Influence of nutrient asynchrony on finisher growth performance and feed efficiency

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Report prepared for the Co-operative Research Centre for an Internationally Competitive Pork Industry

By

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Executive Summary

Maximising the efficiency for which pigs utilise nutrients for weight gain is critical for profitable pork production. There is evidence from investigations in other species that the asynchronous availability of amino acids and glucose in the small intestine may reduce protein utilisation and therefore the efficiency for which feed nutrients are utilised for lean tissue deposition. The primary aim of this investigation was to determine if finisher feed efficiency could be altered by selecting ingredients with differing rates of starch and protein digestion resulting in diets with synchronous or asynchronous nutrient supply.

An initial screening study was conducted at the University of Queensland to determine the differences in starch and protein digestion rates across a range of ingredients. Based on these results, combinations of three starch sources (slowly digestible starch (SDS, sorghum), moderately digestible starch (MDS, wheat) and fast digestible starch (FDS, barley)) and two amino acid sources (highly digestible amino acids (HDAA, casein) and moderately digestible amino acids (MDAA, vegetable protein - soyabean/canola meal)) were used to create 6 diets differing in their synchrony of glucose and amino acid supply to the small intestine. The test diets were used in two animal feeding studies to determine the effect of nutrient synchrony on whole body nitrogen retention rate during the grower/finisher period and on finisher growth performance, feed efficiency and carcass composition.

The first animal investigation tested the hypothesis that nitrogen retention rate will be greater in pigs fed a nutrient- synchronised diet (fast digestible starch + highly digestible AA or slow digestible starch + moderately digestible AA) compared to pigs fed an asynchronous diet (fast digestible starch + moderately digestible AA; or slow digestible starch + highly digestible AA). A 2 x (6x6) Latin Square experiment was conducted with the six dietary combinations to determine nitrogen balance and nitrogen utilization efficiency in grower/finisher pigs. The outcomes from the investigation showed that a combination of fast digestible starch and highly digestible amino acids improved nitrogen retention rate compared with the combination of slowly digestible starch and moderately digestible amino acids. All other combinations of starch and amino acid sources showed an intermediate nitrogen retention rate.

The second investigation tested the impact of these diets on finisher performance when fed from 64 kg live weight. Pigs were individually housed and offered one of the six test diets in two meals per day to 95 % of calculated *ad libitum* intakes. Individual live weights and feed disappearance were recorded on a weekly basis for a 35 day test period and feed efficiency calculated. At the conclusion of the test period, pigs were slaughtered in a commercial abattoir with carcass weight and back fat depth at the P2 site measured. Superior growth performance and feed efficiency over the full 35 day test period was observed when pigs were offered the MDS+HDAA diet (wheat/casein based diet). Final live weight at day 35 was significantly greater in the treatment group offered the MDS+HDAA diet compared to those offered the FDS+HDAA diet, with the other treatment groups displaying intermediate final live weights. There was a trend for carcass weight to be greater in the MDS+HDAA treatment group (P=0.063), while there was no significant influence of diet on live or carcass P2. Given the commercial use of casein as a protein source for finisher pigs is unlikely in the foreseeable future, comparison of the three diets containing MDAA (vegetable proteins) provided an insight into commercially relevant combinations. Comparison of the performance outcomes from these diets indicated that rate of gain, feed efficiency and the change in P2 back fat over the finisher period was numerically improved (not significant) when pigs were offered the MDS+MDAA diet. Blood urea nitrogen analysis from samples obtained at day 21 of the test period supported the outcomes from the nitrogen retention study, with pigs offered the SDS+MDAA displaying the highest BUN concentration, while those offered the FDS+HDAA the lowest concentration. Together, the outcomes from these investigations suggest that the

common dietary combination of MDS+MDAA (diet based on wheat plus vegetable protein sources) ensures a supply of nutrients to the small intestine that can produce reasonable growth performance and feed efficiency without major negative impacts of nutrient asynchrony.

In conclusion, a combination of fast digestible starch and highly digestible amino acids improved nitrogen retention rate compared with the combination of slowly digestible starch and moderately digestible amino acids. However, superior growth performance and feed efficiency outcomes were obtained with the use of moderately digestible starch (wheat) with either the highly digestible amino acids (casein) or the moderately digestible amino acids (canola meal/soyabean meal). Based on these results and the unlikely commercial use of casein in finisher pig diets, the MDS+MDAA diet combination is recommended to minimize any negative effects of nutrient asynchrony on the performance of finisher pigs.

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1. Introduction

The cost of feed represents approximately 50-60 % of the cost of pig production. Given the high feed usage (approximately 40% of all feed) during the finisher period, maximising the efficiency for which pigs utilise nutrients for lean tissue deposition is critical. If there is a lack of synchrony in nutrient supply, amino acids may be catabolised for use as an energy source, thereby reducing the efficiency for which amino acids are incorporated into lean tissue. Evidence from poultry studies suggest that an asynchronous availability of amino acids and glucose in the small intestine from ingested ingredients will reduce protein utilisation and therefore the efficiency for which nutrients are used for lean tissue deposition (Weunng *et a1.* 2003). In this study broilers were fed either a pea/corn based diet (slowly digestible starch) or a tapioca/ corn diet (rapidly digestible starch). Differences in starch digestion rates were determined through *in vitro* studies in which pea starch was found to be most slowly digested, followed by corn starch and then tapioca pellets. Birds offered the pea/corn diet grew faster and more efficiently than those offered the tapioca/corn diets, suggesting that the use of slowly digestible starch sources improves the synchrony of nutrient supply. In addition, these authors also suggested that energy efficiency may also be improved as glucose is able to be utilized more effectively for direct body processes without the need for energy consuming conversions of glucose to fat and then back to glucose.

A preliminary study in pigs investigated the impact of feeding virtually all of the animals daily protein allowance in one meal in the morning, followed by virtually all of the animals carbohydrate intake in one meal in the afternoon (van den Borne *at a1.* 2007). The results from this study clearly showed a negative impact of asynchronous nutrient supply on faecal apparent digestibility of energy, organic matter and non-starch polysaccharides, as well as a negative impact on the efficiency of utilisation of digestible protein. This design did not however provide any assessment of the impact that nutrient asynchrony may have on finisher feed efficiency when commercial, nutritionally balanced diets differing in ingredient composition and therefore the potential rate of starch and protein digestion are fed. Observations from commercially housed finisher pigs fed via electronic feeders suggests that there are some animals that consume their daily feed intakes in only 2 or 3 meals throughout the day. The number of feeding events per pig per day can range from 2 to 35 in pens of 30 pigs with access to 3 electronic feeders. The number of feeding events per pig per day will also depend on the stocking density of the pen and therefore feeder space per pen. The impact of nutrient asynchrony on animals that consistently graze throughout the day is anticipated to be much less than the impact on animals that only feed a few times a day, with an overlap of nutrient availability from each of the meals reducing any negative effects.

This investigation will firstly screen the *in vitro* rates of starch and protein digestion across a range of ingredients in order to formulate diets differing in their nutrient availability profile. Diets will subsequently be prepared and *in vivo* experiments undertaken to assess the impacts on nitrogen retention rate and finisher growth performance, feed efficiency and carcass composition.

2. Methodology

Experiment 1 – In vitro ingredient screening

An initial screening study was undertaken by the University of Queensland's Queensland Alliance for Agriculture and Food Innovation to determine the rates of protein and starch digestion across a range of dietary ingredients (commercial starch A, cooked rice, wheat, sorghum and barley). Eighteen samples (Table 1) were obtained from Rivalea (Australia) and the University of Sydney (Poultry Research Foundation, Faculty of Veterinary Science,

425 Werombi Road, Camden, NSW 2570). The samples from the University of Sydney consisted of wheat, sorghum and barley grains, which were hammer-milled (2.0 or 3.2 mm sieve) to produce a mash that was conditioned at 85°C, and pelleted. Part of the pellets were (laboratory) hammer-milled using a 1-mm sieve before analysis. The rice was cooked (Rice Master Model RC12, Breville Rice Master, Locked Bag 2000 Botany, NSW 1455) using 3x's its weight of water before drying (50°C; 24 hr). The cooked and uncooked rice, and canola and soyabean meals were (laboratory) hammer-milled with the 1-mm sieve prior to analysis.

Table 1 - Sample information and moisture content

Sample	Hammer mill sieve size (mm)	State	Moisture content (%)
Barley*	2.0	Mash	10.5
Barley*	3.2	Mash	10.5
Barley*	2.0	Pellet	11.0
Barley*	3.2	Pellet	11.2
Sorghum*	2.0	Mash	11.4
Sorghum*	3.2	Mash	11.4
Sorghum*	2.0	Pellet	11.5
Sorghum*	3.2	Pellet	11.9
Wheat*	2.0	Mash	10.8
Wheat*	3.2	Mash	11.1
Wheat*	2.0	Pellet	11.5
Wheat*	3.2	Pellet	11.9
Commercial starch[#]	na[†]	na	10.0
Uncooked Rice[#]	na	na	12.1
Cooked Rice[#]	na	na	8.1
Canola meal[#]	na	na	7.1
Soyabean meal[#]	na	na	11.9
Hydrolysed casein[#]	na	na	4.4

Samples were collected from the University of Sydney (*) or Rivalea (#)
[†]na = not applicable

Moisture content

The moisture content was determined after drying overnight (about 16 hr.) in a vacuum oven (<100mHg, 13.3kPa) at 70°C. The moisture content was used to calculate reported parameters on a dry- or solids-basis.

Protein content

The total nitrogen content was determined in a LECO CNS 2000 combustion analyser, where a known quantity (about 150mg) of representative sample was burnt with pure oxygen at 1100°C which volatilizes all forms of nitrogen. The various nitrogen oxides produced (NO, O_2) were reduced to nitrogen (N_2) gas by passing a small aliquot of the collected gas through a copper catalyst column heated to 700°C. All other gases except the carrier helium gas were scrubbed from the aliquot prior to passage through a thermal conductivity cell, producing a change in the output voltage of the cell which was related back to the response from the Ethylenediaminetetraacetic acid (EDTA) standard used to calibrate the instrument (Press, 1992). A factor of 6.25 was used to compute the protein value.

In-vitro protein digestion

An appropriate weight of each sample, calculated using Eqn.[1], was dispersed in 10 mL of distilled water to give 6.25 mg protein/mL. The dispersion was adjusted to pH ≈ 8.0 with HCl and/or NaOH, and incubated at 37°C for 1 hr in a reciprocating (85 rpm) water bath. A mutienzyme solution (10 mL) was prepared, containing 16 mg trypsin (Type IX, bovine pancreas), 31 mg chymotrypsin (type II, bovine pancreas) and 13 mg bacterial protease (Type XIV, *Streptomyces griseus*); the enzymes were obtained from Sigma-Aldrich (Castle

Hill NSW 1765). The pH of the enzyme solution was also adjusted to ≈ 8.0 with HCl and/or NaOH. One mL of the mutienzyme solution was added to each sample suspension after rehydration, and the pH was recorded automatically every 5 s, over 15 min. Casein (Sigma-Aldrich, C7078) was run as the reference, and a conversion factor was calculated assuming complete (100%) digestion of the casein.

Weight of sample = (6.25 x 10 x 100)/P 1

where P = protein content (g/100 g solids)

The *in-vitro* protein digestibility (IVPD) can be estimated using Eqn.[2]:

$IVPD = 210.46 - 18.10\ pH_{10\ min}$.....2

where $pH_{10\ min.}$ = pH at the 10^{th} minute.

However, during method developments, it was observed (PA Sopade) that Eqn.[2] can be reframed in terms of the change in pH between the starting pH and the pH at 10 min. to yield

Eqn.[3]:

$IVPD = 65.66 + 18.10\ \Delta pH$ 3

where $\Delta pH = pH_{10\ min.} - pH_o$, and pH_o = the starting pH, which is about 8.0.

It was also observed (PA Sopade) that the change in pH with time during protein digestion approximates a first-order kinetic, and with a starting pH (time, t = 0), Eqn.[4], a modified first-order kinetic model, was proposed (PA Sopade) to model protein digestograms:

$pH_t = pH_o + pH_{\infty-o}\ (1 - \exp\ [-K_{protein}\ t])$ 4

where pH_t = pH at time t during protein digestion, pH_∞ ($pH_o + pH_{\infty-o}$) = pH at $t \rightarrow \infty$ and Kprotein = rate of protein digestion.

Starch content

The total starch content of the samples was analysed using a method derived from Megazyme(Megazyme International Ireland Ltd., Ireland). About 50 mg of the sample was wetted with 400 µL of 80% ethanol before it was heated in a boiling water bath in the presence of 2 mL of dimethyl sulphoxide (DMSO). The solubilised and gelatinised starch was digested with 3 mL of thermostable α-amylase (1:30) in MOPS (Sigma M-9381) buffer before 4 mL of sodium acetate buffer and 100 µL of amyloglucosidase (Megazyme E-AMGDF) were added and incubated at 50°C. The glucose content was determined using an enzymatic glucose reagent (TR15104 Enzymatic glucose oxidase reagent; Microgenics Diagnostic Pty Ltd, Lidcombe NSW 2141, Australia) and measuring absorbance (Pharmacia LKB – Ultrospec III) at 505 nm (λmax).

In-vitro starch digestion

About 500 mg of the sample was treated with 1 mL of artificial saliva containing porcine α-amylase (Sigma A-3176 Type VI-B; 250 U per mL of carbonate buffer) for 15-20 sec. before 5 mL of pepsin (Sigma P-6887, from gastric porcine mucosa; 1 mL per mL of 0.02M HCl) was added and incubated at 37°C for 30 min. in a water bath (SWB20; Ratek Instruments Pty. Ltd. Boronia VIC 3155, Australia) reciprocating at 85 rev min-1. The digesta was neutralised (5 mL of 0.02 M NaOH) before adjusting the pH to 6 (25 mL of 0.2M sodium acetate buffer) prior to the addition of 5 mL of pancreatin (Sigma P1750 from porcine pancreas; 2 mg per mL of acetate buffer) and amyloglucosidase (Sigma A-7420 from *Aspergillus niger*; 28 U per mL of acetate buffer). The mixture was incubated for 4 hr., during which the glucose concentration in the digesta was measured with an Accu-Check® Performa® glucometer (Roche Diagnostics Aust. Pty. Ltd., Caste Hill, NSW 2154).

For systems that exhibit monophasic starch digestograms during *in-vitro* starch digestion, a modified first-order kinetic model (Eqn.[5]) can be used to describe the digestograms:

$D_t = D_o + D_{\infty-o} [1 - \exp(-K_{starch}\, t)]$ 5

where D_t = digested starch (g/100g dry starch) at time t (min.), D_o = digested starch (g/100g dry starch) at time t = 0, which is equivalent to salivary-gastric digestion, D_∞ (D_o + $D_{\infty-o}$) = digested starch (g/100g dry starch) at infinite time ($t \rightarrow \infty$), which is equivalent to maximum digested starch, and K = rate of digestion (min^{-1}). It has been widely estimated that digestion in the small intestine (pancreatic digestion) can be up to 4 hr. Therefore, D_{240}, which is the predicted digested starch at 240 min., as well as the area under the starch digestogram, AUC (g.min/100 g dry starch) were used as additional digestion parameters. AUC, which estimates the amount of glucose released into the blood (glycemic index), was calculated using Eqn.[6]:

$$AUC = \left[D_\infty t + \frac{D_{\infty-o}}{K}\exp(-K t)\right]_{t_1}^{t_2} \quad \text{.....6}$$

<u>Experiment 2 - Nitrogen balance study</u>

Animals and treatments

A 2 x (6x6) Latin Square experiment was conducted with six combinations of nutrient-synchronised and asynchronous diets, which were repeatedly tested for nitrogen retention efficiency using 12 individually-housed pigs. Over 3 collection periods (10 days each: 5-day adaptation + 5-day total collection) the experimental design aimed to collect 6 replicate samples per treatment. All pigs were individually housed from 39.5 kg ± 0.36 to 58.0 kg ± 0.60 kg live weight in adjustable metabolism pens. All procedures outlined in this study were approved by the Department of Agriculture and Food Western Australia (DAFWA) Animal Ethics Committee.

Twelve entire male pigs (with an extra three spare pigs) were transported to Medina Research Station at 39.5 kg ± 0.36 kg. The six dietary treatments (Table 2) were randomly assigned to 2 pigs per 10-day metabolism study (5-day adaptation + 5-day collection), which was repeated 3 times to collect 6 samples per diet (Table 3). Diets were formulated to contain similar amounts of available nutrients (13.8 MJ DE/kg and 0.53 g available lysine/MJ DE), but the timing of nutrient availability differed due to the differing ingredient compositions (Table 4). Selection of starch and protein sources for the six test diets were made based on the results of the *in vitro* starch and protein digestion studies in Experiment 1. Based on these results, combinations of three starch sources (slowly digestible - sorghum, moderately digestible - wheat and fast digestible - barley) and two amino acid sources (highly digestible – casein and moderately digestible – vegetable protein (canola and soyabean meal)) were used to create a range of synchrony in availability of glucose and amino acids. Dietary formulations were designed to minimise as much as possible any confounding effects of rate of passage, with fat and NDF concentrations maintained as close as possible across diets. Wheat starch was utilised predominately in the barley diets to achieve the target digestible energy (DE) concentration without overuse of added fat. Oat hulls were also utilised in two of the diets to balance as much as possible NDF concentrations across treatments. The nutrient profile of each of the test diets is displayed in Table 5, while the breakdown of energy and available lysine supply from the major dietary components is displayed in Table 6. Energy was predominately supplied by the grain component in each diet, with some top up from wheat starch and millmix/oat hulls where necessary. The contribution of the protein source to dietary DE was maintained below 25% for all diets. Similarly, available lysine was predominately supplied by the protein source in each diet, with the contribution of synthetic lysine maintained at or below 16% and the contribution of available lysine from

the grain source also minimised as much as possible. The six test diets were common to both experiments 2 and 3.

Table 2 - Dietary treatments

Treatment	Starch	Amino acids
1	Fast digestible starch (FDS) +	Highly digestible AA (HDAA)
2	Fast digestible starch (FDS) +	Moderately digestible AA (MDAA)
3	Moderately digestible starch (MDS) +	Highly digestible AA (HDAA)
4	Moderately digestible starch (MDS) +	Moderately digestible AA (MDAA)
5	Slowly digestible starch (SDS) +	Highly digestible AA (HDAA)
6	Slowly digestible starch (SDS) +	Moderately digestible AA (MDAA)

Table 3 - Treatment allocation for individual pigs for sample collection

Pig #	Collection 1	Collection 2	Collection 3
1	3	1	2
2	6	5	4
3	2	3	1
4	5	4	6
5	4	6	5
6	1	2	3
7	5	4	6
8	3	1	2
9	6	5	4
10	1	2	3
11	2	3	1
12	4	6	5

Table 4 - Composition (g/kg as is basis) of the experimental diets1

	FDS+ HDAA	FDS+ MDAA	MDS+ HDAA	MDS+ MDAA	SDS+ HDAA	SDS+ MDAA
Barley	775	554				
Wheat			684	642		
Sorghum					738	600
Mill mix			49.7	100	67.2	113.4
Wheat starch	70	100	50			
Oat hulls			100		82.9	
Sugar	23.5	55.8				
Casein	68.7		64.8		68.4	
Canola meal		124		150		135
Soybean meal		70		47.7		87.0
Meat meal		50		20		35
Tallow	30	30	18.2	17	10	10
Limestone	17.3	10.7	15	15	16	13.4
DCP	9.8		12.1	2.5	11.3	1.1
Cu-proteinate	0.75	0.75	0.75	0.75	0.75	0.75
Choline chloride 60%	1.3		1.6		2.1	
Lysine	0.13	1.50	-	1.50	0.50	1.10
Threonine	0.33	0.47	0.29	0.13	-	-
Mineral premix	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin premix A	0.125	0.125	0.125	0.125	0.125	0.125
Vitamin premix B	0.2	0.2	0.2	0.2	0.2	0.2
Salt	2	2	2	2	2	2

[1]FDS: fast digestible starch; MDS: moderately digestible starch; SDS: slowly digestible starch; HDAA: highly digestible amino acids; MDAA: moderately digestible AA

Table 5 - Nutrient profile of the experimental diets[1]

	FDS+ HDAA	FDS+ MDAA	MDS+ HDAA	MDS+ MDAA	SDS+ HDAA	SDS+ MDAA
DE (MJ//kg)	13.8	13.8	13.8	13.8	13.8	13.8
Protein (g/kg)	139.7	165.3	147.9	179.0	149.4	189.7
Fat (g/kg)	40.7	46.3	30.0	34.7	35.6	39.5
NDF (g/kg)	139.9	135.6	171.3	159.0	210.0	189.1
Starch (g/kg)	473.2	393.5	487.3	426.3	455.6	387.9
Calcium (g/kg)	9.0	9.0	9.0	9.0	9.0	9.0
Available Phosphorus (g/kg)	3.0	3.3	3.4	3.0	3.1	3.1
Choline g/kg	1.4	1.7	1.4	1.8	1.4	1.6
Available lysine /DE (g/MJ)	0.53	0.53	0.52	0.52	0.53	0.53
Methionine: Lysine (g/g)	0.41	0.30	0.41	0.33	0.43	0.34
Methionine+ Cysteine: Lysine (g/g)	0.64	0.63	0.70	0.74	0.67	0.72
Threonine: Lysine (g/g)	0.70	0.70	0.72	0.72	0.73	0.76
Isoleucine: Lysine (g/g)	0.69	0.64	0.74	0.71	0.82	0.80
Tryptophan: Lysine (g/g)	0.25	0.22	0.27	0.26	0.25	0.25
Valine: Lysine (g/g)	1.02	0.85	1.02	0.93	1.10	1.01
*Analysed composition g/kg^*						
Dry matter	898	910	920	897	888	888
Protein	133.0	164.0	158.0	169.0	178.0	201.0
Fat	40.0	47.0	44.0	34.0	37.0	40.0
Lysine	7.6	8.9	8.4	9.2	8.7	9.1
Methionine	2.6	2.2	2.9	2.6	2.6	2.6
Threonine	5.6	6.6	6.1	6.4	6.3	7.0
Valine	7.9	7.7	8.7	8.2	9.1	9.3

[1]FDS: fast digestible starch; MDS: moderately digestible starch; SDS: slowly digestible starch; HDAA: highly digestible amino acids; MDAA: moderately digestible AA

^Nutritional profile and amino acid analysis undertaken by George Weston Technologies, Enfield NSW 2136

Table 6 - Breakdown of dietary energy and available lysine supply by ingredient type

	FDS+ HDAA	FDS+ MDAA	MDS+ HDAA	MDS+ MDAA	SDS+ HDAA	SDS+ MDAA
Digestible energy						
% DE - Grain	71.3	50.9	71.4	67.0	78.0	63.5
% DE - Starch	7.9	11.2	5.6			
% DE - Millmix/hulls			8.5	8.0	9.1	9.0
% DE - Sugar	2.7	6.4				
% DE - Protein	10.2	23.0	9.6	20.4	10.2	24.8
% DE - Tallow	7.8	7.8	4.8	4.4	2.6	2.6
Available lysine						
% Av lysine - Protein	71.8	64.7	67.7	51.8	71.5	66.7
% Av lysine - Grain	26.9	19.3	32.3	32.5	23.3	21.7
% Av lysine - Synthetic	1.4	16.0		16.0	5.3	11.8

Pigs were retained in an adjusted metal metabolism pen fitted with facilities for total faeces and urine collection. Daily feeding levels were adjusted to three times maintenance [3x(0.5MJ DE/kg Bodyweight$^{0.75}$)/diet DE]. Pigs were weighed before commencement of each collection period to adjust the feeding level based on the individual pig's metabolic bodyweight. On weigh days, pigs were taken out of the pen and placed in a large group pen for 2-3 hours for exercise and interaction with other pigs. After 2-3 hours of exercise pigs were returned to the metabolism pens. This procedure allowed 2-3 hours of exercise time every 5 days. Daily rations were halved and fed twice daily at 08:00 and 16:00 h. Fresh water was available *ad libitum* through nipple drinkers set in the pens. For each collection period, total collection of faeces and urine samples were made for 5 consecutive days following a 5 day adaptation to the experimental diets. Urine was collected in a tub containing 100 mL 4 M HCl to minimize urinary ammonia loss

and sub samples were analysed for N content. Faeces were pooled for 5 days, mixed, sub-sampled and analysed for dry matter and N content. Nitrogen retention rate and faecal and urinary nitrogen excretion rates were calculated using the total collection method.

Statistical analysis

Data were analysed by One-Way Analysis of Variance using Genstat 15 (VSN International Ltd). Individual pig was the experimental unit and collection and pen were used as random factors. As body weight is negatively associated with N retention rate (Carr *et al.*, 1977), N output and N retention data were expressed as g/kg body weight $(BW)^{0.75}$/day. As N intake was positively associated with N retention, N intake was used as covariate for data analysis. Significance was accepted at 5% of probability. Least Significant Differences of means were used to differentiate between treatment means.

Experiment 3 – Influence of nutrient asynchrony on finisher growth performance

Animals and treatments

All procedures and protocols utilised in this investigation were approved by the Rivalea Animal Care and Ethics Committee. A total of 240 male pigs (Large White x Landrace) were selected at 16 weeks of age (57.4 kg ± 0.37 kg) and housed in individual pens, with pigs selected over three replicates. All pigs were offered a commercial grower diet *ad libitum* during a seven day acclimatization period, after which time pigs were individually weighed and allocated to one of six test diets as described in Table 4:

A. Fast starch, highly digestible amino acids (FDS+ HDAA)
B. Fast starch, moderately digestible amino acids (FDS+ MDAA)
C. Moderate starch, highly digestible amino acids (MDS+ HDAA)
D. Moderate Starch, moderately digestible amino acids (MDS + MDAA)
E. Slow starch, highly digestible amino acids (SDS + HDAA)
F. Slow starch, moderately digestible amino acids (SDS+ MDAA)

Management and measures

The test diets were fed at 95 % of estimated *ad libitum* intakes according to a scale that increased from 2.0 kg/d at 50 kg live weight to 3.0 kg/d from 100 kg onwards. Feeding levels were adjusted weekly depending on live weight and feed was offered in two equal portions per day (07:00 and 14:00) to reduce the impact of a continuous nutrient supply should pigs choose to graze on the feed throughout the day. All animals had *ad libitum* access to water via nipple drinkers for the entire experimental period. The facility was naturally ventilated with automatic side curtains.

Individual pig weights were recorded on a weekly basis while P2 back fat depth and leg fat depth were measured on the live animal at day 0 and 35 of the test period. Feed intake was estimated from feed disappearance on a weekly basis and feed efficiency calculated. Blood samples were obtained from 12 animals per treatment group at the start of the test period (Day 0) and again at day 21 for analysis of blood urea nitrogen (BUN) concentration. BUN analysis was undertaken by IDEXX Laboratories, Mount Waverley Vic 3149.

Pigs were slaughtered in a commercial abattoir at the conclusion of the 35 day experimental period. Individual hot standard carcass weight (HSCW) and fat depth at the P2 site (60mm from the midline, obtained using a Hennessy Chong) were obtained. Dressing percentage was calculated from the individual live weight and carcass weight.

Statistical analyses

Data was analysed by REML with the individual animal the experimental unit for all analyses. Replicate was included as the blocking factor for all analysis. All analyses were performed using Genstat 16th Edition, VSN International Ltd, Hertiz, UK (Payne *et al.* 2005).

3. Outcomes

Experiment 1- In vitro ingredient screening

The full experimental report for Experiment 1 (including additional figures) is contained in *Appendix A*. The key outcomes from this report are however detailed below. Table 7 shows the protein content and *in-vitro* protein digestion parameters of the samples. As expected, and being different materials, the protein content of the samples was significantly ($P<0.05$) different. The hydrolysed casein had the highest protein content, while the commercial starch had a protein content of less than 1g/100g solids. Among the cereals (barley, rice, sorghum, and wheat), protein content was least in the rice, while the soybean meal had a higher protein than the canola meal. Despite having the least protein content among the cereals, the uncooked rice exhibited the highest rate of protein digestion, similar to the hydrolysed casein which had the highest protein content. Even though the proteins in the cooked and uncooked rice eventually digested to the same extent, the lower rate of protein digestion with the cooked rice could have resulted from hydrothermally induced molecular/structural changes to the proteins during cooking. Although there are techniques (e.g. electrophoresis) to investigate molecular weight distributions of proteins, the present study did not investigate this. This opens the need to complement *in-vitro* protein digestion with electrophoresis. Since *in vitro* protein digestibility is dependent on the rate of protein digestion, regression analysis revealed both were directly related ($IVPD = 83.9 + 0.02\ K_{protein}$; $r^2 = 0.237$, $p = 0.04$).

Table 7 - Protein content and *in vitro* protein digestibility (IVPD) of the samples

Sample	Sieve	State	Protein (g/100g solids)		$K \times 10^{-3}$ (min^{-1})	IVPD (%)
			ASReml-R[#]	GLM*	GLM*	GLM*
Barley	2.0	Mash	12.8	12.8 ^{EF}	457.8 ^{AB}	92.8 ^{AB}
Barley	2.0	Pellet	12.7	12.6 ^{F}	443.3 ^{AB}	99.9 ^{A}
Barley	3.2	Mash	11.9	11.9 ^{GHI}	357.1 ^{BCD}	88.9 ^{B}
Barley	3.2	Pellet	12.4	12.3 ^{FG}	457.9 ^{AB}	93.0 ^{AB}
Canola Meal	na[†]	na	44.0	44.0 ^{C}	153.0 ^{F}	89.0 ^{B}
Commercial starch	na	na	0.6	0.6 ^{K}	268.1 ^{CDEF}	78.1 ^{C}
Cooked Rice	na	na	10.4	10.4 ^{J}	286.3 ^{CDEF}	93.5 ^{AB}
Hydrolysed casein	na	na	95.4	95.4 ^{A}	536.0 ^{A}	94.0 ^{AB}
Sorghum	2.0	Mash	11.5	11.4 ^{I}	387.6 ^{BC}	86.9 ^{B}
Sorghum	2.0	Pellet	11.8	11.7 ^{HI}	203.4 ^{EF}	90.1 ^{B}
Sorghum	3.2	Mash	12.1	12.0 ^{GH}	208.3 ^{EF}	86.2 ^{BC}
Sorghum	3.2	Pellet	11.8	11.8 ^{GHI}	362.9 ^{BCD}	89.9 ^{B}
Soyabean Meal	na	na	54.0	54.0 ^{B}	241.3 ^{DEF}	89.8 ^{B}
Uncooked Rice	na	na	9.8	9.8 ^{J}	542.3 ^{A}	91.9 ^{AB}
Wheat	2.0	na	14.6	14.6 ^{D}	377.9 ^{BC}	94.5 ^{AB}
Wheat	2.0	Pellet	14.4	14.4 ^{D}	347.1 ^{BCD}	93.5 ^{AB}
Wheat	3.2	Mash	13.3	13.2 ^{E}	360.5 ^{BCD}	91.0 ^{B}
Wheat	3.2	Pellet	14.2	14.2 ^{D}	336.9 ^{BCDE}	92.1 ^{AB}
SEM			0.11	na	na	na
Casein[‡]				96.0 ± 0.49	403.1 ± 6.52	100.0

[#]From the Pork CRC statistical analysis

*For the GLM, figures with the same letters are not significantly ($p>0.05$) different

[†]na = not applicable

[‡]Casein was used as a reference sample, and figures are means ± standard deviations

While hydrothermal treatments during cooking (high moisture and long residence time) were suspected to be detrimental to the rate of protein digestion in the rice, pelleting, another hydrothermal treatment, as a main effect, did not exercise a significant effect (Table 8) on the rate of protein digestion among barley, sorghum and wheat hammer-

milled with the 2.0 mm or 3.2 mm sieve. This could be, compared to cooking, due to the relatively low moisture and short residence time during pelletisation, even though temperatures in both processes were close. Generally, heat-induced molecular/structural changes to protein and starch can be different in low and high moisture environments.

Table 8 - Summary of ANOVA for the digestibility of the grains

Source	df#	Protein		Starch				
		$K_{protein}$	IVPD	D_0	D_∞	D_{240}	K_{starch}	AUC*
Grain (Barley, Sorghum, Wheat)	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sieve (2.0, 3.2mm)	1	0.121	0.005	<0.001	<0.001	<0.001	0.006	<0.001
Stage (Mash, Pellet)	1	0.977	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Grain x Sieve	2	0.555	0.079	<0.001	<0.001	<0.001	0.048	<0.001
Grain x State	2	0.113	0.044	<0.001	<0.001	<0.001	<0.001	0.069
Sieve x State	1	<0.001	0.895	<0.001	<0.001	<0.001	0.038	<0.001
Grain x Sieve x State	2	<0.001	0.432	<0.001	<0.001	<0.001	0.014	<0.001

Although there was no significant effect of (laboratory-) milling the pellets prior to analysis (Table 9), the effects of pelleting on the rate of protein digestion would be grain-specific. This is expected in view of differences in grain molecular architecture, and sensitivity to hydrothermal treatments (heat and mass transfers). Among the cereals, sorghum generally exhibited the least rate of protein digestion, and this might be connected with the well-known pronounced starch-protein interactions in sorghum, which are detrimental to starch and protein digestion in the grain. There are other components (e.g. phytate) of sorghum that are claimed to affect its digestibility, but the exact direction of these effects is not particularly clear. The rate of protein digestion was generally higher in a 2-mm milled grain than a 3.2-mm milled grain because, in the absence of detrimental effects, particle size and digestion are inversely related. Moreover, pelleting would enhance the rate of protein digestion more in a 3.2-mm milled grain than a 2.0-mm milled grain irrespective of the form (milled or non-milled) of the pellets.

Table 9 - Effects of sieve size and laboratory-milling on protein and starch digestibility of the pellets from barley, sorghum and wheat

Sieve (mm)	Form	Protein		Starch				
		$K_{protein}$ x 10^{-3} (min^{-1})	IVPD	D_0	D_∞	K_{starch} x 10^{-3} (min^{-1})	AUC* x 10^3	D_{240}
2.0	Milled	382	93.2	4.5	96.3	17.0	17.6	93.9
2.0	Non-milled	331	94.5	4.6	89.8	14.5	15.9	87.0
3.2	Milled	448	91.2	5.5	96.1	14.1	16.6	91.7
3.2	Non-milled	386	91.7	4.5	82.8	14.2	14.5	79.7

*AUC = Area under the starch digestogram (g.min/100g dry starch); a measure of glycemic index

This report concentrates on the non-milled pellets because pigs are not known to chew their diets during ingestion. Hence, the bulk of the pellets are swallowed whole before disintegration in the stomach at a rate depending on salivary-gastric fluids and pellet texture (e.g. durability). Irrespective of the form of the pellets, the grains were significantly different in their *in-vitro* protein digestibility (Table 8) with sorghum being the least digestible while pelleting or hammer-milling with the 2-mm sieve enhanced *in-vitro* protein digestion. The peculiar characteristics of sorghum as it relates to digestibility have been highlighted above.

Effect of different samples on starch digestion

The starch digestion parameters are summarised in Table 10. Generally, unlike with protein digestion, hydrothermal treatments (cooking and pelleting) enhanced starch

digestion. This is because such treatments favour starch gelatinisation, which increases starch digestion because of desirable molecular and structural changes. Although high-moisture treatments could lead to starch retrogradation under favourable storage/handling conditions, with its detrimental effects on starch digestion, the rice was immediately dried (50°C) after cooking, and this could have limited the retrogradation process.

Table 10 - Starch content and *in vitro* digestibility parameters of the samples

Sample	Sieve (mm)	State	Starch (g/100g solids)		D_0*	D_∞*	D_{240}*	K_{starch} x 10^{-3}#	AUC x 10^3†
			ASReml-R	GLM	GLM	GLM	GLM	GLM	GLM
Barley	2.0	Mash	41.3	41.3 FG	0.0 D	100.0 A	86.8 BCD	8.5 EF	13.7 C
Barley	2.0	Pellet	42.1	42.0 EFG	0.8 D	100.0 A	98.5 A	17.4 AB	18.4 A
Barley	3.2	Mash	41.6	41.6 FG	0.0 D	100.0 A	80.3 CDE	6.8 FG	12.1 DEF
Barley	3.2	Pellet	40.9	40.8 FG	0.3 D	100.0 A	99.0 A	19.2 A	18.9 A
Commercial Starch	na	na	68.5	68.4 A	0.0 D	100.0 A	88.6 B	9.1 EF	14.2 C
Cooked Rice	na	na	60.5	60.4 B	2.4 C	90.5 BC	87.9 BC	15.0 BC	15.9 B
Sorghum	2.0	Mash	44.5	44.5EF	0.0 D	94.9 AB	50.3 G	3.1 HI	6.8 G
Sorghum	2.0	Pellet	47.0	47.0 DE	6.0 AB	77.2 D	73.8 E	12.6 CD	13.2 CD
Sorghum	3.2	Mash	43.2	43.2 EFG	0.0 D	100.0 A	34.7 H	1.8 I	4.5 H
Sorghum	3.2	Pellet	50.4	50.4 CD	6.5 A	64.5 E	61.2 F	12.1 CD	10.9 F
Uncooked rice	na	na	55.2	55.2 C	3.3 C	96.6 AB	79.0 DE	6.9 FG	12.3 DE
Wheat	2.0	Mash	39.3	39.3 G	0.0 D	100.0 A	78.4 E	6.4 FG	11.7 EF
Wheat	2.0	Pellet	39.4	39.4 G	7.0 A	92.2 ABC	88.8 B	13.5 CD	16.0 B
Wheat	3.2	Mash	45.1	45.0 EF	5.3 B	52.6 F	38.8 H	5.1 GH	6.1 G
Wheat	3.2	Pellet	44.2	44.2 EFG	6.8 A	84.0 CD	78.9 DE	11.4	13.8 C
SEM			0.86						

*The units of D_o, D_∞ and D_{240} are g/100g dry starch

#The units of K_{starch} are min^{-1}

†The units of AUC are g.min/100g dry starch

With respect to the barley, sorghum and wheat:

- Hammer-milling with the 2-mm retention sieve produced better starch digestion because of a higher surface area as explained.
- Improvements of starch digestion by pelleting were more with the grains milled with the 3.2-mm sieve. This was also observed for protein digestion, and it could be due to more opened structures with pellets than mash.
- Changes in starch digestion due to the hammer-milling and hydrothermal treatments were grain specific, with the sorghum generally exhibiting the least starch digestion among the grains (including rice). Protein digestion in the sorghum was the least too, and the pronounced starch-protein interactions in sorghum could have contributed to this.
- The non-heat-processed grains (including the commercial starch) had low (average 1g/100g dry starch) salivary-gastric digestion, while the heat-processed grains were 4X better digested during the salivary-gastric stage.

Starch and protein digestion

The different materials and processing conditions investigated in the present study provide a variety of samples that could assist in projecting how starch and protein digestion would progress if they were to occur simultaneously as the case in the gastro-intestinal tract. Gastrointestinal enzymes (e.g. proteases and amylases), like other enzymes, are substrate-specific, and each digestion might occur with minimum interference from the other. With materials such as sorghum, where protein bodies are believed to encapsulate starch granules, protein digestion would enhance starch digestion as impediments to starch-amylase contacts would have been reduced or removed.

The present study revealed how material and processing factors influenced starch and protein digestion similarly or differently. For example, among the cereals studied, sorghum generally exhibited the least starch and protein digestibility. However, with rice, high-moisture heat processing (e.g. cooking) was detrimental to protein digestion, but starch digestion was enhanced. This possibly shows differences in the susceptibility of the macronutrients to high-moisture heat processing. High-moisture heat processing is reported to be detrimental to sorghum digestibility because of protein cross-linking. In the absence of any adverse molecular/structural changes, it is most likely that material and processing factors that favour starch digestion would favour protein digestion.

From the present *in vitro* studies, protein digestion proceeded at a much faster rate (360 ± 93.6 min^{-1}) than starch digestion (10 ± 5.1 min^{-1}). While this could indicate situations in the gastro-intestinal tract (both techniques were calibrated against *in vivo* conditions), it could also be because of differences in the enzyme: substrate ratios or the indicators (changes in acidity for protein, and release of glucose for starch) of the digestion products. While regression analysis revealed no significant ($p>0.05$) relationship between the rates of digestion of both macronutrients, excluding the commercial starch as an outlier, estimates of the *in vitro* protein (IVPD) and starch (AUC) digestion were significantly related (AUC = 0.97 IVPD – 76.6; r^2 0.586, $p = 0.001$). The importance of this relationship, although a mathematical exercise, lays in the fact that material and processing factors that promote starch digestion would favour protein digestion if there were no component-specific detriments.

Protein and starch digestion are dependent on material and processing factors. Generally, hydrothermal treatments will favour digestion if the process moisture is not high enough to promote undesirable molecular/structural changes. Moreover, reducing particle size of milled grains to an economical level that produces a durable pellet will enhance digestibility, irrespective of the macronutrient of interest. The pronounced starch-protein interactions in sorghum, in comparison with other cereals, can adversely affect both starch and protein digestion. Despite the higher rate of protein than starch digestion, maximising digestibility, and, therefore, high feed efficiency or animal performance, demand a careful choice of processing conditions. The present study revealed that hammer-milling with the 2-mm sieve prior to pelleting generally gave high starch and protein digestibility. Possibly because of more intact cell structures that were opened, weakened or disrupted by pelleting, both digestion processes were more favourably sensitive to pelleting when the 3.2-mm sieve was used in milling the grains than when the 2-mm sieve was used. With most of the samples studied, starch and protein digestion were directly related suggesting that material and processing factors that favour starch digestion will enhance protein digestion. The practical significance of this finding is that, in the absence of component-specific detrimental factors, high starch digestion is beneficial to animal performance because protein digestion will be high too.

Experiment 2 - Nitrogen balance study

Two pigs (one each from FDS + HDAA and FDS + MDAA treatments) were removed from the experiment due to diarrhea and death from intestinal torsion during adaptation, respectively.

Diets contained significantly different amounts of nitrogen (from 21.7g to 29.4g /kg as is). This variation in dietary N content significantly influenced the N intake ($P<0.001$) and N retention ($P<0.001$) in this study. As a significant positive relationship between dietary protein content and N retention has been reported (Carr et al., 1977; Zervas and Zijlstra 2002a;b), corrected mean values for N-intake was used for statistical evaluation of treatment difference for dependent variables. Data have been expressed per kg metabolic body weight as body weight is a significant source of variation for whole body N retention.

It is also reported that inclusion of acid detergent fibre or insoluble non-starch polysaccharides did not alter N excretion pattern, whereas neutral detergent fibre or fermentable non-starch polysaccharides shifted N excretion from urine to faeces (Zervas and Zijlstra 2002a;b). Increased dietary fibre content is also known to increase endogenous N flow and decrease N digestibility in grower/finisher pigs (Low, 1989; Nyachoti et al., 1997). Therefore, cautious interpretation is recommended for urinary and faecal N excretion data presented in this report as the experimental diets contain variable fermentable and total fibre contents (Table 5).

Whole body nitrogen balance

Whole body N retention data expressed as g per kg metabolic body weight per day are presented in Table 11. Whole body N retention rate was not different between dietary treatments, although there were alterations in N excretion route from faeal to urinary or vice versa depending on the combination of starch and protein digestibility. The N excretion patterns are confounded with the effect of dietary fibre content and treatment effect and therefore it is difficult to interpret them correctly.

Nitrogen utilization efficiency

Nitrogen digestibility was significantly higher in pigs fed HDAA regardless of the source of starch (Figure 1). Faecal and urinary N excretion, and N retention as a percent of intake and percent of digested, are presented in Figures 2 and 3. Although there was no statistical significance, N retention was greater in pigs fed the FDS+HDAA, while the lowest N retention was observed in pigs fed the SDS+MDAA (Figure 2). Nitrogen retention as a percentage of intake was significantly lower in pigs fed the SDS+MDAA diet compared with pigs fed the FDS+HDAA and SDS+HDAA diets (Figure 3). Nitrogen retention as a percentage of digested was significantly lower in pigs fed the SDS+MDAA diet compared with pigs fed the FDS+HDAA and FDS+MDAA diets (Figure 3).

Table 11 - Whole body nitrogen balance in grower pigs fed the experimental diets1

	FDS+HDAA	FDS+MDAA	MDS+HDAA	MDS+MDAA	SDS+HDAA	SDS+MDAA	SEM	*P*=
PERFORMANCE OF PIGS								
Body weight, kg	51.7^{a}	53.2^{b}	53.3^{b}	52.7^{ab}	51.0^{a}	52.0^{ab}	0.53	0.030
Final weight, kg	57.0	58.5	56.5	57.3	56.6	57.2	0.66	0.310
Daily gain, kg/d^{2}	0.93	1.25	0.85	1.00	0.86	0.98	0.131	0.310
DM intake, kg/d^{2}	1.83	1.85	1.79	1.83	1.80	1.78	0.025	0.282
NITROGEN BALANCE^{3}								
N intake, g/ kg $BW^{0.75}$/day	2.26^{a}	2.74^{cd}	2.62^{b}	2.81^{d}	2.58^{b}	2.99^{e}	0.037	0.001

	FDS+HDAA	FDS+MDAA	MDS+HDAA	MDS+MDAA	SDS+HDAA	SDS+MDAA	SEM	*P*=
Faecal N output, g/ kg $BW^{0.75}$/day	0.58^{ab}	0.74^{c}	0.51^{a}	0.68^{bc}	0.57^{ab}	0.73^{c}	0.052	0.002
Urinary N output, g/ kg $BW^{0.75}$/day	0.59^{a}	0.60^{a}	0.85^{b}	0.68^{a}	0.75^{ab}	0.84^{b}	0.073	0.002
Total N output, g/ kg $BW^{0.75}$/day	1.16	1.34	1.36	1.35	1.31	1.56	0.081	0.216
N retention, g/ kg $BW^{0.75}$/day	1.51	1.33	1.31	1.32	1.35	1.10	0.081	0.216

[1]FDS: fast digestible starch; MDS: moderately digestible starch; SDS: slowly digestible starch; HDAA: highly digestible amino acids; MDAA: moderately digestible AA

[2]Initial weight was used as a covariate for statistical analysis

[3]Nitrogen intake was used as a covariate for statistical analysis

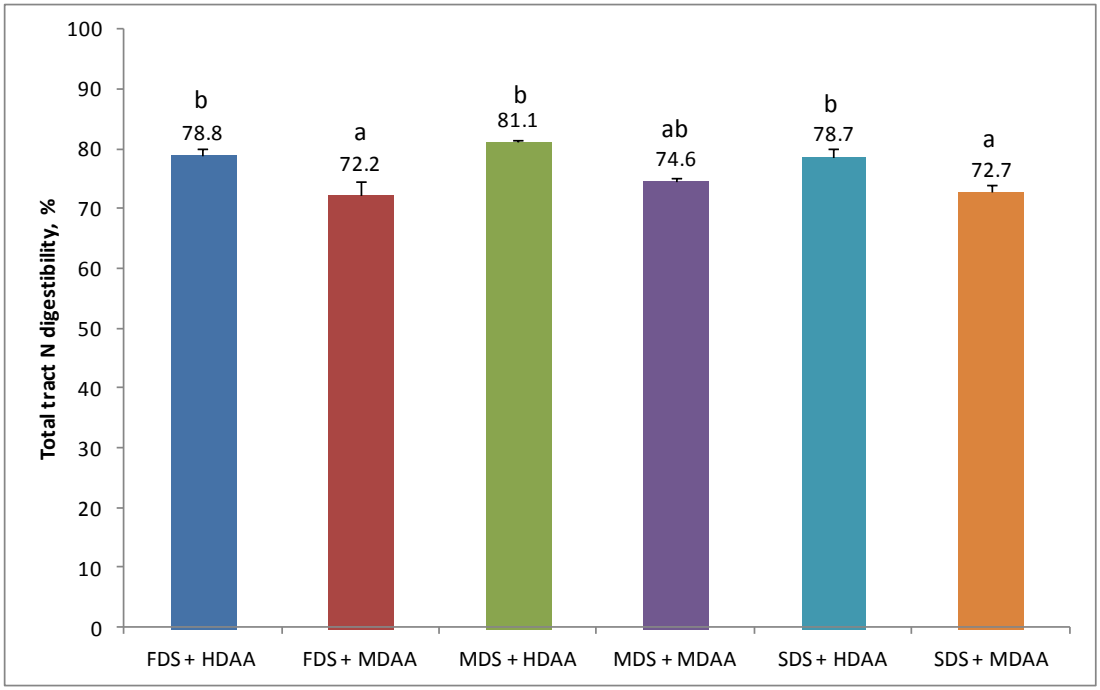

Figure 1 - Total tract nitrogen digestibility of the experimental diets. Diet effect P<0.001.

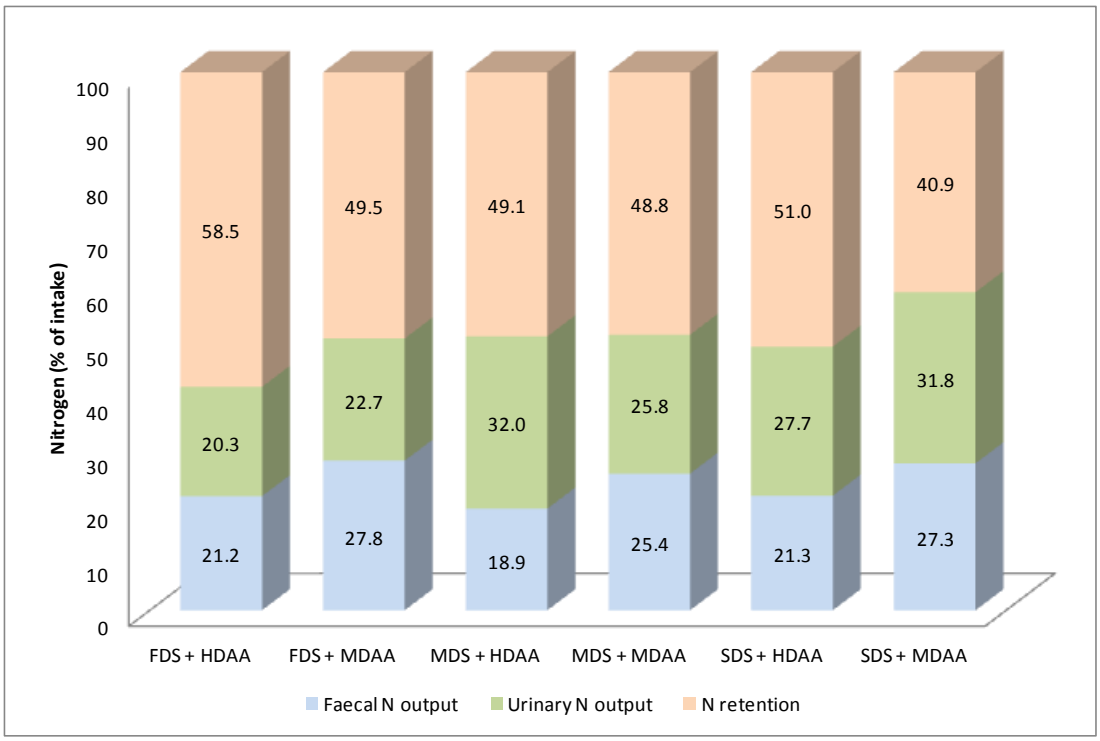

Figure 2 - Nitrogen intake-adjusted faecal and urinary nitrogen output and nitrogen retention (% of intake) determined in grower pigs fed the experimental diets. Diet effect for faecal N output (P<0.001), urinary N output (P<0.01) and N retention as % of intake (P>0.05).

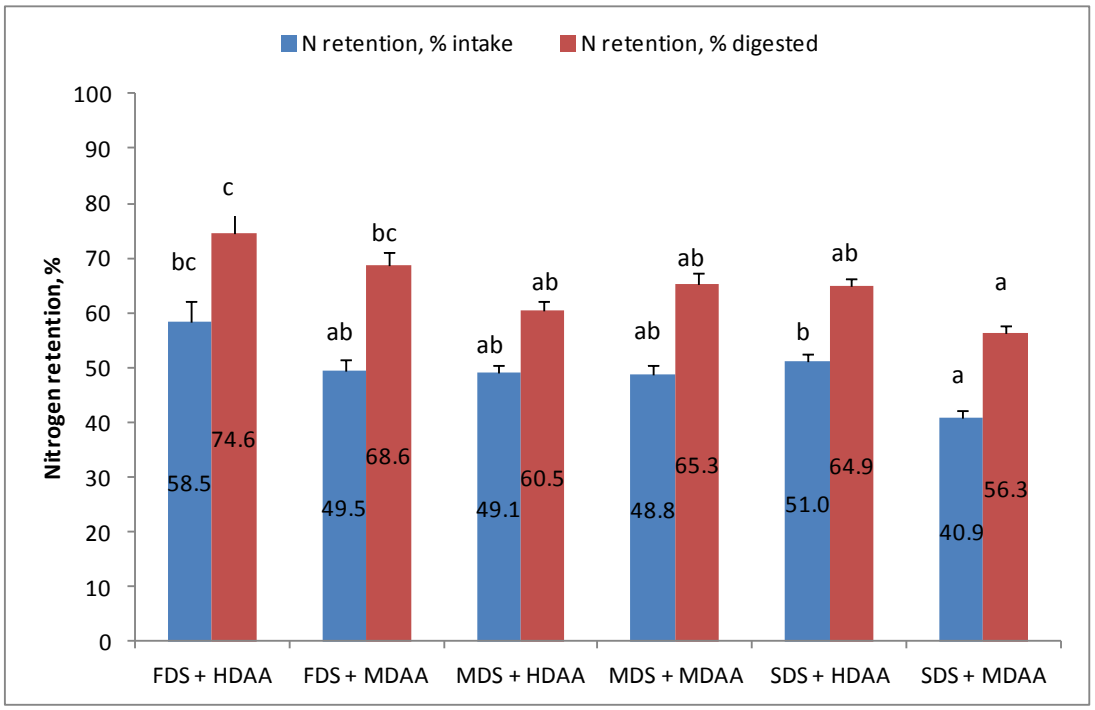

Figure 3 - Nitrogen retention as percent of intake and percent of digested determine in grower pigs fed the experimental diets. Diet effect for N retention as % of intake (P>0.05) and as % of digested (P<0.01).

Experiment 3 - Influence of nutrient asynchrony on finisher growth performance

Two hundred and forty pigs were selected to begin the test period at an average weight of 57.4 kg ± 0.37 kg. There was one death during the test period due to *Actinobacillus*

pleuropneumonia. There were also several animals that were removed during the study for illthrift/ off feed (FDS + HDAA – 2 pigs, FDS + MDAA – 1 pig, MDS+ HDAA – 2 pigs, SDS + MDAA – 3 pigs). The impact of dietary treatment on weekly feed intake, growth performance and feed efficiency is displayed in Table 12. Feed intake was similar across the dietary treatment groups within week, reflective of the restrictive feeding program. The feeding levels allocated were however sufficient to enable satisfactory growth performance in all treatment groups, with average daily gain over the entire test period above 1 kg/t for all treatments (Table 13). Rate of gain during the initial 7 day feeding period was variable across the treatment groups, with pigs offered the FDS+HDAA and the SDS+ HDAA gaining weight more slowly than pigs offered the other dietary treatments despite similar feed intakes. As such, feed efficiency was poorer in these treatment groups ($P<0.001$) during the first week on the test diets. Between 7 and 14 days, pigs offered the MDS + HDAA and the SDS + HDAA grew the fastest and utilized feed for weight gain more efficiency than the other treatment groups. Growth performance and feed efficiency fluctuated on a weekly basis between the dietary treatment groups, with feed intake remaining similar between treatment groups.

The influences of dietary treatment on growth performance and feed efficiency over periods of two to three weeks are displayed in Table 13, providing a clearer indication of the impact of treatment on finisher performance. During the initial two week period, pigs offered the FDS+HDAA and the SDS+HDAA gained weight more slowly and displayed poorer feed efficiency than pigs offered the other diets. During the subsequent period from 14 to 35 days, the pigs offered the FDS+HDAA continued to display poorer performance to the other treatment groups. It is likely that this poorer performance was directly related to the lower analysed lysine content of the FDS+HDAA diet (Table 5) and therefore the pigs were not able to perform to their full potential. During the same period (14-35 days) pigs offered the FDS+MDAA also gained weight more slowly and were less efficient than the highest performers during this period (MDS+HDAA). Growth performance and feed efficiency over the first 28 days and the entire 35 day test period was superior in the MDS+HDAA treatment group. In comparison, the pigs offered the FDS+HDAA displayed the slowest growth performance and the poorest feed efficiency with this result a likely effect of the lower total lysine and protein concentration in this diet.

The influence of dietary treatment on average live weight on a weekly basis and measures of P2 back fat and leg fat depth are displayed in Table 14. Average live weight was statistically similar across the dietary treatments up to day 28 (although the FDS+HDAA treatment group was slipping behind the other treatment groups). Pigs offered the MDS+HDAA were heavier at day 35 than those offered the FDS+HDAA, with the other treatment groups numerically lighter than the pigs offered the MDS+HDAA diet. Measures of P2 back fat and leg fat depth on the live animals did not differ significantly between dietary treatments with or without the adjustment for day 35 live weight (Table 14). The influence of treatment on carcass weight, dressing percentage and carcass P2 are displayed in Table 15. As expected, there was a trend ($P=0.063$) for a higher carcass weight in the pigs offered the MDS+HDAA compared to those offered the FDS+HDAA diet. Dressing percentage was reduced in the MDS+MDAA and the SDS+MDAA treatment groups ($P<0.001$), while carcass P2 back fat depth was not significantly influenced by dietary treatment.

Measures of blood urea nitrogen (BUN) at day 21 supported the nitrogen retention results in Experiment 2. Including BUN concentration at day 0 as a covariate in the analysis, BUN at day 21 was greatest in pigs offered the SDS+MDAA diet, and lowest in the pigs offered the FDS+HDAA diet (Table 14). Care should be taken in interpretation of these results with the differences in analysed lysine and protein concentration of the test diets. Nitrogen retention in experiment 2 was greatest when pigs were offered the FDS+HDAA diet, while BUN concentration was the lowest with this treatment group. These results likely reflect a lower than optimal lysine supply in this treatment group and therefore use

of all available nitrogen by the animal. In contrast the higher BUN concentration in the pigs offered the SDS+MDAA may also be a reflection of the slightly higher total protein content of that diet.

Table 12. Influence of dietary treatment on growth performance and feed efficiency

	FDS + HDAA	FDS+ MDAA	MDS+ HDAA	MDS+ MDAA	SDS+ HDAA	SDS+ MDAA	SED	P-value
Day 0-7								
ROG (kg/d)	0.745	1.005	1.003	0.978	0.792	0.949	0.045-0.046	<0.001
ADFI (kg/d)	1.95	2.02	2.00	2.02	1.98	1.98	0.042-0.043	0.67
FCR (kg/kg)	3.04	2.17	2.13	2.16	2.83	2.17	0.184-0.187	<0.001
Day 7-14								
ROG (kg/d)	1.12	1.14	1.26	1.18	1.20	1.16	0.043-0.044	0.020
ADFI (kg/d)	2.33	2.37	2.37	2.32	2.32	2.32	0.037-0.038	0.50
FCR (kg/kg)	2.14	2.16	1.93	2.03	1.98	2.08	0.079-0.081	0.030
Day 14-21								
ROG (kg/d)	1.047	1.183	1.187	1.147	1.099	1.152	0.052-0.054	0.081
ADFI (kg/d)	2.43	2.51	2.52	2.52	2.50	2.55	0.042-0.044	0.19
FCR (kg/kg)	2.43	2.19	2.41	2.28	2.67	2.28	0.281-0.289	0.61
Day 21-28								
ROG (kg/d)	1.206	1.266	1.361	1.279	1.299	1.254	0.059-0.061	0.21
ADFI (kg/d)	2.60	2.66	2.71	2.65	2.66	2.65	0.046-0.047	0.42
FCR (kg/kg)	2.25	2.20	2.17	2.22	2.13	2.20	0.139-0.143	0.97
Day 28-35								
ROG (kg/d)	1.099	1.079	1.211	1.216	1.143	1.262	0.064-0.067	0.034
ADFI (kg/d)	2.89	2.95	2.99	2.96	2.99	2.98	0.046-0.048	0.37
FCR (kg/kg)	2.77	3.00	2.92	2.49	2.74	2.43	0.202-0.209	0.034

Table 13 - Influence of dietary treatment on periodic growth performance and feed efficiency

	FDS + HDAA	FDS+ MDAA	MDS+ HDAA	MDS+ MDAA	SDS+ HDAA	SDS+ MDAA	SED	P-value
Day 0-14								
ROG (kg/d)	0.926	1.070	1.133	1.081	0.996	1.052	0.030-0.031	<0.001
ADFI (kg/d)	2.15	2.19	2.18	2.17	2.15	2.15	0.034-0.035	0.64
FCR (kg/kg)	2.37	2.08	1.95	2.05	2.22	2.08	0.064-0.066	<0.001
Day 14-35								
ROG (kg/d)	1.114	1.176	1.251	1.213	1.180	1.219	0.027-0.028	<0.001
ADFI (kg/d)	2.64	2.71	2.74	2.71	2.71	2.72	0.036-0.038	0.20
FCR (kg/kg)	2.39	2.33	2.21	2.25	2.31	2.25	0.050-0.053	0.009
Day 0-28								
ROG (kg/d)	1.023	1.147	1.203	1.147	1.098	1.122	0.026-0.027	<0.001
ADFI (kg/d)	2.33	2.39	2.40	2.38	2.37	2.37	0.032-0.033	0.47
FCR (kg/kg)	2.31	2.10	2.01	2.09	2.17	2.13	0.043-0.045	<0.001
Day 0-35								
ROG (kg/d)	1.038	1.133	1.204	1.161	1.107	1.148	0.021-0.022	<0.001
ADFI (kg/d)	2.44	2.50	2.52	2.49	2.49	2.49	0.032-0.033	0.40
FCR (kg/kg)	2.37	2.22	2.10	2.16	2.26	2.18	0.037-0.038	<0.001

Table 14 - Influence of dietary treatment on average live weight and adipose tissue (P2/leg fat) throughout the test period

Live weight (kg)	FDS + HDAA	FDS+ MDAA	MDS+ HDAA	MDS+ MDAA	SDS+ HDAA	SDS+ MDAA	SED	P-value
Day 0	64.7	64.5	64.7	64.8	64.7	64.7	1.30	0.99
Day 7	69.9	71.3	71.7	71.6	70.2	71.2	1.34-1.35	0.68
Day 14	77.5	79.1	80.1	79.9	78.6	79.2	1.45-1.47	0.52
Day 21	85.1	87.4	88.7	87.9	86.3	86.9	1.51-1.54	0.24
Day 28	93.8	96.3	98.3	96.9	95.4	95.7	1.60-1.65	0.14
Day 35	101.5	103.8	106.7	105.4	103.4	103.9	1.60-1.66	0.041
Adipose tissue								
Leg fat day 0 (mm)	10.8	10.8	10.9	11.1	10.9	10.9	0.43-0.44	0.98
Leg fat day 35 (mm)	12.9	13.3	13.4	13.6	13.4	12.8	0.38-0.40	0.25
Leg fat day 35 (mm)^	13.1	13.3	13.2	13.5	13.5	12.9	0.37-0.39	0.44
Change leg fat day 0-35 (mm)*	2.02	2.42	2.56	2.69	2.58	2.00	0.366-0.377	0.27
P2 day 0 (mm)	9.2	9.2	9.2	9.2	9.1	9.1	0.32-0.32	0.99
P2 day 35 (mm)	11.9	12.1	12.1	11.9	12.2	11.9	0.32-0.34	0.92
P2 day 35 (mm)^	12.2	12.1	11.8	11.8	12.3	11.9	0.30-0.31	0.56
Change P2 day 0-35 (mm)*	2.78	2.96	2.91	2.78	3.10	2.84	0.301-0.312	0.90
Blood urea nitrogen								
Day 0 mmol/l	2.55	2.17	2.85	2.45	2.37	2.78	0.252	0.077
Day 21 mmol/l^^	2.59	2.79	3.41	2.97	3.32	3.62	0.222	<0.001

*Fat depth at day 0 included as a covariate

^ Live weight day 35 included as a covariate

^^ Day 0 BUN concentrations used as a covariate

Table 15 - Influence of dietary treatment on carcass measures

	FDS + HDAA	FDS+ MDAA	MDS+ HDAA	MDS+ MDAA	SDS+ HDAA	SDS+ MDAA	SED	P-value
HSCW (kg)	74.5	76.5	78.6	75.9	75.9	75.1	1.33-1.38	0.063
Dressing %	73.4	73.7	73.6	72.3	73.4	72.1	0.42-0.44	<0.001
P2 (mm)	10.9	11.4	11.8	11.2	11.5	10.7	0.43-0.45	0.17
P2 (mm)*	11.1	11.3	11.5	11.3	11.6	10.8	0.41-0.42	0.52

*HSCW included as a covariate in the analysis

4. Application of Research

The results from this investigation provide an insight into the impact of an asynchronous nutrient supply on finisher growth performance and carcass composition. Previous studies have shown that growing pigs offered their protein and carbohydrate requirement in two separate meals during the day utilised protein less efficiently than those offered balanced meals twice a day (van den Borne *et al*. 2007). The aim of this investigation was to extend these results and determine how nutritionally balanced diets differing in ingredient composition and therefore rates of protein and starch digestion (supply of glucose and amino acids to the small intestine) would impact on nitrogen retention, growth performance, feed efficiency and carcass characteristics.

Outcomes from the first *in vivo* investigation suggested that whole body nitrogen retention rate was superior when pigs were offered the combination of fast digestible starch and highly digestible amino acids compared with the combination of slowly digestible starch and moderately digestible amino acids. All other combinations of starch and amino acid sources showed intermediate N retention rate. The blood urea nitrogen analysis from samples obtained in the second animal study confirmed this result, however the FDS+HDAA treatment group did not grow faster or more efficiently than the other treatment groups. While every effort was taken to ensure the diets were as balanced as possible, the use of vastly different ingredients did result in some variability around

dietary protein content, amino acid balance and fibre content. The disparity between the nitrogen retention/ BUN analysis and the growth performance and feed efficiency results are likely confounded by the difference in analysed lysine concentration of the test diets. It is probable that the pigs offered the FDS+HDAA diet were slightly lysine deficient and were therefore not able to grow at the same rate as the other treatment groups.

It is also acknowledged that the use of casein as an ingredient in commercial finisher diets would be cost prohibitive and is unlikely to occur in the foreseeable future in commercial production systems. Taking this into consideration, it is interesting to look at the performance and carcass composition from the three treatments offered the diets containing the moderately digestible amino acids (vegetable protein sources) which are the norm across commercial finisher diets in Australia and elsewhere. Although performance did not differ significantly between treatments, the pigs offered the diet with the most well matched supply of glucose and amino acids (MDS +MDAA) displayed the highest growth rates and the top feed efficiency over the entire experimental period. In addition, pigs in this treatment group also displayed the smallest change in P2 between day 0 and 35 of the test period. These results support the hypothesis that a synchronised supply of glucose and amino acids to the small intestine will provide the best opportunity for optimal growth, feed efficiency and carcass outcomes. Combinations of either fast or slowly digestible starch with moderately digestible amino acids are likely to produce inferior performance depending on the feeding patterns and access to feed under commercial conditions.

In a commercial environment when feed is offered *ad libitum,* pigs are likely to have many feeding bouts over a 24 hr period and the impact of dietary nutrient asynchrony may be markedly lower. Data obtained from commercially housed finisher pigs fed via electronic feeders clearly shows that the number of feeding events for individual finisher pigs can vary from 2 to 35 feeding bouts in a day (Collins, data unpublished). In situations where access to feed is limited by stocking density or feeder space then the synchrony of dietary nutrient supply is likely much more important and producers/ nutritionists should take this into consideration.

5. Conclusion

The outcomes from this investigation suggest that on balance, the most commercially applicable ingredient combination for optimal growth, feed efficiency and carcass characteristics was the use of a finisher diet containing the moderately digestible starch (wheat) and moderately digestible amino acids (canola/soyabean meal). The use of sorghum with vegetable protein sources (SDS+MDAA) significantly reduces nitrogen retention efficiency and should be taken into account in situations where sorghum is the predominant dietary energy source.

6. Limitations/Risks

It is extremely difficult to set up diets differing widely in ingredient composition while maintaining all nutrient specification in a similar range. The interpretation of the results should be viewed carefully due to the variable concentration of dietary lysine, protein and fibre in the finalized diets.

In a commercial situation, finisher pigs would generally have adequate feeder access to enable grazing throughout the day rather than feeding in two distinct meals as was the case in this investigation. The outcomes from this study should therefore be interpreted cautiously as commercially housed finisher pigs may have a more continuous supply of nutrients to the small intestine and therefore the effects of nutrient asynchrony may be more marginal. In situations where feeder access may be limited or pigs are restrictively fed, the outcomes from this investigation should be taken into consideration when formulating diets.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- The use of commercial finisher diets containing moderately digestible starch (wheat) and moderately digestible amino acids (canola/soyabean meal) will maximise growth, feed efficiency and carcass characteristics based on current commercially viable ingredient options.

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