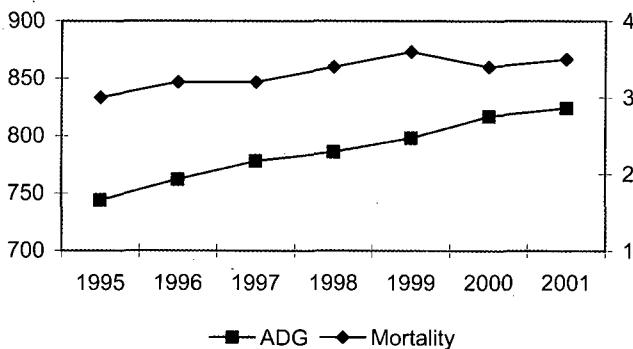
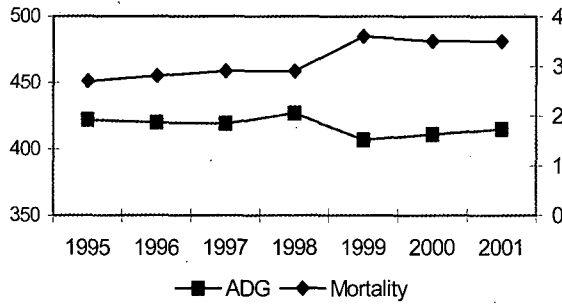


Figure 3. Productivity of finisher-pigs (average daily weight gain). (% Mortality right axis; Average daily weight gain (ADG) left axis in g. (WHO 2003).



**Figure 4.** Productivity in weaner pigs (average daily weight gain): (% Mortality right axis; Average daily weight gain (ADG) left axis in g. (WHO 2003).

#### What can we do to limit the amount of antibiotic resistance that occurs?

There will always be new antibiotics and there will always be controversy about the economic and medical costs of their use compared to their benefits (both in humans and in animals). It is important that some antibiotics are available for use to treat sick animals. However, we need to limit the ways that antibiotics are used in food production animals. In particular, antibiotics should not be used for growth promotion and they should be used only sparingly for prophylaxis. Antibiotics that are 'critical' or 'last-line' for human use should not be used in food production animals at all. These 'critical' antibiotics are only a small percentage of the total amount of antibiotics that are used in humans in Australia. If these 'last-line' antibiotics were reserved for human use alone this would be unlikely to compromise animal welfare.

Antibiotics (or similar agents in the same class as antibiotics) that are 'critical' or 'last-line' antibiotics for serious human infection, should not be used in animals or agriculture. There are many serious infections in humans where there are few or, in some cases, no alternate antibiotics that can be used if antibiotic resistance develops to these agents. These can therefore be classified as 'last-resort' or 'critical'. As a group, most of these antibiotics are only used in people in Australia in small volumes by weight. There are also many alternatives to these antibiotics that can be used to treat animals successfully that are sick (for example, penicillins, tetracyclines). Antibiotic classes that can be classified as 'critical', 'last-resort' or 'reserve' include:

Class of antibiotic	examples (mainly human but with some animal antibiotics)
glycopeptides	(vancomycin, teicoplanin, avoparcin)
3 <sup>rd</sup> and 4 <sup>th</sup> generation cephalosporins	(eg cefotaxime, ceftriaxone, ceftiofur, cefipime),
anti pseudomonal penicillins	(eg piperacillin, ticarcillin),
anti tuberculosis drugs	(eg rifampicin, isoniazid, ethambutol, pyrazinamide),
fluoroquinolones	(eg ciprofloxacin, levofloxacin, enrofloxacin),
aminoglycosides**	(eg amikacin),
carbapenems	(eg imipenem, meropenem),
streptogramins	(eg Synercid, virginiamycin),
oxazolidones	(eg linezolid)

\*\* (only those with a relative extended spectrum and/or a much lower susceptibility to enzymatic destruction eg amikacin)

Because of increasing antibiotic resistance in many important human pathogens new antibiotics have recently been developed and released (eg linezolid). It is critical that if any other new classes of antibiotics are developed for human use that these are not used in animals unless it is established they are not 'critical' for human use.

These 'critical' antibiotics (or others in the same class) should not be used for therapy or any other purpose in food producing animals. Fluoroquinolones have been approved for use in food production animals in many countries. The use of enrofloxacin has resulted in the development of ciprofloxacin-resistant strains of *Salmonella spp* and *Campylobacter spp*. These resistant bacteria have subsequently caused human infections. When the glycopeptide, avoparcin, was used as a growth promoter in food animals in Europe this resulted in the development and amplification of vancomycin resistant enterococcus (VRE) and subsequent colonisation by a significant percentage of the human population via the food chain (between 2 and 17%). After the ban of avoparcin use in food animals in the EU, the percentage of the general population carrying VRE in their bowel showed a marked reduction (WHO 2003).

The basic principles we need to follow in order to maintain or facilitate this approach not only now, but also in the future are given below.

- Antibiotics that are ‘critical’ or ‘last-line’ antibiotics for serious human infections should not be used in food production animals or agriculture.
- The use of antibiotics for prophylactic purposes in animals should be kept to a minimum. The current usage for this purpose should be significantly reduced. The use of methods (other than antibiotics) to prevent infections should be expanded and developed.
- Antibiotics should not be used as growth promoters.

### Conclusion

Antibiotics are a precious but non-renewable resource. They are of major benefit to people who have serious and life threatening bacterial infections. We are currently squandering this resource by using antibiotics much more widely than we need to and in inappropriate ways (both in people and in animals). This has resulted in antibiotic resistance developing and then spreading not only from person to person but also via the food chain from animals to humans. It is essential that we use antibiotics wisely and prudently, otherwise these miracle drugs of the 20<sup>th</sup> century will lose their effect with the wide spread development and amplification of resistant bacteria and the genes that encode for this resistance.

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## THE WELFARE OF GESTATING SOWS IN CONVENTIONAL STALLS AND IN DEEP LITTER SYSTEMS

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Housing sows in stalls is a controversial welfare issue. While exercise during gestation is associated with a reduced incidence of lameness, grouping unfamiliar animals is associated with aggression (Hale *et al.* 1984). Barnett *et al.* (2001) estimated that 26% of sows are stall-housed in Australia for most of their reproductive cycles and up to 62% may be in stalls for part of their pregnancy. In this experiment, we examined the impact on injuries and gait of housing pregnant sows either in stalls (0.6 x 2.1 m, including the feeder) or as large groups in deep litter pens (20 x 10 m, with external-locking feeding stalls located 40-60 m from the pen). All sows had previously experienced their housing treatment.

About 85 recently-mated sows (Landrace x Large White) were allocated weekly to each treatment over eight weeks and measurements were done on 18 focal sows (six each of parities 1, 2 and 3+) in each of the eight groups in each treatment. Injuries were classified as scratches, abrasions and cuts and assessed as described by de Koning (1985). Gait was measured using a four-point locomotion score where a score of zero indicated a normal gait and a score of three indicated a lame animal unable to walk. Gait, along with the number and the severity of injuries were assessed at pre-mating and weeks 1, 9 and 15 of gestation. Analysis of covariance, using pre-mating data, was used to examine treatment effects.

Locomotion score was significantly higher ( $P < 0.001$ ) for sows in the stalls than in the groups (0.68 vs 0.18, SED=0.061; 0.63 vs 0.18, SED=0.071; at 9 and 15 weeks of gestation).

**Table 1. Effect of housing on incidence of injuries (mean value  $\pm$  SED)**

Injuries		Stalls	Pens	SED
Week 1	Scratches	0.60	1.38	0.058*
	Abrasions	0.21	0.03	0.045*
	Cuts	0.05	0.05	0.024
Week 9	Scratches	0.38	0.89	0.076*
	Abrasions	0.26	0.03	0.043*
	Cuts	0.05	0.03	0.020
Week 15	Scratches	0.34	0.84	0.111*
	Abrasions	0.22	0.004	0.051*
	Cuts	0.10	0.03	0.040

\* Significant difference between means ( $P < 0.001$ ).

Locomotion was improved and the incidence of abrasions, but not cuts, was lower in group-sows on deep-litter (Table 1). However, incidence of scratches was higher in these sows. Aggression is likely to be responsible for the higher incidence of scratches in group sows while restriction of movement is implicated in the poorer locomotion and higher level of abrasions in the stall sows, but this needs to be determined. Whether exercise and/or the presence of bedding in the deep litter system improved locomotion needs to be assessed.

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## MALE PIGS WITH ACTIVE TEMPERAMENT HAVE INCREASED PLASMA LACTATE STATUS FOLLOWING RESTRAINT

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Pigs classified as having either an active (many escape attempts) or passive (few escape attempts) temperament when given the 'back test' before weaning, have also displayed glucocorticoid sensitivity (see review by Koolhass *et al.* 1999). However, the effect of temperament on sympathetic activation and catecholamine sensitivity in the pig has not been reported. Change in plasma lactic acid provides an indirect measure of stress-induced catecholamine release. In this experiment we tested the hypothesis that restraint and transport stress would elevate plasma lactate levels in active pigs relative to adrenocortical sensitivity and cortisol release.

Thirty-six hybrid male pigs (Large White x Landrace) were categorised at weaning (28 days of age) as having either an active (more than six escapes) or passive (zero escapes) temperament when held in the supine position for one-minute ('back test'). Animals were kept in group-pens until  $88 \pm 7.2$  kg (mean  $\pm$  SD) liveweight and then sorted by temperament (active and passive) and housed in individual pens in two rooms for 13 days. The pigs were fed a commercial, pelleted diet and water *ad libitum*. On day seven, each pig was restrained with a nose snare (20 seconds) and a blood sample (8 ml) was collected by venipuncture. Restraint continued for two minutes and a further blood sample was collected at the end of this restraint period and again eight minutes later. On day 13 animals received an ACTH injection (1 IU per kg liveweight in 1 ml of saline intramuscularly) and a blood sample was collected 60 minutes post-injection by venipuncture. On day 14 all pigs were transported by truck for 6 h to an abattoir. A blood sample was collected by venipuncture at lairage. Plasma cortisol was analysed by radioimmunoassay and lactate levels by autoanalysis. Treatments were analysed statistically by analysis of variance (Table 1).

**Table 1. Effect of temperament<sup>1</sup> on plasma cortisol and lactate levels in 36 male pigs grown from 88 to 104 kg liveweight and subject to restraint<sup>2</sup>, ACTH<sup>3</sup> injection and transport<sup>4</sup>.**

Temperament	Cortisol (ng/ml)		SEM	Probability	Lactate (ng/ml)		SEM	Probability
	Active	Passive			Active	Passive		
Restraint <sup>2</sup>								
0 minutes	31.6	29.2	3.36	0.563	0.39	0.39	0.07	0.950
2 minutes	36.9	32.5	4.13	0.543	0.72	0.57	0.09	0.392
10 minutes	60.5	55.4	4.43	0.430	0.68	0.42	0.08	0.056
ACTH <sup>3</sup>	100.4	95.5	5.97	0.569	0.45	0.40	0.06	0.533
Transport <sup>4</sup>	81.1	74.9	2.85	0.455	0.67	0.50	0.07	0.119

<sup>1</sup>Active (> 6 escapes) or passive (zero escapes) temperament at weaning when held in the supine position for one minute.

<sup>2</sup>Restrained with a nose snare for two minutes, blood sampled and again eight minutes later. <sup>3</sup>Adenocorticotrophin hormone. <sup>4</sup>Transport for 6 h and restrained in lairage.

The rapid elevation of plasma lactate concentration in active pigs following restraint suggested a more sensitive catecholamine response in active animals than passive animals. A similar response was measured following transport stress. As lactate status did not vary with temperament following ACTH injection, this response is clearly not related to adrenocortical function. Glucocorticoid status was not related to temperament in any of these tests. Thus the increased struggling behaviour of animals assessed in the 'back test' at weaning appears to be positively associated with adrenergic sensitivity in response to restraint and transport stress and not adrenocortical activity.

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## DIETARY NARCOLEPTICS CAN IMPROVE THE EFFICIENCY OF GROWTH IN GROUP-HOUSED BOARS

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The growth potential of boars may be only partially realised commercially because of aggressive and/or sexual activity. Tryptophan, magnesium and bromide are all dietary narcoleptics that have the potential to reduce aggressive and sexual behaviours (Adeola *et al.* 1991; Genicot *et al.* 1991; Pethick *et al.* 1997; D'Souza *et al.* 1998). The aim of this experiment was to determine the effect of dietary narcoleptics on growth performance of group-housed boars.

Two hundred boars and 40 barrows were allocated to six groups of four pens of 10 pigs per treatment. Control and immunocastrate (Improvac<sup>®</sup> CSL, vaccinated at 13 and 17 weeks, Imp) boars and barrows were fed a commercial finisher ration while the other dietary treatments were finisher-diet supplemented with magnesium (5 g magnesium proteinate/kg, Mg), bromide (140 mg bromide chloride/kg, Br) and tryptophan (5 g tryptophan/kg, Trp). Feed was offered *ad libitum* and intake, liveweight and feed conversion ratio (FCR) per pen were determined weekly from 17 to 22 weeks.

**Table 1. Effect of sex and dietary additives on performance over the finisher phase**

	Imp	Barrow	Entire Boars			
			Control	Mg	Br	Trp
Daily gain 17–22 wks, g/d	977 <sup>a</sup>	869 <sup>b</sup>	778 <sup>b</sup>	806 <sup>b</sup>	849 <sup>b</sup>	834 <sup>b</sup>
Feed intake 17–22 wks, g/d	2738 <sup>a</sup>	2880 <sup>a</sup>	2201 <sup>bc</sup>	2351 <sup>b</sup>	2335 <sup>b</sup>	2137 <sup>c</sup>
FCR 17–22 wks, g/d	2.95 <sup>b</sup>	3.32 <sup>c</sup>	2.84 <sup>b</sup>	2.95 <sup>b</sup>	2.77 <sup>b</sup>	2.56 <sup>a</sup>
Carcass weight, kg	73.7	76.8	69.0	71.3	74.1	71.0
Dressing rate, g/kg	754.8 <sup>bc</sup>	773.0 <sup>a</sup>	750.9 <sup>c</sup>	761.0 <sup>b</sup>	761.4 <sup>b</sup>	760.1 <sup>b</sup>

<sup>abc</sup>Means with different superscripts are significantly different ( $P > 0.05$ ).

Daily gain was not affected by dietary treatment although the Imp boars grew significantly faster than controls throughout the experiment (Table 1). Feed intake tended to be higher in all treatment groups than in controls except for the Trp boars. However, these effects were only significant for the Imp boars and barrows. Feed conversion was significantly higher in barrows, and lower in Trp boars over the experiment. Carcass weight tended ( $p=0.053$ ) to increase in barrows and Imp and Br boars while dressing rate was increased by all dietary narcoleptics. These data suggest that Trp and Br show promise as dietary treatments for the improvement of growth performance in group-housed boars and confirm the performance enhancing effect of immunocastration using Improvac<sup>®</sup> on normal boars.

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## TEMPORAL CHANGES IN THE FAECAL MICROBIOTA OF WEANED PIGLETS

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The composition and activity of the gastrointestinal microbiota can profoundly affect the health and performance of the host. As a result, considerable research efforts have focused on dietary methods to manipulate the gastrointestinal microbiota to the benefit of the host. This experiment was designed to determine the 'natural' stability of specific components of the faecal microbiota of pigs, between weaning and slaughter, as this may impact upon the assessed response of the microbiota to dietary manipulation.

Fourteen crossbred piglets from four litters were weaned at  $7.1 \pm 0.9$  kg ( $\pm$ SEM) and  $21.5 \pm 1.3$  days of age, into commercial flatdeck accommodation. The pigs received, on an *ad libitum* basis, standard UK weaner (day 0-20), grower (day 21-60) and finisher (day 61-118) diets. The weaner diet contained 3100 mg/kg zinc oxide and 40 mg/kg avilamycin. The transfer of the pigs to conventional grower and finisher accommodation coincided with the diet changes. Piglets were individually faecal-sampled at weaning and days 5, 12, 19, 25, 32, 60, 65 and 118 post-weaning. One hundred micro-litres of serial decimal dilutions of the faecal samples were cultured on specific bacteriological media to enumerate particular bacterial types. Data were log transformed and analysed using the GLM procedure of Minitab 12.2.

Faecal bacterial counts are shown in Table 1. Temporal changes in the faecal counts of aerobes ( $P < 0.001$ ), anaerobes ( $P < 0.05$ ) and *E. coli* ( $P < 0.001$ ) were measured during the experimental period. Faecal lactobacilli counts were similar at each sampling point.

**Table 1. Pig faecal aerobic, anaerobic, *E. coli* and lactobacilli counts (log CFU/g faeces) between weaning and day 118 post-weaning.**

Day (post-weaning)	Aerobes	Anaerobes	<i>E. coli</i>	Lactobacilli
0	7.51a	7.83ab	5.28ab	7.70
5	7.41a	7.74ab	5.93a	7.32
12	5.55b	7.71ab	4.44b	7.25
19	5.62b	7.42ab	4.86b	7.36
25	6.93a	8.10a	4.85b	7.63
32	7.11a	7.83ab	4.54b	7.50
60	6.90a	7.70ab	5.76a	7.46
65	6.95a	7.75ab	5.86a	7.65
118	6.88a	7.23b	4.57b	7.08
SEM	0.20	0.19	0.28	0.21
Significance	***	*	***	NS <sup>1</sup>

NS<sup>1</sup> non significant, \* $P < 0.05$ , \*\*\* $P < 0.001$ . <sup>ab</sup>In each column, means without a common superscript differ significantly.

These results suggest that 'natural' variations in the faecal populations of aerobes, anaerobes and *E. coli* occur during the period between weaning and slaughter for pigs. These population fluctuations could potentially mask responses to dietary attempts to manipulate the gastrointestinal microbiota. Lactobacilli populations appear to remain more stable through the 'production life' of slaughter pigs, suggesting that lactobacilli may be more resilient to any attempts to manipulate their numbers.

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## STOCKPERSON TRAINING TO IMPROVE PRE-SLAUGHTER HANDLING OF PIGS

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Fear is considered an undesirable emotional state of suffering and a farm animal's fear of humans is markedly affected by the behaviour of stockpeople. Relationships observed at abattoirs between stockperson attitudes and behaviour and pork quality (Coleman *et al.* 2000; Hemsworth *et al.* 2002) suggest that improvements in pre-slaughter handling may improve pig welfare and meat quality. In this experiment we examined the impact of a stockperson training program on stockperson behaviour and pork meat quality.

Two treatments were imposed: an Intervention treatment (n=10) consisting of cognitive-behavioural training designed to improve stockperson attitudes and behaviour, and a Control treatment (n=11) where no intervention was attempted. Stockpeople were observed for 1 h while moving pigs from a forcing pen into a CO<sub>2</sub> stunner on a total of 20 half-day shifts over 10 days. Stockperson behaviour such as pats or strokes was classified as positive; slaps with a hand or an electric goad switched off were rated moderately negative, while prods with an electric goad were rated highly negative. Meat quality was assessed on 1000 carcasses after slaughter by measuring pH, temperature and light scatter at 6-8 h in the loin (*M. longissimus dorsi* at the P2 site) and the ham (*M. semimembranosus* adjacent to the *tuber ischii*). Initial analyses indicated little or no effect of treatment on meat quality, but marked effects of other factors such as shift (morning vs afternoon). Therefore, mixed model regression analyses, using a 'restricted maximum likelihood approach', were used to examine the effects of stockperson behaviour on meat quality.

Trained stockpeople used more (P<0.05) positive behaviours and less (P<0.001) highly negative behaviours to move pigs than stockpeople in the Control treatment (0.001 vs 0.01 positive behaviours per pig and 3.82 vs 1.77 highly negative behaviours/pig, respectively). The only stockperson variable that was clearly related to meat quality was the use of positive behaviour. Positive behaviour was associated with ham colour (P<0.05) and there was tendency for an association with ham pH (P<0.12), loin temperature (P<0.06), and ham temperature (P<0.11). The use of positive behaviour was associated with a reduction in paleness and temperature and an increase in pH.

The cognitive-behavioural training improved the behaviour of the stockpeople. The effects of treatment on meat quality were relatively minor. There was some indication that the use of positive behaviours was associated with slight improvements in meat quality, presumably due to less handling stress before slaughter.

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## ENVIRONMENTAL ENRICHMENT FOR PIGLETS – IMPACT ON BEHAVIOUR AT WEANING

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Farrowing pens generally lack any form of social and environmental stimulation. This may have long-term negative effects on piglets (Grandin, 1988). Pigs in sterile environments may be more prone to stress and more aggressive at weaning. Aggression gives rise to behavioural vices, reduces growth rate and causes injuries (Beattie *et al.* 2000). In this experiment we investigated the impact of providing 'toys' for piglets on post-weaning (24 h after weaning) behaviour and growth to 140 days of age.

Forty Large White litters (nine piglets per litter) were randomly allocated to four pre-weaning treatments. The treatments were (i) No enrichment (NE), (ii) Toys (T; either a small soccer ball, small rubber tyre, PVC pipe, empty two-litre plastic drink bottle or ice cream container), (iii) Handling (H; picking up, patting and stroking piglets as soon as possible after birth, and then for 10 min per litter four times each week until weaned), and (iv) Toy plus handling (TH). Toys were placed in a farrowing pen from five days of age and rotated every three days until weaning at 24 days of age, to ensure that piglets did not lose interest in the toys. Following weaning, the pigs were allocated to weaner pens within treatment and blocked for sex and weight. Piglet interactions throughout lactation and for the first 24 h post-weaning were recorded via video camera using time-lapse video (3 frames/s). The pigs' aggressive and play behaviours were then quantified and analysed using the Chi-Square test. Aggression was defined as: tail and ear biting; bites and mouth hits directed towards the head and shoulder; belly nosing and circling other pigs and body thrusts directed sideways and upwards. Play activity included: jumping up and down on the spot; quick solo circling and gambolling, pushing or biting on toys.

Bouts of play activity were spread over a 24-h period. Most activity was confined to 5 min periods but occasionally 15-min of vigorous activity was seen. There was no difference in growth performance between treatments from birth to weaning. There was an association ( $P < 0.05$ ) between treatment and play-aggressive behaviour split post-weaning (Table 1). There was a positive relationship ( $P < 0.05$ ) between the provision of toys and the behaviour displayed by the pigs post-weaning (NE+H=821 aggressive bouts and 274 play bouts; T+TH=514 aggressive bouts and 1476 play bouts). There was no association ( $P = 0.48$ ) between handling (658 aggressive bouts, 885 play bouts) and no handling (677 aggressive bouts, 865 play bouts). Handling did not improve pig behaviour. Post-weaning growth rate was not affected by pre-weaning treatment.

**Table 1. Incidence of aggression and play behaviour of pigs over the first 24-h post-weaning.**

	Number of Aggressive Bouts	Number of Play Bouts	With-in treatment
No Enrichment	411 <sup>a</sup>	121 <sup>a</sup>	*
Toys Only	266 <sup>b</sup>	744 <sup>b</sup>	*
Handling Only	410 <sup>a</sup>	153 <sup>a</sup>	*
Toys + Handling	248 <sup>b</sup>	732 <sup>b</sup>	*

\* $P < 0.05$  within treatments; <sup>ab</sup> means in a column with the same superscript do not differ ( $P > 0.05$ ).

Play-activity increased markedly when a new toy was introduced. Provision of toys decreased the incidence of post-weaning aggression. In the T and TH groups aggressive behaviour in the 24 hrs after weaning often turned to play when a toy was encountered during a fight.

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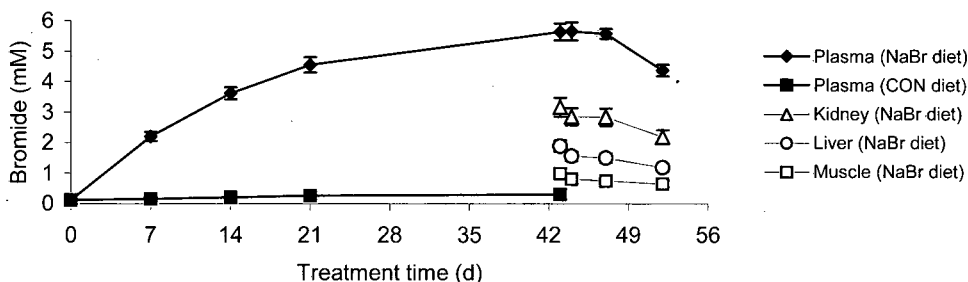
## THE KINETICS OF ACCUMULATION AND CLEARANCE OF BROMIDE FOLLOWING DIETARY SUPPLEMENTATION

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Bromide as a narcoleptic dietary additive in finisher-pig feed has the potential to improve growth performance in boars (McCauley *et al.* 2003). Narcoleptic-effects of bromide in humans (mild sedation, decreased libido) require sustained levels in plasma of about 5 mM. Conversely, bromide consumption from animal products must not be above the acceptable daily intake (ADI) of 1 mg/kg/day. In this experiment we determined the practicability of bromide supplementation by measuring the rates of accumulation and depletion during and after dietary treatment.

Twenty-five gilts aged 16 weeks were randomly allocated to either a standard finisher diet (CON, n=5) or the same diet supplemented with 300 g of sodium bromide (Consolidated Chemical Co., NaBr, n=20) per tonne of feed. Diets were supplied *ad libitum*. Ten pigs (five CON and five NaBr) were slaughtered after six weeks, and the remaining pigs fed the control diet. Further groups of five pigs were slaughtered 2, 5 and 10 days after the cessation of NaBr treatment. Tissue samples were obtained for bromide analysis according to the method of Dijkstra-ten Bolscher *et al.* (2000).



**Figure 1.** Bromide in blood and tissues of pigs fed either CON or NaBr diet from day 0-42, then placed on CON diet. Values are means  $\pm$  SD (n=25 day 0-42; n=5 day 43-52).

Therapeutic levels of bromide were reached after three weeks and were maintained until the end of treatment (Figure 1). Bromide concentrations decreased slowly after bromide supplementation ceased such that after 10 days, the concentrations in blood, kidney, liver and muscle had declined by 22, 31, 37 and 35%. The amount of bromide in tissues in pigs slaughtered the day after treatment stopped was 250, 150 and 80 mg/kg of tissue for kidney, liver and muscle. To consume enough to reach the ADI for bromide (which, itself, would not produce a therapeutic dose), a 60 kg adult would have to consume 320, 530 or 1000 g of kidney, liver or lean meat each day. These data suggest that due to the slow increase in circulating bromide, earlier introduction or use of larger supplements at the start of treatment may increase the duration of the narcoleptic effect. Despite the slow clearance of bromide, it is likely that no withholding period would be required for its use as a supplement.

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IX Biennial Conference  
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## **A SYMPOSIUM – THE ROLE OF SCIENCE IN THE ASSESSMENT OF WELFARE**

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### **Introduction**

The welfare of farm animals is becoming progressively more important for the livestock industries. Changes in the way we farm animals, particularly the move towards more intensive systems, has led to a perceptible shift in the ethical values and concerns of the community such that society is beginning to question its responsibilities to animal production. Attitudes of society towards animal welfare can influence government, industry and community decisions on the way we treat animals in farming systems. In particular, these attitudes may impact adversely on livestock production both directly through what consumers choose not to buy and indirectly through public and consumer pressure on policy set by governments, and the standards set by processors and retailers. It is important that the welfare standards set for farm animals are met by industry and this will occur through industry education, codes of practice and quality assurance programs. It is equally important to demonstrate to the public, consumers, and government that these standards of welfare are acceptable and being achieved.

It is crucial that there is a sound scientific base for assessing the welfare implications of modern farming practices. There is a clear role for governments and the livestock industries to support this scientific base and its research, to generate the scientific knowledge that will allow society to have an informed debate, and to inform, debate and discuss what is known and what needs to be known from the scientific point of view. Science has a major challenge ahead if it is to be a credible contributor to the welfare debate. The exclusion of science will allow emotive arguments from sectional interests to dominate community debate. This is not to say that people's emotional responses are not relevant to the debate – such responses reflect, in part, current personal and community values; however, they should contribute to, not preempt the debate.

Currently there is no definition of animal welfare that is accepted unanimously. For example, the European view that seems to be gaining acceptance places more weight on the importance of 'normal' behaviour than normal biological functioning of the animal. This subjective and arguably uncritical approach is likely to have a major impact on intensive livestock production and paradoxically could jeopardise animal welfare.

In this symposium we will review and identify strengths and weaknesses in the capacity of the present research methodology to evaluate animal welfare.

## THE USE OF ANIMAL PREFERENCE AND BEHAVIOURAL DEMAND TO ASSESS ANIMAL WELFARE

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### Abstract

As the profile of farm-animal welfare has increased, there has been a focus on what constitutes animal welfare and how we can measure it. One approach has been to try to measure how the animal itself perceives its situation through the expression of preference. A problem with examining animal preference is that the expression of a preference does not tell us much concerning how important the resource is to the animal. Accordingly, research methodologies have been developed that measure the strength of an animal's behavioural demand for access to a resource or avoidance of a stressor. This can be done by using operant responses or by forcing the animal to 'trade-off' the test situation against varying levels of another commodity, such as food. Studies using preference and demand approaches to draw conclusions about animal welfare have not always been successful because of problems associated with: 1) determining the relative importance of different resources; 2) doubts about whether animals are making choices that are in their long-term interests and; 3) animals apparently choosing or responding for reasons other than those anticipated by the investigator. Although preference and demand studies in themselves may not be suitable for drawing conclusions about an animal's long-term welfare, if properly designed this research approach can inform us of what the animal feels is important at a particular time. The challenge is to integrate the animal's behavioural demand with its biology so that we are better able to draw conclusions about the consequences for an animal of not fulfilling its significant behavioural demands. It is our hypothesis that while animals may exhibit a preference for the most desirable environment, the price that they are prepared to pay to change their environment increases significantly at the point when the environmental challenges represent a significant biological cost for adaptation.

### Introduction

During the past century, community views on animal welfare in western countries have moved from being concerned only about acts of wanton cruelty toward animals, to concerns about standards of animal care. An area of focus of this public concern for animal welfare has been in systems where animals are kept for profit, such as agriculture. Intensive farming systems, where animals are managed in man-made environments at high densities, have received particular attention from interest groups of animal welfare and were the subject of the first farm-animal welfare campaigns and regulatory scrutiny in the modern era (Harrison, 1964; Brambell, 1965). Correspondingly, the assessment and justification of the welfare of animals in farming systems has moved from simple measurements of system productivity to a broader evaluation of the state of the animals within a system. Such measurements include animal health, physiological measures of stress and the measurement of perturbations in behaviour.

Scientifically, most models which attempt an integrated view of animal stress and welfare incorporate behavioural, physiological and health components (Broom and Johnson, 1993; Moberg *et al.* 2000). The behavioural response of an animal to its physical or social environment, or to management procedures, has often been measured as an indicator of animal welfare. For example, the vocalisation and behavioural activity of piglets has been shown to be indicative of the level of pain response following castration (McGlone *et al.* 1993; White *et al.* 1995). However, as our view of what constitutes animal welfare has become more sophisticated, we have come to appreciate that we may be able to use animal behaviour to inform us better of the behavioural needs of animals and to identify how animals perceive their environment and management procedures. In this paper we review the approaches used to understand how farm animals themselves can tell us what may be good welfare, specifically studies of animal preference and behavioural demand. In this paper, the term 'animal preference' refers to studies where subjects are described as exhibiting a preference



for one situation over others, and 'behavioural demand' pertains to studies where there is an attempt to quantify the level of motivation to access or avoid the situation being tested.

Traditionally, most approaches used to study behavioural responses of animals to assess animal welfare, originated from the field of classical ethology - the observation of the behaviour of animals in their natural state. An alternative strand has come from experimental psychology and it is largely from this field that the use of animal preference and behavioural demand to assess welfare has developed. Today, the two strands have become moderately integrated in the applied field of using animal behaviour to assess animal welfare. Yet, it is our contention that although studies of behavioural response have been useful in furthering our understanding of animal welfare, and in evaluating the welfare impacts of farming environments and practices, the study of animal preference and behavioural demand currently represents an unfulfilled promise.

### **What can animal preference and behavioural demand tell us about animal welfare?**

#### *Welfare concepts*

It is an under-recognised fact that the concepts and definitions involved in animal welfare provoke almost as much debate and disagreement among scientists working in the field as the welfare issue does within society at large. In recent years, rather than perpetuate discussions to determine 'the best' concepts of animal stress and welfare, researchers have generally adopted a preferred model and moved on with the task of understanding the biological mechanisms involved and evaluating animal environments and management practices. Nonetheless, in any consideration of the value of animal preference and behavioural demand in contributing to our understanding of animal welfare, it is important to examine how these types of studies may interact with the differing concepts of welfare.

Some animal welfare models act essentially as checklists that may be used as simple screening or evaluation tools, or to support 'tick-box' type welfare assurance schemes. The 'Five Freedoms' (Farm Animal Welfare Council, 1993) which incorporate elements relating to nutrition, health, normal behaviour, comfort and psychological stress come into this category. More complex models of animal stress and welfare attempt to get beneath the surface by trying to understand what constitutes normal levels of these welfare components and what the consequences may be for the animal if they are not normal.

At this level, most researchers prefer the model of animal welfare as a biological state (Broom, 1986) with homeostatic mechanisms acting in response to stressors to try to maintain an optimal state and with ultimate consequences for animal fitness if these control mechanisms do not cope. Within this model, an additional concept can be incorporated from rodent and human research - that of allostatic load (McEwen, 2002a). Allostatic load is the cost to the organism of maintaining homeostasis in the face of stressors, and can result in subsequent perturbations in behaviour, immune function and body metabolism (McEwen, 2002b).

The model of animal welfare based on the homeostasis/biological state readily incorporates the various challenges to animal welfare that can occur such as infectious disease, tissue trauma, thermal conditions, and perturbations in the social environment. The biological responses that result can be used to assess animal welfare. Such responses include alterations in behaviour, clinical signs of disease, reductions in weight gain and so forth. In the absence of clinical disease, the animal may enter a 'pre-pathological state' (Moberg, 2000) in which its control systems are taxed to the limit by the stressor challenge.

Supporters of the alternative model of animal welfare argue that how an animal feels is the prime determinant of its welfare (Duncan and Petherick, 1991). In this model an animal, as a sentient organism, is aware of its own mental state and feelings of wellbeing or otherwise, and suffers distress as a result of both physical and psychological perturbations. The proponents of the 'feelings' model argue that when an animal is sick or in pain then its conscious response to its condition renders its welfare as poor. If the animal is biologically at risk but has no perception of this and is not likely to perceive its condition then its welfare is not compromised because it is not suffering.

Many people reject the 'feelings' animal welfare model because they associate animal welfare only with animal health despite unconsciously working within this 'feelings' model whenever they argue that there is no animal welfare issue in killing animals for food because the animals are unconscious when they have their throats cut.

*Animal preference, behavioural demand and welfare*

How do the concepts of animal preference and behavioural demand relate to our models of animal welfare? At a simplistic level, it is clear that the closest association is with animal feelings as the determinant of welfare. If animal preference can tell us *what* an animal wants and behavioural demand tells us *how much* it wants it, then it would seem reasonable to assume that by providing an animal with what it greatly desires, we will be making it feel better and thus improving its welfare. The problems with this approach, however, are two-fold. First, there are methodology issues (covered in the next section) that call into question whether many studies of preference and behavioural demand are actually informing us of what an animal really wants, or how much it wants it. Second, preference and demand studies may not be informing us of what is in the animal's best interests either in the short term (a methodology problem) or in the long term (a failing of the feelings model of animal welfare).

Given that the homeostasis/biological state model of welfare appears in general to be more robust, it is appropriate to consider in greater detail how preference and behavioural demand studies may contribute to animal welfare within this model. At first sight, the connection may appear to be very loose because what an animal wants or does not want may not tell us anything about the challenges to the animal if it is placed in a less-preferred situation (Duncan, 1978). However, the mental state of an animal is a valid component of the biological state model (Dantser and Mormede, 1983) and psychological and physical stressors can perturb homeostasis. If preference and demand can help inform us which environments are least aversive to animals and which ones are most preferred, then we may be able to select intensive systems and environments that minimise stress due to boredom, social stressors, discomfort and frustration. If we can use preference and behavioural demand to determine how much animals want to avoid a certain situation, then it may help to determine the practices animals find least painful and what type of stockperson interaction and facilities are most appropriate to minimise animal fear and distress during handling. The main barriers to achieving these goals have been problems with the methodology and interpretation of preference and behavioural demand studies and a failure to integrate this type of research into our understanding of the biology of animal welfare within the homeostasis/biological state model.

*How have animal preference and behavioural demand been used in welfare research?*

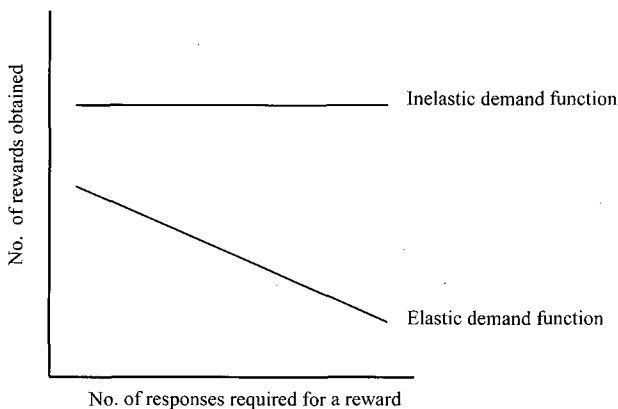
The earliest studies examining preference and behavioural demand of farm animals, including pigs, were concerned with the palatability of feedstuffs. Feeds were offered as free choice tests and the amount eaten by the pigs was recorded (Wahlstrom *et al.* 1974). Later studies required animals to make operant responses to obtain differing feeds with the authors suggesting that this allowed a more accurate quantification of relative preference (Klopfer *et al.* 1981).

More relevant to animal welfare, were studies with pigs that were done by Baldwin and colleagues from the late 1960s (Baldwin and Ingram, 1967ab; Baldwin, 1979). This research was concerned with identifying aspects of the physical environment that were preferred by growing pigs, particularly temperature. Young pigs were trained to switch on infrared heaters that would then operate for a short period before needing to be activated again (Baldwin and Ingram, 1967a). The results showed that the pigs preferred to maintain a temperature of around 25°C - right in their thermoneutral zone. Although this research did not provide dramatic new insights into animal welfare, as the biology of the thermoneutral zone for pigs had been well established, it was interesting because it indicated that the animal preference was aligned with the homeostatic midpoint for the stressor involved. What the studies did not quantify was how important it was to the pigs, behaviourally, to maintain that temperature. Although when the research was transferred to an outdoor context there was evidence that the pigs were prepared to trade-off heater activation in order to undertake other activities such as rooting and exploration (Baldwin, 1979).

The next significant development in the application of preference and demand research to animal welfare was the measurement of animal demand for a resource in response to an increasing 'price' that had to be paid (Dawkins, 1983). In this research, conducted with battery hens, the birds

were forced to choose between litter-floored cages with no food and wire-floored cages that had food present. The price to be paid for litter access was varied by increasing the duration of feed withdrawal before the test. In a second experiment (Dawkins, 1983) the price for litter or food access was varied by shortening the amount of time in a day available to the hens for using either resource. This type of research is based on consumer demand theory, in which demand for a resource is said to be inelastic if it is still purchased as the price increases (for example, food). A resource that is purchased less as the price is increased is described as having an elastic demand curve (defined as luxury items). In animal welfare research, this type of research has typically examined a resource that was 1) limiting in an intensive environment and 2), presumed to have the potential to cause animal stress if absent or present in only very limited amounts.

In extending the concept of measuring behavioural demand for a particular environment or resource to optimise animal welfare, later studies used the performance of operant behaviours to attempt to quantify the level of demand. This approach had its origins in psychological research performed with rats (Skinner, 1938) in which the rats had been trained to press a lever in order to obtain a portion of feed. The use of schedules (the animal performing the operation more than once) in order to obtain a reinforcer (feed) showed that the rats were prepared to work extremely hard for a feed reward when feed was limiting. As the number of operations required to obtain feed increased, so did the rats' preparedness to perform the required operant steps. If feed was plentiful or the animals' demand for a less important resource was tested, the access to the resource declined as the schedule was increased (Figure 1). In research on animal welfare in intensive production systems, this approach was used to measure how many times a hen would peck a key in order to enlarge its cage size (Faure, 1986, 1991). In pigs, Matthews and Ladewig (1994) used the slope of the demand curve to quantify the relative importance of food, access to a social partner, and simply opening the access door to a visibly empty pen. A slope of 0.95 for food, when graphed as  $\log$  [pig operant responses] against  $\log$  [fixed ratio operant schedule], confirmed the inelastic demand for food in pigs. By contrast, access to a social partner had a more elastic demand slope of 0.51 and simply opening the door had a demand slope of 0.37. Farm animals can be trained to perform a variety of operant behaviours for use in animal welfare research such as pushing through weighted doors, with the cost increased by increasing the counterweight on the door (Widowski and Duncan, 2000).



**Figure 1.** Examples of demand functions showing changes in consumption of a commodity where the demand is inelastic (e.g. food) compared with a commodity where demand is elastic (e.g. social contact).

Throughout the past 30 years, researchers have continued to use simple preference tests in animal welfare research, whether as choice tests (such as Y-maze) or providing differing environments (such as flooring or bedding types) and measuring the time spent using each environment. For example, Rushen and Congdon (1986) tested the relative aversiveness of electro immobilisation for sheep compared with shearing, using repeated Y-maze testing and measuring the choices made by the sheep. Natske *et al.* (1982) provided different bedding types for cow cubicles and measured cubicle selection and time spent by the cows lying in a multiple free choice test.

### **The use of animal preference and behavioural demand in pig welfare research**

#### *Housing and flooring*

Given that issues related to the physical environment and restraint in intensive pig production have generated the most interest from an animal welfare perspective (Barnett *et al.* 2001), it is not surprising that the majority of pig preference and behavioural demand studies have focused on these topics. Both preference and demand studies have been used to determine what sort of flooring is ideal from a pig's perspective. In a experiment comparing metal mesh flooring with plastic coated mesh, fibreglass slats and moulded plastic slats, Pouteaux *et al.* (1983) kept groups of weaner pigs in pens with all four floor types. Results showed that the pigs exhibited no preference while standing but spent more time lying on the plastic coated mesh floor than on the other types. There was, however, an effect of air temperature on the results, an effect also noted in other studies. Work by Fraser (1985) found that in a free choice test with both straw and bare concrete available, weaning pigs preferred to lie on the straw at 18 to 21°C but lay down more on the concrete at 25 to 27°C. In a separate experiment conducted under variable temperature conditions (Fraser, 1985) pigs showed no preference for either flooring although they spent time playing with the straw. These results illustrate the dangers in interpreting the data of such studies without considering other factors that can impact upon animal preference. Play and exploratory behaviour by young pigs appears to be an important component of their time budgets spent on different flooring substrates. A time budget study conducted by Beattie *et al.* (1998) found that growing pigs spent most of their time on peat, compost and sawdust, somewhat less time on sand, and only preferred wood bark and straw over concrete. In this experiment it is likely that rooting behaviour and the texture of the substrates in relation to that of dirt, were the main factors influencing the time budgets of the pigs rather than lying comfort.

Operant studies examining the behavioural demand of pigs for earthen substrates have not been particularly successful. In one experiment (Hutson, 1989) weaner-pigs housed in groups, were required to lift a lever 10 times to gain access to an earthen substrate or no substrate. The amount of operant responses by the pig groups was not affected by the presence of the substrate compared with the control condition. Furthermore, in most pig-groups only one 'worker' pig performed most of the operant responses while the remainder joined in the interaction with the substrate once it was available. This suggests that only one pig in each group was reinforced and indicates a potential problem in using this type of methodology in groups of pigs. In a separate experiment (Hutson and Haskell, 1990) six individually-managed sows were initially given free access to an earth pen for farrowing but then had to operate a lever to access the pen for their next farrowing. While all the sows learned to access the earth pen, farrowing in the pen fell from all six sows under free access to just two sows farrowing under the operant condition. The authors suggested that the sows may have been perturbed by the fact that the door closed automatically behind them whenever they left the earth pen. Again, this highlights the importance of controlling for factors that we may take for granted but which exert a strong effect on animal choice. It is also possible that farrowing in the earth pen was not very important to the sows but it is not possible to draw conclusions from the experiment.

A better controlled experiment (Pedersen *et al.* 2002) examined the motivation of pigs to perform operant responses for feed or for a small amount of straw, with or without the presence of a companion pig in an adjoining pen. The experiment was designed not so much to determine how much the pigs wanted straw but as a methodological study to identify whether changes in the social environment affected behavioural demand. The presence of a companion increased the demand for food and the intensity of demand for straw. This was possibly due to social facilitation, leading to the conclusion that the priority pigs place on being able to perform different behaviours varies

according to the social context in which the testing is performed. It should also be noted that the pigs pressed a panel up to a fixed ratio schedule of 60 to obtain 26 g of feed but only worked up to a fixed ratio of 15 to receive the straw reinforcer (Pedersen *et al.* 2002). An experiment by Ladewig and Matthews (1996) used operant schedules to compare the demand curve slopes of individually housed pigs for feed, bedding substrates, social contact and exercise. Not surprisingly, feed prompted the highest level of demand followed by all the bedding materials. Both social contact and exercise had low levels of demand.

The design and flooring of farrowing crates have been examined in preference studies with sows. In one experiment (Phillips *et al.* 1991) sow preference for fully enclosed, solid-sided and open tubular-design farrowing crates was examined in a free-choice preference test. The results varied with age and experience with younger sows preferring to farrow in the more enclosed crates and older sows exhibiting no clear preference. It should be pointed out that the older sows had previously farrowed a greater number of times in the standard, open tubular crates and that it is not possible to determine the strength of preference of the younger sows to farrow in a visually shielded environment. In a similar experiment, sows were offered free choice of farrowing crates with either concrete, plastic-coated metal mesh, or bare mesh floors (Phillips *et al.* 1996). The sows, which had previously been housed on concrete, preferred this flooring although by the end of the experiment the use of the other floor types had increased. A second experiment, in which sows were pre-exposed to the other floor types, resulted in an increase in their usage (Phillips *et al.* 1996). It is difficult to form conclusions from these studies other than previous experience strongly influences pig choice.

An experiment by Spinka *et al.* (1998) examined how averse gilts were to confinement. Twelve group-housed gilts were offered a free choice of individual feeding stalls at the daily feeding time. The gilts could choose between stalls in which they were then confined for 30 minutes or stalls in which they were confined for 240 minutes. Although a majority (eight) of the gilts preferred to enter the short confinement stalls, two gilts exhibited no preference and two gilts preferred long confinement. In this case it is possible that the factor influencing the choice of these animals was not confinement *per se* but the opportunity to escape the unwelcome social attention of con-specifics.

#### *Air quality and gas stunning*

Because ammonia, dust and bacterial contaminants can represent a health and welfare risk in intensive pig production environments, air quality (in terms of ammonia concentration) has been examined in a number of preference and demand studies. In order to measure the strength of pig motivation to avoid ammonia contamination Jones *et al.* (1999) used a trade-off model in which pigs had access to heated compartments with 40-ppm ammonia and unheated compartments containing fresh air. Pigs spent more time in the heated environments as the temperature in the unheated compartments dropped towards the lower critical temperature suggesting that the aversion to 40 ppm ammonia was weaker than the pigs' preference for thermal comfort. In an experiment by Smith *et al.* (1996), which was designed to examine the effects of higher ammonia concentrations, pigs were given free-choice access to otherwise identical pens containing either fresh air or 100 ppm ammonia. Four out of six pigs demonstrated a pronounced preference to spend time in the pen with fresh air leading the authors to conclude that the 100 ppm ammonia was aversive (Smith *et al.* 1996). Jones *et al.* (1996) did a similar longer-term preference experiment and found that pigs were averse, in a free-choice test compared with fresh air compartments, to 10, 20 and 40 ppm ammonia (increasing with time). In apparent contrast to these findings is an experiment in which operant responses were used to quantify the strength of pig aversion to puffs of air containing 100 ppm ammonia (Jones *et al.* 1998). Results from this experiment indicated that the initial aversion to the ammonia was weak and soon declined. It is likely that the operant experiment apparatus, which did not completely expose pigs to an atmosphere of 100-ppm ammonia, is responsible for these discrepancies. It is probably better to quantify the behavioural demand of pigs to remove themselves from a contaminated environment than to use the approach of Jones *et al.* (1998).

To examine the relative aversion of pigs to gas mixtures used for stunning of pigs before slaughter, Raj and Gregory (1995) used an aversion test with feed as a trade-off. Pigs that were feed deprived for up to 24 hours were presented with compartments containing feed and either 90% argon in air, 30% carbon dioxide in air, or 90% carbon dioxide in air. No pigs were reluctant to

enter the 90% argon atmosphere and a majority of pigs entered the 30% carbon dioxide compartment. In contrast, despite 24 hours of feed deprivation, the majority of pigs refused to enter the compartment containing 90% carbon dioxide. The authors' conclusion was that pigs were averse to the 90% carbon dioxide. We would argue that the conclusions and implications drawn from this experiment could be considered as more robust than those from a short-term ammonia preference experiment because the pigs lived in a potentially ammonia-contaminated environment for a much longer period. Thus, their initial aversion may not have been a major component of their overall welfare. In contrast, the animal's aversion and initial perception and stress response to the stunning atmosphere is quite clearly everything that matters from a welfare perspective, assuming the atmosphere is sufficiently anoxic.

#### *Feed*

Pigs are very highly motivated to obtain feed as evidenced by the use of feed as either a trade-off commodity or as a gold-standard inelastic demand commodity in operant studies (Ladewig and Matthews, 1996). Various studies have examined pig preferences and behavioural demand for feed (Baidoo *et al.* 1986; Kyriasakis and Emmans, 1992, 1993). More interestingly, there have been attempts to address the issues of feed restriction and hunger in dry sows and boars, using behavioural demand methodologies. Lawrence *et al.* (1988) examined the number of operant responses that boars would make to obtain a seven grams of feed reinforcer while being maintained at varying fractions of their *ad libitum* intake. Boars fed at even  $1.3 \times$  maintenance performed 266 panel-press responses to receive an additional seven grams of feed. In a subsequent experiment (Lawrence *et al.* 1989), the effects of increasing dietary bulk during feed restriction on behavioural demand for feed were studied. Increasing the dietary bulk of the restricted diet did not reduce the overall operant response rates for additional feed. The results of this experiment suggest that feeding motivation and possibly hunger of adult pigs that are feed-restricted, is not reduced by increasing the physical bulk of the feed provided.

One criticism of this type of methodology is that it may underestimate the hunger felt by the pigs because they may be less motivated to work for additional feed due to the feeling of gastrointestinal distension. To address this issue, Day *et al.* (1996) used a second-order operant schedule in which the animal worked for a reinforcer that was not feed itself but a conditioned stimulus that the animal has previously learned to associate with feed. In this experiment, growing-pigs on the same level of feed restriction but with a greater feed bulk, performed the same number of responses before daily feeding but fewer responses for three hours after feeding. These results indicate that a greater feed-bulk may temporarily promote satiety in feed-restricted pigs but that this effect diminishes after a few hours.

#### *Thermal comfort*

When it was established that young pigs could learn to operate supplemental heating sources (Baldwin and Ingram, 1967a), a range of studies were done on heat preference and demand in pigs (Baldwin, 1979; Verstegen *et al.* 1986, 1987; Morrison *et al.* 1987, 1989; Swiergiel, 1998). It is beyond the scope of this review to cover these studies in detail but it is worth noting when this research indicated that the behavioural demand of young pigs for heat corresponded closely to their established thermo-regulatory requirements. Biological studies have demonstrated that the lower critical temperature for pigs of a given weight is reduced as group size increases due to a greater effect of shared body heat (Bruce and Clark, 1979). Similarly, the greater huddling of young pigs at night reduces the need for additional heat. Morrison *et al.* (1989) investigated the operant demand of weaner pigs for supplemental heat when managed at different group sizes. In accordance with biological knowledge, the pigs demanded less heat at larger group sizes and during the night.

Other studies have used operant heat demand in young pigs to quantify the effects of wind velocity or time of day on heat requirements (Verstegen *et al.* 1986, 1987). In these cases, heat demand was used as a convenient and biologically relevant method of measuring the effects of treatment.

### Conclusions on preference and demand use in pig welfare studies

The application of animal preference and behavioural demand methodologies have been most successful in providing information relevant to pig welfare when they have been used to examine situations in which:

- there is a relatively short-term biological impact;
- the behavioural motivation being tested is linked to biological impact and;
- the pigs are probably responding to what the experimenter hopes they are responding and are not being influenced by other factors.

Thus, operant heat switching can be used as a useful additional indicator of the heat requirements of pigs under different situations and, when used with care, feed preference and feeding motivation can reflect the animal's dietary preferences and drive to eat. In contrast, the complex methodologies, the effect of previous experience, and the long-term nature of the biological consequences involved, render the conclusions for animal welfare that can be made from the published use of preference and demand methodologies in housing and confinement research, rather more tenuous. These issues are covered in more detail in the next section.

### Problems with the use of preference and behavioural demand in welfare research

A commonly voiced criticism of the application of animal preference and behavioural demand methodologies to the assessment of animal welfare is that animals may not necessarily prefer, or be motivated to obtain, variables that are truly in their best interests (Duncan, 1978). For example, in a experiment examining preference and operant demand of pigs for varying concentrations of sweet solutions (Kennedy and Baldwin, 1972), a number of pigs were reported as drinking so much sugar solution that they became ill. After recovering, these animals continued to drink large amounts of sugar solution. Usually these problems occur when the provision of a short-term choice may be appealing to an animal but may have adverse consequences for its longer-term welfare. Animals, unlike humans, are not thought to be able to project into the future. Although evolutionary biologists may argue that animals should generally make choices that improve their fitness, this is more likely to hold true for wild animals presented with choices that represent natural states. It is less likely that domestic animals presented with artificial environments and choices will always select what is in their long-term interests. For example, the confined pigs studied by Ladewig and Matthews (1996) showed minimal demand for exercise yet a degree of exercise has been shown to have a longer-term beneficial effect on pig welfare (Marchant and Broom, 1994).

A second problem to consider is that animals may be choosing or responding for reasons other than those assumed by the investigator. Often, conditioned responses in animals may result in behaviours that are quite different to those desired by the trainer (Breland and Breland, 1961). Similarly, great care needs to be taken by the experimenter to ensure that the animal is responding to the appropriate cues in preference and demand studies. The greater the complexity of the study or the experimental apparatus, the greater the risk of misinterpreting the results. Animal behaviour and preference studies are littered with *post hoc* explanations of why the subjects did something that may have been contrary to all logic and expectation (Hutson and Haskell, 1990; Grandin *et al.* 1994; Spinka *et al.* 1998). Previous experience can be a major factor in influencing animal choice. At the simplest level, this has involved animals developing a preference for one side of a Y-maze (the side with the 'good' treatment) to the point where the animals persist in selecting this path even when it is switched to the 'bad' treatment (Grandin *et al.* 1994). In other studies, hens with experience of battery cages preferred this environment to a free range run (Dawkins, 1977).

Another caveat in interpreting the results of preference studies is that they tend to only show the absolute preference of the animal and do not provide information on the relative importance of the different options. A less preferred flooring substrate, for example, may still be quite acceptable to pigs and not detrimental to their welfare. The generation of demand curves for different commodities for animals has a better chance of providing information on the relative strength of preference. However, in these studies there also needs to be caution in interpreting relative preference. For example, Ladewig and Matthews (1996) tested the demand of pigs for either sawdust or straw substrates, and suggested it was likely that the pigs preferred straw but since the operant response procedure tested only one substrate at a time, the demand curve for sawdust was almost as great. Ladewig and Matthews (1996) suggest that one approach to overcoming this problem is to develop 'two dimensional' operant demand testing. In this system the pig would

press a lever on one side for access to one substrate and a lever on the other side for access to the other substrate, thereby combining elements of both preference and behavioural demand methodologies.

It should be pointed out that there is still a significant level of debate concerning the best methodology to use for behavioural demand testing (for example operant *versus* trade-off) as well as the interpretation of demand curves. A majority of researchers in the field use the slope, or elasticity, of the demand curve (Dawkins, 1997) while others argue that the area under the curve is more important (Houston, 1997ab).

Although such arguments may be very important to those working at a detailed level in the field, the key question is how we can appropriately use animal preference and demand studies to inform us of animal welfare status within the homeostasis/biological state framework. To this stage, many welfare-oriented preference and demand studies have effectively existed within their own framework, without reference or connection to the biology of the animal. We need to determine what the expression of an animal preference or behavioural demand actually means for its welfare if that choice is denied. To this end, most preference and demand studies have remained merely a useful or interesting adjunct to welfare research based on animal behavioural, physiological and other responses to different conditions. At worst, the methodology flaws and counter-intuitive findings of some preference and demand studies have brought scepticism upon the value of this approach as a whole. When behavioural preference and demand research demonstrates robustly that a situation is greatly desired by animals, and complementary research shows that a detrimental biological response occurs if the animals are denied this desired situation, the results may be taken to have genuine implications for animal welfare (Mason *et al.* 2001).

#### **Conclusions and thoughts for the future**

It is probably unreasonable to presume that a domestic animal will always exhibit a preference or significant level of behavioural demand for something that is always in the interests of its long-term welfare. Yet, properly designed, this research approach can tell us what the animal feels is important at that time. For some situations, such as the avoidance of painful stimuli, this is sufficient to make direct conclusions about animal welfare. Even in other situations, where the behavioural demand of an animal for another commodity is measured, there is the possibility that the thwarting of that demand can adversely impact on animal welfare. Stressors acting externally can affect the biological state of a domestic animal both physically and psychologically. However, in addition to rigorous design and interpretation of preference and demand studies, a closer integration of this approach with the biological consequences for the animal is required to address the question, 'Yes, but what does it mean?'

Our suggestion is that there is a need for research that links more directly the animal's behavioural demand to avoid a potential stressor with the biological costs of that stressor. In this case, the stressor can also be the absence of a highly valued commodity. Furthermore, it would be appropriate to examine, for increasing levels of a stressor, whether the level of behavioural demand for 'stressor avoidance' increases at a similar stressor level at which the biological costs also increase. We hypothesise (within the biological model of animal welfare) that, while animals may exhibit a preference for the most desirable environment, the price that they are prepared to pay to change their environment increases significantly at the point when the environmental challenges represent a significant biological cost for adaptation.

A sceptic may ask why we should bother including preference and demand studies at all in our biological assessment of welfare. While it may not always be appropriate to test preference and demand, we argue that it would be wrong to neglect totally what this research has to offer. Some answers to the sceptic's question are contained earlier in this paper, and another response would state that until our understanding and measurement of neural function is much more advanced, animal behaviour remains the best way we have of identifying how a sentient animal perceives its situation. One last reason relates to our final audience. This paper commenced by highlighting how the welfare of domestic farm animals has become increasingly important to the general public. To best promote the adoption of improved farming practices or to highlight good animal welfare within existing practices, the strongest argument that scientists can present to the community in the future may be framed as 'the biological state of the animal is optimal and the



animal strongly prefers...'. While the first component of the argument may contain more biology, we all know which part will be the most convincing to the audience.

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## STUDYING DIFFICULT AND INADEQUATE ADAPTATION TO ASSESS ANIMAL WELFARE

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### Abstract

Animals use a wide range of behavioural and physiological responses to regulate their lives and deal with difficulties that they encounter. Although biological regulation is occurring constantly, adaptation is not always possible and consequently these behavioural and physiological responses can have serious biological costs for the animal, including inefficiencies in growth and reproduction, injury, disease or even death if maladaptation persists. It is these broad, long-lasting responses and their effects on the biological fitness of the animal that have clear implications for animal welfare. A number of authors have therefore proposed that difficult or inadequate adaptation will generate welfare problems for animals.

A continuing difficulty confronting scientists who study animal welfare has been the definition of animal welfare. The failure to achieve a consensus on an adequate definition has limited the role that science has played in developing rigorous standards of welfare for domestic animals. In this paper it is proposed that the most pressing role for science in identifying welfare standards is to identify those situations in which animals have difficulty coping. The issue of whether or not animals require environments that provide more than their 'basic' biological requirements is another level of discussion that needs to occur. An agreement that those conditions that create biological dysfunction are the most serious for animal welfare will enable scientists to study and identify in an agreed and established manner those housing or husbandry practices that create biological dysfunction. Although this is a conservative approach, it will allow significant progress to be made in improving animal welfare by identifying those conditions that create biological dysfunction since most agree that such biological dysfunction seriously limits animal welfare. In this review I will consider the proposal that failure to adapt creates serious welfare problems for animals.

### Introduction

#### *Adaptation and welfare*

The relative constancy of conditions that exist within the body of a healthy animal is achieved by the operation of many homeostatic controls (Broom and Johnson, 1993). Animals use a wide range of behavioural and physiological responses to regulate their lives and deal with difficulties. These homeostatic control systems are activated once a physiological displacement arises and thus they react to environmental and endogenous stimuli to correct or prevent displacements from the optimal range.

Traditionally, homeostasis has been used to denote the equilibrium of the internal environment of an individual animal with respect to its physiological requirements such as body temperature, blood sugar concentrations and oxygen levels. However, in addition to displacements in the animal's physical requirements, departures in the animal's expected or optimal external environment such as opportunity for social contact or avoidance of fear-provoking stimuli, will also lead to adjustments designed to restore 'homeostasis'. Thus the regulation of the animal's optimal or expected physical and psychological requirements are achieved by the operation of numerous homeostatic controls.

Although biological regulation is occurring constantly, adaptation is not always possible and when homeostasis fails, there are biological costs for the animal that may include growth and reproductive failure, injury, disease or even death (Cannon, 1914; Selye, 1976). Death is the ultimate consequence of failure to adapt, however it should be appreciated that less severe challenges to homeostasis, through activation of behavioural and physiological responses, can result in less serious biological costs such as impaired growth, reproduction and health. Thus

difficult or inadequate adaptation will generate welfare problems for animals (Broom and Johnson, 1993; Hemsworth and Coleman, 1998).

A continuing difficulty confronting scientists studying animal welfare is the lack of an agreed definition of animal welfare. Without a clearly accepted definition, animal welfare cannot be measured and studied in an agreed manner that enables the results of research to be widely recognised and used. For the debate about welfare standards provided to domestic animals to progress, it is important for science to provide a framework in which welfare risks can be studied and thus considered by individuals. For example, an individual's opinion of the acceptability of a husbandry or housing practice is influenced by a number of factors including knowledge about effects of the imposition on the biology of the animals and ethics. The failure to achieve a consensus on an adequate definition of animal welfare has limited the role that science has played in developing rigorous standards of welfare for domestic animals.

Although some may disagree, an immediate solution to this stalemate is that the most pressing role for science is to determine how well animals cope with their environment (Barnett and Hemsworth, 2003). That is, whether or not the animals are experiencing difficult or inadequate adaptation. The issue of whether or not animals require environments that provide more than their 'basic' biological requirements is another level of discussion that needs to occur but is unlikely to be solved with our present state of knowledge and discussion on the topic. Nevertheless, agreement that those conditions that create biological dysfunction are the most serious for animals will enable scientists to study and identify, in an agreed and established manner, those housing or husbandry practices that create biological dysfunction. Notwithstanding the possible conservative nature of this approach it will allow significant progress to be made in improving animal welfare by identifying and, if agreed by Governments and/or the animal users, excluding those conditions that create biological dysfunction.

In this I review will consider in more detail the proposal that failure to adapt creates serious welfare problems for animals. The discussion will consist of a brief description of how animals deal with difficulties in their lives and the biological consequences of such difficulties for the animals. The case will then be developed that those conditions that create biological dysfunction also create serious welfare issues for animals. Examples of the approach will be used to demonstrate its value and possible criticisms of the approach will be considered. It should be clearly recognised that this approach extends the views of numerous scientists such as Broom and Johnson (1993), Hemsworth and Coleman (1998) and Barnett and Hemsworth (2003).

#### **The role of science and ethics in developing welfare standards**

An individual's concern about the acceptability of a husbandry or housing practice is influenced by a number of factors including knowledge about the imposition on the animals in question and ethical values. The individual's understanding of how the practice imposes on or challenges the animals, together with the individual's ethical values in relation to whether such an imposition is reasonable, will assist the individual in making an ethical judgement on whether the practice is acceptable. Ethical judgements must always be based on good science, but science is not ethics (Barnett and Hemsworth, 2003; Comstock, 2000). Science therefore clearly has a critical role in the welfare debate. The exclusion of science will result in emotive arguments from sectional interests dominating community debate. This is not to say that people's emotional responses are not relevant to the debate – such responses reflect, in part, current personal and community values however, they should contribute to, not preempt, the debate.

#### **Challenges to homeostasis and their consequences for the animal**

The vitality of all animals depends ultimately on the efficient operation of the regulatory systems that control the conditions within their bodies, termed homeostasis (Broom and Johnson, 1993). These regulatory systems control, for example, body temperature, nutritional state, water balance, social interactions and fear, and react to environmental and endogenous stimuli to correct or prevent displacements from the optimal range. To maintain homeostasis, animals use behavioural and physiological responses and these biological responses are usually proportional to the challenge to homeostasis (Broom and Johnson, 1993). The implications for animal welfare when homeostasis is challenged are best illustrated by recognising the adaptive responses that animals may use in attempting to cope and their consequent biological costs.

Once the central nervous system perceives a potential challenge (stressor) to homeostasis, it develops a biological response or defence that consists of some combination of the four general biological defence responses - behavioural responses, responses of the autonomic nervous system, responses of the neuroendocrine system and responses of the immune system (Moberg, 2000). The central nervous system integrates these responses to provide the animal with the principal resources to cope with the stressor. For example, when an animal is attempting to maintain its body temperature in a cold environment it may respond behaviourally by seeking shelter or building a nest and physiologically by reducing peripheral blood flow and increasing metabolism. The type and combination of the behavioural and physiological responses are dependent on the characteristics of the stressor such as its magnitude and duration.

For many stressors, the first and, at times, the most biologically economical and effective response is a behavioural one. If the challenge, for example, is the close presence of a dominant group-mate or a potential predator, either freezing or avoidance following the startle and orientation responses may be effective strategies to deal with the threat. Alternatively, defensive responses such as growling and a threatening stance or even attack may be appropriate. However, behavioural responses may not be appropriate or effective for all situations, particularly when the behavioural options are limited or thwarted (Moberg, 2000). Nevertheless, some component of behaviour is likely to be involved in every stress response.

Along with behavioural responses, physiological responses can be used by the animal and are elicited in three series of events (outlined below). Full elicitation of these physiological responses depend on the time of exposure to the stressor and the success of the biological responses in coping with the challenge (see Hemsworth and Coleman, 1998). The first series of physiological events is characterised by a rapid, specific response by the autonomic nervous system and consequent secretions of catecholamines (adrenalin released from the adrenal medulla and noradrenalin released from the adrenal medulla and the nerve endings of the sympathetic nervous system). This immediate or 'emergency' response is the 'fight or flight' response proposed by Walter Cannon (Cannon, 1914). This is the principal regulatory mechanism that allows the animal to meet physical or emotional challenges by its impact on metabolic rate, cardiac function, blood pressure, peripheral circulation, respiration, visual acuity, and energy use and availability. A particularly important biological effect is the adrenalin-dependent production of glucose from liver glycogen (glycogenolysis) for an immediate energy supply. This initial reaction lasts for only a short period of time and if the stressor is not removed, a second series of events occurs.

The second series of events, called the acute stress response, is part of Hans Selye's 'general adaptation syndrome' (Selye, 1946; 1976) and is a corticosteroid-dependent mechanism. Corticotrophin-releasing factor (CRH) released from the hypothalamus stimulates adrenocorticotrophic hormone (ACTH) release from the pituitary, which, in turn, stimulates the release of corticosteroids or glucocorticoids from the adrenal cortex. Arginine vasopressin (AVP) from the hypothalamus has a role in some species in stimulating ACTH secretion (Matteri *et al.* 2000). This acute response may last from minutes to hours and has the major function of providing glucose from food or muscle protein (gluconeogenesis) for the required increased metabolic performance. Therefore, during this stage a steady state is achieved in which the increased demand for energy is met by increased metabolic performance. This physiological state of stress disappears on removal of the stressor with generally no ill effects other than a depletion of energy reserves. This is an effective mechanism whereby the animal can adapt to changes in its environment.

While acute stressors are short acting, there are situations in which they could have detrimental effects on the animal. There is a number of examples where an acute stress response at specific times in the reproductive cycle has interfered with different aspects of reproduction (Clarke *et al.* 1992). Because of the importance of the series of carefully orchestrated endocrine events required for oestrus, ovulation and conception and the known effects of stress on these endocrine events, it is perhaps not surprising that activation of the hypothalamic-pituitary-adrenal (HPA) axis before mating may adversely affect female reproduction. Furthermore, there are examples where a presumably painful husbandry procedure such as dehorning of calves results in a substantial acute stress response with a growth check in the animals (Goonewardene and Hand, 1991; Sylvester *et al.* 1998).

If the stressor continues, the response proceeds to the third series of events, which is the chronic stress response. Again, this series of events is a corticosteroid-dependent mechanism but, while in the acute phase the effects are potentially beneficial, this chronic activation of the HPA axis comes at a physiological cost to the animal such as a decreased metabolic efficiency, impaired immunity and reduced reproductive performance. It is well known that the long-term activation of the HPA axis can have marked effects on efficiency of growth with, for example, the catabolic effects of ACTH and corticosteroids (Elsasser *et al.* 2000). Corticosteroids also support the synthesis and action of adrenalin in stimulating gluconeogenesis and lipolysis (Matteri *et al.* 2000). Stress-induced changes in the secretion of pituitary hormones have been implicated in failed reproduction (Clarke *et al.* 1992; Moberg, 2000) and immune competency (Blecha, 2000). How serious these costs are, depends on how long the animal is required to divert physiological resources to maintain homeostasis. Some of the features of a chronic activation of the HPA axis include increased basal secretion of glucocorticoids with a loss of diurnal regulation of the axis and reduced responses to some stressors (Harbuz and Lightman, 1992). While the role and actions of corticosteroids in acute and chronic stress responses are well known, the HPA axis is not the only neuroendocrine axis affected by stressors. The secretion of prolactin and somatotrophin (growth hormone) are equally sensitive to stress and thyroid-stimulating hormone and the gonadotrophins (luteinizing hormone and follicle-stimulating hormone) are either directly or indirectly modulated by stress (Moberg, 2000).

It is becoming increasingly well understood that the immune system in its own right is one of the major defence systems responding to a stressor. The increased incidence of disease in animals suffering from stress has long been recognised as a consequence of modulation of the immune system principally by the HPA axis. However, the central nervous system has a direct role in regulation of the immune system (Moberg, 2000). Furthermore, while the adrenal, somatotrophic and thyroid axes have a critical role in shaping metabolism under the influence of stress, it is clear that endocrine-immune interactions are also important (Elsasser *et al.* 2000). Some of the immune parameters associated with a chronic activation of the HPA axis include an overall reduction in numbers of white blood cells and reduced antibody production, neutrophil function, lymphocyte proliferation, and natural killer cell activity. In addition, there is an increase in acute phase protein response and levels of pro-inflammatory cytokines (Black *et al.* 2001). Chronic stress does not simply suppress the immune system but induces a shift in the cytokine balance resulting in immune dysfunction. Such changes increase susceptibility to infection, fever, and hypersomnia and depress social behaviour.

Thus the hormones secreted from the HPA axis have broad, long-lasting effects on the body and challenges to homeostasis that result in such long-lasting neuroendocrine responses clearly have implications for animal welfare. While some component of behaviour is likely to be involved in every stress response, behavioural responses may not be appropriate or effective for all situations. Indeed, long-term behavioural responses, as with long-term neuroendocrine responses, may indicate difficult or inadequate adaptation. For example, a lack of a nutrient requirement or a situation in which the animal is highly motivated but is unable to perform an appropriate behavioural response may lead to either redirected behaviour or stereotypies. These reactions may be associated with physiological responses indicative of a chronic stress response (Broom and Johnson, 1993; Hemsworth and Coleman, 1998).

While it should be recognised that our understanding of both the causation and function of stereotypies is poor (see Hemsworth and Coleman, 1998), the existence of a stereotypy is at least indicative of a past problem for the animal in coping with its conditions. Furthermore, stereotypies that result in physical damage or illness to the animal, such as the development of lesions in stall-housed sows that persistently rub their tail roots from side to side against stall fittings (Ewbank, 1978), or wind-sucking in horses leading to gastrointestinal catarrh and colic (Fraser and Broom, 1990), clearly have obvious and immediate implications for the welfare of the animals. Behavioural change in which there is abnormality either in the pattern, frequency or context of the behaviour from that which is generally expected, and which results in adverse effects on the morbidity or mortality of the individual or others, also has welfare implications.

For example, tail biting in pigs, for which the cause(s) are poorly understood, results in restlessness, poor growth, and possible paralysis and mortality due to infections in the recipients (van Putten, 1969). Similarly, chewing of wood, soil, hair or faeces may have serious welfare implications for the animal if they ingest such material.

Thus the behavioural and physiological responses used by animals to deal with challenges to homeostasis can have broad, long-lasting effects on the body when such challenges are substantial. It is these broad, long lasting effects on the fitness of the animal that have clear implications for animal welfare.

### **Biological dysfunction leads to serious animal welfare problems**

Difficult or inadequate adaptation will affect the fitness of the animal through a range of long-lasting behavioural and neuroendocrine responses. Therefore, with this background, it is proposed that welfare risks can be considered at two levels as described below. It should be recognised that this framework is underpinned by some sound biological principles and has been recognised and proposed by other scientists such as Dawkins (1983) and Broom and Johnson (1993), albeit slightly differently.

The first level of welfare risk involves the situation in which the environment fails to meet the fundamental or primary biological requirements of the animal. It is these conditions that create biological dysfunction and, as discussed earlier, are the most serious welfare risks for the animal. This view is underpinned by the following definition that 'the welfare of an individual is its state as regards its attempts to cope with its environment' (Broom, 1986). The 'state as regards its attempts to cope' refers to both how much has to be done in order to cope with the environment and the extent to which these coping attempts are succeeding. Attempts to cope include the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses. The extent to which coping attempts are succeeding refers to the lack of biological costs to the animal such as deterioration in growth efficiency, reproduction, and health and freedom from injury. Broom and Johnson (1993) refer to these requirements as 'needs' as they are fundamental to the biology of the animal.

The second level of welfare risk involves the situation in which the environment meets the so-called fundamental biological requirements (as described above) but fails to provide resources that for which animals may show a distinct preference. It is proposed that since these conditions do not create biological dysfunction for the animal, they pose less welfare risk than do those that create biological dysfunction. In this context, a deficiency is generally manifested as a homeostatic maladjustment and when an animal has an unsatisfied requirement, its motivational state will usually elicit behavioural and physiological responses that aim to remedy the situation to allow the individual to cope with its environment. Broom and Johnson (1993) suggest that most of what is strongly avoided is harmful and most of what is strongly preferred is beneficial. However, Broom and Johnson (1993) also recognise that some of what is wished for is not necessary, in the sense of essential for life, that is, 'fundamental to the biology of the animal'. These secondary requirements therefore could also be considered 'wants' or 'wishes', since they are preferred or sought but are not fundamental to the animal's biology.

Fundamental and secondary requirements can be identified by studying whether or not failure to provide these preferred resources or requirements creates difficult or inadequate adaptation for the animal (biological dysfunction) and also by determining what resources animals prefer and in particular the strength of this preference (Dawkins, 1983).

This approach to animal welfare assessment emphasises difficulties caused by long-term stimulation such as housing and routine and regular handling and husbandry practices. Because it generally involves short-term stimulation, the single imposition of a husbandry procedure is obviously less serious to animal welfare and recovery normally occurs after a short-term biological cost. However when a husbandry procedure results in a substantial biological response with consequent adverse effects on fitness, for example mulesing and dehorning, the effects on behaviour, physiology and fitness will indicate the magnitude of the challenge to the animal. This challenge reflects the welfare consequences and such information can be used to identify alternative techniques or pain relieving procedures to reduce welfare implications of the practice on the animal.

There are some husbandry practices that are only imposed once on animals but may create long-term behavioural and physiological responses with consequent prolonged effects on fitness. Traumatic neuromas, (often considered as being indicative of chronic pain), may develop in beak stumps after trimming of domestic poultry (Breward and Gentle, 1985; Gentle, 1986; Lunam *et al.* 1996), and in the tail stumps of dairy cows after tail docking using a knife when 12-18 months old (C. Lunam and J. Barnett, unpublished data).

The framework proposed here considers welfare risks at two levels. The most serious risks are those that create biological dysfunction, while the less serious risks involve requirements (resources or behaviours) that animals show distinct preferences for but failure to obtain these (or failure to be able to display the behaviours) does not create biological dysfunction. The value of this framework is that there is likely to be reasonable consensus, both within and outside science, that the former conditions create more serious welfare risks and thus an agreed approach can be used to identify such issues. The latter risks involve another level of discussion and in the immediate term there is unlikely to be consensus on whether or not many of these situations pose a genuine risk to welfare and indeed whether or not society should eliminate such conditions from the lives of domestic animals. These latter risks concerning the animal's secondary requirements thus fall within the area of personal ethics (Rollin, 2000) in which the issues are left to the individual to resolve. For example, whether or not the individual supports or purchases products from an industry that farms animals in a particular way. In contrast, consensus social ethics (Rollin, 2000) involve those issues that have clear implications for the vast majority of the general public and are generally incorporated into laws and regulations and not left to the individual's decision. Therefore, with agreement on this approach to welfare assessment, those conditions which provide the fundamental welfare requirements of animals would be considered for incorporation into laws, regulations or codes of practice to protect the welfare of domestic animals.

#### **Examples of the value of studying maladaptation as evidence of seriously poor welfare**

Research on two factors that are likely to affect the welfare of commercial pigs, fear and space, will be considered here to demonstrate the value of studying maladaptation to assess welfare risks in animals. Research conducted in the pig industry has shown that human interactions can have surprising effects on animal welfare (see Hemsworth and Coleman, 1998 for details). While many of these interactions may appear mild and harmless to the animals, this research has shown that the frequent use of some routine behaviours by stockpeople can result in pigs becoming highly fearful of humans. Furthermore, handling studies on pigs generally indicate significant effects of fear on the growth and reproductive performance of pigs (see Hemsworth and Coleman, 1998). The mechanism responsible for the adverse effects of high fear on the productivity of pigs appears to be a chronic stress response. Handling treatments in a number of studies that resulted in high fear levels, have also produced either a sustained elevation in the basal free-cortisol concentrations or enlargement of the adrenal glands, together with depressions in growth and reproductive performance (see Hemsworth and Coleman, 1998). These extensive studies indicate that negative handling is a potent stressor for pigs, with marked effects on behaviour (fear or avoidance responses to humans) and stress physiology (both acute stress responses in the presence of humans and chronic stress responses) which in turn can markedly limit a number of fitness variables such as growth and reproduction. Similar results have been obtained with other farm animal species (see Hemsworth and Coleman, 1998).

There is clear evidence of a chronic stress response and reduced reproductive performance in gilts if space allowance is insufficient (for 1 m<sup>2</sup>/pig, Hemsworth *et al.* 1986; and less than 1 m<sup>2</sup>/pig, Barnett *et al.* 1992). Taylor *et al.* (1997) found that reducing space allowance for groups of 10 sows from 2.0 to 1.2 m<sup>2</sup>/pig increased aggression. Similarly, Weng *et al.* (1998) reported increased aggression and injuries with decreasing space allowance. These studies show that space allowance in group-housing is an important factor affecting both the behavioural and physiological responses of pigs and consequently their fitness.

These studies demonstrate the potential value of studying the effects of housing and husbandry practices on both the behavioural and physiological responses and fitness effects to assess the welfare implications of these practices. It is difficult to argue that if such practices affect the fitness of the animal through a range of long lasting behavioural and neuroendocrine responses that the welfare of the animal is not seriously at risk.

## Conclusion

In conclusion, it is proposed that welfare risks can be considered at two levels. The most serious risks are those that create biological dysfunction, while the less serious risks involve requirements (resources or behaviours) that animals show distinct preferences for but failure to obtain these (or failure to be able to display the behaviours) does not create biological dysfunction. Agreement that the conditions that create biological dysfunction are the most serious for animals will enable scientists to study and identify in an agreed and established manner those housing or husbandry practices that create biological dysfunction. While it is accepted that this may be a conservative approach, it will allow significant progress to be made in addressing the, potentially, most serious welfare issues.

There are two main contentious issues with this approach. One involves the definition of animal welfare and the other is whether assessing fundamental requirements adequately includes feelings. This approach uses the definition of animal welfare proposed by Broom (1986) and is based on the premise that maladaptation generates animal welfare problems. Adaptation is considered to involve behavioural and physiological responses that assist the individual to cope with its environmental conditions. There may be some disagreement within science on the appropriateness of this definition. For example, some argue that animal welfare only concerns animal feelings. Nevertheless, I contend that Broom's definition is more widely accepted both within science and by the wider community. Without a consensus on a definition of animal welfare there can be no consensus on the scientific assessment of animal welfare.

In relation to the issue of feelings, Broom (1998) has emphasised the evolutionary advantage of feelings. As others have proposed (Barnett and Hemsworth, 2003; Broom, 1998; Cabanac, 1979), feelings are part of the body's regulatory system and together with a range of learning processes function to either remove animals from harmful situations or attract animals to beneficial situations. Thus, an animal's attempts to cope with difficulties include an integration of feelings into its behavioural and physiological responses.

It is obvious that this proposal requires evaluation. By studying the relationship between fundamental biological requirements and preferences of animals, the validity of this can be tested. For example, finding that the resources most preferred by animals are the same resources that animals show the most severe attempts to adapt to when deprived of them, would indicate that both approaches are valuable and complementary in assessing welfare. However, those resources identified as fundamental are the most important for the animal's welfare. In contrast, the finding that there is little correspondence between the most preferred resources and those resources where deprivation creates biological dysfunction would indicate that some of what is preferred is not necessary in the sense of being fundamental to the biology of the animal. Indeed, some authors have argued that an animal's short-term choice may reflect its proximate (immediate) needs rather than the animal's ultimate needs or those needs necessary for survival, growth and reproduction (Lawrence and Illius, 1997). The latter finding would support the view that deprivation of the fundamental biological requirements create the most serious welfare problems and it is these requirements that science needs to identify promptly if the welfare of domestic animals is to be safeguarded.



## STUDYING STRESS TO ASSESS ANIMAL WELFARE

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### Abstract

This part of the symposium focuses on three aspects, what is animal welfare and how do we measure it? What is stress? And what are the welfare implications of housing systems for pigs using the preferred (homeostasis) approach to welfare assessment? With our present knowledge, the 'homeostasis' approach appears to offer science the best assessment of the welfare of animals. As a research tool, this approach involves comparing and assessing risks to welfare on the basis of relative changes in biological (behavioural and physiological) responses and corresponding decreases in fitness, that is, the ability of a pig to survive successfully, grow and/or reproduce. The hormones involved in stress responses can affect metabolic, reproductive and immunological function and depending on their severity and duration, can also adversely affect homeostasis. These disruptions to homeostasis link the stress response with welfare assessment and provide an important tool, to be used in an holistic approach, to assess risks (such as those imposed by housing systems and husbandry practices), to the welfare of animals. The physiological data on individual and group housing can be used to provide evidence of compromised welfare in all systems as well as identifying some factors that contribute to improved welfare. One reasonable interpretation of the data is that it is the design of the housing system that is important to welfare rather than the housing system *per se*. We need to better understand the myriad of factors involved in welfare issues so that we can minimise any adverse effects and maximise animal welfare.

### Introduction

Since Barnett and Hutson (1987) reviewed the issue of animal welfare of pigs 15 years ago, some of the original misconceptions associated with welfare issues remain while others have disappeared. For example, there now seems to be less of a tendency to ascribe any inexplicable experimental result to stress (thermal, nutritional or social) where, at one time, it seemed to be a common 'catch-all'. However, a typical misconception still remaining is that total cortisol concentrations reflect biological activity and some scientists appear to be of the opinion that an increase in cortisol concentrations is indicative of a welfare problem. Yet, during the last trimester of pregnancy, when there is no evidence of chronic stress, total cortisol and corticosterone concentrations markedly increase in humans (and some rodents). During this time, there is also a concomitant change in transcortin (a plasma globulin that binds cortisol/corticosterone) concentrations, but little change in free (biologically active) cortisol concentrations (see Westphal, 1971).

In this paper I attempt to answer three questions:

- What is animal welfare and how do we measure it?
- What is stress?
- What are the welfare implications of welfare assessment methodology of housing systems for pigs? This last question is used as an example of the usefulness of stress in welfare assessment.

### What is animal welfare and how do we measure it?

Readers are referred to a recent publication (Barnett and Hemsworth, 2003) that expands on the content of this section of the review. Our value systems for animals tend to operate at two levels (Broom and Johnson, 1993). Private attitudes, which may be difficult to enunciate, may be ascribed to belief, intuition, learning or experience. Communal value systems, which may arise from family, culture or some other social belief, are rarely upheld unanimously in the population, but may be supported by a sizeable proportion of it, for example bull fighting. These communal beliefs might also be reinforced by legislative control or codes of practice.

Some have argued that science and ethics cannot be separated in any discussion of animal welfare. For example, some authors have used the term 'animal welfare' to refer to an animal's quality of life (Duncan and Fraser, 1997). Furthermore, they consider that our conception of animal welfare involves values as well as information, and others consider that a conventional definition of animal welfare does little more than establish the general area of discourse (Duncan

and Dawkins, 1983). The widespread variation in science, philosophy and the general community in the definition of animal welfare has created considerable confusion and controversy and hindered attempts to study animal welfare. Without a clear definition, welfare cannot be studied because it cannot be measured either directly or indirectly.

Animal welfare has been defined as 'the state of an individual with regards to their attempt to cope with their environment' (Broom, 1986a). Using this definition, welfare risks can be assessed in terms of 1) how much has to be done by the animal to cope with the environmental imposition and 2) the extent to which the animal's coping attempts are succeeding. The rationale for this definition is considered in more detail later. Scientists should aim to provide the facts on how well animals adapt (that is the welfare risks) to a housing or husbandry practice. Such information, together with the individual's value system, will assist in deciding whether or not a particular practice that imposes on an animal's welfare is acceptable.

As indicated above, a continuing difficulty confronting scientists studying animal welfare has been the definition of animal welfare. The role of science is to provide facts on how well animals cope with their environment and includes the issues of emotions, natural behaviours in natural settings and preferences. The issue of whether or not animals require environments that provide more than those that address their 'basic' biological requirements (that is, pleasure) is another level of discussion that needs to occur. A consensus needs to be reached amongst scientists regarding the conditions that create biological dysfunction in animals so that these conditions can be promptly addressed. This will allow significant progress to be made in improving animal welfare.

While there has been, and continues to be, disagreement over what is important for the welfare of animals, this disagreement has nevertheless led to attempts to study and conceptualise animal welfare in more scientific ways. It is generally accepted that there are three broad approaches used by scientists in studying animal welfare: the 'feelings-based', the 'nature of the species' and the 'functioning-based' approaches (Duncan and Fraser, 1997). A more descriptive title for the last approach, which will be used here, is the 'homeostasis' approach. A fourth approach, the 'animal preferences' approach, is sometimes included in the feelings approach but does not necessarily provide direct information on feelings or emotions. This approach involves studying the animal's choice for resources. A fifth approach is that represented in the concept of the 'Five Freedoms' (see Webster and Nicol, 1988). This approach incorporates the concept that animals should be able to perform their 'natural' behaviours. However, this approach has the least scientific credibility because it fails to define both natural and welfare risks if such natural conditions are not provided. Until these attributes are rigorously defined for elements of the five freedoms, such an approach may be used to reflect an ethical position but are not open to scientific scrutiny.

There is no reason why animal emotions cannot be incorporated into the homeostatic approach as they would have evolved on the basis of their survival values and contribution to biological fitness. This concept of biological fitness generally applies to natural populations and refers to 'fitter' animals having a greater genetic contribution to subsequent generations (Pianka, 1974) and is based on their ability to survive, grow and reproduce successfully. While the last attribute may not always apply to individual farm animals (since their reproduction is either controlled or absent), their ability to grow, survive and reproduce could be considered measures of 'fitness' within the limits of the management system. Most production systems in agriculture have breeding and growing components and these can generate considerable data on reproductive success of individuals. For example, conception rates and mortality, morbidity and growth of offspring can be used as a measure of 'fitness'. Similarly, Beilharz and Seeb (1981) and Beilharz (1982) have linked reproductive performance of domestic species with welfare.

An attribute of the 'homeostasis' approach that provides it with credibility within scientific circles is that it contains some widely accepted criteria of poor welfare such as health, immunology, injuries, growth rate and nitrogen balance. There are some excellent examples of the value of this homeostasis approach in assessing animal welfare (Hemsworth and Coleman, 1998) including the handling studies on pigs that have shown fearful pigs to have a sustained elevation of plasma-free corticosteroid concentrations (Hemsworth and Barnett 1991; Hemsworth *et al.* 1981, 1986a). The consequences of this chronic stress response in these fearful animals included

depressions in growth and reproductive performance (Hemsworth and Barnett, 1991; Hemsworth *et al.* 1981, 1986a).

A counter argument is that our current knowledge may not allow detection of other more-subtle or less-serious risks to welfare. Nevertheless, less serious challenges should be reflected in biological changes, admittedly of lower magnitude, with consequent effects on fitness variables such as growth, reproduction, injury and health. Short-term challenges can also be studied with this approach. Hemsworth *et al.* (1996) used behavioural and physiological responses together with growth performance to assess the welfare implications of the husbandry procedure of daily injections on pigs.

#### *Conclusions on welfare assessment*

With our present knowledge, the 'homeostasis' approach appears to offer science the best assessment of the welfare of animals. As a research tool, this approach involves comparing housing systems or husbandry procedures. Using this approach, risks to welfare are assessed on the basis of relative changes in biological responses (behavioural and physiological) and corresponding decreases in fitness. Assessing motivation using preference testing has the potential to measure the animal's important underlying needs, and thus provides a valuable addition to the homeostasis approach in studying animal welfare.

While there is a wide acceptance of the scientific method in problem solving, its ability to contribute to our understanding of the factors that contribute to welfare is sometimes questioned if the data do not 'fit' with an individual's perceptions. It would be unfortunate, in relation to improving animal welfare, if agreement could not be reached on a single definition of animal welfare. There would appear to be no benefits in having a scientific definition and another that includes aspects that cannot be resolved by the scientific method. Public perceptions are not ignored in the welfare debate. Public concerns are quite rightly a significant driver in raising questions but, from a scientific perspective, they are not part of the answer. With our present knowledge, the most scientifically credible approach to welfare assessment involves measuring the magnitude of the biological responses to the challenge and also the consequences of these behavioural and physiological responses on the animal's ability to grow, reproduce and remain healthy. Information on the animal's preferences for resources should provide valuable information complementing this approach and this approach is used later in this paper when examining the welfare implications of housing systems for adult pigs.

#### **What is stress?**

As noted in the previous section, an important feature of the homeostasis approach is the animal's use of the stress response to deal with challenges. However, it must be emphasised that in terms of welfare it is the consequences for the animal that are important. There is general agreement that a multi-factorial approach to welfare assessment is required and thus determining the stress response plays an important role in determining the magnitude and duration of any challenge to an animal and its consequences for fitness and welfare. A useful definition of stress is that 'stress is the sum of the non-specific responses to environmental disturbances (stressors)' (Selye, 1946). Non-specific responses refer to those responses that are independent of the nature of the stressor and thus this definition of stress always considers a whole range of responses. Admittedly, in Selye's day, the non-specific responses tended to focus on a broad range of physiological responses including those of the nervous, immunological, circulatory and hormonal systems (particularly those of the hypothalamic-pituitary-adrenal axis) whereas now, behavioural and psychological responses also fit within the conceptualised framework of Selye's General Adaptation Syndrome. Thus, while I consider it advantageous to use stress and its consequences in a multi-factorial approach to welfare assessment, there are obviously some barriers that seem to preclude its wider use and these are the issues I wish to focus on in this section of the paper. As an indicator of the usefulness, or lack of usefulness, of stress in assessing welfare, I have briefly examined the research articles in the journal *Animal Welfare* for the last 10 years (1993-2002). Using the assumption that most of the research articles were related to welfare, 30% of the papers used stress as an indicator of welfare. Half of these papers involved aspects of pig welfare. This means that 70% of papers were assessing welfare using a limited range of criteria that is, excluding stress and its consequences.

There are several good articles that give different points of view of the concept of stress and the problems of its measurement (see Barnett and Hutson, 1997; Moberg, 1985). I will draw on these in this section particularly to look at some of the issues that tend to interfere with its use as a tool in welfare assessment. For a broad overview of the stress response, readers are referred to the section subtitled 'challenges to homeostasis and their consequences for the animal' in the paper by Hemsworth (2003) in this symposium. The major barriers to adoption involve arguments over terminology, measurement techniques, acute *versus* chronic responses and psychological *versus* physical stressors.

Most of the criticism of the concept of stress arises because of confusion over terminology, particularly a definition of stress and the cavalier attitude of ascribing undefined problems to stress. Nevertheless it is important to remember that, while elevated corticosteroid concentrations may be a consistent feature of the stress response, they do not represent the totality of the response. Thus, while a sustained elevation of corticosteroid concentrations provides *prima facie* evidence of a chronic stress response, the stress response and elevated corticosteroid concentrations are not synonymous, although they are often implied to be. Additional responses include changes in other hormones, cardiovascular function, metabolism and the immune system. One reason for the focus being on cortisol/corticosterone rather than some of the other changes is the enormous body of literature on this variable. In terms of welfare then, we can use cortisol concentrations only as *prima facie* evidence of a real or actual risk to welfare. An actual risk to welfare can only be identified on the basis of the magnitude of the response and the detrimental consequences of elevated corticosteroid concentrations, or some other parameter. For example, if a particular situation results in a loss of body protein, a reduced reproductive performance, a sustained increase in metabolic rate or a suppression of the immune system, it is reasonable to suggest that the welfare of animals in that situation is at risk. Another aspect of the stress concept that has received some criticism is whether the stage of adrenal exhaustion occurs as the final part of the General Adaptation Syndrome (GAS). This doubt has arisen because death of animals can occur despite high concentrations of corticosteroids. However, it is a misconception to think of the stage of exhaustion as suggesting under activity of the adrenal cortex and consequently low concentrations of circulating corticosteroids. In Selye's account of the GAS it is the ability to resist that is exhausted and the characteristics of the stage of exhaustion are similar to the early responses of GAS and include hyperactivity of the adrenal cortex.

Two criticisms are often raised with regard to the measurement of physiological criteria. The first is deciding how large a physiological change has to be to indicate reduced welfare. I have tried to emphasise that it is the consequences of stress that are important to welfare. Thus, if animal responses, including the stress response, are such that there are adverse effects on fitness, then the change has been large enough to indicate reduced welfare. Nevertheless, because it is not currently possible to ascribe a specific change in, for example cortisol concentrations, that causes reduced welfare, there is some validity to this criticism. The data presented in the following section on housing systems for pigs indicates effects of elevated cortisol concentrations on indicators of fitness.

The second criticism in relation to the measurement of stress is due to the difficulty of obtaining blood samples with minimal disturbance to the animal and also sampling problems associated with diurnal rhythms of the parameter being measured. However, these are just technical problems and providing we have a good understanding of the diurnal patterns of hormone release in the particular species we are studying, good experimental technique can account for these variables. For example, we can sample blood remotely if necessary from behind blinds or we can use remote back-pack samplers. In pigs we know that taking samples once an hour during daylight is highly correlated with samples taken every 20 minutes over 24 hours and thus these problems just represent a challenge to the scientist and are not really a valid criticism (Barnett *et al.* 1981). Other measurement issues relate to what is being measured and an understanding of the stress response. Thus, it is important to distinguish between the concentration of total and free cortisol as the former is not related to biological activity.

A third criticism of the stress concept is the interpretation of acute stress responses that can occur in response to pleasant as well as unpleasant stimuli. For example, mating in rats (Ssechtman *et al.* 1974) and perhaps voluntary exercise in man (Sutton and Casey, 1975), could be described as

being pleasant but both result in increased corticosteroid concentrations, indicating care must be taken not to interpret all acute stress responses as signifying reduced welfare. Notwithstanding this limitation, it is possible to use the duration and intensity of the acute stress response to address specific management procedures that minimise acute stress responses. Acute stress responses are, by definition, short-term and do not generally have long-term detrimental consequences and thus they are often difficult to interpret in terms of welfare. It is also currently difficult to distinguish between acute and chronic stressors. Indeed, chronic stressors have been viewed as a series of intermittent acute stressors (Ladewig, 2000). In chronic stress there is some evidence that the sensitivity of the pituitary gland to CRH is reduced, although the release of ACTH is maintained by arginine vasopressin (Dallman, 2000). The distinction between acute and chronic stress is moot if the focus is on the consequences of the stress response rather than the mechanisms involved.

Another criticism of the concept of stress is on the relative importance of physical and psychological factors. There is no doubt that emotions are potent stimulators of the HPA axis (Mason, 1968) although Selye tended to concentrate on the more easily recognisable physical factors in his description of the stress concept. However, while it may be difficult to be certain of the precise nature of the stressor if emotions are involved, emotional factors can easily be fitted into the stress concept. As previously emphasised, it is the consequences of the stress response that are important to welfare and not the cause(s) of the response.

#### *Conclusions on stress*

While there are difficulties with the concept of stress, there is sufficient understanding of the mechanisms involved to indicate its usefulness in welfare assessment. In particular, for welfare, the focus should be on the consequences of the stress response rather than the response *per se*. It is difficult to understand how welfare can be assessed in a multi-factorial manner without proper use of the concept of stress.

#### **What are the welfare implications of housing systems for pigs using the homeostasis approach to welfare assessment?**

In this section I will use the homeostasis model of animal welfare to describe the welfare implications of one of the most contentious issues associated with pig production – the individual housing of dry sows. I will also focus on the more common housing systems for which there is a considerable body of literature, namely stall and conventional (indoor) group housing. This is not to ignore more recent innovations such as deep litter and large-group systems for weaner, grower/finisher pigs and dry sows. However, there are relatively few data, particularly physiological, on such systems. Hopefully this will change over the coming years.

There has been community concern over a number of years for the welfare of pregnant sows confined in stalls. The two major types of stalls are: 1) tether stalls (tethers) where pigs are housed in a partial stall and attached by the neck, or less frequently by the girth, to the stall by a moulded collar and chain, and 2) cage/individual stalls (stalls) which are small rectangular pens/cages that house individual pigs. Tethers are generally being phased out and are little used in Australia. In the Netherlands about 20% of pregnant sows are housed in tethers, although they are to be phased out by 2006 (Anonymous, 1999). A feature frequently associated with individual housing is stereotypies; these behaviours, defined as repetitive and relatively invariant sequences of movement which have no obvious function, are more common in both tether and stall-housed sows (Broom, 1983; Wiepkema, 1983; Arellano *et al.* 1992) than in groups. However, for individually-housed sows it has been shown that nutrition is a major contributor to stereotypies (Lawrence and Terlouw, 1993) and group housed sows engage in oral-nasal activities for a similar amount of their time budgets (Dailey and McGlone, 1997), whether housed indoors or outdoors. Nevertheless, current scientific thinking is that irrespective of the function of stereotypies, the existence of a stereotypy is indicative of a past problem for the animal in coping with its conditions (see reviews by Mason, 1991; Rushen, 1993). Stereotypies that result in physical damage to, or illness in, the animal have obvious and immediate implications for the welfare of farm animals. An example in pigs is the lesions that can develop in stall-housed sows who persistently rub their tail roots from side to side against stall fittings (Ewbank, 1978). Thus, while stereotypies should not be used alone, they can be used together with other biological responses and their consequent effects on biological fitness, to assess risks to animal welfare.

### *Individual housing*

A common housing system for pregnant pigs are stalls that, like tethers, were introduced predominantly to control feed-intake and reduce aggression. Limited survey data suggests that 26% of sows are stall-housed in Australia for most of their reproductive cycles (except for farrowing in crates and a period of group housing around mating). In addition, up to 62% may be in stalls for part of their reproductive cycle (that is, in stalls for a restricted time followed by group housing, farrowing in crates and group housing around mating) (Paterson *et al.* 1997). The majority of sows are also housed in groups for about one week after weaning, for the purpose of being re-mated. In The Netherlands about 75% of pregnant pigs are housed in stalls. Community concern still exists for this practice (Anonymous, 1992; Baker, 1996).

Early work with stalls indicated that there was no physiological evidence that stalls (of certain designs) were associated with a risk to the welfare of pregnant pigs. This contrasted with other studies that showed evidence of a chronic stress response in pigs housed in tethers and consequential effects on fitness indicators for reduced welfare. These fitness indicators included a change in nitrogen metabolism indicative of gluconeogenesis and a metabolic cost, an increased metabolic rate, immunosuppression and a reduced reproductive performance (see Barnett *et al.* 2001 for details). Barnett *et al.* (1989) found that compared to pigs housed in groups, pigs housed in stalls with either vertical bars or wire mesh on the front section of the stall divisions, had a moderate but significant increase in basal free-cortisol concentrations. However, this increase was markedly less than that of pigs housed in tethers. Furthermore, while glucose concentrations were elevated (indicating a metabolic cost) in pigs housed in tethers, no increase was evident in pigs in the two stall treatments in either experiment. Thus, while some of these data provide *prima facie* evidence of a stress response in stalls, there was no apparent adverse consequence and thus no significant risk to welfare. In an experiment over a number of parities, Broom *et al.* (1995) concluded that, by the fourth parity, welfare was reduced in stalls compared to group-housed sows, on the basis of increased stereotypies, increased aggression and reduced bodyweight. However, they were unable to find any differences on the basis of physiological, immunological or reproductive measures. Similarly, Borell *et al.* (1992) found no differences in responsiveness to adrenocorticotrophic hormone (ACTH) between sows in stalls and groups (increased responsiveness to ACTH, is an indicator of a chronic stress response (see Hennessy *et al.* 1988; Rushen, 1991 for rationale and review of ACTH responsiveness). A comparison of differential lymphocyte counts from sows housed in stalls and groups during gestation showed no treatment effects at farrowing (Nind *et al.* 1997a) suggesting that the immune systems were not differentially activated. Sows or gilts in stalls are less responsive than group-housed pigs to external stimuli including water poured on their back, sow grunts, piglet squeals and an electronic buzzer (Broom, 1986b; Barnett, 1995), although the reasons for this reduced responsiveness are unknown. Muscle mass and bone strength were reduced in pigs housed in cage stalls over successive pregnancies (Marchant and Broom, 1996a) and joint damage was increased in individually housed compared to group-housed pigs (Fredeen and Sather, 1978). Similarly, higher resting heart rates (Marchant *et al.* 1997) and the longer time that sows take to lie down (Marchant and Broom, 1996b) in stalls compared to group-housed pigs have been interpreted as a lack of physical fitness (Marchant *et al.* 1997). However, the longer time to lie down in stalls is also considered a consequence of the spatial requirements of the sow (Baxter and Schwaller 1983). Other than the limited data on joint damage and heart rate, the majority of the physiological and immunological data suggest that pig welfare is not adversely affected by stall housing.

There is some evidence that stall design may adversely affect the welfare of pregnant pigs (Barnett *et al.* 1991). Pigs in stalls with only horizontal bars on the stall divisions showed evidence of a chronic stress response, based on a sustained elevation of basal free-cortisol concentrations, similar to that seen in tethered pigs, and active avoidance of neighbouring pigs. Pigs housed in stalls comprised of vertical bars showed cortisol concentrations similar to group-housed pigs (and lower than pigs in both tethers and stalls with horizontal bars). Surprisingly these latter stall-housed pigs had high levels of aggressive interactions with their neighbours.

Some recent design innovations have resulted in a stall that allows pigs to turn around (McFarlane *et al.* 1988; Johnson *et al.* 1990). One commercial type of turn-around-stall, the Moorcomfort<sup>®</sup> gestation stall, has side panels that are hinged at about 60 cm from the front creating

a swing partition between the rear two-thirds of adjacent stalls allowing neighbouring pigs to 'borrow' space from each other and thus turn-around. A small experiment of this type of stall showed that cortisol concentrations were similar to group-housed pigs (Barnett and Taylor, 1995).

#### Group housing

Indoor group housing is a common housing system for pregnant pigs. While some attention has been given to factors such as space allowance and group size (Jensen *et al.* 1970; Ford and Teague, 1978; Kuhlert *et al.* 1985; Barnett *et al.* 1986; Hemsworth *et al.* 1986b), less consideration has been given to other factors such as social contact, dominance order and design features in pens that may affect welfare. A common criticism of individual housing systems for pigs is that social contact is disrupted. However, the effects of social rank on reproductive success of group-housed sows indicate potential problems for certain animals. For example Mendl *et al.* (1992), reported that socially intermediate pigs had higher concentrations of salivary cortisol, were more responsive to an ACTH challenge (indicating a chronic stress response) and had lighter piglets. Social rank during pregnancy can also affect maternal behaviour with subordinate sows subsequently displaying more stereotypies, increased restlessness and more interrupted suckling bouts than dominant sows after farrowing (Csermely and Nicosia, 1991). Similarly, Nicholson *et al.* (1993) reported that, compared with dominant and submissive sows in the same group, socially intermediate sows showed specific signs of stress (elevated cortisol and reduced natural T killer-cell activity) had lower farrowing rates and smaller litters.

Recommendations for space requirements for adult pigs are few, probably based on current practice, and are in the range of 1.4-1.8 m<sup>2</sup>/pig (Cale, 1979; Anonymous, 1998a, b). There is clear evidence of a chronic stress response and reduced reproductive performance (a fitness indicator) if space allowance is insufficient (for example, 1 m<sup>2</sup>/pig, Hemsworth *et al.* 1986b; < 1 m<sup>2</sup>/pig, Barnett *et al.* 1992). While the former experiment indicated that there may be advantages to reproductive performance of housing at 3 m<sup>2</sup>/pig than at 2 m<sup>2</sup>/pig, the physiological criteria indicated no differences between these space allocations. None of the recommendations take into account the amount of additional 'free space' available to pigs kept in large groups and the potential to reduce space allocation per pig in such group pens. This aspect warrants research. Some limited research by Taylor *et al.* (1997) showed that varying group sizes of 5, 10, 20 and 40 sows with a space allowance of 2 m<sup>2</sup>/sow, had no effects on reproductive performance (proportion of sows that farrowed, piglets born/sow and piglets born/sow alive, stillborn or mummified). Although aggression, which was measured on days one and two after grouping, increased as group size increased, the number of lesions, measured on days 5 and 53, were similar across treatments. In the same experiment, reducing space allowance for groups of 10 sows from 2.0 to 1.2 m<sup>2</sup>/sow, increased aggression. Similarly, Olsson *et al.* (1994) reported increased injuries as group size increased and Weng *et al.* (1998) reported increased aggression and injuries with decreasing space allowance. The latter experiment recommended a space allowance between 2.4 and 3.6 m<sup>2</sup>/sow for groups of six pregnant sows. The latter experiment also emphasised that the results could not be extrapolated to other group sizes and space allowances.

There are no recommendations on group size for adult pigs in the Codes of Practice relating to welfare (Anonymous, 1998a, b). Nevertheless, this management factor varies widely in commercial practice and may affect both welfare and sexual behaviour. Studies by Barnett *et al.* (1984, 1986), housing sexually mature gilts in pairs, resulted in a chronic stress response compared to housing in groups of 4-8. Both large group size (24 vs 8 pigs) and small group size (3 vs 9, 17 or 27 pigs) may have detrimental effects on oestrus expression (Christenson and Ford, 1979; Christenson and Hruska, 1984). Increasing group size and concomitantly decreasing space allowance may have detrimental effects on oestrous expression (Cronin *et al.* 1983). Broom *et al.* (1995), compared sows in groups of five fed in stalls and a group of 38 sows fed via an electronic feeding station. While there was increased aggression in the larger group, particularly after initial mixing, any differences in aggression and stereotypies had disappeared by the fourth parity. Further research is required to determine the optimum group size for pregnant pigs. There are no data on space allowance/group size interactions for adult pigs.

Aggression among recently grouped and unfamiliar gilts and sows is seen as a welfare disadvantage of group housing. Aggression can be reduced in gilts by: i) modifying pen size and shape, ii) modifying pen design, iii) pre-exposing pigs to their new pen, iv) grouping after dark or

providing feed *ad libitum*, v) using masking odours and vi) using 'mood-altering' drugs (see Barnett *et al.* 2001 for details). However, all or some of these methods may only be effective in postponing aggression rather than reducing it. There are few rigorous recommendations and this subject needs further research.

The evidence that reduced space allowance can compromise welfare (Hemsworth *et al.* 1986b) suggests a potential compromise between space and pen design. Barnett (1997) compared feeding in full or partial stalls and feeding troughs and space allowances of 1, 1.4 and 2 m<sup>2</sup>/pig (that were additional to the area occupied by the stalls/troughs). The results showed that full stalls reduced the level of stress and aggression around feeding and that 1 m<sup>2</sup>/pig was inadequate on the basis of elevated cortisol concentrations and reduced immunological responses. Barnett recommended a minimum space allowance of 1.4 m<sup>2</sup>/pig in addition to the provision of full stalls for gilts weighing 117 kg. If space is a limiting factor in such situations, partial stalls appear to confer some welfare benefits. It is not known if more space is required for larger sows.

These data on group housing suggest that there are a number of factors that can adversely affect sow welfare in this housing system. These include the social status of the sow, the space allowance and group size as well as design features of the pen. The welfare consequences are evident on the basis of changes in chronic stress, reproductive and immunological responses, either during gestation and/or the subsequent lactation.

#### *Conclusions on housing systems*

The data on stress and concomitant or consequential changes in behaviour, immunology and reproduction in pigs housed individually and in groups, can be used to provide evidence of compromised welfare in all systems, depending on the factors involved. The data also help to identify some factors that contribute to improved welfare. More realistically, the data challenge some conventional thoughts on the focus of welfare-related criticism of housing systems. One reasonable interpretation of some of the data is that the design of the housing system is more important to welfare than the housing system *per se*. This can be a difficult message to 'sell' as, historically, animal welfare and animal rights campaigns have focussed on production systems. The reality is that pressure on individual housing of pigs is likely to remain a controversial issue from the view of public perception. However, the industry has always been innovative and because it sees stall housing having reproductive and welfare advantages, compromises may be reached by housing in stalls for a defined period that is considerably less than the period of gestation or by using 'turn-around' stalls. From a scientific perspective, we need to better understand the myriad of factors affecting welfare in both individual and group housing systems so that we can minimise any adverse effects and maximise the opportunities for improving welfare.

#### **Conclusions**

In this paper I have shown there are difficulties in defining welfare and stress and that there are a number of conceptual issues limiting the wider use of the stress response as an indicator of welfare. Thus, there has been a less than holistic approach to some welfare issues and this has resulted in difficulties in interpretation of data as they relate to welfare. This has led to questions on the role of science in the welfare debate. The hormones involved in the stress response can affect metabolic, reproductive and immunological function and, depending on their severity and duration can adversely affect homeostasis. It is these disruptions of homeostasis that link the stress response with welfare assessment and provide an important tool, to be used in an holistic approach, to assess the risks to the welfare of animals.

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## SYMPOSIUM CONCLUSIONS

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The contributors to this symposium generally agree on the assessment of animal welfare but their views are not universal and other scientists may disagree. However, the majority of scientists studying welfare do agree that good science in this discipline must seek to understand how animals deal with difficulties in their lives and that this requires a multidisciplinary approach.

The assessments of animal welfare put forward by Barnett and Hemsworth complement one another. The assessment of the response to physiological stress as proposed by Barnett is part of Hemsworth's discussion on the biological responses used by animals to deal with difficulties. Animals adapt to difficulties that confront them and the view presented in this symposium is that these adaptive responses are clear measures of reduced welfare.

Fisher and Hogan propose that, in addition to understanding the biological state of the animal, it is important to measure preference as an indicator of how the animal perceives its situation. This should advance our capacity to interpret welfare risks. Most importantly, the three papers highlight the need to integrate the animal's preference with its biology in the overall assessment of animal welfare.

Until scientists working on animal welfare can both broadly agree and convince others on the best methodology or methodologies to evaluate animal welfare, these two approaches, response to physiological stress and biological response to stress, should guide methodology in welfare research. While those conditions that create biological dysfunction will create serious risks for the animal's welfare, research needs to identify conditions that the animal prefers since as Fisher and Hogan note, 'we all know which part (approach) will be the most powerful argument for the audience'.

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## A PRELIMINARY STUDY OF OIL SPRAYING TO IMPROVE AIR QUALITY IN RICE-HULL BASED SHELTERS

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Previous monitoring of air quality in Australian piggeries has been 'static' (that is, in a fixed position within the animal housing). Static monitoring cannot be extrapolated to determine the exposure of livestock workers to detrimental air quality as: (1) the breathing-zone height of animals is different from humans; (2) animal-house workers spend a relatively short time in the housing, whereas static results reflect long-term exposure; and (3) workers may move between different housing. Thus, personal sampling, with equipment attached to workers' collars near the breathing zone, is a more reliable guide to human exposure. Banhazi *et al.* (1999) demonstrated that oil spraying improved air quality in pig sheds where straw was used as bedding. However, there are no data available on the effectiveness of oil spraying in pig sheds where rice-hulls are used. In this experiment we hypothesized that spraying rice-hull bedded sheds with a canola oil/water emulsion would improve air quality for pigs and humans.

Two weaner sheds were sprayed with a 15% canola oil/water emulsion at 6 L/m<sup>2</sup> floor area before pigs were moved in. Static and personal exposures (total and respirable dust, ammonia, carbon dioxide, bacteria) were measured for at least four hours per shed, one and three weeks after spraying, as described previously by (Donham *et al.* 1995).

**Table 1. Effect of oil spraying on personal and static monitoring parameters**

Sheds	Oil treatment	Weeks post-spraying	NH <sub>3</sub> (ppm)	CO <sub>2</sub> (ppm)	Total dust (mg/m <sup>3</sup> )	Respirable dust (mg/m <sup>3</sup> )	Bacteria (cfu/m <sup>3</sup> )
Personal monitoring							
2&10	No	1	1.5	1500	10.82	1.39	-
1&9	Yes	1	0	2025	8.56	0.84	-
2&10	No	3	6.75	2113	12.3	1.32	-
1&9	Yes	3	6.88	1600	10.1	1.32	-
Static monitoring							
2&10	No	1	2	1250	13.9	1.2	835 500
1&9	Yes	1	4.5	2425	7.29	0.49	557 750
2&10	No	3	11.75	1500	11.06	0.81	1 039 250
1&9	Yes	3	21.5	1750	5.19	0.44	746 250

Data were blocked for monitoring method (static and personal) and week to attain adequate replication for an analysis of variance. In week 1, the predicted mean for respirable dust (static and personal, combined) was significantly higher in the control (1.29 mg/m<sup>3</sup>) than the treated group (0.66 mg/m<sup>3</sup>) (SED=0.23, P=0.04). Concentrations of airborne bacteria were reduced post-spraying, but this was not significant (P>0.11), perhaps due to low replication. There were no other significant associations between air quality and oil treatment. Spraying an emulsion of 15% oil/water on rice-hull bedding reduced respirable dust in the short-term and slightly decreased airborne bacteria. Oil spraying may improve air quality in rice-hull bedded systems, but further work is needed to refine the method, rate and frequency of application.

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## DIET COMPOSITION OF GROWING PIGS HOUSED IN A DEEP LITTER (RICE HULLS) SYSTEM

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Information on feed intake and diet composition is fundamental to modern pig production and our ability to measure these parameters has a significant effect on the efficiency of production and the quality of carcasses produced for an increasingly discerning market. Deep litter housing systems are an attractive option for the Australian pig industry, but consumption of deep litter material (straw, rice hulls, etc.) by pigs makes it difficult to formulate diets accurately to meet pig requirements and hence optimise the nutrition of these animals. The aim of this experiment was to use a range of plant-wax alkane markers to measure the diet composition of pigs housed in a deep litter system, with rice hulls provided as the litter, and thus quantify litter ingestion.

Two hundred female pigs (commercial genotype; 22-25 kg) were allocated to two pens (100/pen) within a commercial deep litter shed, both containing rice hulls as the deep litter material. Pigs were offered a commercial grower diet that included 200 mg/kg of n-hexatriacontane (C-36) as an alkane marker. After a 7-day adaptation period, faecal samples were collected from 10 pigs in each pen on day 8. Pigs were then removed from the pens, the bedding material replenished, the pigs randomly remixed (now at 25-30 kg) and the procedure repeated (total experimental period 16 days). Alkane profiles in the rice hull, diet and faecal samples were determined by gas chromatography. Diet compositions were estimated by matching the alkane profiles of the faeces and diet components (grower diet, rice hulls) using the least-squares software package EatWhat (Dove and Moore, 1995). Estimates (Table 1) were made with and without correction of faecal alkane concentrations for the incomplete recovery of alkanes of chain length <C36 (H. Dove, unpublished data).

**Table 1. Rice hulls (% of total diet) consumed by growing pigs housed in a deep litter system.**

Pen	Collection	Rice hulls (%) in diet - uncorrected (SEM) <sup>b</sup>	Rice hulls (%) in diet - corrected <sup>a</sup> (SEM) <sup>b</sup>
1	1	4.2 (0.34)	5.5 (0.36)
1	2	8.1 (0.91)	9.5 (0.95)
2	1	4.5 (0.46)	5.8 (0.48)
2	2	8.2 (0.53)	9.7 (0.55)

<sup>a</sup>Faecal concentrations corrected for alkane recovery (C25-C36 = 0.80-0.95). <sup>b</sup>SEM, standard error of mean.

Rice-hull intake was consistent across pigs within pens and between pens. Pigs (20-25 kg) with seven days of exposure to the deep litter material consumed a diet that consisted of about 5.6% rice hulls, with consumption increasing to 9.6% after 16 days. Adjusting for incomplete faecal alkane recovery increased the estimated proportion of rice hulls in the diet, but not markedly. The results suggest that consumption of rice hulls by pigs housed in deep litter systems is significant. This fact, combined with evidence that rice hull consumption increases with time and that rice hulls contribute no energy to the overall diet (R.J. van Barneveld, unpublished data) suggests that new approaches need to be developed for the formulation of diets for pigs housed in these systems if nutritional efficiency is to be optimised. Further research is required to establish the upper limit of deep litter consumption, the consumption of other deep litter materials, and the influence of deep litter consumption on the overall utilisation of dietary nutrients.

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## AN EVALUATION OF DIFFERENT COOLING METHODS FOR GROWING PIGS IN DEEP-LITTER SHEDS

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In Australia, the production of pork from deep-litter, low-cost buildings has expanded in recent years. In most instances these buildings have uninsulated roofs and pigs can become heat-stressed even though space allowances are generally more liberal ( $>1 \text{ m}^2/\text{pig}$ ) than conventional sheds ( $0.65 \text{ m}^2/\text{pig}$ ). Our production records show that there is a seasonal decline in carcass weight for age over summer in the order of 5-10 kg. Deep-litter systems are also less efficient in feed conversion and produce fatter pigs compared to conventionally housed pigs (Sargent *et al.* 1999). In this experiment we evaluated the growth and carcass performance of grower pigs when provided with different cooling environments over summer in an uninsulated shed with rice hulls as bedding.

Pigs were housed in a steel-roofed shed with a concrete sub-floor at Corowa, NSW, from December to February. Nine hundred Large White x Landrace cross entire male pigs (100 pigs/pen,  $1 \text{ m}^2/\text{pig}$ ) were allocated by weight, at nine weeks of age ( $21.2 \pm 0.8 \text{ kg}$ ; mean  $\pm$  SE), between three treatments. A control treatment consisted of 300 mm of hulls with spray cooling set at 5-20 minutes on-off when temperature exceeded  $26^\circ\text{C}$ . Treatment 2 used minimal cover of 150 mm of hulls with more frequent spray cooling at 10-10 minutes on-off and included a shallow wallow ( $2100 \times 2000 \times 350 \text{ mm}$  depth) in each pen. This treatment was designed to reduce the build up of heat in the bedding caused by composting and to increase skin wetness of the pigs. Treatment 3 was similar to the control, except that the litter was replaced after nine weeks with fresh hulls to a depth of 300 mm to reduce heat caused by composting in the finisher phase. There were three pen replicates per treatment. Pen daily gain, feed intake and individual carcass weight and backfat P2 depth were recorded and treatment differences analysed by one-way ANOVA.

**Table 1. Mean daily gain (ADG), feed intake (ADI), feed efficiency (9-22 weeks) and carcass performance<sup>1</sup> of male pigs over summer.**

	ADG (kg/d)	ADI (kg/d)	FCR (feed:lw gain)	Carcass wt <sup>1</sup> (kg)	Carcass P2 <sup>1</sup> (mm)
Deep litter	0.803 <sup>a</sup>	1.95 <sup>a</sup>	2.43	80.4 <sup>a</sup>	11.9 <sup>a</sup>
Minimal/wallow	0.862 <sup>b</sup>	2.06 <sup>b</sup>	2.38	83.2 <sup>b</sup>	12.0 <sup>a</sup>
Deep/replaced	0.783 <sup>a</sup>	1.90 <sup>a</sup>	2.43	78.0 <sup>a</sup>	11.0 <sup>b</sup>
SEM	0.017	0.04	0.02	0.3	0.1
P value	0.011	0.029	0.524	0.001	0.001

<sup>1</sup>Analysis from individual carcasses. \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ . NS, treatment effect not significantly different. <sup>abc</sup>Mean values with different superscripts differ  $P < 0.05$ .

Growth performance and carcass weight were significantly improved in the pen environment with minimal litter and wetter pen conditions. When standardised to carcass weight, the adjusted mean P2 value ( $\pm$  SE) tended to be lower ( $P=0.136$ ) in the pens of minimal litter with wallow ( $11.6 \pm 0.18 \text{ mm}$ ) compared to the control deep-litter pens ( $12.0 \pm 0.18 \text{ mm}$ ). Efficient lean growth may be limited in deep-litter environments unless adequate cooling systems are in place.

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## LITTER TEMPERATURE CAN BE MODIFIED TO POSSIBLY REDUCE HEAT LOAD ON PIGS IN DEEP-LITTER SHEDS

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Deep-bedded litter systems have been part of mainstream Australian pig production since the early 1990s. Payne *et al.* (2000) recommended spray cooling for deep-litter systems after more than 85% of respondents stated they used normal spray cooling systems developed for conventional piggeries. We evaluated the effect of modifying the pen environment on growth and carcass performance (Smits *et al.* 2003) and also recorded surface and deep-litter temperature and litter wetness. Pens were designed with either A) deep-litter to a depth of 300 mm of fresh rice hulls at day 0 and spray cooling of 5-20 minutes on-off set at 26°C; B) minimal litter to a depth of 150 mm fresh rice hulls at day 0 and a wallow and spray cooling at 10-10 minutes on-off set at 26°C; C) used rice hulls to a depth of 300 mm that were replaced after nine weeks with fresh hulls and spray cooling at 5-20 minutes on-off set at 26°C. Litter temperature was recorded using a hand-held thermometer (Raypak®) on the surface of the litter and at a depth of 100 mm in five locations per pen that covered the lying and dunging areas. The temperatures were taken around midday when ambient temperatures were maximal. Litter samples were pooled per pen and moisture content (wetness) was determined. Data were analysed by one-way ANOVA.

**Table 1. Change in litter temperature on the surface or at depth.**

Treatment	Surface temperature (°C)			Deep temperature (°C)		
	Week 1	Week 7	Week 14	Week 1	Week 7	Week 14
A. Deep-litter	27.8 <sup>a</sup>	29.5 <sup>c</sup>	31.5	36.8 <sup>a</sup>	39.7 <sup>b</sup>	39.7 <sup>b</sup>
B. Minimal/wallow	27.0 <sup>a</sup>	26.7 <sup>a</sup>	29.8	33.9 <sup>a</sup>	31.4 <sup>a</sup>	31.2 <sup>a</sup>
C. Deep/replaced	34.2 <sup>b</sup>	28.6 <sup>b</sup>	31.3	51.1 <sup>b</sup>	37.7 <sup>b</sup>	42.7 <sup>b</sup>
SED	0.80	0.36	0.40	1.36	0.96	1.14
P value	0.001	0.003	0.185	0.001	0.001	0.001

<sup>abc</sup>Mean values within columns with different superscripts differ P<0.05.

Compared to the deep-litter pens, the surface temperature of the litter in Treatment B was significantly lower over the first seven weeks and tended to be lower at the end of 14 weeks. Temperature of the litter was related to the litter moisture content. After seven weeks, the moisture content of the litter with standard spray cooling was 29.5% and 33.3% for Treatments A and C. This was significantly lower (P<0.01) than the pens in Treatment B with high spray cooling and wallow (56.5%). After 14 weeks of occupation, moisture content was higher (P<0.05) in treatment B pens (54.6%) compared to Treatment A pens (40.7%). Replacing the litter after nine weeks tended to reduce the moisture content of the bedding in Treatment C (31.2%) but it did not lower surface-or deep-litter temperature compared to Treatment A. Altering the depth and/or moisture content of the litter can reduce bedding temperature.

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## WEANER PIGS PRODUCED OUTDOORS OUTPERFORM COUNTERPARTS PRODUCED INDOORS

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Pigs produced in outdoor farrowing systems can outperform those produced in intensive indoor systems (Gentry *et al.* 2002). In this experiment we hypothesised that outdoor pigs would experience less of a growth check at weaning regardless of the post-weaning housing system (conventional or deep-litter pens).

Two groups of 80 (n=160) female crossbred pigs were obtained at weaning (~21 days,  $5.6 \pm 1.1$  kg liveweight, mean  $\pm$  SD) from an indoor (IP) and an outdoor (OP) production system of similar health status and genetic composition. In a 2 x 2 factorial experiment, IP and OP piglets were allocated to either conventional (C) or deep-litter (DL) rearing treatments. 'C' pigs were housed in part-slatted weaner pens (10 pigs/pen, 0.4 m<sup>2</sup>/pig) equipped with a heated kennel, two nipple drinkers, and 115 mm/pig of feeding space. After seven weeks, they were transferred to another building into part-slatted grower/finisher pens (10 pigs/pen, 0.77 m<sup>2</sup>/pig) equipped with a single space feeder, two nipple drinkers, and spray cooling. 'DL' pigs were housed in deep-litter pens (10 pigs/pen, 4.6 m<sup>2</sup>/per pig) in two EcoShelters<sup>®</sup>, each containing four pens equipped with 160 mm/pig of feeder space, four drinkers, and spray cooling. After 47 days, DL pigs were moved into diagonally opposite pens, and feeders replaced to simulate environmental changes experienced by C pigs at this time. Although floor space, feeders and drinkers differed between C and DL pens, allowances liberally exceeded requirements and were not considered limiting. Pelleted feed was offered *ad libitum* in a six-diet, phase-feeding program. The phase 1 diet (6 kg/pig) contained olaquinox at 100 ppm and zinc oxide at 3000 ppm. Barley straw was added to DL pens as necessary to maintain 50% of the bedded area clean and dry. Pigs were weighed weekly and slaughtered in the week that they reached 105 kg liveweight. The experimental unit was the pen and differences between treatments tested by analysis of variance.

**Table 1. Performance of piglets from indoor (IP) and outdoor production (OP) systems reared in conventional (C) or deep-litter (DL) pens from weaning to market (5–105 kg)**

	Production (P)		Rearing (R)		SED	Significance (Pvalue)		
	IP	OP	C	DL		P	R	PxR
Gain 0-47 d (g/d)	416	467	423	460	9.13	<0.001	<0.001	0.73
Gain 0-market (g/d)	740	744	727	758	8.67	0.60	<0.001	0.52
Feed intake (g/d)	1772	1773	1711	1834	37.6	0.99	0.007	0.81
Feed:gain ratio	2.38	2.36	2.32	2.42	0.03	0.56	0.01	0.90
Carcass weight (kg)	79.2	81.0	79.1	81.0	0.24	<0.001	<0.001	0.44
Dressing %	74.6	75.8	74.4	76.0	0.24	<0.001	<0.001	0.58
Carcass P2 (mm)	13.6	14.2	14.2	13.6	0.43	0.16	0.21	0.09

Overall, growth rate was similar although OP pigs initially grew faster than IP pigs, perhaps indicating a greater tolerance of OP pigs to multiple stressors that occur at weaning (e.g. diet change). OP pigs had opportunity to ingest soil, pasture, straw, and sow feed before weaning, possibly enhancing gut development. OP pigs may also have developed beneficial foraging behaviours resulting in decreased post-weaning anorexia. DL pigs ate more and grew faster than C pigs at all stages. OP and DL pigs had higher dressing percentages but not fat levels, suggesting that environmental enrichment affected the physiological development and lean tissue deposition of OP and DL pigs.

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## THE EFFECT OF CHRONIC HIGH AMBIENT TEMPERATURE ON THE GROWTH PERFORMANCE OF GROWING PIGS

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Pigs reared in commercial enterprises may encounter multiple and concurrent stresses that have a negative impact on growth performance. In this experiment we hypothesised that chronic exposure of pigs (in otherwise ideal conditions) to a stress such as high ambient temperature, would reduce growth rate to a level equivalent to cohorts reared in commercial piggeries. The aims of this preliminary experiment were to investigate the growth performance of reference pigs reared in a negative (high temperature) and a positive (ideal) environment and compare their growth rates with pigs of the same genotype and health status in two different commercial piggeries.

The crossbred entire male pigs used in this experiment were sourced from the same producer. The reference base consisted of twenty-four weaner pigs that were allocated at weaning (21 days) to one of two temperature treatments. The pigs were individually penned in a controlled environment facility in two separate air spaces that were operated identically for the first four weeks (week 1 at 28°C, week 2 at 27°C, week 3 at 26°C, week 4 at 24°C). Thereafter, one air space was maintained at a 22°C (thermoneutral) and the other at 30°C for 20 h and 22°C for 4 h (heat stress) until about 23 weeks of age. Water (Drik-O-Mat® drinkers) and pelleted diets that met nutritional requirements were offered continuously in single space feeders. Individual liveweight, feed intake and room water usage were recorded weekly. Carcass weights and P2 backfat were measured at slaughter in the reference pigs. The growth rates of 25 focus pigs were measured on each of two commercial grow-out facilities, in a deep-litter pen of 250 pigs from start to finish on Farm A and the same on Farm B for 10 weeks and then in open-flush gutter pens of 15 pigs until sale.

**Table 1: Growth performance (mean ± SE) of pigs grown in individual pens at 22°C and 30°C and growth rates (mean ± SE) of pigs at two commercial farms.**

	22°C	30°C	Farm A	Farm B
Weight at start (kg)	6.10 ± 0.20 <sup>a</sup>	6.00 ± 0.20 <sup>a</sup>	7.00 ± 0.21 <sup>a</sup>	6.00 ± 0.18 <sup>a</sup>
Weight at finish (kg)	105 ± 2.1 <sup>a</sup>	102 ± 2.2 <sup>a</sup>	89.9 ± 1.7 <sup>b</sup>	94.9 ± 2.27 <sup>b</sup>
Days on trial (d)	112 ± 2.5	126 ± 3.5	119	119
Growth rate start to finish (g/d)	864 ± 11.8 <sup>a</sup>	765 ± 16.7 <sup>b</sup>	697 ± 2.85 <sup>c</sup>	747 ± 18.6 <sup>b</sup>
Carcass Wt (trim 13) (kg)	69.8 ± 2.1 <sup>a</sup>	68.4 ± 1.2 <sup>a</sup>		
Dressing %	66.6 ± 0.9 <sup>a</sup>	66.6 ± 0.9 <sup>a</sup>		
P2 back-fat (mm)	15.9 ± 0.8 <sup>a</sup>	13.6 ± 1.0 <sup>a</sup>		
Feed used (kg/pig/d)	1.94 ± 0.04 <sup>a</sup>	1.47 ± 0.04 <sup>b</sup>		
Feed:gain (kg/kg)	2.25 ± 0.04 <sup>a</sup>	1.91 ± 0.03 <sup>b</sup>		

<sup>abc</sup>Results in the same row with different subscripts differ (<sup>ab</sup>P<0.001; <sup>c</sup>P<0.05).

Of the reference pigs, the heat-treated pigs had lower feed intakes, higher water intake, were more feed efficient and leaner, but took longer to reach market weight than pigs reared at 22°C. These results agree with Trezona *et al.* (2002) except that we found no difference in carcass weight or dressing percentage. Chronic exposure to heat stress reduced growth performance of individually housed pigs to similar levels of pigs on Farm B, but not Farm A. This suggests that Farm A pigs may have encountered accumulated stress factors that were more significant than the chronic high ambient temperature imposed on the reference pigs.

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## A REVIEW - FAT DEPOSITION AND METABOLISM IN THE PIG

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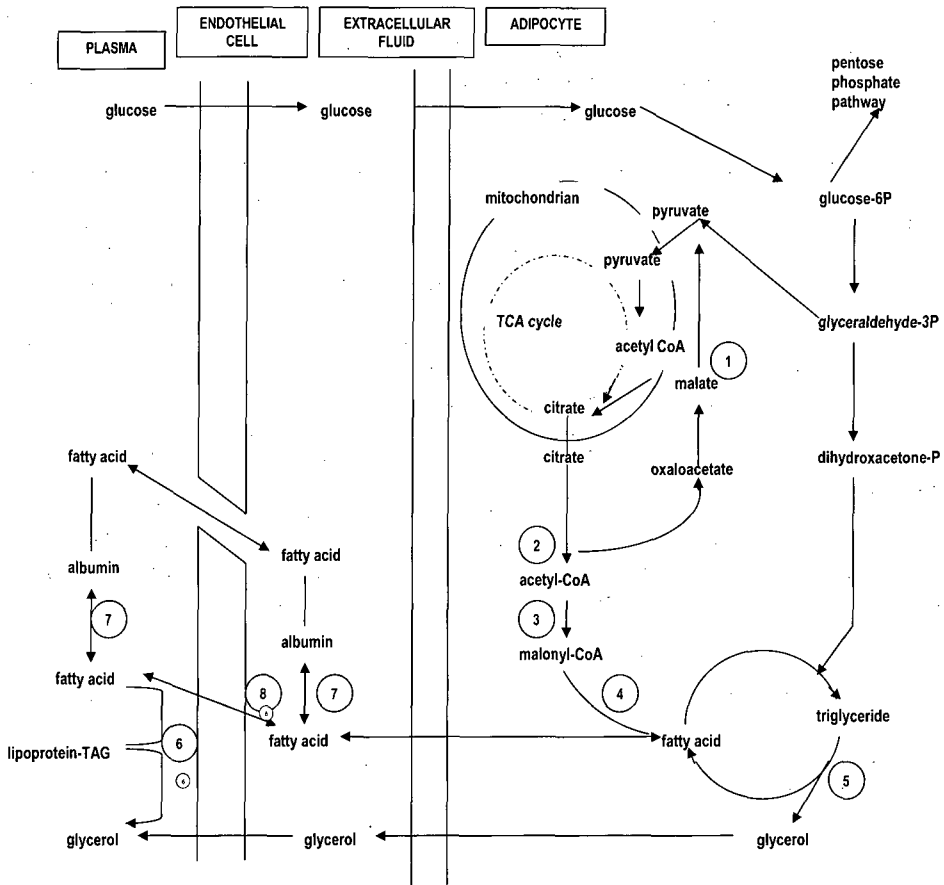
### Introduction

The pig has an enormous capacity for growth of both lean and adipose tissue. In a pig consuming a high carbohydrate diet, the major source of energy for both oxidation and storage as fat is glucose. The partitioning of glucose between oxidation and lipogenesis is influenced by a number of factors including nutrition, sex, age, liveweight, ambient temperature and genetic background. There have been significant improvements in carcass composition over the past 30 years as measured by the amount of fat that is now deposited in the carcass of the pig. However, recent observations suggest that the relationship between backfat at the P2 site, the site generally used for genetic selection, is not as strong as it once was and that current selection pressure is resulting in redistribution of fat to other parts of the body, particularly the belly. These changes in fat distribution have focused attention on the fat content of various primal cuts and the importance of producing primals with appropriate levels of fat, including intramuscular fat, for various market segments. The purpose of this review is to provide some background on the metabolic events involved in fat deposition and to understand the hormonal regulation of these pathways. The review also deals with some of the factors influencing fat deposition and some of the technologies that are available to manipulate body fat and their mechanisms of action. The reader is guided to additional reviews on fat metabolism (Mersmann, 1986), quantitative aspects of fat and carbohydrate metabolism (Danfaer, 1998) and metabolic modifiers (Dunshea and Walton, 1995) for additional information.

### Biochemical pathways associated with fat deposition

The major pathways within and adjacent to the adipocyte are shown in Figure 1 (see Mersmann, 1986). Fat is stored as triglyceride which consists of a glycerol moiety and three long-chain fatty acids. Glucose is the source of the glycerol backbone while fatty acids can be either synthesised *de novo*, again principally from glucose, or are pre-formed. In pigs, glucose is usually considered the major carbohydrate precursor for *de novo* fatty acid synthesis. Glucose is metabolised via glycolysis to pyruvate, which can then enter the mitochondrion. Pyruvate is subsequently converted to citrate via the initial stages of the tricarboxylic acid pathway. Citrate can then exit the mitochondrion where it is then cleaved to acetyl CoA and oxaloacetate by ATP-citrate lyase. Acetyl CoA is carboxylated to malonyl CoA by acetyl CoA carboxylase which is then polymerised into fatty acids by fatty acid synthetase. These latter two enzymes are often used as markers of the lipogenic state in adipose tissue (Harris *et al.* 1993). These fatty acids can then be incorporated into triglycerides. The reducing equivalents required for *de novo* fatty acid synthesis are provided by the pentose phosphate pathway and other dehydrogenases. Pre-formed fatty acids can arise from the uptake of plasma non-esterified fatty acids (NEFA) or after hydrolysis of circulating lipoprotein triglyceride by lipoprotein lipase (LPL). In addition, an intracellular source of fatty acids can arise during lipolysis, a process regulated by hormone sensitive lipase (HSL). Fatty acids arising from lipolysis can be released into the circulation or else re-esterified into triglyceride. In contrast, the lack of glycerol kinase within the adipocyte ensures the glycerol is quantitatively released into the circulation. Therefore, glycerol and NEFA entry into the plasma pool should reflect lipolysis and fat mobilisation respectively. The relative balance of the anabolic (lipogenesis) and catabolic (lipolysis) processes is largely determined by energy balance. Adipose tissue in the well-fed, rapidly growing pig is largely in a lipogenic state whereas during fasting or under stress conditions adipose tissue is in a net lipolytic state to provide energy for peripheral tissues.

Tracing carbon atoms after injecting pigs with radioactive glucose and acetate has shown that adipose tissue is the major site of *de novo* lipogenesis (O'Hea and Leveille, 1969). The preferred substrate for *de novo* lipogenesis in adipose tissue is glucose although acetate and lactate can also be incorporated into newly formed triglycerides.



**Figure 1.** Lipogenesis and lipolysis pathways in the adipocyte. 1. NADP malate dehydrogenase, 2. ATP citrate lyase, 3. Acetyl CoA carboxylase, 4. Fatty acid synthase, 5. Hormone sensitive lipase, 6. Lipoprotein lipase, 7. Fatty acid equilibration, 8. Membrane transport of fatty acids. (Mersmann, 1986; Pethick et al. 2003).

Given that pigs are generally fed a high-carbohydrate diet (Dunshea *et al.* 1992a), the mechanism for lipid synthesis is by *de novo* lipogenesis that involves the transformation of carbohydrate to fatty acids. However, under some conditions such as when pigs are fed a high fibre diet that undergoes extensive hindgut fermentation, acetate may be a significant source of carbon for *de novo* fatty acid synthesis. Also, acetate rather than glucose is the preferred substrate for hepatic lipogenesis suggesting that under these conditions the liver may make an appreciable contribution towards *de novo* lipogenesis. The major source of pre-formed fatty acids is dietary fat. Thus, pigs that are consuming a high proportion of fat in the diet these fatty acids can be extensively used for lipogenesis. Also, animals that have a genetic predisposition for a low rate of fat deposition or that have been treated with a metabolic modifier such as porcine somatotropin (pST) will have a high ratio of pre-formed to *de novo* synthesised fatty acids incorporated into adipose tissue triglycerides.

Using a novel *in vivo* approach involving infusion of radiolabelled glucose and oleic acid, Dunshea *et al.* (1992abc) constructed a carbon balance sheet for adipose tissue from control and pST (to be discussed later) treated pigs (Table 1). Focussing on the control barrows, it is apparent that *de novo* fatty acid synthesis from glucose is the predominant source of fatty acids, accounting for 74% of fatty acids deposited in adipose tissue triglycerides. Using a modification of the technique similar rates of *in vivo* lipogenesis from glucose were observed in boars and gilts by Dunshea *et al.* (1998c). It also appears from this balance sheet that most of the fatty acids liberated

during lipolysis are recycled back into adipose tissue triglycerides. Madsen *et al.* (1992) estimated that over the growth phase from 20 to 90 kg that 75% of adipose tissue lipid was synthesised *de novo*. Danfaer (1998) summarised much of the available literature on *in vivo* lipogenesis and NEFA and glycerol mobilisation and suggested that *de novo* lipogenesis accounted for 77% of triglyceride fatty acids.

*De novo* fatty acid synthesis in adipose tissues results in the formation of long-chain fatty acids, predominantly palmitic, stearic and oleic with small amounts of palmitoleic acid. The mono-unsaturated fatty acids (oleic and palmitoleic) are synthesised from their saturated precursors via a  $\Delta^9$ -desaturase and the extent of this depends on dietary and environmental factors. Oleic acid is the major unsaturated fatty acid in pig adipose tissues and originates either from the diet or through *de novo* synthesis, predominantly in adipose tissue (O'Hea and Leveille, 1969). Although diet accounts for a substantial amount of oleic acid in adipose tissues, *de novo* synthesis is still the major pathway. The last step in its synthesis involves the desaturation of stearic acid by microsomal stearoyl-CoA desaturase, a  $\Delta^9$ -desaturase. This microsomal enzyme is primarily responsible for ensuring the appropriate fluidity of cellular lipids within cells at various locations. Whilst the enzyme's major function is the desaturation of stearic acid it is also responsible for the conversion of palmitic acid to palmitoleic acid. The enzyme is closely linked with the fatty acyl synthase pathway, where newly formed substrate is more accessible than that of dietary origin. The regulation of the activity of this enzyme is considered to be significant in determining overall fat composition. Where the content of oleic is high (e.g. subcutaneous fat compared with perirenal fat) there is a concomitant higher activity of stearoyl-CoA desaturase (Buller and Enser, 1986).

**Table 1. Balance sheet for lipid accretion (grams carbon/day) in adipose tissue of a growing barrow (from Dunshea *et al.* 1992c)<sup>a</sup>**

Variable	Control	pST
Fatty acids		
<i>De novo</i> synthesis <sup>b</sup>	134	8
Synthesis from pre-formed <sup>c</sup>	49	16
Mobilised <sup>d</sup>	-36	-70
Recycled <sup>e</sup>	33	28
Glycerol		
Synthesised <sup>f</sup>	16	2
Mobilised <sup>g</sup>	-5	-9
Total	191	-25
Observed <sup>h</sup>	242	42

<sup>a</sup>Treatment involved daily intramuscular injections of porcine somatotropin (pST, 120 µg/kg bodyweight) or excipient (control). All rates were adjusted to an animal that is 80 kg bodyweight.

<sup>b</sup>Derived from estimates of the rate of fatty acid incorporation into triglycerides and the amount of chemical lipid found at slaughter after cessation of the study.

<sup>c</sup>Derived using dietary composition and feed intake values (Dunshea *et al.* 1992a) and the assumptions that 1) 90% of dietary lipid is absorbed as fatty acids, and 2) absorbed fatty acids are incorporated into adipose tissue lipid in a proportion similar to that found for labelled oleic acid at slaughter (Dunshea *et al.* 1992b).

<sup>d</sup>Based on the average plasma non-esterified fatty acid (NEFA) concentrations on day 7 of treatment (Dunshea *et al.* 1992a) and the regression equation relating plasma NEFA concentration and NEFA entry rate (Dunshea *et al.* 1992b).

<sup>e</sup>Estimates of the extent to which mobilised NEFA are reincorporated into adipose tissue lipid based on the recovery of infused labelled oleic acid found for each treatment group at slaughter (Dunshea *et al.* 1992b).

<sup>f</sup>Derived from estimates of the rate of incorporation of glycerol into triglyceride and the average chemical lipid found for each treatment group at slaughter (Dunshea *et al.* 1992c).

<sup>g</sup>Based on the average plasma glycerol concentration on day 7 of treatment (Dunshea *et al.* 1992a) and the regression equations relating plasma glycerol concentration and glycerol entry rate (Dunshea *et al.* 1992b).

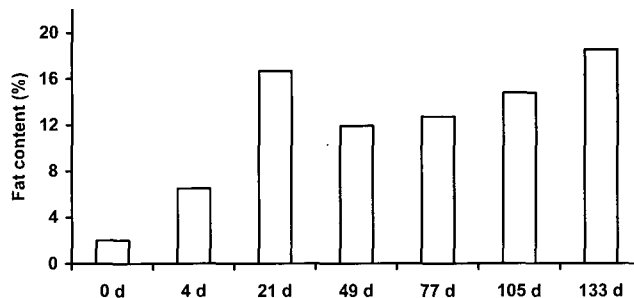
<sup>h</sup>Calculated using lipid accretion rates observed in pigs of the same genotype fed a similar diet and treated with the same dose of somatotropin over an interval beginning at 50 kg bodyweight and ending at 100 kg bodyweight (Boyd *et al.* 1986) combined with the average percentage of carbon in adipose tissue triglycerides as derived from fatty acid composition data (Dunshea *et al.* 1992c).

### Pattern of deposition

Adipose tissue, the main site for storage of fat in animals, is primarily comprised of adipocytes embedded in a connective tissue matrix with a highly developed blood capillary system. As rapidly growing animals approach 110 kg, lipids account for about 75-90% of the total wet

weight of adipose tissue and is located almost entirely inside the adipocytes. Other cells such as fibroblasts and pre-adipocytes are also present, generally in close proximity to the blood capillaries.

The neonatal pig is born with very little body fat but over the suckling period there is a dramatic increase in body fat (Figure 2; Manners and M<sup>c</sup>Crae, 1963; Lodge *et al.* 1978; Dunshea *et al.* 2003) most likely because sow's milk contains a relatively high proportion of fat. After weaning the pig changes from the high fat liquid feed to a high carbohydrate solid feed and there is a decrease in both the rate of lipogenesis (Mersmann *et al.* 1975) and the proportional fat content of the body. Once the pig adjusts to the weaning process and is consuming feed, deposition of body fat increases, as does the proportion of fat in the carcass (Dunshea *et al.* 2003). The mass of adipose tissues increases because of both hyperplasia (increase in cell number) and/or hypertrophy (increase in cell size). Cell numbers increase up until at least six months of age (Anderson and Kauffman, 1973; Hood and Allen, 1977) although the rate of fat cell synthesis decreases rapidly after about 40 days (Kirtland and Gurr, 1980). Studies on adipocyte size and volume show an increase in cell size with age (Anderson and Kauffman, 1973; Hood and Allen, 1977; Akanbi and Mersmann, 1996) although few studies have looked at adipocyte volume at bodyweights much in excess of those of commercial relevance. In general, the greatest proportion of postnatal growth in adipose tissue mass occurs through hypertrophy. However, Enser *et al.* (1976) suggested that even within subcutaneous backfat there can be regional differences in the relative importance of hyperplasia and hypertrophy. For example, the increase in backfat thickness over the shoulder between 39 and 70 kg liveweight was due to both hyperplasia (particularly in the second layer) and hypertrophy whereas in the fat over the loin the increase in backfat depth was primarily associated with hypertrophy (Enser *et al.* 1976).



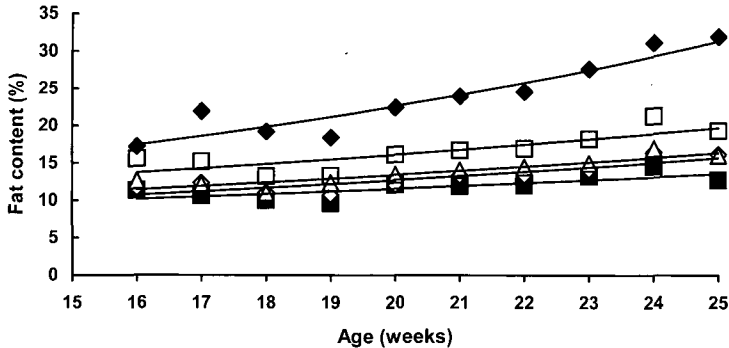
**Figure 2.** Body fat content (%) of boars at various ages (from Manners and McCrae, 1963, Auldust *et al.* 1997; Dunshea *et al.* 2003).

In the pig, adipose tissue is present on the outside of muscle tissues (e.g. subcutaneous fat), located between muscles (intermuscular fat) or between individual muscle fibres (intramuscular fat) and is also associated with organs such as kidney, heart and with the intestine (omental fat). Usually, subcutaneous fat is made up of layers that develop at a different stage of the animals' growth. There is a distinct layer of connective tissue between the generally two layers of subcutaneous adipose tissue. As slaughter weights have increased in Australia occasionally a third inner layer develops over the loin. At 28 kg the first and second backfat layers are approximately the same thickness and the adipocytes are of a similar size (Hood and Allen, 1977). As the animal grows beyond *ca.* 53 kg, the thickness of the second layer becomes larger than the outer layer. A similar pattern applies for adipocyte cell volume. Rates of lipogenesis in general tend to be greater in the second as compared to the first layers of subcutaneous backfat (Anderson *et al.* 1972; Hood and Allen, 1977; Camara *et al.* 1996). However, in one experiment, Rule *et al.* (1989) looked at triglyceride biosynthesis in preparations from various adipose tissue depots and found that triglyceride synthesis was greater in the first than in the second subcutaneous adipose tissue layer. Similarly, Budd *et al.* (1994) suggested that adipocytes isolated from the second layer of subcutaneous tissue were more responsive to hormonal manipulation of lipogenesis and lipolysis



than adipocytes isolated from the first layer. In part these differences may be related to the different stages of growth at which the studies were conducted.

The ontogeny of fat deposition in various primal cuts of the pig is of increasing commercial interest. For the past 30 years genetic selection in Australia and elsewhere has focussed on decreasing subcutaneous backfat depths at the P2 site. Initially, this genetic selection had the desired effect of decreasing subcutaneous fat over the entire body. However, recent observations suggest that the relationship between backfat at the P2 site is not as strong as it once was (D'Souza *et al.* 2002b; Suster *et al.* 2003) and that current selection pressure is resulting in redistribution of fat to other parts of the body, particularly to the belly. For example, D'Souza *et al.* (2002b) conducted an experiment to investigate the ontogeny of fat deposition in contemporary gilts and found that the fat content of the carcass, and the shoulder, loin, belly and ham primal cuts all increased from 16 to 25 weeks of age (Figure 3). The most dramatic increase in fat tissue deposition was observed in the belly primal cut. The amount of fat in the belly primal cut increased from 21 to >30% in female pigs from 16 to 25 weeks of age. The change in the fat to lean muscle ratio in the shoulder, loin and ham gradually increased from 16 to 25 weeks of age, however, the fat to lean muscle ratio in the belly primal cut dramatically increased after 19 weeks of age. Shields *et al.* (1983) investigated the carcass primal characteristics in pigs slaughtered at 54, 73, 91, 109, 127 and 145 kg, and similarly reported that the proportion of fat to lean tissue was greatest in the belly primal cut compared to the shoulder, loin and ham primal cuts. There is presently a trend in Australia to increase carcass weights to maximise production efficiencies. However, the increase in belly fat especially in the late finisher growth phase has ramifications for the current Singapore export market, which desires pork bellies as a premium primal cut. This is one example of how discerning consumers can be with the demands that they place on carcass, specifically fat composition and distribution. There are now a number of technologies that can be used in concert with genetic selection to manipulate body composition (see below).



**Figure 3.** Relationship between age and percentage of fat in the carcass (□), shoulder (■), loin (◇), belly (◆) and ham (▲) primal cuts in gilts (after D'Souza *et al.* 2003).

An experiment by Eggert *et al.* (1996) investigated the effect of genotype, sex and slaughter weight on fat distribution in four subprimal cuts (loin, ham, picnic and Boston – shoulder cuts). The results indicate that the high-lean pigs had less total fat in the subprimal cuts compared to the average-lean pigs, however, the ratio of intermuscular fat to subcutaneous fat was not affected by genotype or slaughter weight. The ratio of intermuscular fat to subcutaneous fat was greater in gilts for the Boston subprimal cut only compared to barrows.

Another adipose tissue site that is becoming increasingly important from a consumer sensory point of view is intramuscular (IM) fat. In addition to reducing fat in the whole body, genetic selection for leaner pigs has also reduced IM fat levels, and the perception is that pork is now tougher, less moist and has reduced flavour. A recent experiment conducted by D'Souza *et al.* (2002a) reported that the IM fat levels in the *Supraspinatis* muscle changed significantly (highest at

21–22 weeks of age), while IM fat levels in the *Longissimus thoracis* and *Biceps femoris* muscle of female pigs did not significantly change between 16 and 25 weeks of age. This would suggest that the rate of IM fat deposition to lean muscle tissue deposition in the *Longissimus thoracis* and *Biceps femoris* muscle in female pigs from 16 to 25 weeks of age remained constant. D'Souza *et al.* (2002a) also reported that IM levels were highest in the *Supraspinatis* muscle (shoulder), and lowest in the *Biceps femoris* muscle (ham), while the level of IM fat in the *Longissimus thoracis* muscle (loin) intermediate. Nold *et al.* (1999), compared the IM fat % in different muscles in boars, barrows and gilts, and also reported higher than average IM fat levels in shoulder muscles compared to that in the ham muscles. The higher IM fat levels in the shoulder muscles compared with the ham and loin may indicate that IM fat levels in pig muscles progressively decrease along the cranial–caudal axis. However, Davies and Pryor (1977) found no significant difference in IM fat deposition at different anatomical locations, which does not support any topographical IM fat deposition pattern in pigs.

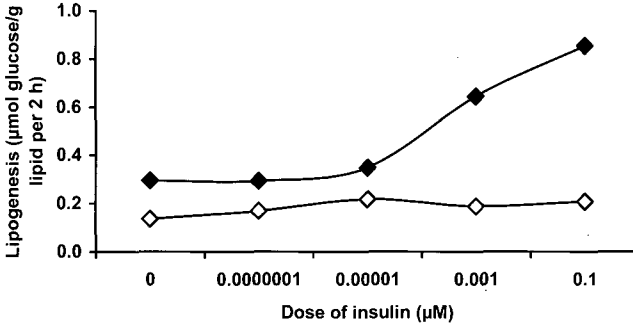
Recent studies (Channon *et al.* 2001) have shown that IM fat content in boars and gilts were as low as 1%. The use of genotypes with high Duroc bloodlines have resulted in increased IM fat and improved pork quality (Blanchard *et al.* 1999). Similarly, D'Souza *et al.* (2002a) reported that crossbred pigs with a high Duroc % had higher IM fat levels in the loin muscle and better eating quality compared to crossbred pigs with low Duroc %. Nutritional manipulation of IM fat levels in pork has been investigated in a number of studies. Cisneros *et al.* (1996) reported that feeding pigs reduced protein:energy diets increased IM fat levels in the *Longissimus thoracis* muscle. Studies by Eggert *et al.* (1998) have shown that feeding pigs diets supplemented with fat during the finisher growth phase increased IM fat levels, and improved the eating quality of pork. D'Souza *et al.* (2003) reported that feeding pigs a diet with either 15 or 30% reduction in the protein to energy ratio during the grower growth phase improved the IM fat levels in the *Longissimus thoracis* muscle from 1.3 to 2% which is near the threshold (2.5%) required for optimal eating quality. Pigs fed the 15 and 30% reduced protein to energy ratio diets had similar fat levels compared to pigs fed the control diet. However, pigs fed the 30% reduced protein to energy ratio diets had an inferior feed conversion ratio compared to pigs fed the control diet. D'Souza *et al.* (2003) also reported that feeding pigs a grower and finisher diet deficient in vitamin A improved the IM fat levels in the *Longissimus thoracis* muscle from 1.3 to 2%. It has been proposed that the effect of Vit A on IM fat deposition is mediated by retinoic acid, a derivative of Vit A, which regulates the adipogenic differentiation of fibroblasts, inhibiting the terminal differentiation of intramuscular adipose tissue in cattle (Kuri-Harcuch, 1982).

#### **Endocrine factors involved in fat metabolism**

##### *Insulin*

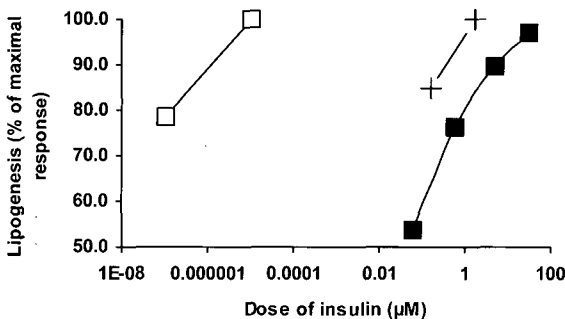
As mentioned previously, the growing/finishing pig is in a net lipogenic state with lipogenesis far outweighing lipolysis and fat mobilisation. Both lipogenesis and lipolysis are regulated by insulin. Insulin stimulates lipogenesis and inhibits lipolysis in incubated rat adipose tissue (Halperin and Denton, 1969; Saggerson and Greenbaum, 1970). Effects of insulin on porcine adipose tissue are more equivocal: most *in vitro* studies have demonstrated a small to modest stimulation of lipogenesis but little effect on lipolysis (Mersmann, 1986; Mersman and Hu, 1987; Budd *et al.* 1994). Blood glucose decreases rapidly after an injection of insulin and then returns to basal levels after about 45 minutes (Wray-Cahen *et al.* 1993; Dunshea and King, 1995; Tomas *et al.* 1997; Ostrowska *et al.* 2002). The decrease in blood glucose is due to simultaneous inhibition of hepatic glucose release and stimulation of utilisation of glucose by muscle and adipose tissue (Dunshea *et al.* 1992c). In the growing pig, Dunshea *et al.* (1992c) estimated that under basal conditions approximately 26 and 16% of glucose turnover was used by subcutaneous adipose tissue for lipogenesis and oxidation, respectively. Infusion of insulin, while maintaining blood glucose concentrations with exogenous dextrose infusion, resulted in 51 and 65% increases in the incorporation of glucose into the fatty acid and glycerol moieties of adipose triglycerides, respectively. Whole body glucose turnover was increased by 117%. The dose of insulin infused (6.0 mU/kg of BW per min) has been shown to be sufficient to maximise whole body glucose utilisation (Wray-Cahen *et al.* 1993) and so should represent the maximum rate of insulin-stimulated lipogenesis. Budd *et al.* (1994) found that insulin stimulated lipogenesis was increased by a similar magnitude (+58%) in cultured adipocytes obtained from porcine subcutaneous tissue.

However, these authors also found that adipocytes from different tissue sites have disparate rates of basal lipogenesis as well as responses to insulin (Figure 4).



**Figure 4.** Effect of dose of insulin on lipogenesis in adipose tissue from subcutaneous (◇) or omental (◆) sites (after Budd *et al.* 1994).

Thus, the data of Budd *et al.* (1994) suggest that basal and insulin-stimulated rates of lipogenesis are greater in omental adipocytes but that subcutaneous adipocytes appear to be more sensitive to insulin. The reason being the rate of lipogenesis was maximised at a lower (>100-fold lower) insulin concentration. Given that subcutaneous adipose tissue is by far the greatest adipose tissue depot, these data would suggest that under normal variations in circulating insulin, subcutaneous adipose tissue would be the major adipose tissue site of glucose utilisation. Based on whole body glucose responses to exogenous insulin infusion a number of researchers have proposed that the pig is more sensitive to insulin than the ruminant (Pethick and Dunshea, 1996). While it is always difficult to compare across studies and laboratories, collation of the data of Budd *et al.* (1994) and from studies with growing ruminants (Yang and Baldwin, 1973; Vernon, 1982) suggest that subcutaneous adipose tissue from pigs is much more sensitive to the lipogenic actions than that of ruminants (Figure 5). The effects of pST on lipogenesis are mediated by reducing insulin sensitivity (see later) and this relative difference in sensitivities between the species may partially explain why somatotropin is more effective in decreasing fat deposition in pigs than in ruminants.



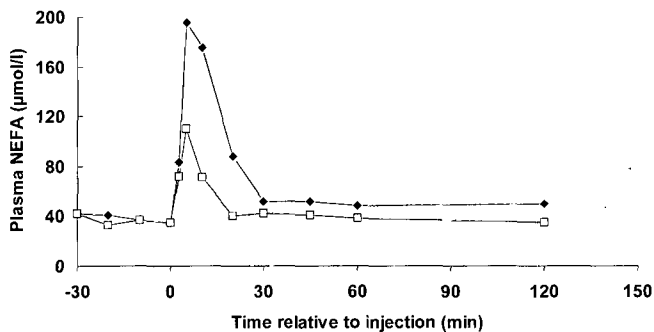
**Figure 5.** Effect of dose of insulin on lipogenesis in subcutaneous adipose tissue from pigs and ruminants. Values for incubations containing no insulin were 63, 48 and 42% of maximal for pig (□), steer (■) and sheep (+) adipose tissue, respectively (after Yang and Baldwin, 1973; Vernon, 1982; Budd *et al.* 1994).

Infusion of insulin decreases glycerol and NEFA concentrations and entry rate in ruminants and other species, particularly in underfed animals exhibiting low insulin and high NEFA and glycerol concentrations (Bergman, 1968; Petterson *et al.* 1994; Dunshea *et al.* 1995). However, adipose tissue from the rapidly growing pig exhibits very low rates of lipolysis and fat mobilisation and, under these conditions, it is very difficult to demonstrate an anti-lipolytic effect of insulin (Dunshea *et al.* 1992c; Dunshea and King, 1995; Ostrowska *et al.* 2002). Likewise, insulin had no effect on lipolysis in cultured porcine adipocytes that had been isolated from a number of tissue sites (Budd *et al.* 1994). However, effects of insulin on plasma NEFA concentrations can be seen when plasma NEFA are elevated as can occur during pST treatment or treatment with dietary conjugated fatty acid (Dunshea *et al.* 1992b; Ostrowska *et al.* 2002). The antilipolytic action of insulin is concentration dependent and evident within the physiological range in sheep (Petterson *et al.* 1994) and pigs (D. Wray-Cahen, unpublished). It appears likely that under most conditions the relatively high energy intakes and insulin secretion will ensure low rates of lipolysis and fat mobilisation, perhaps just sufficient to ensure that the metabolic pathway is maintained in case it needs to respond to adrenergic or other stimulation.

#### Adrenergic hormones

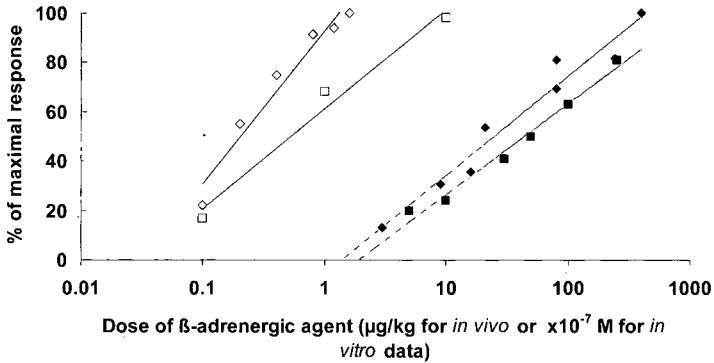
As mentioned earlier, lipolysis within the adipocyte is under the control of HSL, which catalyses the initial hydrolysis of triglyceride (Figure 1). HSL is activated by cAMP via a cascade system after initial stimulation by the membrane bound adenylate cyclase complex. Adenylate cyclase is comprised of at least three proteins: a catalytic protein, one or more hormone receptors and a nucleotide-binding protein with both stimulatory (Ns) and inhibitory (Ni) GDP binding components (Fain and Garcia-Sains, 1983; Ross and Gilman, 1990). Activation of the Ns component of adenylate cyclase by catecholamines or glucagon is very rapid and generally of short duration, with elevated lipolysis occurring only as long as cAMP levels are high.

Examples of rapid lipolytic responses include exercise, cold stress and the stress associated with negative handling. These effects are rapid in onset and duration and highlight the central role played by catecholamines in the acute control of lipolysis and fat mobilisation. An example of the rapidity with which catecholamines can increase fat mobilisation is provided in Figure 6, where the plasma NEFA response to an intravenous injection of fenoterol in control and ractopamine-treated pigs is shown (Dunshea and King, 1995). The NEFA response is rapid in onset, short in duration and, as will be discussed later, decreased in ractopamine-treated pigs. However, at a more chronic level of regulation, fat metabolism does not appear to be mediated completely via the sympathetic nervous system although there can be quite clear changes in responsiveness and sensitivity to catecholamines as the animal moves from one physiological state to another (see below). For example, the new born pig has a very low lipolytic capacity which then increases over the first two weeks postnatally before declining again by five months of age (Mersmann *et al.* 1975, 1976). For example, the sensitivity of adipocytes to adrenergic stimulation was decreased 10-fold between 10 and 75 days of age (Akanbi and Mersmann, 1996). This decline mirrors to some extent the pattern of endogenous somatotrophin secretion. Somatotrophin regulates the lipolytic responses to catecholamines (Sechen *et al.* 1990; Wray-Cahen *et al.* 1993; Boisclair *et al.* 1997).



**Figure 6.** Plasma NEFA response to a fenoterol ( $2 \mu\text{g/kg}$  of bodyweight) challenge in pigs. Treatments were either a control diet ( $\blacklozenge$ ) or a diet containing  $20 \text{ mg/kg}$  ractopamine ( $\square$ ). Values are means for challenges conducted on days 4, 10 and 24 of treatment.

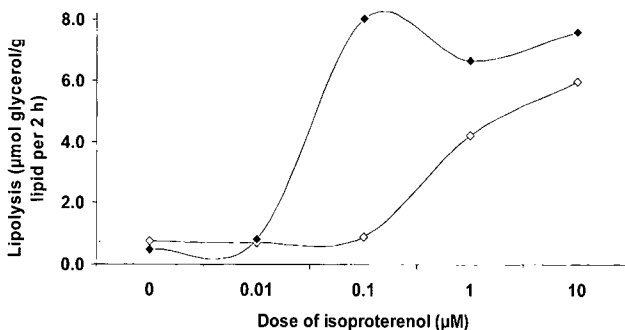
Fat mobilisation in the pig appears to be relatively insensitive to adrenergic stimulation (Pethick and Dunshea, 1996; Pethick *et al.* 2003). *In vivo* NEFA responses to epinephrine in growing pigs have suggested an effective dose that gives 50% of the maximal NEFA response ( $ED_{50}$ ) of approximately 12 to 25  $\mu\text{g}/\text{kg}$  (Dunshea *et al.* 1998b) which is much higher than any estimate of  $ED_{50}$  for synthetic  $\beta$ -agonists in ruminants (0.5-0.7  $\mu\text{g}/\text{kg}$ , Sechen *et al.* 1990; Burmeister *et al.* 1992) (Figure 7). Similar species differences were observed in cultured subcutaneous adipose tissue explants. Although there may be differences between physiological states and test  $\beta$ -adrenergic agents it does raise the possibility that dietary  $\beta$ -agonists are less efficient in reducing fat deposition in pigs than in ruminants because of their lower adipose tissue adrenergic sensitivity.



**Figure 7.** Effect of dose of *in vivo*  $\beta$ -adrenergic challenge ( $\mu\text{g}/\text{kg}$ ) on plasma NEFA responses and *in vitro*  $\beta$ -adrenergic incubation ( $\times 10^{-7}$  M) on glycerol release from adipose tissue explants. Data are for *in vivo* lactating dairy cow ( $\diamond$ ), *in vivo* lactating dairy cow ( $\square$ ), *in vivo* growing pig ( $\blacklozenge$ ) and *in vitro* growing pig ( $\blacksquare$ ). All data were generated using epinephrine with the exception of *in vivo* pig study where fenoterol, which has a similar potency to epinephrine (Mersmann, 1987), was used (from Pethick *et al.* 2003 using data of Mersmann *et al.* 1974; Sechen *et al.* 1990; Dunshea *et al.* 1998b).

However, it should be noted that there are also differences between adipose tissue from different sites. For example, Budd *et al.* (1994) found that omental adipose tissue was more sensitive (by *ca.* 100-fold) to isoproterenol than subcutaneous adipose tissue (Figure 8).

Effects of adrenergic stimulation on lipogenesis are less marked but in general there appears to be either no change or a modest reduction in the rate of lipogenesis in adipose tissue explants cultured in the presence of catecholamines (Williams *et al.* 1987; Mersmann *et al.* 1987; Budd *et al.* 1994).



**Figure 8.** Effect of dose of isoproterenol on lipolysis in adipose tissue from subcutaneous ( $\diamond$ ) or omental ( $\blacklozenge$ ) sites (after Budd *et al.* 1994).

## Factors affecting fat deposition

### Nutrition

Knowledge of the relationship between tissue deposition and energy intake is crucial to determining optimum feeding strategies for different classes of pigs. Excellent descriptions of these relationships can be found in SCA (1987). In this model, total energy deposition increases linearly with increasing energy. Energy retained as protein also increases linearly up to a maximum at an intrinsically determined energy intake, beyond which further energy has no effect on the rate of protein deposition. Fat deposition also increases linearly until the energy intake at which protein deposition is maximised where a sharp increase in the rate of fat deposition occurs. The potential impact of energy intake upon body composition is very much related to what intake, if any, for a particular pig corresponds to the energy intake at which protein deposition is maximised. Therefore, whether protein deposition continues to respond linearly up to the limit of appetite or reaches a plateau at an intermediate energy intake can have profound effects upon weight gain, body composition and feed conversion efficiency (FCR). A management practice that ensures that pigs do not deposit excessive fat is to know if and where this inflection point occurs and to feed at or around this energy intake. The inflection point will vary with genotype, sex, age and environment and whether pigs are receiving metabolic modifiers (see Dunshea, 1994; Hill *et al.* 2003 for reviews). In general, young pigs (<60 kg) of improved genotypes cannot consume enough to maximise protein deposition whereas older pigs, particularly gilts and barrows, can consume energy in excess of that required to maximise protein deposition.

The ratio of protein to energy and the amino acid balance of the protein also can have a profound effect on the rates of tissue deposition. For any level of feed intake below that which maximises protein deposition, the relationship between protein deposition and the ratio of protein to energy is best described by a linear-plateau model (SCA, 1987). In this model, protein deposition increases with increasing protein:energy until it reaches a maximum beyond which there is no further increase in protein deposition. Conversely, fat deposition decreases with increasing protein:energy until an inflection point after which there is a plateau. Clearly, feeding a diet containing a protein:energy ratio below this inflection point will result in excessive fat deposition.

Numerous studies have shown that feeding protein:energy values below the requirement will increase carcass fat content (Campbell *et al.* 1984,1985a; Dunshea *et al.* 1993b; King *et al.* 2000). The slope of the ascending portion of the relationship between protein deposition and dietary protein:energy is the efficiency with which dietary protein is deposited. This slope is determined by the digestibility of dietary protein and by how well the pattern of absorbed amino acids matches the pattern of requirements for tissue deposition and maintenance. Any imbalance in amino acids profile will lead to catabolism of the excess amino acids with the carbon skeletons from relatively minor amino acid excesses incorporated into additional fat deposition.

### Sex

There are well established differences in growth performance between boars, gilts and barrows (Campbell and Taverner 1988; Whittemore *et al.* 1988; Campbell *et al.* 1989a, 1990a; Dunshea *et al.* 1993a, 1998a, 2001; King *et al.* 2000). Boars deposit more protein and generally less fat than either barrows or gilts. Also, the maximal rates of protein deposition and the liveweight at which it occurs differ between the sexes (Whittemore *et al.* 1988; Suster *et al.* 2001a). Another important observation is that the slope of the relationship between energy intake and protein deposition (see above) is steeper for boars than for gilts or barrows (Campbell *et al.* 1985b; Campbell and Taverner, 1988; King *et al.* 1997; Dunshea *et al.* 1998a). While older data suggest that in finisher pigs (>55 kg liveweight) a plateau in protein deposition was achieved in all sex classes of pigs at an intake of around 35 MJ DE/d (Campbell *et al.* 1985b), studies conducted with genetically improved pigs suggest that the plateau occurs at higher feed intakes or, zero in the case of elite boars. (Rao and McCracken, 1992; King *et al.* 1997; Dunshea *et al.* 1998a). The practical message from these studies is that improved boars and some gilts can be fed *ad libitum* to maximise protein deposition without excessive fat deposition. This is particularly so when it is realised that feed intake is generally lower under commercial conditions than it is in the individually housed pigs that have been used for many of these studies (Black *et al.* 2001). The situation is not so clear with finisher barrows since they have a similar maximal rate of protein deposition as gilts but a higher voluntary feed intake (Dunshea *et al.* 1993a). At liveweights below

about 50 kg liveweight, improved boars and barrows have a similar potential to deposit lean tissue (Suster *et al.* 2001a) but barrows are still fatter because of their greater feed intake.

### Genetics

There have been enormous improvements in the pig industry over the last three decades and many of these can be attributed to heavy selection pressure for lean tissue and reduced fat growth. The selection pressure has most likely been on mature bodyweight although pigs are rarely grown out to mature body size to confirm this. Generally, genotypes that are heavier at maturity grow faster and contain less fat than do animals of smaller mature size. The discussion to date has already alluded to the impact of improved genetics on the effects of nutrition and sex on the relationships between protein deposition and protein and energy intake. To summarise, improved pigs deposit more protein and less fat at any particular energy intake than unimproved pigs (Campbell and Taverner, 1988). Some improved genotypes cannot consume sufficient energy to maximise protein deposition even during the finishing phase (Rao and McCracken, 1992; King *et al.* 1997; Dunshea *et al.* 1998a). As a consequence, growth performance and rate of lean tissue deposition of the improved pig is vulnerable to any factors that reduce feed intake, particularly since genetic pressure on reduced carcass fatness has indirectly meant selection pressure against feed intake. Also, as mentioned previously, one of the consequences of sustained genetic selection against backfat at the P2 site has been the recent observations that subcutaneous fat is being redistributed to other regions in the body.

### Ambient temperature

Season has been shown to have an impact on the growth and hence deposition of fat in pigs. This has mainly been due to the effect of ambient temperature on feed intake and its subsequent effect on growth and fat deposition (Close, 1978). Heat-stressed pigs decrease their feed intake to minimise their metabolic heat production, which is also accompanied by changes in their adipose tissue with an increase in abdominal fat (review of Le Dividich *et al.* 1998). Kouba *et al.* (2001) reported that heat-stressed pigs (31°C) had significantly higher flare fat compared to pigs at 20°C. Trezona *et al.* (2002b) reported that pigs fed *ad libitum* and housed at 21°C had thicker subcutaneous fat depth compared to pigs housed at 21°C and restrictively fed (feed intake determined by pig fed *ad libitum* and housed at 32°C for 20 h and 21°C for 4 h). Also, pigs fed *ad libitum* and housed at 32°C had thicker subcutaneous fat depth at the shoulder region along the midline compared to pigs housed at 21°C and restrictively fed. Hence it can be concluded that pigs grown under the same nutrition but in different thermo-environments had similar growth paths but have differences in fat deposition.

Manipulation of feed intake in pigs to simulate the effect of temperature on growth performance and fat deposition has also been investigated. Trezona (2001) reported that when the growth of pigs was manipulated through changes in feed intake, the backfat depth at the P2 site significantly changed even though total fat and lean tissue content remained similar, perhaps indicating a redistribution of fat within the carcass. A recent experiment by Trezona *et al.* (2002a) confirmed that the manipulation of feed intake to simulate the effect of temperature resulted in a redistribution of fat and lean muscle within the belly primal cut.

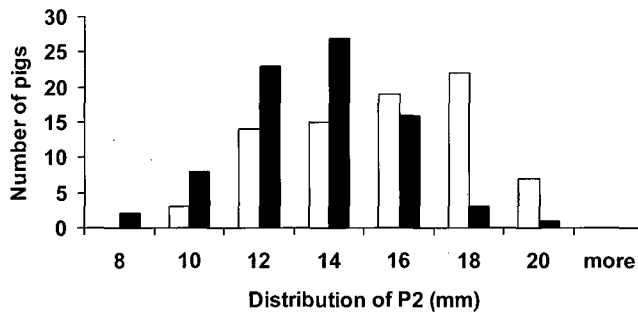
Conversely, Lefaucheur *et al.* (1991) reported that pigs grown at 12°C (cold exposure) had higher dissected total fat and subcutaneous fat and a decrease in leaf fat compared to pigs grown at 28°C. This would suggest a shift in fat deposition from internal to external depots in pigs maintained in cold environments/seasons.

### Porcine somatotropin

Somatotropin is a peptide hormone produced in the pituitary of mammals and other species. It has long been known that injection of pigs with pituitary tissue extracts containing pST increased lean tissue deposition and decreased fat accretion in growing pigs (Turman and Andrews, 1955). Advances in biotechnology have now provided a means of producing pST on a commercial scale and the efficacy of daily injection of recombinantly-derived pST for improving productive performance and reducing fat content of pigs is beyond doubt. For example, exogenous pST treatment consistently improves average daily gain, FCR and reduces fat deposition and its efficacy is unquestioned (Etherton *et al.* 1987; Campbell *et al.* 1988, 1989a, 1990ab, 1991; King *et al.* 2000; Dunshea, 2002; Dunshea *et al.* 2002a). Dose-dependent increases in lean deposition and reductions

*et al.* 1992). Porcine somatotropin is effective in increasing protein deposition and decreasing fat deposition in boars, gilts and barrows (Campbell *et al.* 1989a) of both poor and improved genotypes (Campbell *et al.* 1990a, 1991; Krick *et al.* 1992). Although the greatest responses occur in finisher pigs (60 to 120 kg), exogenous pST also improves performance in younger pigs (30-60 kg) (Campbell *et al.* 1989b, 1990b; Krick *et al.* 1993). As a result of the reduction in fat deposition, there is a consistent dose-dependent reduction in feed intake (Krick *et al.* 1992).

There has been a suggestion that pST treatment may reduce the variation of backfat under commercial conditions. This is an important consideration for producers since a reduction in variation will mean the P2 of all market pigs will be closer to the average aiding in satisfying market specifications and predicting where the majority of their animals will be on the processor's grid. A further investigation of the individual animal data from the experiment of Dunshea *et al.* (2002a) does seem to suggest a decrease in both the average and variation in P2 of heavy (*ca.* 120 kg liveweight) pigs (Figure 9). Almost three-quarters (74%) of the pigs treated with pST had a P2 backfat of 14 mm or less, while only 41% of control pigs were under 14mm. This corresponds to a reduction in average P2 of 2 mm for pST treated pigs. In addition, the variation in P2 was reduced by pST treatment (6.8 vs 4.5 mm). To extend this observation, pooled data from 16 on-farm commercial studies was examined and it was found that pST decreased the average, median and maximum P2, P2 deposition and the range in P2 (Dunshea and Trainor, 2003). Importantly, the variance in P2 was also reduced by pST treatment. The implication for producers is that more pigs will be closer to the required backfat level, and therefore more will fall into the processors required range.



**Figure 9.** Distribution of P2 backfat in control pigs (□) and pigs injected with porcine somatotrophin (pST) (■) (raw data from Dunshea *et al.* 2002a).

As mentioned previously, there is concern about the amount of fat being deposited in the belly of heavy contemporary pigs. In a recent experiment designed to investigate the effect of pST on regional fat deposition it was found that pST dramatically reduced the amount of fat in the belly of pigs (Suster *et al.* 2001b). Pigs from an improved genotype were treated with either 5 or 10 mg/d of recombinant pST for four weeks from 80 kg liveweight. Whole body fat deposition over the four week treatment period was reduced by 45 and 57% in pigs treated with 5 and 10 mg pST/d, respectively. As a consequence, body fat mass was reduced by 21 and 26% whereas belly fat mass was reduced by 24 and 46% at the same respective doses.

One of the mechanisms by which pST alters fat deposition is by altering responses to the homeostatic signals, insulin and catecholamines (see Dunshea 1993 for a review). Basal lipogenesis in adipose tissue from pigs treated with pST is decreased by up to 85%, both *in vivo* (Dunshea *et al.* 1992c) and *in vitro* (Walton *et al.* 1987; Magri *et al.* 1990; Wolverson *et al.* 1992; Harris *et al.* 1993). Consistent with these changes in lipogenesis, the activity of key lipogenic enzymes (e.g. acetyl CoA carboxylase, fatty acid synthase, malic enzyme) is similarly reduced in adipose tissue from pigs treated with pST (Magri *et al.* 1990; Wolverson *et al.* 1992; Harris *et al.* 1993). In addition to these effects on basal lipogenesis there is also evidence that pST can modify porcine adipose tissue response to insulin. The ability of insulin to stimulate lipogenesis and glucose transport is similarly reduced in adipose tissue obtained from pST-treated pigs (Walton *et*



*al.* 1986; Magri *et al.* 1990; Harris *et al.* 1993) as it is when tissue from control pigs is co-incubated with pST (Walton *et al.* 1986; Walton and Etherton, 1987). The decreased stimulation of lipogenesis by insulin is due to both decreased sensitivity and responsiveness to insulin (Walton *et al.* 1987). Many authors have reported elevated plasma glucose and insulin concentrations in pigs treated with pST (Chung *et al.* 1985; Etherton *et al.* 1986; Dunshea *et al.* 1992abc; 2002a; Dunshea, 2002; McCauley *et al.* 2003) which is indicative of *in vivo* insulin resistance. Also, pST treatment causes a reduction in the ability to clear glucose from the system in response to an insulin challenge or glucose load (Gopinath and Etherton, 1989; Wray-Cahen *et al.* 1993; Kerber *et al.* 1998). In addition, there is an augmented plasma insulin response to a glucose load (Gopinath and Etherton, 1989; Wray-Cahen *et al.* 1993). By using a hyperinsulinemic-euglycemic clamp it has been demonstrated that, at least for glucose utilisation, whole body sensitivity, but not responsiveness to insulin, is reduced during pST treatment (Dunshea *et al.* 1992bc; Wray-Cahen *et al.* 1993, 1995). Dunshea *et al.* (1992c) also demonstrated that pST treatment decreased feed intake, glucose production/utilisation and lipogenesis by 34, 35 and 85%, respectively with the reduction in glucose utilisation being completely accounted for by reduced adipose tissue lipogenesis and oxidation. Insulin infusion at a rate sufficient to maximise glucose utilisation dramatically increased lipogenesis and glucose utilisation but there were no differences in the incremental responses of the control and pST-treated pigs. Thus, the differences that existed under basal conditions were maintained during hyperinsulinemia. Dunshea (1993) suggested that the insulin resistance and resultant reduced adipose tissue lipogenesis and glucose oxidation were largely responsible for the reduction in feed intake observed in response to pST treatment.

Treatment with somatotropin also causes an increase in lipolytic response to adrenergic stimulation in pigs (Wray-Cahen *et al.* 1993) and cattle (McCutcheon and Bauman, 1986; Sechen *et al.* 1990; Boisclair *et al.* 1997). Treatment with pST generally causes a modest increase in plasma NEFA suggesting that adipose tissue fat mobilisation is increased (Wray-Cahen *et al.* 1991; Dunshea *et al.* 1992ab). Using a combination of tracer techniques and characterisation of plasma NEFA and glycerol concentrations over the day, it was estimated that fat mobilisation was doubled from 56 to 109 g/d during pST treatment (Dunshea *et al.* 1992bc). Although this is a substantial increase it is clearly insufficient to account for the difference in fat deposition that occurs during pST treatment (see Table 1). This is even more apparent when it is realised that most of the mobilised fatty acids are reincorporated into adipose tissue triglycerides (Dunshea *et al.* 1992b).

#### *β*-agonists

The *β*-agonist ractopamine (Paylean<sup>®</sup>) has recently been approved by the United States Food and Drug Administration's Centre for Veterinary Medicine for use as an in-feed ingredient to increase lean tissue growth and improve production efficiency in pigs. Treatment of pigs with *β*-agonists, particularly ractopamine (RAC), generally has given dose-dependent improvements in ADG, FCR and carcass lean content (see Dunshea and Gannon, 1995). Unlike for pST, feed intake is typically unchanged (Adeola *et al.* 1990; Gu *et al.* 1991; Yen *et al.* 1991) or decreased slightly (Adeola *et al.* 1990; Watkins *et al.* 1990; Mitchell *et al.* 1991) during *β*-agonist treatment. Other *β*-agonists which have improved performance in finisher pigs are salbutamol, cimaterol, clenbuterol, Ro 16-8714, BRL-47672 and L-644,969 (see Dunshea and Gannon, 1995) although none of these other *β*-agonists have been approved for use in pig production. While there is general agreement that protein deposition is increased during *β*-agonist treatment, effects on fat deposition have been more equivocal. For example, while RAC increased protein deposition in boars, gilts and barrows of an improved genotype by 15, 42 and 41% respectively, there was little effect on fat deposition (Dunshea *et al.* 1993a). Dunshea *et al.* (1998a) looked at the effects of sex, dietary ractopamine and dietary energy intake on tissue deposition rates and found no effect of ractopamine on fat deposition or backfat. Dunshea *et al.* (1998a) summarised the available data and concluded that ractopamine did not appear to decrease backfat measured along the midline (Aahlus *et al.* 1990; Gu *et al.* 1991; Yen *et al.* 1991; Dunshea *et al.* 1993ab, 1998a) whereas backfat depths measured off the midline have been either decreased (Aahlus *et al.* 1990; Adeola *et al.* 1990; Watkins *et al.* 1990; Yen *et al.* 1990; Gu *et al.* 1991; Dunshea *et al.* 1993b) or unchanged (Mitchell *et al.* 1991; Yen *et al.* 1991; Dunshea *et al.* 1993a, 1998a; Sains *et al.* 1993). Since ractopamine has been approved in the USA and elsewhere, additional data have become available. Schinckel *et al.* (2001) presented a summary of proprietary information from 20 studies conducted in the late 1980s to early 1990s that indicated a reduction in

backfat at the 10<sup>th</sup> rib in relatively fat animals. In a more recent experiment with leaner pigs there was a reduction in 10<sup>th</sup> rib backfat with ractopamine feeding although the response was not as great (Schinckel *et al.* 2001). However, Smits and Cadogan (2003) presented a number of recent Australian studies with ractopamine using a variety of treatment regimes in different classes of pigs and found that although lean meat yield was increased, there were no significant effects of ractopamine on backfat measured either at the P2 site or over the leg.

Initial screens for prospective  $\beta$ -agonists involved evaluation of their lipolytic activity (Veenhuisen *et al.* 1987) so it may be expected that treatment of pigs with  $\beta$ -agonists would increase lipolysis, decrease lipid synthesis and, consequently decrease fat deposition. While species and tissue specificity exists, most  $\beta$ -agonists are acutely lipolytic when administered at adequate concentrations (Mersmann, 1991). However, porcine adipose tissue does have stringent specificity requirements for stimulation of lipolysis and inhibition of lipogenesis. Ractopamine is antilipogenic and lipolytic in isolated rat adipocytes (Hausman *et al.* 1989) and porcine adipose tissue explants (Peterla and Scanes, 1990) but not in simple preparations of porcine adipocytes (Liu *et al.* 1989; Mills and Liu, 1990). However, a difficulty of interpreting data from some *in vitro* systems, is that there is an accumulation of adenosine in the medium (Honner *et al.* 1985). However, if adenosine is inactivated or its actions blocked, the lipolytic and anti-lipogenic activities of ractopamine and clenbuterol on isolated porcine adipose tissue can be demonstrated (Lui *et al.* 1989; Mills and Lui, 1990). Limited studies have been conducted with *in vitro* cultures of adipose tissue from  $\beta$ -agonist treated pigs. Basal *in vitro* lipogenesis was lower (-8%) than control values in adipose tissue from ractopamine treated pigs in one experiment (Williams *et al.* 1987) but not in another (Adeola *et al.* 1992). While basal *in vitro* lipolysis was not affected by dietary ractopamine in the latter experiment, isoproterenol stimulated lipolysis was reduced (Adeola *et al.* 1992). *In vitro* lipolysis and lipogenesis in adipose tissue from grower pigs treated with cimaterol was not different from controls (Mersmann *et al.* 1987). However, there was also no effect of cimaterol on growth or backfat in these pigs. Also, dietary RAC had no effect upon the activity of acetyl CoA carboxylase and malic enzyme or glucose-transport proteins in adipose tissue obtained from pigs fed dietary RAC for up to 24 days (Liu *et al.* 1994). However, in another experiment by the same group, dietary RAC decreased fatty acid and synthetase and malic enzyme (Mills *et al.* 1990). Finally, Dunshea *et al.* (1998c) found that although dietary ractopamine increased whole body glucose turnover there was no effect on *in vivo* lipogenesis in subcutaneous backfat of either boars or gilts.

As mentioned earlier, intravenous injection or infusion of many  $\beta$ -agonists into pigs increases fat mobilisation and lipolysis as evidenced by elevated plasma NEFA concentrations (Figure 6; Mersmann, 1987; Dunshea and King, 1995; Dunshea *et al.* 1998b; Ostrowska *et al.* 2002). Ractopamine and clenbuterol, which only exhibit lipolytic activity in porcine adipose tissue under well defined conditions *in vitro* (see above), increase plasma NEFA concentrations when infused or injected into pigs (Veenhuisen *et al.* 1987; Mersmann, 1989). However, there have been relatively few studies examining *in vivo* lipid metabolism in pigs fed  $\beta$ -agonists. Hancock and Anderson (1990) found that plasma NEFA concentrations were acutely elevated (+37%) when ractopamine was included in the diet of restrictively fed pigs. However, by day 8 of ractopamine treatment plasma NEFA concentrations had returned to pre-treatment levels. In contrast, in pigs fed close to *ad libitum* there was no significant effect of RAC on basal plasma NEFA concentrations suggesting that RAC did not stimulate lipid mobilisation or lipolysis, or if it had that these effects had disappeared by day 3 (Dunshea and King, 1994). Interestingly, there was an interaction between day of treatment and ractopamine such that basal plasma NEFA decreased with time on treatment (Dunshea and King, 1994). In another experiment with pigs fed close to *ad libitum*, Dunshea *et al.* (1998b) saw no effect of chronic (34 d) ractopamine treatment on plasma NEFA concentrations. Taken together, these data suggest that dietary ractopamine may modestly increase fat mobilisation for up to 2-3 days but that this effect quickly disappears.

A key as to why the lipolytic effect of dietary ractopamine may only last a few days was provided by Dunshea and King (1994) who found that the lipolytic response to an exogenous adrenergic challenge was inhibited in pigs fed ractopamine (Figure 6). The attenuation of response was evident 4 days after the commencement of ractopamine feeding when the lipolytic response to fenoterol was only 44% that of control gilts. On days 9 and 24 the NEFA response to fenoterol

challenge was only 25 and 34% that of the control pigs, respectively. In a subsequent experiment Dunshea *et al.* (1998b) demonstrated that dietary ractopamine treatment reduced sensitivity (as indicated by ED<sub>50</sub>) to *in vitro* challenges with both fenoterol and isoproterenol with no change in responsiveness (as indicated by R<sub>max</sub>). Dietary RAC treatment for 10 days reduced the sensitivity to fenoterol by approximately 1.5-fold whereas feeding RAC for 34 days resulted in an almost 10-fold (Expt 1) reduction in sensitivity. In support of their findings *in vivo*, Spurlock *et al.* (1993) reported that feeding RAC for 24 days reduced the density of adrenergic receptor in adipose tissue by 50% (as assessed with isoproterenol) with differences being detectable as early as 1 day after feeding RAC. Also, decreased sensitivity of  $\beta$ -adrenergic receptors with unchanged responsiveness was evident in acute co-incubations of porcine adipocytes with RAC or clenbuterol and epinephrine (Liu and Mills, 1989). In this context, dietary clenbuterol decreased *in vitro* adipose tissue  $\beta$ -adrenergic sensitivity without changing responsiveness (Mills and Orcutt 1989). Also, as mentioned previously the pig appears to be much less sensitive to the lipolytic actions of adrenergic agents than ruminants (Pethick and Dunshea, 1996; Figure 7) which is reflected in the reduced efficacy, at least with respect to reducing fat deposition, of exogenous dietary  $\beta$ -agonists in the different species. Unfortunately, a tactic adopted by unscrupulous users of illegal  $\beta$ -agonists to counteract the low  $\beta$ -agonist sensitivity of pigs and the rapid down-regulation that occurs in response to  $\beta$ -agonist exposure is to increase the dose of  $\beta$ -agonist over the latter part of the finishing period. Quite apart from the dangers to animals of feeding high levels of  $\beta$ -agonists, this practice also results in even higher levels of residues in the carcass and organs. However, it is quite possible that a controlled incremental approach with ractopamine may be one way of ensuring a more consistent and sustained response, particularly with respect to ensuring a sustained effect on fat deposition. Also, effects may be more pronounced if ractopamine is only used for a short period (<3 weeks) if an incremental program is not used.

#### *Conjugated linoleic acid*

Conjugated linoleic acid (CLA) represents a mixture of positional and geometric isomers (18:2 n-6) of linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid) with conjugated double bonds located at positions 7,9-, 8,10-, 9,11-, 10,12- or 11,13- on the carbon chain. The double bonds can be in the *cis*- (*c*) or *trans*- (*t*) configuration depending upon the special arrangement of the hydrogen atoms attached to the carbon atoms of the double bond (Parodi, 1997). Over the past decade there has been escalating interest in this group of fatty acids, most particularly because of potent antioxidative and anticarcinogenic properties attributed to one or more of these isomers. Another biological effect of CLA, particularly the *trans*-10, *cis*-12 isomer, relates to fat deposition and nutrient partitioning.

In general, inclusion of CLA in the diet has reduced backfat (Ostrowska *et al.* 1999, 2003; Thiel-Cooper *et al.* 2001; Dunshea *et al.* 2002b; Wiegand *et al.* 2001, 2002; Tischendorf *et al.* 2002) although some workers have measured no change (Ramsay *et al.* 2001; Eggert *et al.* 2001; Dunshea *et al.* 2002b). The most comprehensive experiment was conducted by Ostrowska *et al.* (1999) who used comparative slaughter techniques to determine the effects of various doses of CLA on actual rates of fat deposition. Ostrowska *et al.* (1999) found that while dietary CLA had no significant effect on average daily gain or feed intake throughout the experiment, FCR was improved by 0.2 kg/kg (6.5%) in pigs fed diets containing CLA. The carcass protein deposition response to dietary CLA was quadratic in nature with protein accretion maximised at a dietary CLA supplement of 5.0 g/kg. Carcass fat accretion decreased linearly with increasing CLA supplement rates. At the highest level of CLA there was a 30% reduction (-86 g/d) in carcass fat accretion and a 25% reduction (-6 mm) in P2 backfat. These were quite dramatic decreases, albeit the experiment was conducted in a fat genotype. Using a similar genotype, Ostrowska *et al.* (2003) confirmed these data using dual energy X-ray absorptiometry and importantly demonstrated that the effects were most pronounced over the first 4 weeks of an 8 week feeding program. Recently, a experiment was conducted with a leaner genotype of pig housed under commercial conditions to assess the effects of feeding 4.0 g/kg CLA. CLA had no significant effect upon feed intake and daily gain but the small changes in both reduced FCR (-0.10 g/g, P=0.10) (Dunshea *et al.* 2002b). While there was no significant effect of CLA on ultrasonic backfat depths, there was a significant decrease in carcass P2 (-1.0 mm, P=0.014) and estimated carcass fat (-7 g/kg, P=0.049) with responses being greater in gilts than in boars.

Ostrowska *et al.* (2002) examined the metabolite profile and acute responses to homeostatic signals in pigs fed diets containing CLA, and low or high fat, in an effort to understand the mechanism of CLA. Pigs fed the diets containing CLA had higher plasma NEFA concentration than the pigs fed diets without CLA, regardless of dietary fat content, and this response appeared to be most pronounced over the first 2 days of CLA feeding. These authors hypothesised that one of the contributing factors could be a reduced uptake of the NEFA resulting from hydrolysis of circulating triglycerides catalysed by lipoprotein lipase (LPL) at the epithelial cell surface. A reduced uptake of pre-formed fatty acids was clearly indicated by the higher levels of circulating triglycerides in pigs fed diets containing CLA, despite the tendency towards a reduced feed intake, and thus dietary fat intake, in these pigs. Likewise, others have also found a substantial, although non-significant increase (24%) in serum triglycerides in pigs fed CLA (Stangl *et al.* 1999). An increase in plasma triglyceride levels in pigs fed supplemental CLA could be an indication of reduced activity of LPL. In this context, Park *et al.* (1997) found that CLA decreased the heparin-releasable LPL activity in 3T3-L1 cultured murine adipocytes. Therefore, it is reasonable to hypothesise that a major action of CLA on fat accretion is *via* decreased lipid synthesis from pre-formed fatty acids possibly through reduced lipoprotein lipase activity. Also, dietary CLA can inhibit the activity of the  $\Delta^9$ -desaturase in adipose tissue (Smith *et al.*, 2002) which may in turn inhibit lipogenesis.

Investigations with lactating cows indicate that the major action of CLA on milk fat synthesis is a reduced rate of *de novo* synthesis (Baumgard *et al.* 2000, 2001; Chouinard *et al.* 1999). CLA was also shown to reduce the mRNA abundance of lipogenic enzymes including acetyl-CoA carboxylase and fatty acid synthetase, two key enzymes in *de novo* fatty acid synthesis, in adipose tissue of growing mice (Tsuboyama-Kasaoka *et al.* 2000) and in lactating cows (Baumgard *et al.* 2002ab). However, there was no change in enzyme activity in weaned pigs fed CLA (Bee, 2000). In the experiment by Ostrowska *et al.* (2002), dietary CLA had little effect upon plasma insulin concentrations. Furthermore, no hyperglycemic responses were evident throughout the experiment indicating that glucose production and utilisation were not markedly changed by dietary CLA. However, the ratio of insulin:glucose, which is used as a measure of insulin sensitivity in rodents and humans (Harder *et al.* 1999; Legro *et al.* 1998), tended to be increased ( $P=0.13$ ) with dietary CLA, particularly in pigs fed the low-fat diet (Ostrowska *et al.* 2002). While this may be indicative of slight insulin resistance, it was definitely nothing like that observed in rodents (DeLany and West, 2000; Tsuboyama-Kasaoka *et al.* 2000). Ostrowska *et al.* (2002) also found that plasma glucose clearance after insulin challenge was not altered by CLA treatment indicating no change in whole body response in glucose uptake. Studies in pre-diabetic Sucker fatty rats have shown only at higher concentrations of supplemental CLA isomer mix was there any improvement in glucose tolerance and insulin sensitivity (Houseknecht *et al.* 1998). It should also be noted that effects of CLA on insulin sensitivity are more likely to be observed in animals with metabolic disorders, such as in the Sucker rat, rather than in normal animals as used in experiment of Ostrowska *et al.* (2002). In this context, recent studies in lactating cows showed that abomasal infusion of *trans*-10, *cis*-12 isomer of CLA had no effect on the plasma glucose response to an insulin challenge (Baumgard *et al.* 2002a).

The small, but significant increase in plasma NEFA in the CLA-fed pigs observed by Ostrowska *et al.* (2002) could also be due to increased fat breakdown and enhanced rate of movement of fatty acids into  $\beta$ -oxidation for ATP production. The *in vitro* work with 3T3-L1 adipose cells supported the evidence that CLA potentially enhanced lipolysis and it was attributed to an increase of the carnitine palmitoyl transferase activity, both in the adipose tissue of the fed animals and in the skeletal muscle of fasted mice (Park *et al.* 1997). It is most unlikely that increased fat mobilisation explains the reduced fat deposition of 86 g/day because when concentrations and turnover of NEFA are allowed for (Dunshea *et al.* 1992b), it only accounts for about 6 g/day (Ostrowska *et al.* 1999). Hence, it is unlikely that the major component of the reduced fat deposition due to CLA supplementation is a result of increased lipolysis. In this context, there was no change in plasma NEFA in lactating cows infused with the *trans*-10, *cis*-12 CLA isomer of CLA (Baumgard *et al.* 2002a).

An additional indication of an increase in adipose tissue fat mobilisation during CLA feeding was provided by the greater increase (+126%) in plasma NEFA after an intravenous epinephrine

injection (Ostrowska *et al.* 2002). In dairy cows, infusion of *trans/cis*-10,12 CLA, the biologically active isomer that causes a marked reduction in milk fat synthesis, had little effect on NEFA concentrations (basal lipolysis) or circulating leptin concentrations. However, modest reductions (24-33%) in the NEFA response to an epinephrine challenge were observed in two investigations where cows were treated with the *trans*-10, *cis*-12 CLA isomer compared to the control and treatment with *cis*-9, *trans*-11 CLA (Baumgard *et al.* 2002a). Hence, there was no indication of increased fat mobilisation in lactating cows abomasally infused with *trans*-10, *cis*-12 CLA isomer. In the present experiment, pigs received a mixture of CLA isomers that was particularly enriched with both *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 CLA isomers which may in part explain the differences. Also, adipose tissue from lactating dairy cows is much more sensitive (10-100 fold) to catecholamines than adipose tissue from growing pigs (Figure 7, Pethick and Dunshea, 1996). Thus, although the studies used similar doses of epinephrine they would have been conducted on the ascending phase and plateau phase of the dose response curve in pigs and cows, respectively. Therefore, dietary CLA may have increased sensitivity to epinephrine in pigs (Ostrowska *et al.* 2002) while decreasing maximal responsiveness in cows (Baumgard *et al.* 2000, 2002a), two scenarios which are not mutually exclusive. Regardless, it is still unlikely that increased fat mobilisation is a major proportion of the reduction in fat deposition during dietary CLA supplementation in pigs.

#### GnRH vaccine

Another technology that has the potential to alter fat deposition in the pig is the immunocastration vaccine, Improvac<sup>®</sup>. Over recent years, the average weight of pigs at slaughter in Australia has increased being driven by the efficiencies associated with the slaughter of heavier pigs. Because boar taint increases with sexual maturity, the increase in slaughter weight has been associated with an increase in the risk of boar taint. One method of inhibiting sexual development and boar taint is immunisation against gonatotropin releasing hormone (GnRH) with Improvac, which contains a modified form of GnRH in an aqueous adjuvant system (Dunshea *et al.* 2001; McCauley *et al.* 2003; Oliver *et al.* 2003). The vaccine formulation and approved protocol allows the pigs to receive the secondary vaccine around four weeks before slaughter. Any taint substances already present are progressively metabolised, allowing the entire boar to be slaughtered at a heavier bodyweights without taint and after having benefited from the effects of its own testicular steroids on growth. Boars vaccinated with Improvac consume more feed and grow faster than either control boars or barrows of a similar liveweight. Improvac-treated boars deposited more fat and had a greater backfat-depth than boars because of their increased feed intake and a gradual decrease in steroid production over the first two weeks after the booster vaccination (Dunshea *et al.* 2001; D'Souza and Mullan, 2002; McCauley *et al.* 2003; Oliver *et al.* 2003). However, recent studies have shown that simultaneous use of pST and Improvac increased growth performance without pigs getting over fat. Another potential benefit of Improvac is that pork may be more tender since IM fat increased by 60% in boars treated with Improvac (D'Souza and Mullan, 2002).

#### Conclusion

The major site of fat deposition in the pig is in subcutaneous adipose tissue and the major source of substrate for *de novo* lipogenesis is glucose. In pigs consuming a typical high-carbohydrate diet, *de novo* lipogenesis from glucose accounts for about 75% of lipid synthesis. The remaining fatty acids arise from pre-formed fatty acids derived from either the diet or fat mobilisation. In the growing pig, lipogenesis is much greater than lipolysis and, under most conditions, the relatively high-energy intakes and insulin secretion of pigs will ensure low rates of lipolysis and fat mobilisation. The rate of fat deposition is influenced by a number of factors including nutrition, sex, age, liveweight, ambient temperature and genetic background. Genetic selection in Australia and elsewhere has focused on decreasing subcutaneous backfat depths at the P2 site with resultant decrease in fat over the entire body, including IM fat. However, recent observations suggest that the relationship between backfat at the P2 site is not as strong as it once was and that current selection pressure is redistributing fat to other parts of the body, particularly the belly. These changes in fat distribution have focused attention on the fat content of various primal cuts and the importance of producing primals with appropriate levels of fat, including IM fat, for various market segments. Fortunately, Australian pig producers have, or soon will have, access to a number of technologies such as pST, ractopamine and GnRF vaccination that will allow manipulation of carcass fat to suit various markets.

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## THE LACTOSE REQUIREMENTS OF MALE WEANER PIGS

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The fermentation of lactose, an important carbohydrate source for young pigs, plays a crucial role in the regulation of gastric pH and gut health in pigs (Yen, 2001). However, the growth response and dietary lactose requirements of piglets below 50-days of age is undefined.

In this experiment, we hypothesised that increasing dietary lactose would improve growth performance of weaner pigs. One hundred male QAF-genotype weaners, at 21 days of age, were allocated to five experimental diets containing increasing levels of whey powder (60% lactose). Diets were offered *ad libitum* and formulated to contain 15 MJ/kg DE and 0.9 g/MJ DE of available lysine. Pigs were housed in individual pens immediately after weaning and feed intake and liveweight were measured weekly over a 21-day period.

**Table 1. Effects of dietary lactose on the growth performance of weaners starting at 6.5 kg.**

	Days	Lactose g/kg					SEM	Significance	
		0	50	100	150	200		Linear	Quadratic
Wt	0-14	8.83	9.21	9.34	9.61	9.96	0.119	**	*
(kg)	0-21	10.59	11.73	11.49	12.55	12.68	0.177	**	**
ADG	0-14	167	194	203	218	247	8.28	**	*
(g/d)	0-21	195	249	238	285	294	8.29	**	**
FCR	0-14	1.36	1.13	1.15	1.08	0.99	0.094	NS	NS
	0-21	1.49	1.19	1.21	1.12	1.12	0.031	**	**
ADI	0-14	227	219	234	235	245	7.63	NS	NS
(g/d)	0-21	291	296	287	318	328	8.03	*	NS

NS, not significant, \* $P \leq 0.05$ , \*\* $P \leq 0.001$ . ADG= average daily gain; FCR=feed conversion ration; ADI=average daily intake.

Increasing the lactose content resulted in a linear ( $P \leq 0.001$ ) and quadratic ( $P \leq 0.05$ ) increase in ADG and liveweight for both 0-14 days and 0-21 days. In addition, an improvement in FCR up to 150 g/kg lactose (linear  $P \leq 0.001$ ; quadratic  $P \leq 0.001$ ) and ADI up to 200 g/kg lactose (linear  $P \leq 0.05$ ) was measured during 0-21 days. Neither a linear nor quadratic effect was observed over 0-14 days for FCR. The hypothesis was supported as maximum growth performance occurred at 200 g/kg lactose. The large growth response observed during the last seven days suggests that lactose could be a cost-effective dietary inclusion for weaner pigs older than 42 days.

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## AMINO ACID DIGESTIBILITY OF TRANSGENIC PEAS AND PARENT PEAS IS SIMILAR IN PIGS

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Pea weevil (*Bruchus pisorum*) larvae cause damage to field pea (*Pisum sativum* L.) crops by feeding on the starchy pea contents. Transgenic peas (TGP) expressing the bean (*Phaseolus vulgaris*)  $\alpha$ -amylase inhibitor 1 gene are resistant to weevil attack (Morton *et al.* 2000). The present experiment was done to determine the nutritive value of TGP for pigs.

Eighteen individually-housed boars ( $33.4 \pm 0.4$  kg) were randomly allocated to a basal diet containing 966 g/kg wheat and 34 g/kg vitamins and minerals (n=6) or the basal diet plus 500 g/kg of either parent peas (n=6) or TGP (n=6). Diets contained n-hexatriacontane (0.2 g/kg) as an indigestible marker. Pigs were offered 1.6 kg/day for 15 days, after which they were anaesthetised, the ileal and faecal digesta collected and the pigs subsequently euthanased. The dry matter (DM) and crude protein (CP) content of the parent peas and TGP were 877 and 891 g/kg and 195 and 241 g/kg air dry, respectively.

The ileal DM and starch digestibilities of the basal wheat, parent pea and TGP diets were 78.3, 74.2 and 45.8% and 95.9, 95.2 and 42.4% respectively. The apparent nutrient digestibilities of the parent peas and TGP (Table 1) were determined by difference.

**Table 1. Apparent ileal and faecal digestibilities (%) of crude protein, dry matter, starch and amino acids in parent and transgenic peas.**

	Parent Peas	Transgenic Peas	SED <sup>a</sup>	P- Value
Ileal Digestibility				
Dry matter	69.9	12.7	12.65	0.006
Crude Protein	78.5	79.7	3.38	0.74
Starch	95.0	-50.0	29.9	0.005
Amino acids				
Lysine	81.3	82.4	2.91	0.71
Threonine	68.1	71.5	3.94	0.43
Arginine	88.2	90.6	1.85	0.25
Isoleucine	73.6	76.4	3.60	0.47
Faecal digestibility				
Dry Matter	84.4	78.8	4.34	0.25
Crude Protein	77.1	75.0	4.35	0.66
Starch	99.9	100.1	0.69	0.741

<sup>a</sup>SED – Standard error of the differences of means.

The apparent amino acid and CP digestibilities of the TGP were similar to the parent pea. In contrast, the apparent ileal DM and starch digestibilities of the TGP were reduced. A component of the low apparent ileal DM and the negative starch digestibilities in the TGP are likely to be due to the inhibition of endogenous  $\alpha$ -amylase, which may have reduced the starch digestion of the wheat in the diet. The inclusion of TGP is anticipated to affect pig growth by reducing the net energy of the diet but, as amino acid digestibility is unaffected, TGP may still be a valuable protein source for pigs. The TGP may also have a role as a functional food for humans.

Supported by the Grains Research and Development Corporation and Paramounts Seeds, Ltd.

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## INFLUENCE OF THREONINE:LYSINE RATIOS ON GROWTH PERFORMANCE OF LATE FINISHING GILTS FED PAYLEAN® (RACTOPAMINE HYDROCHLORIDE)

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Baker (1997) and Cadogan *et al.* (1998) both reported that the optimal total threonine:lysine ratio for finishing swine was 70%. This ratio is higher than the ratio reported for young pigs (Chung and Baker, 1992). The higher ratio in older pigs is associated with a higher proportion of the total threonine requirement coming from endogenous amino acid losses and reflects the larger proportion of threonine in ileal amino acid secretions compared with that in body protein (Black and Davies, 1991). The aim of this experiment was to determine the optimal threonine:lysine ratio of late finishing gilts fed Paylean® (ractopamine hydrochloride), an adrenergic agonist that increases protein accretion.

Six hundred and thirty PIC gilts (average weight  $100.2 \pm 0.49$  kg), were weighed by pen and assigned to one of five dietary treatments (30 pens, 20-22 pigs per pen). Dietary treatments included four concentrations of digestible threonine (0.50, 0.55, 0.60, and 0.65%). Diets contained 0.93% true digestible lysine which, based on previous studies, should have been marginally limiting. This corresponded to true digestible threonine:lysine ratios of 54, 59, 64, and 70%, respectively. Experimental diets were based on corn-soybean meal and contained 0.30% L-lysine HCl (16% crude protein). Soybean meal was held constant and dietary threonine was increased by adding L-threonine. A high crude-protein control diet (18.2% crude protein) contained 0.10% L-lysine HCl (68% threonine:lysine). Data were analysed by ANOVA and polynomial coefficients were used to determine linearity and non-linearity of appropriate treatment means.

**Table 1. Influence of threonine:lysine ratios on the growth of late-finishing gilts fed Paylean®.**

Item	Control	Threonine:Lysine Ratio				SEM	Treatment	P-values	
		54.0	59.0	65.0	70.0			Linear	Quadratic
Day 0 BW, kg	99.4	100.4	99.9	100.5	100.1	0.62	0.72	0.81	0.91
ADG, g/d	1047	1015	1029	1063	1088	20.8	0.15	0.009	0.77
ADFI, g/d	2765	2797	2808	2821	2896	34.9	0.14	0.02	0.23
G/F, g/g	0.379	0.363	0.367	0.377	0.376	0.006	0.23	0.07	0.68
Day 21 BW, kg	121.6	121.7	121.6	122.8	122.9	0.68	0.43	0.07	0.77

Increasing the threonine:lysine ratio increased ADG and G/F linearly. There was no effect ( $P > 0.22$ ) of crude protein level on any of the growth performance traits measured. These data indicate that the optimum ratio of digestible threonine:lysine for finishing gilts fed Paylean® is at least 70%. This experiment demonstrates that low protein diets can be used when feeding Paylean® without affecting growth performance provided the diets are properly supplemented with synthetic amino acids.

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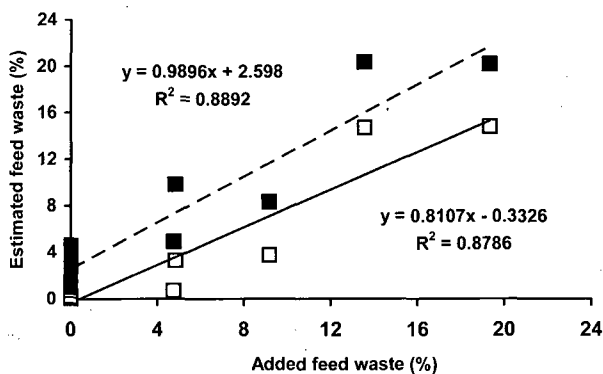
## VALIDATION OF A METHOD TO ESTIMATE FEED WASTE

F.R. Dunshea, M. Watt, D.J. Kerton, C.D. Hofmeyr and P.J. Eason

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A means of estimating feed wastage on a shed or piggery basis is required before feed wastage in the pig industry can be reduced. The aim of this experiment was to determine whether a theoretical approach to estimating feed wastage (Dunshea *et al.* 2003) could be used to estimate known feed wastage.

Fifteen, Large White x Landrace boars (initial weight  $51.9 \pm 3.70$  kg) were housed in a pen containing a 500 mm wide, self-feeder divided in half so that two pigs could feed simultaneously. Finisher feed containing 1.0% and 0.25% of acid insoluble ash (AIA) and chromic oxide was provided *ad libitum*. All effluent was collected from the pen into a pit that contained a submersible pump to mix the effluent. Effluent was collected for four days to provide basal (0% added feed waste) values after which a three-day collection was made with a known amount of feed mixed with the effluent each day. Effluent was collected for 11 periods with each period of waste-feed addition being interceded by a basal period. Feed, faeces (obtained from three pigs per period) and sub samples (3 x 150 g) of mixed effluent were used to determine dry matter, AIA and chromic oxide concentrations.



**Figure 1.** Relationship between feed waste estimated using chromic oxide (□) and acid insoluble ash (■) as markers and added feed waste.

The relationship between added feed and feed waste estimated using AIA provided an estimate of basal feed wastage of 2.6% with a slope of 0.99. In contrast, chromic oxide estimated a basal feed wastage of -0.3% with a slope of 0.81. Using the two markers, there was a strong relationship between feed wastage estimated ( $R^2=0.953$ ,  $P<0.001$ ).

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DUNSCHEA, F.R., WATT, M., KERTON, D.J. and EASON, P.J. (2003). In 'Manipulating Pig Production IX', p. 155, ed. J. Paterson. (Australasian Pig Science Association: Werribee).



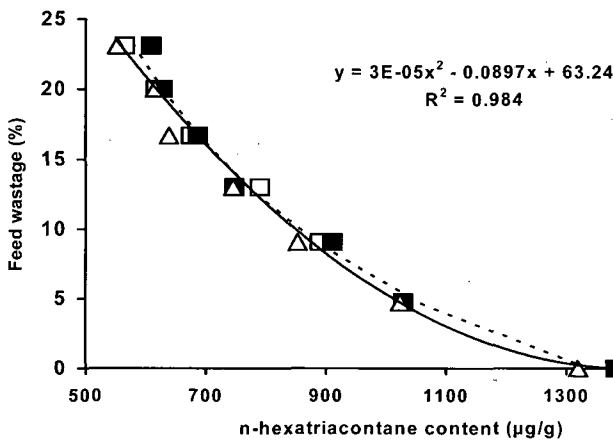
## A MARKER METHOD TO ESTIMATE FEED WASTE

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Subjective estimates of feed wastage have led to up to 30% of food being lost and not consumed. This experiment was designed to determine whether indigestible markers, that only appear in pig effluent through faeces or spilt feed, could be used to estimate feed wastage.

Six boars ( $48.6 \pm 1.21$  kg) in metabolism crates were fed 2.0 kg/day of a finisher diet containing 1.0, 0.02 and 0.25% added acid insoluble ash (AIA), n-hexatriacontane and chromic oxide. The digestibility experiment involved a four-day pre-feeding period followed by a five-day faecal collection period. Faeces ( $41.6 \pm 3.73\%$  dry matter (DM)) were mixed with water and feed to make a series of simulated effluents containing 1, 2 and 4% DM and containing 0, 5, 10, 15, 20, 25, 30% added feed. Each of the 42 simulated effluents contained 6.0 kg of faeces+water before adding the feed. The effluents were mixed in buckets using a rotor attached to an electric drill and 3 x 150 g sub samples were obtained from the mid-depth of the effluent. Samples were freeze-dried before chemical analyses.



**Figure 1.** Relationship between n-hexatriacontane content of simulated effluent with differing initial dry matter (DM) and feed wastage. □ 1% DM, ■ 2% DM, △ 4% DM, dotted line represents theoretical relationship.

AIA, chromic oxide and n-hexatriacontane provided similar estimates of DM digestibility (0.852, 0.860 and 0.850) and compared closely to that determined by total faecal collection (0.872). The relationships between n-hexatriacontane ( $R^2=0.984$ ), AIA ( $R^2=0.969$ ) and chromic oxide ( $R^2=0.972$ ) contents in the dried simulated effluents were described by quadratic equations and were all similar to the theoretical equations. Including effluent DM content in the equation only marginally improved the prediction when n-hexatriacontane was used as a marker. All three markers could be incorporated into the diet and used to predict feed wastage across a wide range of effluent DM.

## WEANER PIG PERFORMANCE IS SIMILAR WHEN FED A DIET CONTAINING EITHER MILLED OR WHOLE CANOLA SEED

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The high oil content in canola seed (about 400 g/kg) avoids the need to add feed oil or tallow to weaner diets. Weaner pigs grew at a similar rate when fed a diet containing either milled canola seed or solvent-extracted canola meal plus canola oil (Brewster *et al.* 2001). This experiment was designed to test the hypothesis that weaner pig performance would be similar when offered diets containing either no canola, milled canola or whole canola seed.

Forty-eight, male, Large White x Landrace pigs were randomly allocated at  $8.2 \pm 1.1$  kg (mean  $\pm$  SD) to three dietary treatments (n=16) for 21-days. Diets were formulated to contain 12.3 g available lysine and 15 MJ digestible energy per kg and included a triticale-based control containing no canola seed; and two identical triticale-based diets containing either hammer-milled or whole canola seed at an inclusion rate of 100 g/kg. The canola seed was analysed using near-infrared reflectance to contain 939 g/kg dry matter, 236 g/kg crude protein, 419 g/kg oil and 7 mmol/kg of glucosinolates. The pigs were housed in individual cages in one room maintained at 27°C. Each cage had an individual feed trough and nipple drinker. Food was offered fresh each day as a mash diet to maintain at least 500 g in each trough. Liveweight at the start and finish of the experiment and food intake were measured. A blood sample (8 ml) was collected by venipuncture from each pig at the end of the experiment to assess plasma tri-iodothyroxine (T3) and thyroxine (T4) concentration by radioimmunoassay.

**Table 1. Mean live performance<sup>1</sup> and plasma tri-iodothyroxine (T3) and thyroxine (T4) concentration for 48 male pigs offered triticale-based diets containing either no canola seed, milled canola seed (100 g/kg) or whole canola seed (100 g/kg) from 8 to 25 kg liveweight.**

Measurement	No canola	Milled canola	Whole canola	SEM	Significance <sup>2</sup>
Daily intake (g)	811	846	835	25.4	NS
Daily gain (g)	514	509	493	14.7	NS
Feed:gain (g/g)	1.58 <sup>a</sup>	1.66 <sup>b</sup>	1.70 <sup>b</sup>	0.028	*
T3 (ng/ml)	0.99	0.95	0.97	0.037	NS
T4 (ng/ml)	45.7 <sup>a</sup>	41.8 <sup>ab</sup>	37.6 <sup>b</sup>	2.05	*

<sup>1</sup>Mean live performance adjusted for initial liveweight. <sup>2</sup>NS, Not significant. \*P<0.05. <sup>ab</sup>Means in rows with different superscripts differ significantly.

Feed:gain was significantly (P<0.05) less when weaner pigs were offered a diet containing no canola (1.58) compared to milled canola (1.66) or whole canola (1.70) diets. There was no significant improvement in live performance from milling canola seed. However, plasma thyroxine concentration was significantly (P<0.05) reduced in pigs offered diets with whole canola seed (37.6 ng/ml) compared to no canola seed (45.7 ng/ml). These results suggest that anti-nutritional factors in canola seed reduce thyroid function and are associated with an increase in feed:gain when canola seed is included in weaner pig diets. There was no advantage in milling canola seed when included in pig diets at 100 g/kg.

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## MEASUREMENT OF TOTAL AND REACTIVE LYSINE CONTENT OF CANOLA MEAL USING NEAR INFRARED SPECTROSCOPY

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Reactive lysine can be used as a measure of heat damage in processed pig feed ingredients (van Barneveld *et al.* 1999; van Barneveld and Ru, 2003). Routine analysis of randomly collected samples of solvent-extracted canola meal of apparently high quality (R.J. van Barneveld, unpublished data) revealed that the sample set contained between 5.4 and 8.4 g/kg DM of reverted lysine (reverted lysine = total lysine – reactive lysine). This implies that between 25-27% of the total lysine on a dry matter basis is unavailable for use by the pig. This will depress pig performance, even when digestible lysine values are used in diet formulations, and adversely affect the nutritional value of canola meal relative to other protein sources. To maintain canola-meal quality before incorporation into pig diets, routine analysis of heat damage is required. In this experiment we assessed the potential for near infrared spectrophotometry (NIRS) to measure the total and reactive lysine content of canola meal.

A calibration sample set of 60 samples of canola meal was assembled via random collection of cold-pressed and solvent-extracted samples or through structured heat treatments of a cold-pressed canola meal sample (van Barneveld and Ru, 2003). NIRS reflectance spectra of all samples were recorded using a NIRSystems Model 6500 Spectrophotometer (FossNIRSystem Inc., Silver Spring, MD, USA) and IntraSoft International (ISI) WINISI software (FossNIRSystem Inc., Silver Spring, MD, USA). Scanning was performed via a sample transport module in reflectance mode over the wavelength range 400-2500 nm at 2 nm intervals using a small ring cup. Examinations of final spectra were done using the second derivative with a standard normal variate and detrend scatter correction and modified partial least squares regression of the derivatised spectra. The superlative math treatment was 2, 5, 5, and 1. The Standard Error of Cross Validation (SECV) was used as a measure of the accuracy of the calibrations in each case.

**Table 1. Calibration statistics for the prediction of total and reactive lysine (g/kg, DM) in cold-pressed and solvent-extracted canola meal samples using near infrared spectroscopy.**

Constituent	Range	SD <sup>1</sup>	RSQ <sup>2</sup>	SECV <sup>3</sup>	SEL <sup>4</sup>	SECV/SEL	SECV/SD
Total lysine	1.57 – 21.45	4.64	0.99	0.42	0.40	1.05	0.09
Reactive lysine	0.62 – 15.93	4.46	0.99	0.76	0.60	1.27	0.17

<sup>1</sup>Standard deviation. <sup>2</sup>Square of correlation coefficient R. <sup>3</sup>Standard error of cross validation. <sup>4</sup>Standard error of laboratory reference.

The results indicate that NIRS has potential to predict accurately both total lysine and reactive lysine contents in canola meal (Table 1). The resultant calibration statistics for cold-pressed and solvent-extracted canola meal samples demonstrated a very encouraging level of measurement accuracy for total lysine (SECV of 0.42 g/kg and R<sup>2</sup>=0.99). The outcomes for the reactive lysine prediction were also very positive (SECV=0.76 g/kg and R<sup>2</sup>=0.99). The ratio between the SECV and the standard deviation (SECV/SD) was below 0.3 which is used as an indicator of calibration robustness. Analysis of canola meal samples for total and reactive lysine content using NIRS could therefore be used as a routine quality control procedure.

Supported by Australian Oilseeds Federation.

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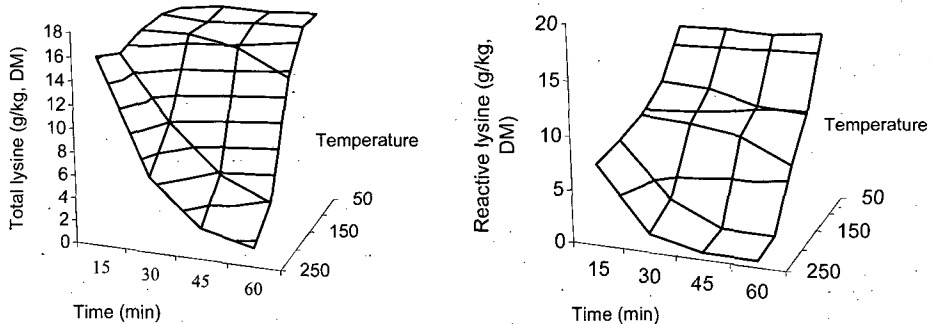
## CHANGES IN THE REACTIVE LYSINE CONTENT OF CANOLA MEAL WITH VARYING HEAT APPLICATIONS

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Definition of the nutritional quality of canola meal can be confounded by reactions that take place when heat is applied during oil extraction. Van Barneveld *et al.* (1999) reported that despite similar total lysine concentrations in cold-processed, expeller-extracted and solvent-extracted canola meals, true ileal digestibility of reactive-lysine in pigs was significantly higher ( $P < 0.001$ ) in the cold-pressed meal. This suggests that heat application during expeller and solvent extraction has a negative influence on the ileal-digestible lysine from these canola meals when fed to pigs. Van Barneveld *et al.* (1999) also demonstrated that gross reactive lysine content was a good indicator of the true ileal-digestible reactive-lysine content for pigs. The aim of this experiment was to assess the influence of varying heat applications on the reactive-lysine content of canola meal and the potential for heat damage during processing before incorporation into pig diets.

Graded levels of dry heat (50, 100, 150, 200 or 250°C) were applied to a sample of cold-pressed canola meal for 15, 30, 45 or 60 min, respectively. Dry heat was selected so that the influences of heat alone could be examined without influences from moisture and pressure. Samples were analysed for total lysine content and reactive-lysine content using the methods described by Rutherford *et al.* (1997) with 10% of samples randomly selected as replicates for analytical assurance purposes.



**Figure 1.** Total lysine (left) and reactive lysine (right) content (g/kg, DM) of cold-pressed canola meal heated to 100, 150, 200 and 250°C for 15, 30, 45 or 60 min, respectively.

Dry heat application had minimal influence on total lysine content when applied at low temperatures or at any temperature for short periods (Figure 1, left). In contrast, heat application above 50°C reduced the reactive lysine content of canola meal when applied for any time period (Figure 1, right). As these temperatures are routinely exceeded during canola meal processing, monitoring of reactive lysine content may be a useful measure of canola meal quality before incorporation into pig diets, as it is more sensitive to the effects of heat than total lysine content.

Supported by Australian Oilseeds Federation.

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## WASTE YIELD RATIOS FROM A NEW ZEALAND PIG UNIT

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We used a mathematical pig-growth model to determine if optimising the diets of pigs to closely match their nutritional requirements would reduce the nutrient-load of piggery effluent. As part of this work, an intensive 'baseline' survey of the existing waste production was undertaken at a 'study farm', which was raising grower/finisher pigs (24 to 90 kg). It is this initial waste survey data that are reported here.

All waste produced at the study farm was collected via under floor drains into a central sump where it was mixed and pumped into a tanker for land application. For every sump of waste, the change in liquid height after removal was measured allowing calculation of the waste volume. A sample of the mixed waste slurry was also taken. The samples were then analysed in duplicate or triplicate for chemical oxygen demand (COD), total solids (TS), total nitrogen (TN) and total phosphorus (TP). Over 1700 individual analyses were done on the waste collected during the 70-day survey period. At the start and end of the survey period, the waste collection system was fully emptied and cleaned to ensure that the total mass of waste produced over this period was measured. The amount of waste produced during the survey depended on the number and size of pigs during this time. Using figures supplied by the farmer, it was possible to determine the total weight of pigs on any one day. During the experimental-period the average daily weight of all pigs was estimated at 146 110 kg. This measure of 'kg of pigs' provided a base unit against which the 'kg of waste components' could be proportioned (Table 1).

**Table 1. Waste production during survey period.**

	COD	TS	TN	TP
Total waste produced (kg)	27590	60940	5615	1299
g waste/kg pigs x day	2.70	5.96	0.549	0.127

The ratios (g waste/kg pigs x days) for TS, TN and TP are similar to ratios that can be derived from data of Vanderholm (1984) for fresh manure which were 6.0, 0.46 and 0.15 g waste/kg pigs x day, respectively. The Vanderholm (1984) value for COD was, however, 5.8 g waste/kg pigs x days. The lower COD value in the current experiment could be attributed to anaerobic decomposition of the waste since it normally spent over a week in the underfloor drains. As the pH was close to neutral and the surface area exposed was relatively small compared to the volume stored, the volatilisation of ammonia was minimised. Analysis of the feed during the monitoring period showed that 11 479 kg of nitrogen and 3536 kg of phosphorus were consumed by the pigs. This means 48.9% of the N and 37.7% of the P contained in the feed is wasted.

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## THE EFFECT OF DIETARY $\alpha$ -TOCOPHEROL CONCENTRATION AND ANTIBIOTIC SUPPLEMENTATION ON WEANED PIGLET PERFORMANCE AND INDICATORS OF OXIDATIVE STRESS

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The use of in-feed antibiotics to enhance performance has been widespread in pig husbandry for many decades but EU legislation will ban such use of the remaining antibiotics by 2006. Alpha-tocopherol is the most biologically active form of vitamin E and its ability to scavenge free radicals makes it a potent antioxidant. Substantially increasing the concentration of dietary  $\alpha$ -tocopherol above NRC (1998) recommendations may have the potential to reduce oxidative stress and improve the health and performance of the host, thereby reducing the need for in-feed antibiotics. The aim of this experiment was to determine whether increased dietary  $\alpha$ -tocopherol enhances weaned piglet performance and could therefore be considered as an alternative to antibiotic growth promoters.

Two hundred and forty crossbred piglets (JSR Healthbred) were weaned at  $22.3 \pm 1.6$  days of age ( $\pm$ SEM) and  $6.4 \pm 0.6$  kg, into commercial flatdeck accommodation. Seven or eight piglets were allocated to each pen ( $1.99 \text{ m}^2$ ) on the basis of litter, weight and gender. Pens were allocated to a  $2 \times 2$  factorial experiment involving two levels of DL- $\alpha$ -tocopheryl acetate (50 mg or 500 mg/kg feed), and two levels of antibiotic (0 mg or 40 mg of avilamycin (day 1-20)/salinomycin (day 21-40)/kg feed). Diets were formulated to be isonutritious and provided on an *ad libitum* basis. On day 20 post-weaning, the pigs were moved to conventional grower accommodation. Piglets were weighed at weaning and on day 40 post-weaning. Eight piglets from each treatment group were slaughtered on day 40 post-weaning. Blood and liver samples were obtained to determine erythrocyte antioxidant enzyme activities and liver malondialdehyde concentrations. Data were analysed using the GLM procedure of Minitab 12.2.

**Table 1. Average daily feed intakes (FI, g/pig/d), average daily liveweight gains (ADG, g/pig/d) and feed conversion ratio (FCR) (day 1-40 post-weaning). Total liver malondialdehyde concentration (MDA, pmols/mg protein) and erythrocyte superoxide dismutase (SOD, U/g Hb) and glutathione peroxidase (GSHPx, U/g Hb) activities (day 40).**

D 1-40	$\alpha$ -tocopherol ( $\alpha$ -t)		Antibiotic (Ac)		SEM	$\alpha$ -t	Ac	$\alpha$ -t*Ac
	50 mg/kg	500 mg/kg	0 mg/kg	40 mg/kg				
FI	467.3	484.3	474.3	477.3	10.72	NS <sup>1</sup>	NS	NS
ADG	351.5	366.3	357.8	360.0	9.99	NS	NS	NS
FCR	1.33	1.33	1.33	1.33	0.01	NS	NS	NS
MDA	219.7	183.6	185.8	217.5	23.29	NS	NS	NS
SOD	901.0	918.3	886.8	932.5	55.21	NS	NS	NS
GSHPx	300.4	359.1	380.2	279.3	64.46	NS	NS	NS

<sup>1</sup>NS, non significant.

Increasing dietary  $\alpha$ -tocopherol did not increase the performance of weaned piglets or reduce the indicators of oxidative stress (Table 1), suggesting that the lower  $\alpha$ -tocopherol concentration was more than adequate for the degree of oxidative stress encountered. Lack of response to in-feed antibiotics is usually associated with 'clean' environments, but we believe the trial conditions represented UK commercial situations.

*This work was funded by Roche Vitamins Europe Ltd.*

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## SORGHUM OR SORGHUM KERNEL CAN REPLACE WHEAT IN WEANER PIG DIETS

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Sorghum is not used widely in weaner pig diets in Australia because of the grain's high tannin concentration, is thought to increase feed:gain (Kondos and Foale, 1983). Dehulling of sorghum grain to produce sorghum kernel removes most of the tannin content. In this experiment we tested the hypothesis that diets based on sorghum kernel would maintain weaner pig performance at a level similar to wheat and superior to sorghum grain.

Forty-eight, four-week-old, male, Large White x Landrace pigs were allocated at  $7.5 \pm 1.2$  kg (mean  $\pm$  SD) to three diets formulated to each contain 15 MJ digestible energy (DE) per kg and 0.82 g available lysine/MJ DE. The three diets included a wheat-based control diet containing no sorghum and two sorghum diets (sorghum grain and sorghum kernel) with similar levels derived from the same sorghum sample (cv. MR32). The sorghum grain was dehulled using commercial pearling equipment. The wheat, sorghum and sorghum kernel samples were analysed by near-infrared reflectance to contain 14.3, 13.7 and 13.8 MJ DE per kg (air-dry basis), respectively. Each sample was analysed for amino acid content. Tannic acid level in the sorghum was screened using the Modified Chlorox Bleach Test and graded as low (<0.25 mg catechin equivalents/100 mg). Each grain source was ground finely through a 5 mm screen. The same protein supplements and free amino acids were added to each diet. Canola meal (solvent extracted) was included in each diet (maximum 76 g/kg) to correct for low cystine concentration (2.0 g/kg) in the sorghum and sorghum kernel diets compared to 2.9 g/kg cystine in the wheat sample. The pigs were housed in individual weaner cages in one room maintained at 27°C and each cage had an individual feed trough and nipple drinker. Feed was offered fresh each day as a mash diet to maintain at least 500 g in each trough. Uncontaminated feed spillage was collected daily and returned to each feed trough. The experiment continued for 21 days. Liveweight at the start and finish of the experiment and food intake were measured. Three pigs (two from the wheat diet and one from the sorghum diet) were removed from the analysis because of illness or lameness.

**Table 1. Mean live performance<sup>1</sup> for 45 male pigs offered diets based on either wheat (n=14), sorghum (n=15) or sorghum kernel (n=16) from 7.5 to 25 kg liveweight.**

Measurement	Wheat	Sorghum	Sorghum kernel	SEM <sup>2</sup>	Significance <sup>3</sup>
Daily intake (g)	882	857	856	31.1	NS
Daily gain (g)	536	542	557	20.0	NS
Feed:gain (g/g)	1.65	1.58	1.54	0.036	NS

<sup>1</sup>Mean live performance adjusted for initial liveweight. <sup>2</sup>Average SE. <sup>3</sup>NS, Not significant.

Diets based on sorghum kernel maintained weaner pig performance similar to wheat, but there was no evidence that sorghum kernel was superior to sorghum grain. There was no significant difference in daily feed intake, daily liveweight gain and feed:gain when weaner pigs were offered either wheat, sorghum or sorghum kernel diets (Table 1). Tannin concentration in sorghum grain (cv. MR32) was low (<0.25 mg/100 mg) and not associated with an increase in feed:gain in weaner pigs. Sorghum can therefore replace wheat in weaner pig diets provided sorghum-based diets are formulated to correct for deficiencies in lysine, threonine and sulphur amino acids and the sorghum is low in tannins.

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## INTERRELATIONSHIP BETWEEN THREONINE REQUIREMENT AND NEUTRAL DETERGENT FIBRE

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The endogenous loss of threonine is significantly higher than other essential amino acids due to the high content of threonine in mucin (Lien *et al.* 1997). Endogenous loss of threonine (and other amino acids) is increased by higher feed intake and fibre level (Taverner *et al.* 1981). AUSPIG simulations also demonstrate that neutral detergent fibre (NDF) influences threonine requirement although dietary threonine is rarely adjusted in formulation of finisher diets.

To establish the interrelationship between threonine and NDF intake on pig performance, 100 entire male and 100 female pigs were allocated to 10 treatments at  $78 \pm 3.5$  kg liveweight. The treatments consisted of two levels of NDF and five increasing levels of available threonine (AT). Diets were formulated to contain 13.8 MJ/kg DE, 10.3 MJ/kg NE and 0.50 g/MJ/DE of available lysine. Pigs were housed in individual pens and offered diets *ad libitum* for 42 days. Average daily gain (ADG), average daily intake (ADI) and feed conversion gain ratio (FCR) were measured weekly. Pigs were killed on day 43 and carcass weight (CWT) and P2 backfat were recorded.

**Table 1. Effects of increasing AT (g/MJ/kg DE) and NDF (%) on ADG (kg), FCR (g:g), ADI (kg/d), CWT (kg) and P2 (mm).**

AT	0.24		0.27		0.30		0.33		0.36		Significance <sup>1</sup>	
NDF	11	22	11	22	11	22	11	22	11	22	SE M	
ADG	872	857	995	892	1026	983	955	992	997	940	0.60	AT**, NDF*
FCR	3.16	3.31	2.91	3.24	2.88	2.92	2.94	2.91	2.92	2.99	0.03	AT**, NDF**
ADI	2.75	2.79	2.87	2.86	2.90	2.86	2.76	2.85	2.83	2.79	0.02	
CWT	92.2	90.6	94.8	90.8	96.4	94.4	93.9	94.4	94.8	93.7	0.47	AT***
P2	13.9	15.8	14.1	14.3	13.9	14.1	14.2	14.6	14.2	14.6	0.23	

<sup>1</sup>\*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

While there were no interactions between AT and NDF on growth performance during the 42-day period, available threonine and NDF significantly influenced ADG and FCR. NDF had no effect on carcass or P2. Increasing AT improved CWT (P<0.001) but had no influence on P2. A 'broken stick' analysis determined that the two-fold increase in NDF increased AT requirement from 0.27 to 0.30, 0.27 to 0.28 and 0.25 to 0.30 g/MJ DE to maximise ADG, FCR and CWT, respectively. These findings support the AUSPIG stimulated requirements and demonstrate AT levels should be adjusted relative to the NDF intake, either through the diet or deep litter of finishing pigs.

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## THE USE AND EFFICACY OF ACIDULANTS IN THE DIETS OF POST-WEANED PIGLETS

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The search for effective alternatives to antibiotic growth promoters has generated a renewed interest in the use of acidulants in the diets of growing/finishing pigs and post-weaned piglets. Organic acids vary significantly in their characteristics. Formic acid, for example, is a powerful acid and can effectively reduce gut pH when used in post-weaning diets (Gabert and Sauer, 1994; Roth and Kirchgessner, 1998). However, it is also corrosive and because of this, it is difficult to handle. Citric acid is not such a powerful acid and is more palatable when used in a post-weaning feed.

In this experiment we compared the efficacy of citric acid and formic acid during the immediate post-weaning phase at the Provimi Ltd. Green Hill Farm Feed Evaluation Unit in the UK. Two hundred and fifty-six Large White x (Large White x Landrace) piglets were weaned at 25 days of age and housed in groups of eight pigs per pen in flat deck accommodation in a controlled environment. Pigs (entire males and females in about equal proportions) were housed in weight-matched groups; small, small/medium, medium/large and large piglets. Piglets were allocated to treatment so that there were four pens of each weight class receiving each treatment and eight pens receiving each treatment. In total, there were 64 pigs per treatment. The experiment was designed as a randomised block design with two separate batches of 128 piglets.

Pigs were offered either a negative control diet (treatment A) or a diet containing an acid salt (formic/lactic) blend at 2 kg/t (treatment B), citric acid at 10 kg/t (treatment C) or a formic acid/calcium formate blend at 3 kg/t (treatment D). During the 25-day experiment all pigs were offered a two-diet program. The stage 1 diet was fed for the first 10 days post-weaning and followed by a stage 2 diet for the remaining 15 days. Piglets were individually weighed at weaning, at the changeover from the first to second stage diet, and at the end of the experiment and all feed intakes for each pen were recorded. The data were analysed using a GLM Analysis of variance using 'Minitab' using weaning weight as a covariate and treatment and weight class as main effects in the model.

**Table 1. Effect of acidulants on the performance of post-weaned piglets from weaning to day 25 post-weaning.**

Treatment	A	B	C	D	SE <sub>D</sub>	Signif.
Feed intake (g/d)	357 <sup>a</sup>	358 <sup>a</sup>	389 <sup>b</sup>	327 <sup>c</sup>	0.021	*
Daily Gain (g/d)	301 <sup>a</sup>	296 <sup>a</sup>	334 <sup>b</sup>	251 <sup>c</sup>	0.011	*
FCR	1.19	1.21	1.16	1.30	0.035	NS

\* P<0.05.

The acids had beneficial effects on both feed intake and growth in the post-weaned piglet with treatment C (citric acid at 1% inclusion) giving the best level of performance (P<0.05). There were no significant treatment x weight-class interactions on growth rates, feed intakes or feed conversion ratios. In this experiment, the costs per kg of liveweight gain were: 43 (\$A1.12), 43 (\$A1.12), 39 (\$A1.01) and 44 (\$A1.14) pence for treatments A,B,C and D respectively, and the margin over feed costs were; 56 (\$A1.45), 56 (\$A1.45), 60 (\$A1.56) and 55 (\$A1.43) pence respectively.

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## IN VITRO ACTIVITIES OF THREE PHYTASES UNDER DIFFERENT PH AND PROTEASE CHALLENGES

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Commercial phytases differ in their bioefficacy when used as supplements in low phosphorus diets of broilers and pigs (Igbasan *et al.* 2001). The differences may be due to the different pH optima and protease sensitivities of phytases as the enzymes will be less effective outside their pH optima and degraded if attacked by endogenous proteases. In this experiment we describe the responses of phytases derived from *Peniophora lycii*, *Aspergillus niger* and *Escherichia coli* to conditions resembling the action of stomach and ileum pH and proteases.

Enzyme activity was measured using 0.2 M buffers from pH 2.0 to 7.0 and 2 mM phytate at 37°C. Release of inorganic phosphate was measured by a standard method (Heinonen and Lahti, 1981). Acid stability was compared by incubating the phytases at 37°C for 2 h in 50 mM glycine-HCl, 5 mg/ml BSA pH 2, 2.5 or 3. The phytase activity was then assayed at pH 5. For protease sensitivity, about 10 µg/ml of phytase in 5 mg/ml BSA was treated for 2 h at 37°C with protease in 50 mM buffers. The residual phytase activity was measured at pH 5.

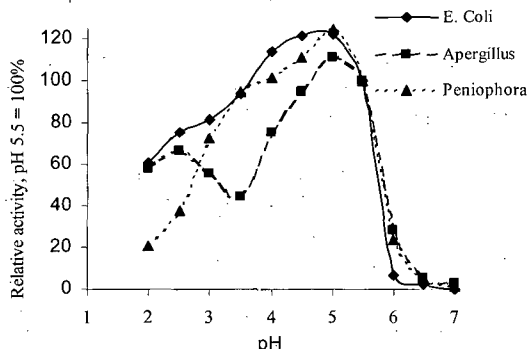
*E. coli* phytase had a broader pH-activity profile than *P. lycii* and *A. niger* phytases (Figure 1). Phytase resistance to protease inactivation was tested against pepsin, trypsin and chymotrypsin. *E. coli* phytase was found to be the most resistant to inactivation (Table 1). The greatest difference was seen with chymotrypsin. *E. coli* phytase retained over 60% activity compared to 5% for *P. lycii* and *A. niger* phytases after treatment at the above conditions. Furthermore, *E. coli* phytase was also more resistant to pepsin and trypsin. Acid stability of the phytases was compared at pH 2, 2.5 and 3. At pH 2.5 *A. niger* and *E. coli* phytases retained about 60% activity while *P. lycii* had only 10% activity.

*E. coli* phytase had greater pH stability and protease resistance which may partly explain differences in bioefficacy between commercial phytases seen in the monogastric animal. More work is needed to confirm the significance of these results *in vivo*.

**Table 1. Percentage residual activities of phytases when treated with protease.**

Phytase	Pepsin	Trypsin	Chymotrypsin
<i>E. coli</i>	76.7 <sup>a</sup>	23.0 <sup>a</sup>	65.8 <sup>a</sup>
<i>A. niger</i>	31.4 <sup>b</sup>	0.45 <sup>b</sup>	2.95 <sup>b</sup>
<i>P. lycii</i>	5.42 <sup>c</sup>	1.25 <sup>b</sup>	5.77 <sup>b</sup>
SEM	0.36	1.28	1.03
Pvalue	<0.0001	0.002	<0.0001

<sup>ab</sup>Figures in columns with different superscripts are statistically different (P<0.05).



**Figure 1.** pH-activity of *E. coli*, *Aspergillus* and *Peniophora* phytases

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## INTERACTIVE EFFECTS OF WHEAT PHOSPHORUS CONTENT AND ENZYMES ON MINERAL DIGESTIBILITY IN WEANER PIGS

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About 70% of total phosphorus (P) and 60% of calcium (Ca) are bound to phytate in wheat (Kim *et al.* 2002; Frolich and Asp, 1985), limiting the amount of P and Ca that is digestible in the gastrointestinal tract. The addition of Ca and P to pig diets is therefore required. Phytic acids are present in the aleurone layers of wheat, indicating a possible association of phytic acids with non-starch polysaccharides (NSP) (Frolich and Asp, 1985) and supplements of phytase and NSP-degrading enzyme may therefore increase P and Ca digestibility in weanling pigs fed a wheat-based diet. The hypotheses tested were 1) wheat P-content will influence mineral digestibility; 2) individual supplementation of xylanase and phytase will increase P and Ca digestibilities; and 3) simultaneous supplementation of xylanase plus phytase will have synergistic effects on P and Ca digestibilities in weanling pigs fed wheats that differ in their P content.

A 2 x 4 factorial experiment was done with the respective factors being wheat (Low-P: 223 mg and High-P: 335 mg/100 g) and enzyme supplementation (no enzyme, xylanase, phytase, and xylanase plus phytase). The enzymes were xylanase and phytase (Porzyme 9300<sup>®</sup>, minimum activity 4000 U/g endo-1,4- $\beta$ -xylanase; Phyzyme XP 5000G<sup>®</sup>, minimum activity 4100 U/g phytase, Danisco Animal Nutrition, UK). The two pre-selected wheats were heat treated before diet manufacture. Eighty male weaner pigs (Landrace x Large White, 5.4  $\pm$  0.07 kg) were fed an identical pre-trial diet for one week and received their respective experimental diet for three weeks, after random allocation based on liveweight. All diets contained 650 g/kg wheat and similar concentrations of calculated digestible energy (14.2 MJ/kg), available lysine (0.78 g/MJ DE), available P (0.32%) and Ca:P (1.46:1). Titanium dioxide (TiO<sub>2</sub>) was added as an inert marker. Faecal 'grab' samples were collected for three consecutive days from day 14. Data were analysed using the ANOVA procedure of Statview.

Wheat P-content had no significant effect on mineral digestibility (Table 1). Xylanase improved P digestibility in the High-P wheat but not in the Low-P wheat. Phytase improved P digestibility alone in the Low-P wheat but improved the digestibility of P and Ca in the High-P wheat. However, interactions occurred between the wheat P content and enzyme supplementation, so that xylanase plus phytase did not improve P and Ca digestibility in the Low-P wheat, but did improve P (P<0.001) and Ca (P<0.01) digestibility in the High-P wheat.

**Table 1. Effects of wheat P content (W) and enzyme supplementation (E) on digestibility coefficient (DC) of P and Ca (%) determined with 42-day-old male weaner pigs<sup>1</sup>.**

Wheat	Low-P wheat				High-P wheat				Pooled SEM	Significance <sup>2</sup>		
	No	Xyl	Phy	X+P	No	Xyl	Phy	X+P		W	E	WxE
DC <sub>P</sub>	43.2 <sup>a</sup>	45.9 <sup>a</sup>	56.0 <sup>b</sup>	45.8 <sup>a</sup>	36.8 <sup>a</sup>	42.8 <sup>b</sup>	51.9 <sup>c</sup>	53.2 <sup>c</sup>	7.82	0.21	***	***
DC <sub>Ca</sub>	59.1 <sup>ab</sup>	59.3 <sup>ab</sup>	61.9 <sup>b</sup>	55.7 <sup>a</sup>	54.8 <sup>a</sup>	59.2 <sup>a</sup>	67.6 <sup>b</sup>	63.2 <sup>b</sup>	7.08	0.12	**	*

<sup>1</sup>Mean digestibility determined from 10 pigs per treatment combination. Values with different superscripts within a row for each wheat are significantly different (P<0.05). <sup>2</sup>\*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

These data indicate that the combined use of xylanase and phytase for P and Ca digestibility might be effective only in wheats having a higher total P-content, and therefore a higher phytate-P level (Kim *et al.* 2002). Phytase alone can enhance the total tract digestibility of P, independent of wheat P-content.

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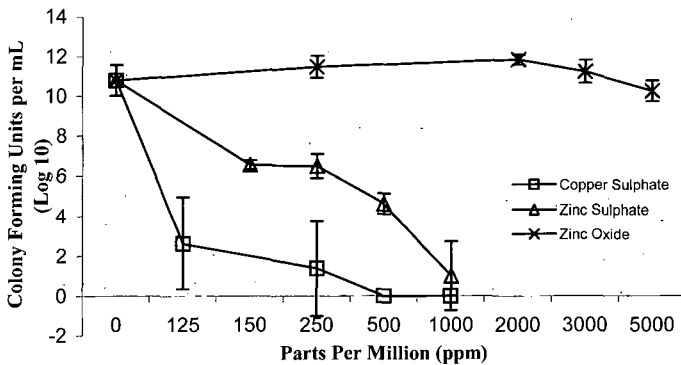
## EFFECT OF VARIOUS TRACE MINERALS ON THE GROWTH OF *E. COLI* K88 *IN VITRO*

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In the first 14 days post-weaning, challenges by pathogenic organisms can result in post-weaning scours, loss of performance and increased mortality. Zinc oxide has been widely used at levels of 2000-3000 ppm zinc to enhance growth and minimise scour in nursery pigs. However this practice is considered in some countries to cause environmental pollution and the use of zinc oxide is being restricted. The aim of this experiment was to investigate the use of copper and zinc sulphate as possible replacement trace minerals for high levels of zinc oxide. An *in vitro* experiment was initially done to assess the effect of the trace minerals on pure cultures of bacterial organisms before doing an *in vivo* experiment. *E. coli* K88 is one of the most frequent bacterial organisms associated with post-weaning scours and the effect of these trace minerals on this bacterium was therefore of particular interest.

The trace minerals used were copper sulphate, zinc sulphate and zinc oxide. Tryptic soy broth was diluted with sterile water in the ratio 1:6 to provide the growth medium. The concentrations of the trace minerals added to the growth medium were copper sulphate (0, 125, 250, 500 and 1000 ppm); zinc sulphate (0, 150, 250, 500 and 1000 ppm) and zinc oxide (0, 250, 2000, 3000 and 5000 ppm). The 30 ml test vessels were inoculated with 1.5 ml (5%) of a 12-18 h bacterial culture of *E. coli* K88 (Newport Laboratories 2-0087-2, field strain). The test vessels were incubated at 37°C for 24 h and then 10-fold dilutions were made and 100 µl were spread on blood agar plates. These were incubated for 24 h at 37°C and then a count made of the colony forming units. The test was repeated three times.



**Figure 1.** The mean number (three replicates) of colony forming units of *E. coli* K88 after incubation with different levels of copper sulphate, zinc sulphate and zinc oxide.

Zinc oxide did not restrict the growth of *E. coli* K88 (Figure 1). Copper sulphate was a better inhibitor of the growth of *E. coli* K88 than zinc sulphate. A dose response was measured for copper and zinc sulphate in terms of the survival of *E. coli* K88. The best response was achieved by using copper sulphate at 125 ppm, reducing *E. coli* K88 numbers by 75%. Zinc oxide can therefore be replaced by copper or zinc sulphate to reduce bacterial growth of *E. coli* K88.

## RESTRICTION OF PROTEIN INTAKE IN POST-WEANING PIGS

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Reducing protein level below accepted requirements reduced weaner performance and carcass fat but did not affect final carcass weight (Henman, 2001 unpublished). When dietary protein was restricted, Skiba *et al.* (2002) showed that pigs increased protein deposition during the realimentation period. The hypothesis tested in this experiment was that restriction of protein intake during three particular periods of growth would reduce backfat depth in finisher pigs.

The experiment consisted of six dietary treatments x two sex treatments in a factorial design. Animals were fed diets above their expected requirement except during the periods of nominated restriction. During these times, protein was restricted at 10% of the available lysine to DE ratio with other amino acids held at the same ratio to lysine. The diets were isoenergetic within each phase across treatments. There were four pens of twenty pigs of each sex for each treatment kept in commercial pens for the first six weeks after weaning, moved to grower pens for seven weeks and then finisher pens for the remainder of the experiment. All pigs were the same age at the end of the experiment. The restricted growth periods were divided into the following phases, Phase 1=0-3 weeks after weaning, Phase 2=3-6 weeks after weaning, Phase 3=0-4 weeks of grower. Pigs were fed unrestricted diets throughout the rest of the experiment. Requirements were determined by the Auspig Pig Model.

**Table 1. Carcass characteristics of pigs on restricted lysine diets during designated phases.**

Treatments	Carcass Weight (kg)	Fat Depth (mm)	Dressing Percent (%)
Control	82.8	14.0	79.6
Phase 1 Restricted only	84.2	14.8	79.6
Phase 2 Restricted only	82.9	12.5*	79.8
Phase 3 Restricted only	84.1	14.1	79.8
Phase 1&2 Restricted	84.8	13.8	79.5
Phase 1,2&3 Restricted	82.7	13.7	79.6
SEM	0.506	0.202	0.102

\* Significant at  $P < 0.05$ .

Protein restriction during Phase 2 (7-10 weeks of age) lowered P2 backfat at the end of the finisher phase (Table 1). Protein restriction during the first three weeks after weaning or from 10-13 weeks of age had no effect on the subsequent performance of the animals. Further work is required to evaluate the changes in metabolism caused by the restriction in dietary protein during the 7-10 week old pig.

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## DIFFERENCES IN *IN VITRO* FERMENTABILITY OF SELECTED CARBOHYDRATES COMPARING ILEAL AND FAECAL INOCULA FROM UNWEANED PIGLETS

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Addition of prebiotics to the diet is assumed to stimulate fermentation in the gastrointestinal tract (GIT), although often no testing is done to validate this assumption. Establishing the difference in the *in vitro* fermentability of potential substrates, both in terms of kinetics and end-products, should indicate where fermentation is likely to occur and whether end-products could be produced that may influence the health of the GIT. We used an *in vitro* method to determine if differences in the fermentability of two ingredients could be detected using inoculum from the ileum (IL) and faeces (FEC) of unweaned piglets.

Cumulative gas production was measured *in vitro* using faecal inocula from nine three-week-old crossbred piglets (no creep feed offered, nor antibiotics given). Unmolassed sugar beet pulp (SBP) and lactulose were used as substrates and fermented *in vitro* for 72 h. Gas production profiles were fitted to a mono-phasic model (Groot *et al.* 1996): where  $G=A/(1+(C/t)^B)$ , where G=total gas, A=asymptote, B=switching characteristic, C=half-time for the asymptote and t=time. After fermentation, volatile fatty acids (VFAs), NH<sub>3</sub> concentrations and organic matter loss (OML) were measured. This procedure was repeated one-week later using ileal contents from the same piglets. Statistical analysis was carried out using the GLM procedure of the SAS Institute (1990).

**Table 1. Fermentation characteristics of two potential prebiotics using ileal and faecal inocula of unweaned piglets.**

Substrate	Inoculum	OMCV <sup>1</sup>	T <sub>1/2</sub> <sup>1</sup>	OML <sup>1</sup>	Tot VFA <sup>1</sup>	NH <sub>3</sub> <sup>1</sup>
Lactulose	Ileal	400.6	10.2	99.6	421.4	82.4
	Faecal	446.2	6.5	97.3	380.9	76.2
SBP	Ileal	266.2	22.2	73.6	376.8	76.5
	Faecal	407.6	21.6	87.9	394.3	60.8
	Substrate	***	***	***	**	***
Probability	Inoculum	***	*	NS	NS	***
	Sub*Inoc	**	NS	*	NS	***
MSD <sup>3</sup>		35.2/18.2	4.2/3.2	2.8/1.4	71/37	5.6/2.9

<sup>1</sup>OMCV=cumulative gas produced (ml/g OM incubated); T<sub>1/2</sub>= half-time of asymptotic gas production (h); OML=M loss (as % of organic matter incubated); Tot VFA=Total volatile fatty acids (mg of acetic acid equivalents per g OM incubated); NH<sub>3</sub> = Ammonia concentration (mg per g OM incubated). <sup>2</sup>NS, not significant, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. <sup>3</sup>MSD minimum significant difference (P<0.05) - the first number refers to substrate and the second to inoculum.

Lactulose was more rapidly fermented than SBP by both inocula, with higher VFA and NH<sub>3</sub> production. Kinetics of the two inocula differed significantly (P<0.01). For both substrates, there was a longer T<sub>1/2</sub> and a lower OMCV when fermented with IL compared with FEC. This might be due to the differences in both the number and diversity of microbes present in different areas of the GIT. The OML for SBP was higher for FEC than for IL. There was no difference in VFA but NH<sub>3</sub> was significantly higher for IL. When designing diets containing prebiotic substrates for unweaned piglets, it is important to determine where in the GIT fermentation will be stimulated.

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## A HIGH LOAD OF RAPIDLY FERMENTABLE CARBOHYDRATES REDUCES WORM BURDEN IN INFECTED PIGS

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The type and level of dietary carbohydrate has a marked influence on the establishment, gut location and fecundity of *Oesophagostomum dentatum* in pigs (e.g. Petkevicius *et al.* 2001). The aim of this experiment was to determine the impact of inulin and/or sugar beet fibre (SBF) on the metabolism in the large intestine of pigs with established *O. dentatum* infection.

Four experimental diets were formulated based on barley flour with added insoluble fibre from oat husk (Diet 1); inulin ( $\beta$ -2,1 fructan) (Diet 2); SBF (Diet 3) or inulin plus SBF (Diet 4). Chromic oxide (2 g/kg dry matter) was added as an insoluble digestibility marker. Thirty-two 10-week-old pigs were randomly divided into four groups of eight pigs. After three weeks adaptation to Diet 1, all pigs were infected with a single dose of 6000 *O. dentatum* infective larvae. At week-7 after infection, one group of pigs was switched to Diet 2, another group to Diet 3 and a third group to Diet 4 with the remaining pigs continuing on Diet 1. Faecal samples were taken during three consecutive days in week 12 to determine the total-tract digestibility of macronutrients. At week 13, all pigs were killed and their gastrointestinal contents collected and analysed for short-chain fatty acids (SCFA) and lactic acids (LA). Worm number was also determined. The digestibility of macronutrients up to the end of the small intestine was estimated in four ileal-cannulated pigs. The effect of diet was assessed by one-way analysis of variance. Worm numbers were analysed after log transformation.

**Table 1. Fermented carbohydrates (CHO) in the large intestine, concentrations of lactic acids (LA) and total short-chain fatty acids (SCFA) in the caecum and colon and worm burden of pigs fed the experimental diets.**

Diet	1	2	3	4	SEM
Total fermented CHO, g/d	157 <sup>c</sup>	478 <sup>a</sup>	332 <sup>b</sup>	395 <sup>ab</sup>	27.3
Readily fermented CHO*, g/d	42 <sup>d</sup>	392 <sup>a</sup>	93 <sup>c</sup>	158 <sup>b</sup>	7.5
Caecum					
LA, mmol/kg	2 <sup>b</sup>	60 <sup>a</sup>	1 <sup>b</sup>	1 <sup>b</sup>	9.5
SCFA, mmol/kg	134	157	159	159	9.0
Colon					
LA, mmol/kg	0	1	1	0	0.5
SCFA, mmol/kg	123 <sup>b</sup>	185 <sup>a</sup>	168 <sup>a</sup>	159 <sup>a</sup>	10.0
Worm number (95 % CI)	3566-5096	2-74	920-1596	338-593	

\*Sum of sugars, starch and fructan. <sup>abcd</sup>values in the same row with unlike superscript letters were significantly different (P<0.05). CI=confidence interval.

The experimental diets induced differences in carbohydrate fermentation in the large intestine. On Diet 2, the amount of fermented carbohydrates was three times higher than on Diet 1. The high load of rapidly fermented carbohydrate with Diet 2 caused accumulation of LA in the caecum and raised SCFA concentration in the colon. This acidified and variable luminal environment could potentially cause unstable conditions for the worms (particularly females) and may be responsible for the significant reduction in intestinal worm burden of pigs consuming Diet 2 compared to Diet 1. Faecal egg counts were also reduced following the switch to Diet 2.

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## EFFECTS OF LYSO-PHOSPHOLIPIDS AND ENZYME SUPPLEMENTATION ON THE GROWTH PERFORMANCE OF WEANER PIGS

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High-energy weaner diets with a high fat content are used to overcome the young pig's low feed intake and help realise its high capacity for growth. However, the ability of the pig to digest added fat is limited in the weeks immediately after weaning (Cera *et al.* 1988). Lyso-phospholipids promote highly effective emulsification and micelle formation (Mine *et al.* 1993) that assist in fat digestibility and absorption (Saunders and Sillery, 1976). In this factorial experiment we evaluated a source of lyso-phospholipids (1 kg/t of Lysoforte™), a source of enzymes (1 kg/t of Kemzyme® containing xylanase,  $\beta$ -glucanase, cellulase, protease, amylase) and their combination, on the growth performance of weaned pigs fed diets differing in cereal base and tallow content.

The diets used were based on de-hulled oats or wheat and with or without 3% added tallow but with the same amino acid:digestible energy (DE) ratios. The with/without tallow DE values were 15.2 MJ/kg and 14.5 MJ/kg respectively for the wheat-based diets and 16.1 MJ/kg and 15.4 MJ/kg for the oat-based diets (diets contained 0.88 g available lysine/MJ DE). Crossbred male pigs were housed in individual cages with 10 pigs/treatment. Each diet was fed *ad libitum* to pigs from weaning at 21 days (7 kg) to 42 days of age.

**Table 1. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) responses from diets without or with tallow.**

	Tallow	De-hulled oats				Wheat				SE	Significance
		C <sup>1</sup>	L	K	LK	C	L	K	LK		
ADFI	-	341	323	345	324	294	344	344	324	6.3	T*
(g)	+	342	394	327	393	337	335	358	360		
ADG	-	270	263	303	285	242	260	290	249	5.7	TxL*
(g)	+	242	280	229	300	215	266	306	274		
FCR	-	1.26	1.23	1.14	1.14	1.21	1.32	1.19	1.30	0.032	T**
	+	1.41	1.41	1.43	1.31	1.57	1.26	1.17	1.31		

<sup>1</sup>C=control; L=Lysoforte; K=Kemzyme; LK=Lysoforte+Kemzyme; T=tallow. \*P $\leq$ 0.05, \*\*P $\leq$ 0.01.

Tallow inclusion significantly increased feed intake but reduced feed conversion efficiency (Table 1). The presence of Kemzyme either alone or with Lysoforte significantly improved growth rate and FCR. There was a significant tallow x Lysoforte interaction for growth rate, so that irrespective of the grain type, Lysoforte either alone or in combination with Kemzyme, resulted in positive growth responses in the presence of tallow but not in the absence of tallow. In the presence of tallow, the growth responses for Lysoforte only and Lysoforte + Kemzyme were 16% (38 g/d) and 24% (58 g/d) respectively in the dehulled oats diet and in the wheat diet were 24% (51 g/d) and 27% (59 g/d) respectively. This source of lyso-phospholipids appears to overcome the limitations of fat digestibility in the young weaned pig and improves growth rate.

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## K-DIFORMATE ENHANCES WEANER PIG PERFORMANCE

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The inclusion of antibiotics in the diets of pigs to manipulate favourably the microbial population of the gut and enhance growth performance (Anderson *et al.* 2000) is declining because this practice may contribute to the development of antibiotic resistance in bacteria that are potential human pathogens. Considerable attention has been paid to organic acids and their salts, or the so-called 'acidifiers', as possible substitutes (Partanen and Mroz, 1999) for antibiotics. Potassium diformate (K-diformate), a crystalline powder with low corrosive properties, is a chemical complex of formic acid and potassium and has potential as a growth promoter. In this experiment, the effects of graded inclusion rates of K-diformate in wheat-based, weaner diets (14.9 MJ/kg DE; 13.4 g/kg available lysine) on the growth performance of pigs were determined. The diets contained exogenous phytase and xylanase but no anti-microbial feed additives. Each of the four dietary treatments was offered to eight pens of either male or female pigs (20 pigs per pen; Bunge genotype sires from Large White x Landrace cross sows) for three weeks, from 8.7 kg to about 14 kg liveweight, on an *ad libitum* basis. Data from pen means were subject to linear regressions and analyses of variance.

**Table 1. Effects of K-diformate (Formi®) on growth performance of weaner pigs from 22 to 43 days of age.**

Parameter	K-diformate (g/kg)				SEM	Significance (P=)
	0	6	12	18		
Daily gain (g)	215 <sup>a</sup>	238 <sup>ab</sup>	249 <sup>ab</sup>	266 <sup>b</sup>	12.18	0.044
Feed intake (g)	307	334	336	347	12.33	0.156
Feed:Gain (g/g)	1.44	1.41	1.36	1.33	0.050	0.438

<sup>ab</sup>Mean values not sharing common superscripts are significantly different (P=0.05); LSD = 35.3.

The linear effects of K-diformate were to increase weight gain (P<0.005) and feed intake (P<0.05) with an associated improvement (P<0.10) in feed efficiency of weaner pigs. On the basis of pair-wise comparisons K-diformate, at 18 g/kg, increased weight gain by 23.7% (P=0.006), feed intake by 13.0% (P=0.030) and tended to improve feed efficiency by 7.6% (P=0.143). These results are supported by other studies in which K-diformate has been shown to improve growth performance of weaner (Paulicks *et al.* 1996) and grower-finisher pigs (Roth *et al.* 1996; Øverland *et al.* 2000). K-diformate has the capacity to reduce total anaerobic bacterial and coliform counts in the gut of young pigs (Canibe *et al.* 2001) and this bacteriostatic effect is probably the main mode of action. In a nitrogen balance experiment, K-diformate improved the apparent use of crude protein (Roth *et al.* 1998), which was attributed to increased absorption of amino acids. While the reduction of gastric pH by organic acids is thought to facilitate protein proteolysis, this effect, as discussed by Partanen and Mroz (1999), has not been consistently demonstrated. The positive influence of K-diformate on weaner pig performance in this initial, local evaluation justifies further studies, which should include its effects on performance of older pigs and carcass traits, possible interactions with other feed additives and clarification of its modes of action.

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