The use of nucleotides, functional amino acids and vitamins to stimulate feed intake, enhance gut development and immunity in the pre- and post-wean piglet for lifetime growth performance

2C-108

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

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December 2009

GOVERNMENT OF WESTERN AUSTRALIA

Department of Agriculture and Food

Pork CRC

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Established and supported under the Australian Government's Cooperative

Executive Summary

The period immediately after weaning can be particularly stressful for the piglet because of the multitude of changes that occur at this time. The growth check that many piglets experience at this time is sufficient to influence growth through to slaughter, having a detrimental impact on profitability. Sow's milk and weaner diets have a number of different components, and those in sow's milk in particular could be beneficial in reducing the stress associated with weaning. If weaner diets could be fortified with at least some of these components then this may help to reduce the growth check post-weaning. This experiment aimed to examine the effect of the addition of nucleotides, inositol or glutamate, either individually or in combination, to weaner diets on gut development, immune function and lifetime pig performance.

The diets were fed for three weeks post-weaning and the pigs were subsequently monitored through until slaughter. There was no significant difference between the treatments in either post-weaning or lifetime performance. It is possible that the lack of a significant difference in growth performance is due to the optimum conditions in which the pigs were housed, as compared to commercial practice. Measures of gut development and immune function were used in an attempt to identify the mechanisms by which each of these additives may be working so as to possibly predict their value for pigs in less ideal environments. Improvements in gut histology and/or immune function were found with the addition of either inositol, glutamate or nucleotides, or the inclusion of all three.

This experiment shows the importance of including more sensitive measures of pig performance where possible. Under commercial conditions it is thought that the most benefit will be obtained when all the additives are included.

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1. Introduction

The period immediately after weaning can be particularly stressful for the piglet because of the multitude of changes that occur at that time. These changes include being separated from the sow and litter mates, mixed with unfamiliar piglets, moved to different accommodation with different environmental conditions, and presented with different feed types and methods of presentation. As a consequence the growth rate of piglets immediately post-weaning is less than that pre-weaning and certainly well below their genetic potential. This growth check at weaning can adversely affect their long-term performance and hence the profitability of commercial piggeries.

A key contributor to the growth check in weaner pigs is the structural and functional changes in the small intestine, such as villous atrophy and crypt hyperplasia, which results in a decrease in digestive and absorptive capacity. The importance of food intake and dietary exogenous growth factors as mediators of intestinal structure and function post-weaning have been widely reviewed and have been shown to reduce the impact of the post-weaning growth check (Cranwell, 1995; Pluske *et al*., 1997). For example, the provision of oral glutamine to the young piglet has been shown to support mucosal growth thereby maintaining villous integrity and the structure and function of the small intestine (Pluske *et al*. 1996). Glutamate also has a distinct flavour which is thought to enhance palatability of the feed resulting in improved feed intake. Another nutrient that has been suggested as having a role in reducing the post-weaning growth check is inositol, a vitamin that is a fundamental component of cell membranes and is necessary for the proper function of nerves, brain and muscles. The inclusion of nutrients may also aid in the development of the immune system which will also help reduce the post-weaning growth check.

Numerous human and animal studies suggest that dietary nucleotides play a role in the development of the gastrointestinal and immune systems, and that collectively they reduce the growth check that occurs in almost all baby mammals post-weaning (Rutz *et al.*, 2006). The nucleotides which are present in sow's milk are 5'AMP, 5'CMP, 5'GMP and 5'IMP. The concentration of these nucleotides in sow's milk is many times greater than that in a high quality creep feed (Mateo *et al.*, 2004), suggesting that if we could increase the levels in creep feed then it might help piglets with the transition at weaning.

While glutamate, inositol and nucleotides may all play a role in reducing the growth check of piglets at weaning, it is also possible that the supply of all three in increased concentrations at the same time may have additive effects. One product that is currently being used in the Australian pig industry that contains all three components is NuPro® (Alltech Inc.), which is derived from the inner cell contents of the *Saccharomyces cerevisae* yeast. One of the first experiments conducted with this product was by Carlson *et al*. (2005) who compared diets that contained either 5% Nupro® or 5% plasma protein to that of a control diet (vegetable diet only) in weaner pigs. The piglets fed the plasma and NuPro diets had significantly higher feed intake, average daily gain (ADG), and liveweight (LW) at the end of the supplementation period (28 days), and those on the Nupro® treatment had improved performance thereafter reaching the target slaughter weight 5-7 days earlier than either the control or plasma protein treatments. Other studies have since shown similar responses, but no studies have been conducted to try and elucidate which of the above mentioned growth factors might be giving this response.

Performance of many piglets in the period immediately after weaning is less than what we believe is satisfactory. The growth check that many piglets experience is sufficient to influence growth through to slaughter, which can thus have a detrimental impact on profitability. Sow's milk contains a number of different components and some of these can be described as growth factors because they do more than supply nutrients, but may be

important for helping reduce the various stressors experienced at weaning. If we can identify those that play a role, then fortification of weaner diets with these components could help reduce the growth check post-weaning. The hypothesis for this experiment was that the addition of nucleotides, inositol or glutamate to creep and weaner diets will improve gut development, immune function and lifetime pig performance compared to a control diet, and that the response would be greatest when these three factors were provided in combination.

2. Methodology

Animals and experimental design

Ninety Large White x Landrace x Duroc surgically castrated male pigs were used in a completely randomized block experiment. The experimental treatments were:

1. Control: Standard weaner 1/weaner 2 diet
2. Inositol: Standard weaner 1/weaner 2 diet + inositol
3. Glutamate: Standard weaner 1/weaner 2 diet + glutamate
4. Nucleotides: Standard weaner 1/weaner 2 diet + nucleotides
5. Combined: Standard weaner 1/weaner 2 diet + inositol + glutamate + nucleotides

Housing

The pigs were transported to the Medina Research Station at weaning (3 weeks of age) where they were randomly allocated to treatment after stratification on LW. They were housed in individual pens (0.6 ×0.6 m) in an environmentally controlled weaner facility. Ambient temperature was maintained at 30°C for the first week after weaning, and then dropped by 1°C each week thereafter. At 6 weeks of age, when the feeding of experimental diets was complete, 8 piglets from each treatment were removed for detailed examination, and the pen area per pig was thus doubled for the next two week period. At this stage (8 weeks of age) all remaining pigs were moved to a naturally ventilated grower-finisher shed, where they were again housed in individual pens (1.8 × 0.9 m) until slaughter. All pigs had *ad libitum* access to feed and water.

Diets

The experimental diets were fed as a mash for 21 days after weaning, with a first stage weaner diet (Weaner 1) being fed for the first 7 days after weaning followed by a second stage weaner diet (Weaner 2) that was fed for the next 14 days. The Weaner 1 diets were formulated to contain 15.2 MJ DE/kg and 0.9 g available lysine/MJ DE while the Weaner 2 diets contained 14.9 MJ DE/kg and 0.85 g available lysine/MJ DE. The composition of the Weaner 1 and Weaner 2 diets are given in Tables 1 and 2, respectively. For the inositol, glutamate and nucleotide diets, the additives were included in the diet and replaced the same quantity of wheat. For the combined treatment, the diet was re-formulated to take account of the larger quantity being added and because the additive was also a source of protein. At the end of the experimental diet phase (i.e. 6 weeks of age), all pigs were fed the same commercial diets until slaughter.

At approximately 20 weeks of age (an average of 101 kg LW) all feed was removed from feeders in the evening, and the following morning all pigs were transported to a commercial abattoir. The pigs were stunned using a carbon dioxide, dip-lift stunner set at 85% CO_2 for 1.8 minutes (Butina, Denmark). Exsanguination, scalding, dehairing and evisceration were performed using standard commercial procedures.

Measurements

The pigs were weighed weekly from 3 weeks of age until slaughter. Voluntary feed intake was recorded on a weekly basis and feed:gain ratio was calculated by dividing the total weight of feed eaten by the LW gain in the same period. Carcass weight and backfat depth at the P2 site were determined on the hot carcass at 45 minutes after slaughter. Backfat depth at the P2 site was measured using PorkScan.

Blood sampling

After the initial 7 day feeding period, 8 piglets from each treatment were selected by identifying the median weight and then randomly selecting 4 pigs from above the median and 4 pigs below the median and blood sampled via jugular vein puncture. Blood from the same 8 piglets was also collected at the conclusion of the 3 week feeding period. The blood samples were centrifuged and the serum was stored at -20°C until analysed. Haptoglobin in serum was quantified using a spectrophotometric method with a commercial kit (Phase™ Haptoglobin Assay, Tridelta Limited, Ireland). The assay was performed on an automated analyser according to the manufacturer's instructions (Olympus AU400; Olympus UK Ltd, Hertfordshire, United Kingdom). Immunoglobulins G, A and M (IgG, IgA and IgM) were determined by Elisa (Bethyl Laboratories Inc., Texas, United States).

Table 1: The composition of Weaner 1 diets fed for the first 7 days post-weaning

Treatment	Control	Inositol	Glutamate	Nucleotides	Combined
Ingredients (g/kg):					
Wheat (12% CP)	433.3	433.2	430.6	431.3	422.9
Cooked oats	150.0	150.0	150.0	150.0	150.0
Full fat soya	100.0	100.0	100.0	100.0	100.0
Soycomil	80.4	80.4	80.4	80.4	52.7
Fishmeal	100.0	100.0	100.0	100.0	100.0
Whey powder	80.0	80.0	80.0	80.0	80.0
Meat meal	41.6	41.6	41.6	41.6	38.7
Vegetable oil	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral[a]	1.00	1.00	1.00	1.00	1.00
Lysine	1.046	1.046	1.046	1.046	1.138
Methionine	0.055	0.055	0.055	0.055	0.022
Threonine	0.491	0.491	0.491	0.491	0.434
Limestone	1.00	1.00	1.00	1.00	2.04
Salt	1.50	1.50	1.50	1.50	1.50
Rovabio	0.50	0.50	0.50	0.50	0.50
Betaine	2.00	2.00	2.00	2.00	2.00
Acid-Pak 4-way	2.00	2.00	2.00	2.00	2.00
Inositol	0	0.122	0	0	0
Glutamate	0	0	2.7	0	0
Ascogen®[b]	0	0	0	2.0	0
Nupro®[c]	0	0	0	0	40.0

Treatment	Control	Inositol	Glutamate	Nucleotides	Combined
Nutrient composition[d]					
DE (MJ/kg)	15.7	15.7	15.7	15.7	15.5
Crude protein (%)	25.7	25.7	25.7	25.7	25.7
Avail. Lysine (%)	1.37	1.37	1.37	1.37	1.36

[a] Each kilogram of vitamin and mineral premix contains 7 MIU Vitamin A, 1.4 MIU Vitamin D_3, 20 g Vitamin E, 1 g Vitamin K, 1 g Vitamin B_1, 3 g Vitamin B_2, 1.5 g Vitamin B_6, 15 mg Vitamin B_{12} 12 g niacin, 10 mg pantothentic acid, 0.19 g folic acid, 30 mg biotin, 10.6 g Calcium pantothenatic, 60 g iron, 100 g zinc, 40 g manganese, 10 g copper, 0.2 g cobalt, 0.5 g iodine, 0.3 g selenium, and 20 g antioxidant.
[b] The source of nucleotides was ASCOGEN® (Chemoforma Ltd)
[c] The combined source of inositol, glutamate and nucleotides was NuPro^{2000} (Alltech Pty Ltd).
[d] Calculated composition.

Table 2: The composition of Weaner 2 diets fed from 7 to 21 days post-weaning

Treatment	Control	Inositol	Glutamate	Nucleotides	Combined
Ingredients (g/kg):					
Wheat (12% CP)	586.0	586.0	584.4	584.8	557.0
Cooked oats	100.0	100.0	100.0	100.0	133.3
Full fat soya	96.4	96.4	96.4	96.4	73.3
Soycomil	10.0	10.0	10.0	10.0	10.0
Fishmeal	100.0	100.0	100.0	100.0	100.0
Whey powder	30.0	30.0	30.0	30.0	30.0
Meat meal	48.7	48.7	48.7	48.7	47.7
Vitamin-mineral[a]	1.00	1.00	1.00	1.00	1.00
Lysine	1.293	1.293	1.293	1.293	1.177
Methionine	0.043	0.043	0.043	0.043	0
Threonine	0.520	0.520	0.520	0.520	0.426
Salt	1.50	1.50	1.50	1.50	1.50
Rovabio	0.50	0.50	0.50	0.50	0.50
Betaine	2.00	2.00	2.00	2.00	2.00
Acid-Pak 4-way	2.00	2.00	2.00	2.00	2.00
Inositol	0	0.073	0	0	0
Glutamate	0	0	1.6	0	0
Ascogen®[b]	0	0	0	1.2	0
Nupro®[c]	0	0	0	0	20.0
Nutrient composition[d]					
DE (MJ/kg)	15.7	15.7	15.7	15.7	15.5
Crude protein (%)	25.7	25.7	25.7	25.7	25.7
Avail. Lysine (%)	1.37	1.37	1.37	1.37	1.36

[a] Each kilogram of vitamin and mineral premix contains 7 MIU Vitamin A, 1.4 MIU Vitamin D_3, 20 g Vitamin E, 1 g Vitamin K, 1 g Vitamin B_1, 3 g Vitamin B_2, 1.5 g Vitamin B_6, 15 mg Vitamin B_{12} 12 g niacin, 10 mg pantothentic acid, 0.19 g folic acid, 30 mg biotin, 10.6 g Calcium pantothenatic, 60 g iron, 100 g zinc, 40 g manganese, 10 g copper, 0.2 g cobalt, 0.5 g iodine, 0.3 g selenium, and 20 g antioxidant.
[b] The source of nucleotides was ASCOGEN® (Chemoforma Ltd)
[c] The combined source of inositol, glutamate and nucleotides was NuPro® (Alltech Pty Ltd).
[d] Calculated composition.

Gut histology

After the three week feeding period the same eight piglets that were blood sampled were euthanized and a mid-line incision was performed to locate the gastrointestinal tract. The entire gastro-intestinal tract was removed and samples of the duodenum, jejunum and ileum collected. The samples were placed in a 70% ethanol solution for subsequent storage. The tissue samples were processed onto paraffin wax blocks and cut into five µm thick cross sections using a microtome. The sections were then mounted onto Polylysine coated slides and stained with hematoxylin and eosin. The villous height and crypt depth were determined using a binocular light microscope at a magnification of x10. The crypt depth was measured from the junction with the villous to the base of the crypt. Villous height was measured from the crypt orifice to the tip of the villous. Approximately 10 measurements were taken on each slide and the mean villous height and crypt depth determined. The villous height to crypt depth ratio was also calculated.

Statistics

One-way analysis of variance (ANOVA) with the Genstat program was used to analyse the main effect of diet. Position within the shed was used as a block in the analysis.

3. Results

Post-weaning period (3 to 6 weeks of age)

The overall health status of piglets in this experiment was excellent and it was not necessary to remove any animals from the experiment for health reasons. There was no significant difference in either the initial weight (P=1.00) nor final weight between the experimental diets at the end of the post-weaning period (P=0.216) (Table 3). There was also no significant difference in ADG with treatment, however there was a trend for the pigs on the glutamate or combined diets to grow approximately 25 g/day faster compared to the other diets over this period (P=0.150). There was no significant effect of treatment on feed intake (P=0.221), but pigs fed the combined diet had a significantly lower feed to gain ratio compared to those fed the control, inositol and nucleotide diets (P=0.028). There was no significant difference in the feed to gain ratio between pigs fed the glutamate diet or the combined diet.

Table 3: Liveweight (LW), average daily gain (ADG), voluntary feed intake (VFI) and feed to gain (F:G) for weaner pigs fed experimental diets for the first three weeks post-weaning (n=18)

Parameter	Control	Inositol	Glutamate	Nucleotides	Combined	SED	P-value
LW (kg):							
Day 0	6.14	6.13	6.13	6.14	6.14	0.097	1.00
Day 21	10.1	10.1	10.7	10.1	10.6	0.702	0.216
ADG (g)	190	187	217	189	214	15.5	0.150
VFI (g/day)	363	360	393	367	348	19.3	0.221
F:G	1.97[a]	2.00[a]	1.84[ab]	2.04[a]	1.72[b]	0.108	0.028

Weight change during the 3 week post-weaning period is shown in Figure 1. There was a trend for pigs on the glutamate and combined diets to be heavier at the end of the three-week period but this was not significant. Daily gain at 7 day intervals during the 3 week post-weaning period is shown in Figure 2. From Day 7 to 14 there was a trend for the pigs fed the glutamate diet or combined diet to grower faster than those fed the other diets (P=0.082).

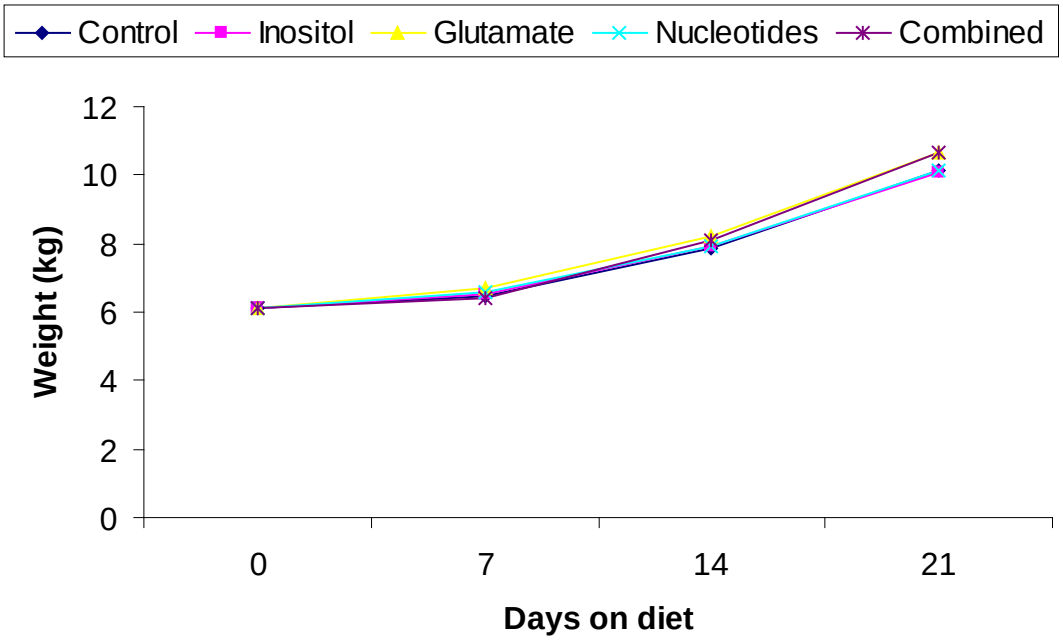

Figure 1: Change in weight for each diet over the three week post-weaning period (n=18).

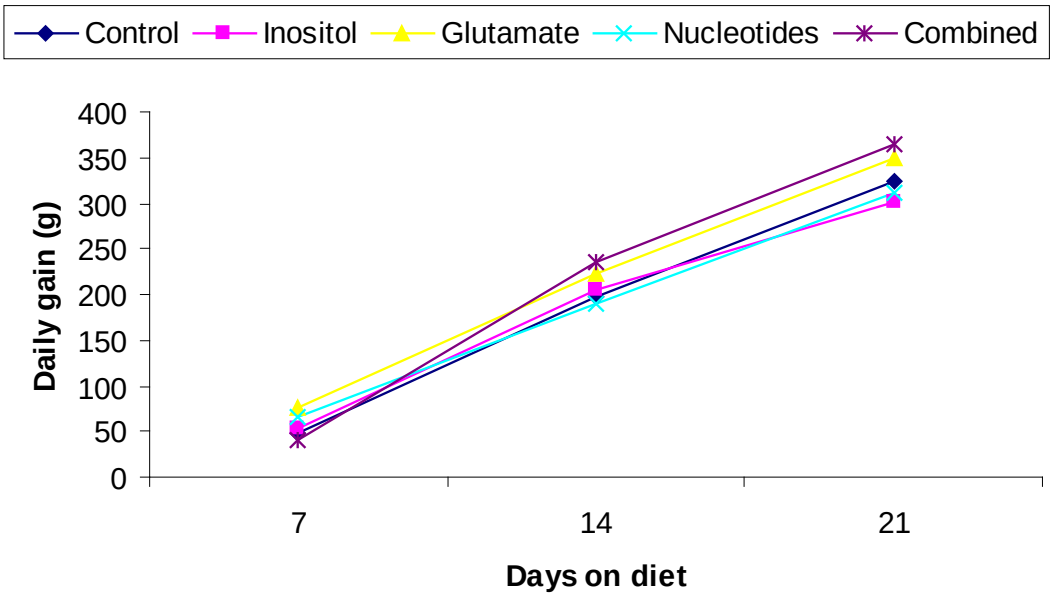

Figure 2: The weekly average daily gain for the three week post-weaning period for each treatment (n=18).

The individual data for ADG over the three-week post-weaning period was broken down into various categories, and the proportion of pigs in each growth category calculated (Figure 3). There was a strong indication that groups fed the glutamate or combined diets had less pigs in the lower growth category (i.e. < 149 g/d) and a higher proportion of pigs growing faster than 200 g/d.

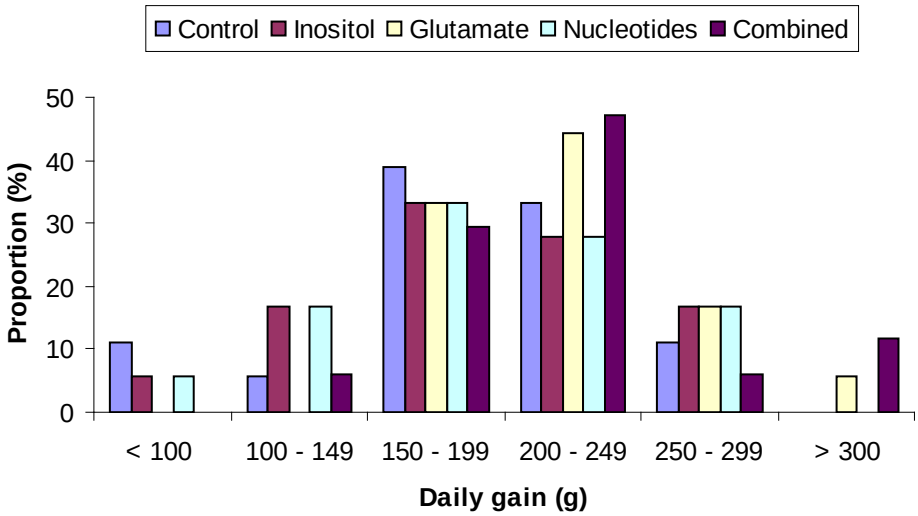

Figure 3: The proportional growth rate of piglets in the 21 days after weaning for each experimental diet (n=18).

In the post-weaning period the co-efficient of variation for ADG appeared to be reduced by at least 5 percentage points when pigs were fed diets containing either the glutamate or

the combination of additives (Table 4). However, from weaning to slaughter the co-efficient of variation was similar for all diets.

Table 4: Co-efficient of variation (%) for ADG for pigs in the 21 day post-weaning period (n=18) and for those pigs that grew from weaning to slaughter (n=10)

Parameter	Control	Inositol	Glutamate	Nucleotides	Combined
Weaning to day 21	25.9	28.7	18.3	30.9	20.3
Weaning to slaughter	6.17	4.15	4.62	6.03	5.89

Gut histology

The duodenum villous height varied between the treatments with pigs fed the combined treatment having a greater villous height (P=0.029) compared to the control and inositol treatments. There was no difference in villous height between pigs fed the glutamate and nucleotide diet compared to the combined diet. There was no difference in any other gut parameter examined (Table 5).

Table 5: Gut histology of pigs after receiving the experimental diets for 21 days post-weaning (n=8)

Parameter	Control	Inositol	Glutamate	Nucleotides	Combined	SED	P-value
Duodenum							
Villous height (µm)	465[a]	461[a]	517[ab]	521[ab]	569[b]	36.0	0.029
Crypt depth (µm)	211	233	259	225	231	24.4	0.422
Villous:crypt ratio	2.39	2.16	2.09	2.50	2.59	0.255	0.247
Jejenum							
Villous height (µm)	410	438	467	423	433	31.0	0.464
Crypt depth (µm)	236	241	227	224	231	14.3	0.752
Villous:crypt ratio	1.85	1.89	2.19	1.99	2.20	0.251	0.501
Ileum							
Villous height (µm)	375	389	405	399	410	26.0	0.674
Crypt depth (µm)	220	201	201	224	207	18.4	0.640
Villous:crypt ratio	1.85	2.13	2.13	1.93	2.22	0.249	0.552

Acute phase proteins

Haptoglobin concentration 7 and 21 days after weaning was not affected by diet (P>0.05, Table 6). Haptoglobin concentration decreased from Day 7 to Day 21. IgA and IgM were not significantly different between each of the experimental diets on either Day 7 or Day 21 after weaning (P>0.05). While there was no significant difference between diets for IgG on Day 7 by Day 21 the IgG levels were significantly increased in pigs that received the inositol and glutamate diet compared to the control and nucleotide diet (P=0.034). There was no difference in the IgG level in the combined diet compared to the other diets. From Day 7 to Day 21 IgA and IgM levels increased while IgG decreased.

Table 6: Haptoglobin and immunoglobulin concentrations in pig serum of pigs after receiving the experimental diets for 7 and 21 days post-weaning (n=8)

Parameter	Control	Inositol	Glutamate	Nucleotides	Combined	SED	P-value
			Haptoglobin (mg/mL)				
Day 7	1.45	2.14	1.24	1.45	1.55	0.479	0.407
Day 21	0.759	1.06	0.514	0.574	0.864	0.230	0.142
			Immunoglobulin A (mg/mL)				
Day 7	0.151	0.157	0.230	0.150	0.158	0.036	0.149
Day 21	0.465	0.384	0.387	0.338	0.399	0.087	0.698
			Immunoglobulin G (mg/mL)				
Day 7	5.11	6.93	7.12	6.27	6.89	0.927	0.204
Day 21	3.57[a]	4.56[b]	4.37[b]	3.47[a]	4.05[ab]	0.395	0.034
			Immunoglobulin M (mg/mL)				
Day 7	1.12	1.05	0.97	1.11	0.96	0.156	0.775
Day 21	1.89	2.12	1.73	1.67	1.91	0.242	0.376

Lifetime performance

Apart from those pigs that were euthanized at 21-days post-weaning, the other pigs remained on the experiment for 118 days. The initial weight and final weight were not different between diets (P=0.984 and P=0.752, respectively; Table 5). Average daily gain, voluntary feed intake and the feed to gain ratio were not significantly different over time. However, there was a trend for pigs receiving the nucleotide diet to have a lower feed to gain ratio from the end of the feeding period until slaughter compared to the other diets (P=0.072). There was also no significant difference in either carcass weight, dressing percentage or backfat between experimental diets (P=0.830, P=0.959 and P=0.927, respectively).

Table 7: Live weight (LW), average daily gain (ADG), voluntary feed intake (VFI), feed to gain (F:G) and carcass quality for those pigs that were on the experiment from weaning until slaughter 118 days later (n=10)

Parameter	Control	Inositol	Glutamate	Nucleotides	Combined	SED	p-value
Days on trial	118	118	118	118	118		
LW d-0 (kg)	6.23	6.07	6.06	6.23	6.18	0.401	0.984
LW d-21 (kg)	10.2	10.2	10.9	9.95	10.9	0.491	0.239
LW d-118 (kg)	101.5	98.6	100.9	100.9	101.5	2.47	0.752
ADG d-0 to d-118 (g)	807	781	803	802	808	19.7	0.644
ADG d-21 to d-118 (g)	943	908	927	937	934	23.4	0.637
VFI d-0 to d-118 (kg/d)	1.95	1.87	1.95	1.84	1.93	0.049	0.116
VFI d-21 to d-118 (kg/d)	2.29	2.19	2.28	2.17	2.27	0.060	0.137
F:G d-0 to d-118	2.41	2.39	2.42	2.30	2.40	0.051	0.135
F:G d-21 to d-118	2.43	2.41	2.46	2.31	2.44	0.053	0.072

Parameter	Control	Inositol	Glutamate	Nucleotides	Combined	SED	p-value
Carcass weight (kg)	65.1	63.2	65.0	64.7	64.4	1.83	0.830
Dressing percentage	64.2	64.1	64.5	64.1	64.0	0.667	0.959
Backfat (P2, mm)	11.9	11.3	12.3	12.2	11.8	1.25	0.927

The average feed to gain ratio for all treatments for each week is given in Figure 4. Feed to gain increased over time but was quite variable between each week. There is some relationship between the average maximum temperature for each week and the feed to gain obtained. In general, at higher maximum temperatures the feed to gain was worse.

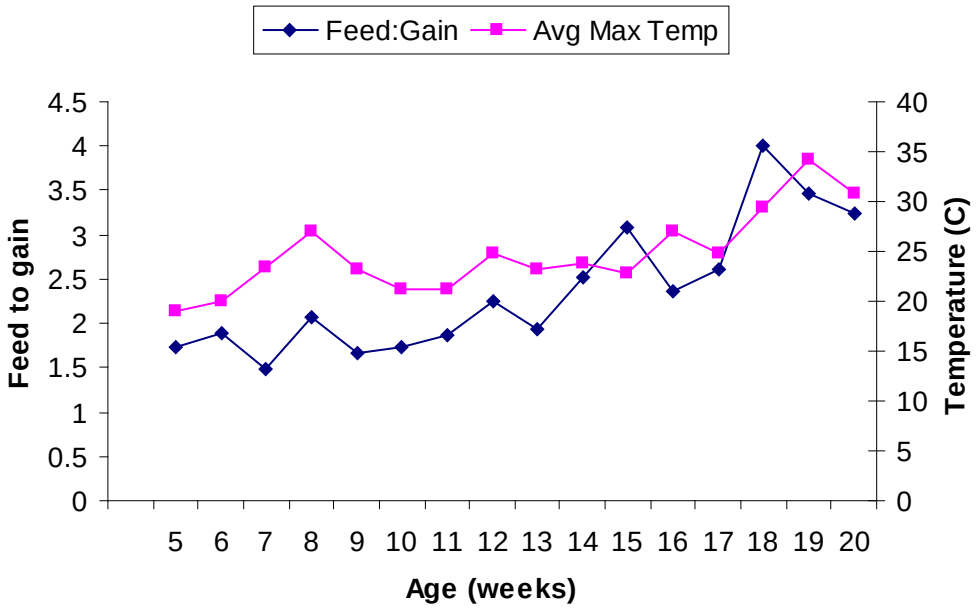

Figure 4: The average feed to gain ratio for all treatments (n=10) and average maximum temperature for each week of the experiment.

4. Discussion

This experiment aimed to determine the effect of the addition of nucleotides, inositol, glutamate or a combination of these on pig performance, gut development and immune function. Pig performance was generally not affected by treatment, although those fed the combined diet had a lower feed to gain in the weaner phase. There was also no difference in overall lifetime performance between the diets. There was an apparent reduction in the proportion of pigs that had an ADG less than 149 g/d for the glutamate and combined treatments in the three weeks post-weaning and this is in agreement with studies that have been conducted on commercial farms by Frey (unpublished). However, in that study there was a long term benefit to growth performance which was not apparent in this study. In agreement with Frey (unpublished), Carlson *et al*. (2005) also found that pigs which received the combined diet were 7 kgs heavier than the control diet at 130 days of age.

The lack of significant difference in growth performance between treatments is unlikely to have been due to insufficient replication as there were a relatively large number of

animals per treatment, and all were accommodated in individual pens. It is more likely that the conditions under which these pigs were reared were close to optimal, during both the period post-weaning and during the grower-finisher phase. While there would have been some stressors related to the practice of weaning the extent to which this affected subsequent performance would have been less then would be experienced in typical commercial piggeries.

It has been suggested that a total intake of 250 g of NuPro® (combined diet) is necessary over the three week post-weaning period for NuPro® to be effective (Close *pers comm*). In this study, the average calculated intake of NuPro® per pig over this period was 178 g and therefore the intake of NuPro® was perhaps not sufficient to provide a significant positive response to its inclusion in the diet. The quantity of inositol and glutamate in the diets were based on the equivalent amount present in Nupro® so again the intake may not have been sufficient to result in a difference in performance.

Pigs that received the combined, glutamate and nucleotides diet had an increased villous height in the duodenum. This finding is in agreement with Mateo (2005) and Martinez-Puig *et al.* (2007) who when feeding nucleotide supplemented diets found an increase in villous height. In contrast, Carlson *et al.* (2005) found no difference in the duodenal villous height but a reduced crypt depth in pigs that received the combined diet. The increase in intestinal tissue growth could be due to the presence of nucleotides in the combined and nucleotide diets as nucleotides are the building blocks of nucleic acids which are required for new tissue to grow (Mateo, 2005). In addition, the increased villous height suggests that more cells were migrating to the villi to aid in digestion and absorption and this may assist the pig in overcoming the negative consequences of weaning (Carlson *et al.* 2005). This may help to explain why there was a trend for pigs from the combined and glutamate diet to have a greater daily gain in the three weeks post-weaning.

The lack of difference in daily gain and feed intake but a greater villous height in the nucleotide diet is in agreement with Martinez-Puig *et al.* (2007) and Kehoe *et al.* (2008) who found that nucleotide supplementation of the diet for pigs and calves, respectively, improved gut histology with no impact on daily gain and feed intake. Martinez-Puig *et al.* (2007) suggests that the incidence of post-weaning diarrhoea was reduced through supplementation with nucleotides although this was not observed in this study due to the low incidence of diarrhoea. Again this may be due to the optimum conditions in which the pigs were housed.

Immunoglobulin G was increased in the serum of pigs that received diets containing inositol and glutamate and in the combined diet indicating that pigs receiving these diets had an increased immune function. It was also thought that IgG levels may also have been increased in pigs that received the nucleotide diet as nucleotides play an important role in an efficient immune system function (Rutz *et al.* 2006). However, Mateo (2005) also found no difference in serum IgG when the diet was supplemented with nucleotides and suggested that this may have been due to an absence of a challenge or that the nucleotides were not included in the diet for a sufficient period.

It had been the intention in this experiment to use a synthetic source of nucleotides and to include these in the Weaner 1 and Weaner 2 diets at a similar concentration to that which is in sow's milk. However, gaining approval from the registration authority to use these products was required and this would have caused a delay in the start of the experiment by several months or more. In addition, it was thought that purified nucleotides may not be as effective due to a loss of bioavailability, and by using the commercially available product then the uptake by industry would have been faster if we got a positive response in performance. Therefore it was decided to use a commercial product (Ascogen) that is already marketed as a source of nucleotides. In a similar vein, it was also decided to use a

commercial product to represent the combination of the three factors under test in this experiment.

An aim of this study was to elucidate which of the factors of the combined diet was contributing to the beneficial response in growth performance and time to slaughter that has been found previously. Although no benefit was found in growth performance in this study when inositol, glutamate and nucleotides were used either separately or together, positive responses were found in gut histology and immune function depending on the additive. The inclusion of all the additives in the combined diet resulted in an improvement in gut histology and immune function. It is hypothesised that in a commercial environment it will be beneficial to include all of the additives, rather than one individually as each additive has a different mode of action.

5. Conclusion

In this study, there were no significant differences in pig performance between any of the treatments, either in the period immediately after weaning or through until slaughter at 101 kg LW. However, there was an indication that the % of slow growing (< 150 g/d) piglets in the 21 days after weaning was reduced when the diet contained either glutamate or the products in combination. There was a positive response in gut histology when pigs were fed a diet containing either glutamate, nucleotides or all of the products in combination. There was also a positive response in IgG concentration when pigs received a diet containing inositol or glutamate, or the product that contained all ingredients. It is therefore likely that the inclusion of some of these products, either individually or in combination, could have positive benefits to the performance of piglets under more challenging conditions such as would be found in many commercial piggeries.

Although no difference in growth performance was found in this study, beneficial effects of including the combined diet was found on feed to gain in the first three weeks post-weaning, gut histology and immune function. Therefore, under commercial conditions it is thought that the inclusion of a product containing inositol, glutamate and nucleotides would be optimal.

6. Limitations/Risks

These results highlight an issue for the conduct of research projects such as this, in which we would like to mimic commercial conditions while at the same time having good control over the conduct of the experiment and measurement of pig performance. It is thus essential to consider more sensitive measures of pig performance wherever possible rather than rely solely on measures such as growth rate and feed consumption. This would indicate a role for the use of nutrigenomic techniques in future experiments of this type.

7. Recommendations

It is recommended that the pigs in this facility may need to be challenged for future experiments due to the optimum conditions. More sensitive measures of indicating pig performance and the mechanisms by which the additives work, as used in this study may need to be considered wherever possible rather than rely solely on performance measures.

The combined addition of glutamate, inositol and nucleotides in weaner diets is recommended to maximise pig performance.

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