



Travel Report

9th International Symposium on Digestive Physiology of Pigs Spain May 2009

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A report prepared for the Pork CRC

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Pre-symposium workshop: *Physiological basis of voluntary feed intake in swine. Pre- and post-absorptive regulation*

The pre-symposium workshop, sponsored by the Spanish Government (INIA) and Lucta S.A., covered current knowledge on nutritional, physiological and endocrine factors involved in the control of feed intake. It is generally perceived that feed intake of pig is influenced by energy and protein content of a diet and also its bulkiness, which affects distension of the stomach and the intestines. A research group at the University of Leeds UK (Forbes, J.M.) proposed an interesting novel theory that an animal's feed intake may be determined from the animal's effort to minimise its total discomfort such as gut fill, energy and protein requirements. This *minimal total discomfort* (MTD) model only provides general outlines of a novel frame work at this stage and requires further development, but was received with interest and muted curiosity by the audience.

A number of presentations were then given summarising taste receptor biology (Professor Kirk Klasing, UC-Davis), olfactory receptor biology (Dr Eugeni Roura, Lucta) and feed preferences of pigs (professor David Torrallardona). This is an early but evidence as to whether these are significant factors for the regulation of overall feed intake of pigs were equivocal. Further research should prove to what extent the taste, oronasal sensing and pigs' feed preferences affect feed intake of pigs.

Another aspect covered in the workshop was the hormonal and metabolic regulation of feed intake, presented by Drs Jeff Carroll (USDA-ARS, Lubbock, Texas) and Dr John Black. For the hormonal control of feed intake, evidence was presented that glucose and cholecystokinin reflect short-term energy balances while insulin and leptin reflect long-term energy stores within the body. The critical factors determining feed intake of pigs were summarized as being (1) the intestinal 'brake', caused by excessive distension of undigested material entering particular sections of the intestine; (2) the effects of metabolic hormones such as adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR), which monitor body energy status and interact with regulatory hormones such as insulin, leptin and ghrelin; and (3) the hypothalamic melanocortin system, which regulates hunger and satiety through many hormones such as AMPK, mTOR, insulin, leptin, ghrelin and pancreatic peptides.

This symposium demonstrated that considerable advances have been made in the understanding of factors that regulate feed intake of pigs in terms of the physiological, nutritional, hormonal and metabolic aspects. However the symposium also showed the complexity involved in the control of feed intake in pigs, and how commercial solutions to the issue require much further research and investigation.

Session I - Methodologies to study GIT function and application of pig models

Indicator AA oxidation (IAAO) method to measure in vivo AA availability

Professor Ron Ball (University of Alberta, Canada) presented an overview of the indicator AA oxidation (IAAO) technique for evaluation *in vivo* of AA availability, which was developed based on the fact that protein synthesis and AA oxidation has an inverse relationship, and AA oxidation reflects the partition of dietary AAs between oxidation and protein synthesis. The advantages of this technique include shorter adaptation period (2 days) and lower cost for measurement of AA availability than conventional ileal digestibility techniques. Most importantly, while ileal digestibility techniques only provide amounts of AA that are digested in the small intestine, the IAAO technique provides *in vivo* estimates of the quantity of AA that are used for body protein synthesis. The technique involves surgery to establish two catheters (femoral vein and inferior vena cava), infusion of a known amount of radio-labelled AA ($\text{L-[1-}^{14}\text{C]Phe}$) and collection of $^{14}\text{CO}_2$ to calculate oxidation (catabolism) rate of AAs. The technique can be employed to measure AA availability of feedstuffs, AA requirement of pigs and the gastrointestinal tract requirement of AA. Professor Ball presented recent experimental data showing that the pigs' GIT utilises about 64%, 40% and 30% of threonine, branched-chain AAs and methionine requirements, respectively, while tryptophan was not used.

This technique could provide a solution for the accurate evaluation of available AA in protein-rich ingredients used in Australia, and eventually for the establishment of NIR prediction calibrations for available AA.

Metabolomics

A number of papers attempted to use metabolomics as a tool for discriminating the pigs' metabolic responses to altered dietary treatments. Metabolomics involves analysing carbohydrate and protein profiles in the samples such as intestinal digesta, serum and urine to characterise what metabolic changes are occurring due to changes in dietary components. Metabolite differences in pigs detected in urine (Yde et al.; Hedemann et al.), serum (Yde et al., He et al.) and lymph (Laerke et al.) after feeding diets containing different cereal and fibre sources were possible. However, interpretation of the results extracted from the principal components analysis needs improvement to be accepted as a valid methodology in nutrition.

Terminal restriction fraction length polymorphism (T-RFLP) technique

Many research groups are using the T-RFLP technique (that is established in the laboratory at Murdoch University) to investigate changes in intestinal microbial community upon dietary treatment. Although most studies found significant differences in microbial profiles, interpretation of the profile (identification of corresponding micro-organism using a gene library) is quite often poor at this stage due to a lack of bacterial gene information.

Round Table Discussion I: Future of NSP degrading enzymes under the new constraints. Application to improve the nutritional values of novel feed ingredients (e.g. DDGS), to improve gut health

In this discussion session, a major question arising was why the addition of exogenous xylanase(s) to diets, which improves nutrient digestibility in wheat- and wheat bran-based diets, does not have the same effect in wheat DDGS-based diets. Alteration of chemical and physical structure of fibres during the ethanol extraction process is thought to most likely be responsible for this, and specific enzyme products for the ethanol industry by-products need to be developed. Further research in this area was urged and cooperation between industry and scientists was deemed as crucial to answer the question.

Session II - Modulation and relevance of GIT microbiota

NSP structure is responsible for intestinal microbial fermentation

Canadian researchers (Metzler-Zebeli et al.) fed a semi-synthetic diet containing either 5% of (1) low-fermentable low-viscous cellulose, (2) low-fermentable high-viscous carboxymethylcellulose (CMC), (3) high-fermentable low-viscous oat β -glucan, or (4) high-fermentable high-viscous oat β -glucan, to 30-kg cannulated pigs, and measured microbial fermentation in the small and large intestine. Results showed that the low-fermentable high-viscous CMC diet increased ileal DM digestibility and the high-fermentable low-viscous diet increased large intestinal fermentation of carbohydrates and protein. In a parallel digestibility study, measurements showed that decreased ileal apparent digestibility of energy and CP was explained by increased fermentability but not by increased viscosity. The results, under test conditions, may indicate that the individual structure of NSP is responsible for microbial fermentation in the intestine rather than shared physical properties such as viscosity or fermentability. Since a semi-synthetic diet was used and varying degrees of viscosity were not examined, further examination is required to challenge the viscosity theory for negatively influencing intestinal digestion and fermentation.

Timing of probiotic application is important

A German research team (Pieper et al.) dosed probiotics orally (5×10^9 cfu, *Lactobacillus plantarum*) either 3 days before weaning (d 25) or at weaning (d 28), and then examined the intestinal microbiota profile using DGGs (denaturing gradient gel electrophoresis). The *L. plantarum* dose at weaning significantly altered the intestinal microbiota compared with dosing the same amount of *L. plantarum* before weaning. In the second experiment the authors challenged piglets with enterotoxigenic *E. coli* (ETEC; O149:K91:F4ac) at weaning and dosed 3×10^9 cfu *L. plantarum* within 2 hr after the *E. coli* challenge. The results showed a significantly decreased diarrhoea incidence with oral dosing of the probiotic. The findings highlighted that the use of probiotic was beneficial but timing of the dose is important. It is important to use probiotics in high potency with the correct timing (generally at weaning) before the microbial (pathogenic) population is established.

Plant extracts and dietary protein interaction reduces protective effects of plant extract

Numerous studies were conducted on plant polyphenolics, which purportedly prevents binding of ETEC to the brush border membrane. However despite *in vitro* blocking abilities of plant polyphenolics for binding of ETEC and LT (heat-labile) toxin to the brush border, the majority of *in vivo* applications of plant polyphenolics for prevention of post-weaning diarrhoea have been less successful. A study by a research group at Belgium (Verhelst et al.) demonstrated that some plant polyphenolics are able to prevent ETEC and LT binding to the brush border *in vitro* but addition of protein in the media markedly decreased the ability to block the binding of ETEC to brush border. This particular study, along with many other *in vivo* studies that failed to prevent PWD using plant polyphenolics, suggests that plant extracts are not yet a reliable alternative or replacement to in-feed antibiotics.

Other major findings in this symposium include:

- Low pH and a high acetic acid concentration are not responsible for reduced feed intake in fermented liquid feeding. This means there are other factors

that reduce the palatability of a fermented liquid diet (Canibe et al., Denmark)

- Using a T-RFLP technique, a research group in The Netherlands demonstrated that intestinal microbial community profiles were markedly changed when pigs were fed a diet containing pea hulls and faba bean hulls. Van der Meulen et al. presented data suggesting that legume fibres could be used for manipulation of intestinal microbial community to prevent intestinal disorders in weaner pigs. These authors did NOT examine lupin hulls or Australian faba beans, which may have beneficial effects on piglets post-weaning through manipulation of intestinal microflora, and is worthy of follow-up in Australia
- An Irish research group (O'Doherty et al.) included laminarin and fucoidan, seaweed-derived polysaccharides, in diets for weaner pigs and found reductions in the intestinal Enterobacteria and increases in Lactobacilli spp. in the large intestine

Session III - Digestive function and nutrient absorption

Immune system stimulation increases sulfur AA requirements

Rakhshandeh et al (University of Guelph, Canada) presented an interesting paper in this session. The authors evaluated the impact of immune system stimulation on apparent ileal digestibility of AA and whole-body nitrogen and sulfur balances in 22-kg pigs fed three levels of sulfur AA intake (1.1, 1.2 and 3.2 g/d; 53% MET and 47% CYS) from sulfur AA-limiting diets. Immune system stimulation was induced by intramuscular injection of *E. coli* lipopolysaccharide every 24 hours for 7 days. The results demonstrated that immune system stimulation (1) did not alter ileal AA digestibility; (2) decreased N retention but not sulfur AA retention; (3) and therefore, whole-body N to sulfur balance ratio was reduced. The data suggests that when the pigs' immune system is stimulated then the sulfur AA requirement will be higher than in healthy pigs. It was an interesting study however the experimental error (standard error) was very high due to a low number of experimental units used (8 pigs per treatment) and illness of pigs due to the disease model used.

Other major findings in this symposium include:

- A Canadian research group fed growing gilts (85-110 kg) with either beef tallow or PUFA derived from flaxseed (C18:2n-2 and C18:3n-3) and collected ileal samples at 100 kg LW. Fatty acid analysis of the ileal samples showed that in growing gilts, microorganisms in the small intestine could synthesise and absorb conjugated linoleic acids (CLA). Although quantification of CLA produced in the small intestine of pigs is required, this finding suggests that feeding PUFA to gilts and sows may have beneficial effects on sow and progeny health (Martinez-Ramirez and de Lange)
- A Danish (Norgaard et al.) and a Swedish (Kluge et al.) research group showed that inclusion of benzoic acid in grower and lactating sow diets decreased urinary and blood pH, which have possible implications in reducing bacterial infections in the reproductive tract of females in the breeding herd

Round Table Discussion II: Mineral digestibility and environmental issues. Efficacy and interactions of phytases

In this discussion session, numerous short-communication papers were presented showing that inclusion of phytase increased P and in some cases, the AA digestibilities. Combinations of phytase with xylanase consistently showed no synergistic effect over the use of phytase alone. A review by Poulsen (Denmark) showed that about 30% of plant P is retained and 70% of P is excreted. Use of phytase improved P availability to 50-65%. Therefore, use of the available P concept in diet formulation and the use of low-phytate grains and phytase supplementation are widely applied in world pork production. Other papers suggested that fermented liquid feeding could be a solution to improve P availability. But the major development should be directed to the development of quick-acting phytase as phytate is predominantly degraded in the stomach and, as retention time in the stomach is short, it acts as a bottleneck for the improvement of phytase efficiency.

Session IV - *The digestive tract as a barrier to maintain pig health*

Insoluble fibre reduces post-weaning diarrhoea (PWD) in E coli infected weaner pigs

Van der Meulen and Jansman (The Netherlands) used a small intestinal segment perfusion model to test whether pea, pea hulls, faba bean and faba bean hulls have any effects on fluid absorption in *E. coli*-infected piglets. Perfusion with pea hulls and faba bean hulls showed significantly increased net fluid absorption compared to pea and faba bean meals. This result indicates that legume hulls may promote net fluid absorption in piglets affected by post-weaning diarrhoea. Molist Gasa et al. (University of Manitoba, CA, and Spain) fed a corn-soybean meal-based diet with and without 4% wheat bran to weaner pigs challenged with *E. coli* K88. The results showed that wheat bran inclusion significantly decreased total coliform and *E. coli* K88 numbers. These findings support our previous work (Kim et al., 2008*) that insoluble fibres could reduce the incidence of PWD. Lupin hulls are produced in relative abundance in Western Australia and are predominantly used in ruminant feeding at the moment, however it would be interesting to examine the effects of lupin hulls in *E. coli*-infected pigs to establish whether this Australian (and relatively cheap) source of legume fibre can be used for control of PWD.

*Kim, J.C., Mullan, B.P., Hampson, D.J., Pluske, J.R. (2008). Addition of oat hulls to an extruded rice-based diet for weaner pigs ameliorates the incidence of diarrhoea and reduces indices of protein fermentation in the gastro-intestinal tract. *British Journal of Nutrition* 99:1217-1225

Other major findings in this symposium include:

- Using a lactulose recovery technique, intestinal permeability was tested between 'eaters' (first day, above 20 g) and 'delayed-eaters' (eating diet the fifth day after weaning) of feed after weaning. The results showed that 'delayed-eaters' had significantly higher lactulose recovery between 2-8 days after weaning, indicating piglets not eating immediately at weaning have higher intestinal permeability and hence are more likely to suffer bacterial translocation from the lumen into the body (Wijtten et al., The Netherlands)
- In-feed use of spray dried plasma reduced ileal macrophage numbers and reduced intestinal permeability and post-weaning diarrhoea (APC; Campbell et al., Spain)

Session V - *Applied aspects of digestive physiology*

Strategic use of feed ingredient and feed additives

Professor de Lange and colleagues (University of Guelph, Canada) addressed the strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. It was emphasized that a complimentary goal in nutrition might be to formulate young pig diets with the specific task of optimizing the growth, function and health of the GIT. Important aspects of gut health-promoting pig diets are: reduced content of fermentable AAs, minimal buffering capacity, minimal content of anti-nutritional factors, and supply of beneficial compounds such as immunoglobulins. The optimum dietary level and type of fiber will vary with the nature of enteric disease challenges and production objectives. These diet characteristics are influenced by feed ingredient composition and feed processing, including feed fermentation and application of enzymes. A large number of feed additives have been evaluated that are aimed at (1) enhancing the pigs' immune responses (e.g. immunoglobulin; ω -3 fatty acids, yeast derived β -glucans), (2) reducing pathogen load in the pigs' GIT (e.g. organic and inorganic acids, high levels of ZnO, essential oils, herbs and spices, some types of prebiotics, bacteriophages, anti-microbial peptides), (3) stimulating establishment of beneficial gut microbes (probiotics, some type of prebiotics), and (4) stimulating digestive function (e.g. butyric acid, gluconic acid, lactic acid, glutamine, threonine, cysteine, nucleotides). When manipulating the gut microbiota, the positive effects of gut health-promoting microbes should be weighed up against the increased energy and nutrient costs to support these microbes.

Sow and their progeny performance

- An Irish research group fed sows (between d 109 gestation and d 24 lactation) a diet containing seaweed extract and fish oil and found that piglets from seaweed extract-fed sows had lower *E coli* populations in the caecum and increased villous height to crypt depth ratio in the ileum compared to piglets from the sows fed a basal diet. However the effect was not significant when sows were fed seaweed extract and fish oil (Leonard et al.).
- A Spanish group (Lizardo et al.) studied the effect of dietary electrolytic balance (dEB) on lactating sow and their progeny performance under hot ambient temperature conditions (23-28 °C). Sows were fed diets containing either a dEB of 120, 200 and 300 mEq/kg. During lactation sows fed a diet with 300 dEB lost less weight (24.4 kg) than sows fed diets with 120 and 200 dEB (27.5 kg, 32.4 kg, respectively). Piglet weaning weight tended to be higher for dEB 300-fed sows (7.92 kg) compared with dEB 120- (7.3 kg) and dBE 200 (7.12 kg)-fed sows. Average daily gain of piglets during the last 14 d of lactation was significantly higher in dEB 300-fed sows (242 g) compared with dEB 120- (212 g) and dBE 200 (191 g)-fed sows. This finding suggests that late gestation and lactation diets in summer should be formulated to contain high dietary electrolytic balance (>200 mEq/kg).
- **Chicory inulin reduces skatole levels in backfat:** this study was conducted by a Norwegian research group (Kjos et al.) in response to a national ban on surgical castration. The aim was to study the effect of chicory inulin (containing 70% inulin) in diets for entire male pigs on the composition of microbiota in the gastrointestinal tract (GIT), and on concentrations of skatole in the GIT and adipose tissue. A total of 48 pigs were fed the same grower diet until four weeks prior to slaughter, and were then switched to the experimental finisher diets until slaughter at approx. 101 kg final BW.

The dietary treatments consisted of a basal finishing diet and the basal diet added with 3%, 6% or 9% of chicory inulin. The skatole levels in backfat were in general low (0.03 µg/g fat), and there were a significant reduction with increasing levels of inulin (linear, $P < 0.006$). The dietary treatments did not influence daily gain and lean percentage, however, there was a tendency towards reduced dressing percentage with increasing levels of inulin (linear, $P < 0.09$). The authors did not conduct sensory tests for boar taint. However, dietary inulin use would need to be cost-effective in comparison to the use of Improvac under Australian conditions to warrant its use, although some producers may favor a dietary method of reducing boar taint to an injection regime.

Moreover, this paper was one of the few (< 5) that dealt with growing-finishing pigs, as the preponderance of papers concerned the weanling pig. The paucity of papers related to GF pigs is due, I believe, to the European situation where there are restrictions on the use of growth promoting antibiotics and other compounds (e.g., ZnO in some countries), virtually forcing these sort of studies to be undertaken. As the DPP conference remains heavily driven by the Europeans, it is therefore not surprising that such a disparity exists.

The value to my attendance at the conference

The DPP conference is the only international pig-specific conference of its kind, hence the obvious value to me as a nutritional digestive physiologist is in keeping up-to-date on research in this and related fields pertaining to pigs. It is also reassuring to know that research being conducted in Australia by the Pork CRC is internationally competitive and relevant.

Implications for the Australian pig Industry and the Pork CRC

I think that the following are worthy of follow-up/discussion in relation to the research programs and directions of the Pork CRC:

1. The IAAO technique has potential for rapid and accurate *in vivo* estimation of AA availability in protein-rich feedstuffs, provided a facility and accompanying laboratory apparatus is available. This technique could be of interest as a corollary to the laboratory-based reactive lysine technique.
2. Metabolomic and T-RFLP techniques, both of which are in use at Murdoch University, are becoming more widely accepted as techniques to monitor metabolic and microbial changes with different dietary treatments. However, interpretation of the data generated from the techniques needs consideration. I believe that techniques such as these are of value for hypothesis testing, but should not drive the research agenda/programs initially.
3. The sheer number of papers and posters dealing with feed additives in weanling pigs cannot be ignored, and undoubtedly will have (and already has started to) a trickle-on effect in the Australian industry. The CRC has generally resisted (wisely) funding a lot of research in this area, but nevertheless and where data are absent, incomplete and (or) obscure, the most promising candidates likely need to have some local testing/verification. Examination of 'Shield Zn' by DAFWA using the ETEC infection model at Murdoch University is a case in point. In this regard, ZnO continues to attract a lot of research interest amongst Europeans despite its seemingly bulletproof positive effects.
4. The finding that the timing of probiotic application could be important for efficacy has potential implications for the DPI-NSW project (Dr Toni Chapman) investigating probiotics after weaning.
5. Sick pigs of all growth stages might have increased sulphur AA (and indeed other AA) requirements than healthy pigs. The CRC has not yet really examined AA requirements of pigs, outside of those associated with the use of metabolic modifiers, to a great degree, and this is a potentially new research area. Profs John Pluske and David Hampson have recently submitted an ARC-Linkage grant examining such a proposition with *E. coli* infection after weaning, however it will be November/December before the outcome of this application is known.
6. The potential roles of insoluble fibre sources (e.g., hulls from lupins) in reducing post-weaning diarrhoea, and possibly other conditions such as PE, are worthy of consideration. Investigations should consider the particle size of the product, given data presented by the University of Manitoba (see comments previously - Molist Gasa et al.)
7. Maintaining dietary electrolytic balance to > 200 mEq/kg for formulation of lactating sows' diets in summer could be of interest for Australian pig industry.
8. The finding that inulin (and potentially other 'fermentable' sources of carbohydrate) could play a role in the control of boar taint is of interest, particularly in light of data from project 2C-107 (carbohydrates and swine dysentery). Duration and cost of feeding and optimum inclusion level would require investigation.

Acknowledgement

Appreciation is given to the Pork CRC and Murdoch University for the financial support of this conference opportunity.

Professor John Pluske

8th June 2009

Appendix 1

Travel Schedule:

Date	Activity
Mon 18 th May	Travel from Perth to Reus, Spain
Tue 19 th May	DPP 2009 meeting
Wed 20 th May	DPP 2009 meeting
Thur 21 st May	DPP 2009 meeting
Fri 22 nd May	DPP 2009 meeting
Sat 23 rd May	Depart for Perth
Sun 24 th May	Arrive Perth

Appendix 2

Publications arising from the conference

Poster: Kim JC, Heo JM, Mullan BP and Pluske JR. The use of trivalent metal markers for estimating the individual feed intake of young pigs. (Abstract 1.20).

Poster: Pluske JR, Hernandez A, Mullan BP and Hansen CF. Determining the weaned pigs' responses to dietary medium-chain triglycerides under conditions of mild challenge with enterotoxigenic *Escherichia coli*. (Abstract 2.31).

Oral presentation: Heo JM, Kim JC, Hansen CF, Mullan BP, Hampson DJ, Maribo H, Kjeldsen N, Pluske JR. Effects of dietary protein level and zinc oxide on the incidence of post-weaning diarrhoea in weaner pigs challenged with an enterotoxigenic strain of *Escherichia coli*. (Abstract 4.04).

Invited Plenary: De Lange CFM, Pluske JR, Gong J and Nyachoti CM. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. (Abstract 5.00).

Poster: Halas D, Hansen CF, Hampson DJ, Mullan BP, Wilson RH and Pluske JR. Effects of benzoic acid and inulin on ammonia-N excretion, plasma urea levels, and the pH in faeces and urine in weaner pigs (Abstract 5.47).

Peer-reviewed 4-page papers will be published in *Livestock Science* late 2009/early 2010