DEVELOPMENT OF SEMI-MOIST EXTRUDED CREEP FEEDS TO PROMOTE GASTRO-INTESTINAL TRACT DEVELOPMENT, FEED INTAKE AND SUBSEQUENT WEANING WEIGHTS.

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

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Executive Summary

This research project was undertaken to:

- Develop a basis for the production of extruded, shelf stable, semi-moist (~15-20% moisture) creep feeds.
- Demonstrate an improvement in creep feed intake and post-weaning intake using a semi-moist feed relative to an existing commercial creep feed.
- Examine the influence of semi-moist creep feeds and subsequent increases in feed intake post-weaning on lifetime performance.
- Assess the influence of semi-moist creep feeding and diet composition on gut development, microbial communities in the gut and the incidence of disease.

The conduct of the project resulted in a set of production parameters being established for the manufacture of a shelf-stable semi-moist (20% moisture) creep feed using high temperature cooking. Once these parameters had been established and a suitable feed manufactured three further experiments were conducted.

Experiment 2 sought to compare the use of a semi-moist extruded creep feed with standard creep feeding programs across multiple production systems. Whilst the results found that the ability to significantly influence the pre-weaning performance of the piglet by the use of a semi-moist extruded creep was limited. Whilst minor improvements in performance were seen, the influence of feeding a creep diet over not feeding any supplemental feed during lactation was greater than the type of feed offered. Semi-moist extruded creep feeds did appear to have significant promise in reducing the post weaning growth check, although studies were conflicted as to whether this was due to increased feed intake or improved feed conversion. The form or composition of the diet didn't significantly affect the enzyme activity, histology or flora within the gastrointestinal tract. Enzyme activity is more influenced by the presence or absence of feed whilst gut histology appears to be influenced by the quality of the diet ingredients - poorer quality ingredients resulting in greater development. The higher quality ingredients in the semi-moist extruded creep feed did however appear to result in somewhat of an "anti-microbial" effect, with the lack of detection of virulence genes associated with diarrheagenic *E.coli* groups in the treatment group.

Whilst a response to dietary treatment was seen immediately post-weaning in experiment 3, the advantage ascertained by consuming the semi-moist extruded creep feed during lactation and for the week immediately post-weaning was not maintained throughout the whole of life. It is hypothesized that this advantage was diluted by the environmental 'noise' that exists within commercial production systems.

Experiment 4 shows that a semi-moist extruded creep feed containing high quality ingredients is a suitable replacement for slurry feeding runt piglets. Whilst growth performance was not significantly enhanced, the clean, shelf-stable nature of the product was preferable to the on-farm production of supplemented slurries and resulted in greater levels of hygiene.

This project has delivered:

- A set of production parameters for the manufacture of shelf-stable semi-moist feeds.
- A highly-palatable product that is suitable as a delivery device for less palatable ingredients, whether they be medications, supplements or poorer quality ingredients that may play a greater role in gut development.

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1. Introduction

Suckling and weaner pigs consume only a small proportion of the total feed utilized in a pig production operation. In modern pig production systems, creep and weaner feed use may represent only 13-15% of total feed use. Despite this, nutrition of the young pig has received a significant amount of research attention and producer focus in years past due to the fact that there is clear evidence that increasing the growth rate of piglets prior to weaning has a marked effect on their lifetime performance and the profitability of the pig enterprise (Mahan and Lepine, 1991).

We are also aware that unless carefully managed, the post-weaning "growth check" has the potential to negatively influence the subsequent production efficiency and the long term viability of the pig. This "growth check" occurs as a result of rejection of the solid feed by the piglet and lower feed digestibility as a result of an inability of the immature gut to absorb nutrients, resulting in retarded growth.

There is a need to utilise a creep feeding regime that improves pre- and post-weaning growth, feed intake, gut integrity and piglet health.

It is common commercial practice to provide piglets with a "creep" or "starter" diet (containing cereals, plant and animal proteins) at 7-14 days of age. By providing piglets with dry feed during lactation it is thought that liveweights will be increased at weaning, and development of the gastrointestinal tract will be improved thus reducing the impact of the dietary change that occurs at weaning.

Despite this, there is equivocal evidence to show that creep feeding is actually beneficial. Pluske *et al.* (1995) reviewed research to show that intake of creep feed is generally small and variable and that direct evidence that creep feeding stimulates voluntary feed intake and liveweight gain at weaning is also limited.

Rather than using dry creep feed in an attempt to improve weight gains and intestinal development of suckling pigs, supplemental liquid feed has been utilized to make up for deficiencies in both volume and composition of sow milk. Sow milk production peaks during the second week of lactation after which time it may limit pig growth (Toner *et al.* 1996). This is compounded by the low protein to fat ratio of sow's milk. Dunshea *et al.* (1997) demonstrated that the provision of supplemental skim milk with a comparatively high protein to fat ratio can readily improve the growth performance of suckling pigs.

While creep feeding with liquids has been shown to be beneficial in terms of performance, it is difficult to undertake in commercial production systems due to:

i. Cost and cost of supplying liquid creep feed to piglets;
ii. Maintenance of hygiene in the farrowing shed;
iii. Capacity to cost-effectively source whole milk or eggs for use in liquid creep feeding regimes;
iv. Need to replenish liquid creep feed on a regular basis to maintain freshness and to prevent deterioration;

v. The provision of liquids during the creep feeding failing to promote development or adaption of the gut to solid feeds.

With the above in mind, the BECAN Consulting Group Pty Ltd funded a preliminary investigation into the development of a semi-moist creep feed. It has been proposed that the provision of a semi-moist creep feed, via an extrusion cooking process, could potentially confer the benefits of a liquid creep feeding regime while maintaining the convenience of a dry feed.

Hypothesis

Extruded, shelf-stable, high nutrient density, low pH, semi-moist creep feeds based on functional proteins and low levels of soluble NSP will cost-effectively promote feed intake and growth pre- and post-weaning, gut development and overall health and well being of piglets.

2. Experiments

Experiment 1 *- Development of a shelf-stable, semi-moist creep feed based on functional proteins and low levels of soluble NSP.*

Background

The provision of a semi-moist feed, via the extrusion cooking process, has been proposed as a transitional feed to reduce the post-weaning "pig check". Through the incorporation of a significant amount of high quality protein (preferably from milk and eggs), it is thought that such a feed may assist in overcoming the issues associated with feeding standard commercial feeds. It is also suggested that the soft texture of this type of product will increase palatability and reduce feed aversion.

A previous attempt to prepare a semi-moist extruded creep feed showed significant promise, with both the palatability and digestibility of the feed being significantly better than standard commercial feed. However, the manufacturing process was not optimal, with a significant amount of the product developing mould when stored.

Process Discussion and Results

The objective of this experiment was to prepare and optimize a process for the manufacture of a semi-moist extruded creep feed with two alternative processes evaluated, high-temperature cooking (HTC) and low-temperature protein forming (LTP).

The HTC process seeks to fully utilize the binding properties of both the protein and starch components of the feed, however, the elevated temperatures in this method limits the use of enzymes. The LTP process exploits the low temperature binding properties of protein, without significant modification to the starch component.

The semi-moist extruded creep feed was formulated (Table 1) with digestibility and palatability in mind. Starch components were in the form of milled rice flour and wheat gluten, whilst protein sources were primarily of animal origin.

Table 1. *Semi-moist extruded creep ingredient list.*

Ingredients (g/kg)	
Rice flour	263
Wheat gluten	64
Full-fat soyabean meal	50
Fish meal	100
Skim milk powder	185
Whole milk powder	49
Whey	38
Whole egg	25
Whole milk	82
Vegetable oil	50
Glycerine	30
Propylene glycol	20
Premix	44

Owing to the commercial-in-confidence nature of the extrusion process details of the screw and barrel profiles (Appendix 1), die geometries (Appendix 2), experimental process parameters (Appendix 3) and the recommended process parameters (Appendix 4) for the production of semi-moist extruded creep feed are contained in the attached appendices.

As a result of these experimental runs it was concluded that HTC was the most effective process. High-temperature cooking resulted in a product that was well bound, yet had a soft and pliable structure, with a diameter of 4mm and a length of 5mm (Figure 1).

Figure 1. Semi-moist extruded creep as produced by high-temperature cooking (HTC).

Whilst conducting these experimental runs a series of issues were identified that should be addressed when manufacturing semi-moist extruded creep feed.

- Fish meal was a major culprit in die blockages. It would be preferable to use steam-dried fish meal or alternatively the fish meal should be pre-milled (<1.5mm screen) or pre-sieved prior to inclusion.
- All solid and liquid ingredients should be prepared as separate complete pre-blends to ensure a homologous mix.
- The production capacity could be increased significantly by the manufacture of a single piece die, with an increased number of die holes. This will reduce the total manufacturing time and thus reduce production cost.

Analysis of the finished product showed it very closely matched the feed as formulated indicating that HTC did not significantly affect product quality (Table 2)

Table 2. Nutritional profile of semi-moist extruded creep.

	As formulated	As analysed
Energy – calculated (MJ/kg)		14.2
Total lysine (%)		1.51
Moisture content (%)	20.0	20.0
Crude protein (%)	24.3	25.0
Crude fat (%)	8.9	7.1
Ash (%)	10.2	5.3

Experiment 2 *- Assessment of semi-moist, extruded creep feeds in commercial production systems using existing creep feeding regimes as a control.*

Hypothesis

Shelf-stable, semi-moist extruded creep feeds will promote superior feed intake and growth performance in pre-weaned piglets, will enhance gut development and will reduce post-weaning growth check.

Methodology

A shelf-stable, semi-moist extruded creep feed was compared to existing creep feeding regimes within a variety of commercial farrowing house environments. The feed intake and growth rate of litters was assessed in addition to weaning weight.

Results

Farm A

Semi-moist extruded creep was compared with a standard dry creep (14.9 MJ Digestible Energy (DE)/kg, 0.90g Available Lysine (AvL)/MJ DE) feeding routine from 7 days of lactation through to weaning at 21 days. Both dietary regimes were offered on an *ad libitum* basis, the practice of *'little and often'* being applied to ensure feed freshness.

There were no significant differences between treatments for any of the traits measured (Table 3). Piglets on the standard regime tended to be both heavier at the start and end of the experiment and gained more during the test period, they also consumed more feed than piglets fed semi-moist extruded creep. Variation in piglet performance was not greatly affected by treatment.

Table 3. *Results of pre-weaning creep feeding regime (mean ± s.e.) comparing a standard creep feed (Standard) with semi-moist extruded creep (SMEC).*

	No. of litters	No. of suckers	Start Weight (kg)	End Weight (kg)	Weight Gain (kg)	ADFI (kg)
Standard	37	368	2.76 ± 0.06	5.83 ± 0.13	3.07 ± 0.12	0.40 ± 0.02
SMEC	37	368	2.70 ± 0.08	5.69 ± 0.16	3.00 ± 0.13	0.36 ± 0.03
SED			0.10	0.21	0.18	0.03
P-value			N.S.	N.S.	N.S.	N.S.

Farm B

Semi-moist extruded creep was compared with a standard dry creep feed and a regime where no creep feed was offered during lactation. Feed was offered on an *ad libitum* basis from 18 days of age through to weaning at 26 days.

There were no significant differences between treatments for any of the performance traits measured (Table 4). Both the standard and semi-moist extruded creep treatments gained more weight during the test period than the *Nil* control, with the standard treatment gaining more than the semi-moist extruded creep treatment. There was no significant difference in average daily feed intake (ADFI) between the standard and semi-moist extruded creep treatments.

Table 4. *Results of pre-weaning creep feeding regime (mean ± s.e.) comparing a standard creep feed (Standard) with a semi-moist creep feed (SMEC) and no creep feeding (Nil) during lactation.*

	No. of litters	No. of suckers	Start Weight (kg)	End Weight (kg)	Weight Gain (kg)	ADFI (kg/d)
Standard	58	571	5.47 ± 0.12	7.45 ± 0.15	1.98 ± 0.06	0.15 ± 0.01
Nil	58	577	5.47 ± 0.12	7.31 ± 0.15	1.84 ± 0.06	
SMEC	58	566	5.46 ± 0.13	7.38 ± 0.15	1.92 ± 0.05	0.18 ± 0.01
SED			0.18	0.21	0.08	0.02
P-value			N.S.	N.S.	N.S.	N.S.

Piglets from this study were maintained in treatment groups for 11 days post-weaning, with the *Nil* control receiving the same standard starter ration post-weaning as the Standard treatment, after this period all pigs were pooled and fed one standard diet. Unfortunately, four of the five replicates of the study were seriously affected by post-weaning scours with medicated feed being substituted for all treatment diets. One replicate was able to be completed prior to the scour outbreak and this is reported below (Table 5).

Weaners consuming semi-moist extruded creep grew significantly faster in the 11 days post-weaning than those on the standard creep treatment, whilst those weaners that received no supplementary feeding during lactation were intermediate.

This faster growth was a response to the increased consumption of feed during this period which was significantly higher than the consumption of the standard creep feed (P=0.038), with the *Nil* treatment in lactation again being intermediate. Feed conversion ratio was marginally worse in the semi-moist extruded creep regime but not significantly so.

Table 5. *Results of post-weaning (days 0-11) creep feeding regime (mean ± s.e.) comparing a standard creep feed (Standard) with a semi-moist creep feed (SMEC) and no creep feeding (Nil).*

	No. of pens	No. of weaners	Start Weight (kg)	End Weight (kg)	Ave Daily Gain (kg/d)	ADFI (kg/d)	FCR
Standard	6	102	7.28 ± 0.48	9.51 ± 0.50	0.19 ± 0.01	0.20 ± 0.02[a]	1.05 ± 0.05
Nil	6	102	7.01 ± 0.44	9.67 ± 0.60	0.22 ± 0.02	0.23 ± 0.01[ab]	1.04 ± 0.04
SMEC	6	100	7.27 ± 0.47	10.07 ± 0.54	0.23 ± 0.01	0.26 ± 0.01[b]	1.11 ± 0.03
SED			0.66	0.77	0.02	0.02	0.05
P-value			N.S.	N.S.	N.S.	0.038	N.S.

As the weaners aged, days 12 to 39, differences between treatments lessened (Table 6), although weaners that received semi-moist extruded creep were still heavier at day 39 than both the standard and *Nil* treatments. However, this difference was not significant and the variation in average daily gain between treatments, during this period, had reduced.

Table 6. *Results of post-weaning (days 12-39) creep feeding regime (mean ± s.e.) comparing a standard creep feed (Standard) with a semi-moist creep feed (SMEC) and no creep feeding (Nil).*

	No. of pens	No. of weaners	End Weight (kg)	Ave Daily Gain (kg/d)
Standard	6	92	26.5 ± 1.12	0.631 ± 0.026
Nil	6	102	26.9 ± 0.89	0.637 ± 0.012
SMEC	6	95	27.2 ± 0.81	0.636 ± 0.017
SED			1.34	0.03
P-value			N.S.	N.S.

Farm C

Semi-moist extruded creep feed was compared to a medicated commercial creep diet (15.68MJ DE/kg, 0.90g AvL/MJ DE) and a non-medicated creep feed (15.67MJ DE/kg, 0.90g AvL/MJ DE). Feed was offered on a stepped *ad libitum* basis in the farrowing house from day 8 (80g/litter/day) of lactation through to weaning at day 28 (1500g/litter/day). Piglets were weighed weekly whilst in the farrowing house. Dietary treatments were continued for the week immediately post-weaning. Five days post-weaning, six pigs per treatment were sacrificed for gut sampling, to assess treatment effects on gut morphology, enzyme and microbiological activity, and Enterotoxigenic *Escherichia coli* (ETEC) gene expression.

There was no significant difference in liveweight between treatments at any weighing event (Table 7). However, those pigs receiving the semi-moist extruded creep gained significantly more weight than those on the standard diet (P=0.008), with the non-medicated treatment being intermediate.

Table 7. *Results of pre-weaning creep feeding regime (mean) comparing a standard medicated creep feed (Standard) with a semi-moist creep feed (SMEC) and non medicated creep feed (Non-medicated) during day 7-28 of lactation.*

	No. of suckers	Start Weight (kg)	Day 14 Weight (kg)	Day 21 Weight (kg)	End Weight (kg)	Weight Gain (kg)	Ave Daily Gain (kg/d)
Standard	194	2.71	4.21	5.90	7.32	4.57[a]	0.218[a]
Non-medicated	188	2.59	4.08	5.78	7.44	4.84[ab]	0.231[ab]
SMEC	185	2.64	4.21	5.98	7.68	5.04[b]	0.240[b]
SED		0.07	0.11	0.16	0.19	0.15	0.007
P-value		N.S.	N.S.	N.S.	N.S.	0.008	0.008

Piglets remained on their different creep fed treatments for the week following weaning to assess the different regime's capacity to overcome the nutritional stress associated with weaning. Feed intake during this period was similar for all three treatment groups however differences in FCR occurred as a result of the poor weight gain of the standard treatment (Table 8), which grew the least, but consumed the most feed.

Table 8. *Performance of weaners (mean) in the week immediately post-weaning (day 29-35) fed semi-moist extruded creep (SMEC) or medicated standard creep (Standard) or a non-medicated standard creep (Non-medicated).*

	Weight Gain (kg)	Feed Intake (kg/day)	FCR
Standard	0.66	0.16	1.87
Non-medicated	0.68	0.14	1.42
SMEC	0.84	0.15	1.37
SEM	0.08	0.01	0.17
P-value	N.S.	N.S.	N.S.

Six pigs per treatment were selected for gastro-intestinal sampling at 5 and 6 days post weaning (9 pigs sampled on each day). Each pig was euthanized by an intra-cardial injection of barbiturate (Lethabarb; 0.5 mL/kg body weight, 325 mg/mL Sodium Pentabarbitone, Virbac Australia Pty Ltd). Pigs were exsanguinated and a midline laparotomy performed.

The gastrointestinal tract was ligated from the pyloric junction to the anus, excised and laid out on the dissection table. The pancreas was excised, weighed, wrapped in foil and snap frozen in liquid nitrogen. The small and large intestines were ligated and separated. The mysentry was removed and the intestinal lengths were recorded. The small intestine was divided into equal thirds with the proximal third assumed to be the duodenum, followed by the jejunum and ileum.

The mid 40 cm section of each intestinal region (i.e. duodenum, jejunum, ileum, and colon) and the caecum were ligated and immediately placed on ice. These samples were

then immediately shipped to Elizabeth MacArthur Agricultural Institute (EMAI, Camden, NSW) for analysis of microbial populations.

A 5 cm sample was collected at a point 5 cm distal from the proximal end of duodenum and was gently flushed with phosphate buffered saline (PBS, pH 7.6), and cut longitudinally before being wrapped in foil and snap frozen with liquid nitrogen for enzyme analysis. Another 5 cm sample was collected at a point 40 cm distal from the proximal end of the duodenum, jejunum and ileum and flushed with PBS and fixed in 10 % (v/v) neutral buffered formalin for histomorphological analysis.

Biochemistry

Duodenal Samples

Methods used for the preparation of duodenal samples to analyse brush border enzymes activity where modified from those of Shirazi-Beechey *et al.* (1991) with further minor modifications by Iji (1998). The preparation involves hypotonic shock and rupturing of cells, followed by two phases of centrifuging. The end products were a homogenate (collected from the initial centrifuging phase) and a purer vesicle solution (collected from the secondary centrifuging phase), both of which were stored at -20 ^{o}C prior to use.

The disaccharidases ß-glucosidase (lactase, EC 3.2.1.23), α-glucosidase (maltase, EC. 3.2.1.20) and fructofuranosidase (sucrase, EC 3.2.1.26) were assayed according to the methods described by Dahlqvist (1964). Vesicles were incubated in duplicate at 37 ^{o}C with their respective substrate buffers (100 mM of lactose, maltose and sucrose in succinate buffer). Once the incubation was complete, a GPD-Perid kit (Boehringer-Mannheim Australia, Castle Hill, NSW) was used to estimate glucose release. Absorbance was read at 610 nm using a Spectrophotometer (150-20 spectrophotometer, Hitachi, Japan).

The methods described by Forstner *et al.* (1968) and Holdsworth (1970) were modified for assaying of alkaline phosphatase (AP). The reaction involved incubating 20 µL of homogenate with 800 µL of 50 mM Tris buffer (pH 10.1), 100 µL of 50 mM MgCl2, and 100 µL of freshly prepared 10 mM phosphatase substrate (10mM paranitrophenol phosphate, Sigma 104, Sigma-Aldrich Pty Ltd, Castle Hill, NSW) at room temperature for 20 minutes. The reaction was terminated with 100 µL of 40 % trichloroacetic acid (TCA). Colour development was achieved by vibromixing 100 µL of the reaction mixture with 2 mL of 0.4 M NaOH. Standards were prepared at concentrations of 0, 50 and 100 µL, using p-nitrophenl (Sigma 104-1, Sigma-Aldrich Pty Ltd, Castle Hill, NSW) and a blank solution (containing 20 µL of Milli-Q water in place of the homogenate). Samples and standards were read at 410 nm on the spectrophotometer.

The analysis of leucine aminopeptidase (LAP) was modified from Miura *et al.* (1983). Homogenate was diluted 1:4 with Milli-Q water in duplicate. A 50 µL diluted sample was combined with 400 µL of Milli-Q water, 200 µL of 200mM phosphate buffer (pH 7.0) and 200 µL of Leucine ß-naphthylamide substrate (in 95 % ethanol) with intermittent vortexing, and then incubated at 37 ^{o}C for 30 minutes in the water bath. The reaction was

terminated with 600 µL of 30 % TCA, before 200 µL of 0.3 % sodium nitrite (in 95 % ethanol), 200 µL of 1.5 % ammonium sulphamate (in 95 % ethanol) and 600 µl of 0.1 % N-1-naphthylethylene diamine-dihydrochloride were added to each tube with intermittent vortexing. Standards were prepared using 0.4 mM ß-naphthylamine and 2N HCl. Absorbance was read in duplicate at 560 nm on a spectrophotometer.

The method for protein assays was modified from Bradford (1976). From the homogenate, a dilution was prepared by combining 100 µL of homogenate with 900 µL of Milli Q water in duplicate. The reaction we achieved by combining 40 µL of diluted homogenate with 2 mL of Bradford reagent (a mixture of Coomassie Brilliant Blue, 95 % ethanol, 85 % phosphoric acid, and distilled water, 2:1:2:15 ratio per mL). Standards containing BSA (Bovine Serum Albumin) in concentrations from 10 – 150 µL were prepared, and 2 mL of Bradford reagent was added. Absorbance of samples and standards were read in duplicate at 595 nm. From the vesicles, 40 µL were transferred in duplicate and diluted with 160 µL of water. Tubes were vortex before 2 mL of Bradford reagent was added. Standards containing BSA (Bovine Serum Albumin) in concentrations from 10 – 200 µL were prepared, and 2 mL of Bradford reagent was added. Their absorbance was read in duplicate at 595 nm.

Absorbance data obtained for protein assay were converted to protein concentration using the Lowry computer software (Elsevier BIOSOFT, Cambridge, UK).

Pancreatic samples

Pancreas samples were homogenized and centrifuged to obtain supernatant, which was stored at -20 ^{o}C prior to use.

The method for the analysis of lipase activity was modified from the original method of Thoner (1973), with modifications by Winkler and Stuckmann (1979). A lipase substrate was prepared by dissolving 30 mg of p-nitrophenylpamitate in 10 mL of isopropanol and mixing this with 90 mL of 0.05 M Sorensen phosphate buffer containing 207 mg Na deoxycholate.

The pancreatic homogenate was diluted 1:99 with Milli-Q water. The reaction was achieved by incubating 25 µL of homogenate (or Milli-Q water for the blank) with 200 µL of pre-warmed (37 ^{o}C) lipase substrate in duplicate. After vortexing the substrate was incubated at 37 ^{o}C for 15 minutes. The reaction was terminated with 4 mL of 0.4 M NaOH. Standards were prepared at 0, 50 and 100 µL using p-nitrophenol (1 µmol/mL). Absorbance was read at 410 nm.

The method used for analysis of chymotrypsin was derived from Serviere-Zaragoza *et al.* (1997). The homogenate was diluted 1:9 with Milli-Q water. A 250 µL sub-sample of diluted homogenate was incubated with 1 mL of substrate containing 450 µL of 0.1 mM N-Succinyl-(Ala)2-Pro-Phe-p-nitroanilide (SAPNA) into 59.55 mL of the buffer (50 mM Tris-HCl and 20 mM $CaCl_2$ (pH 7.5)). Standards were prepared using 2.5 mM p-nitroaniline. Samples and standards were vortexed and then incubated at 37 ^{o}C for 1 hour before the reaction was terminated with 500 µL of 30 % acetic acid. Samples and standards were cooled to room temperature and vortexed. Their absorbance was then read at 410 nm.

Protein content was analysed according to a modification of the method by Bradford (1976). The pancreatic homogenate was diluted 1: 9 with Milli Q water and was analysed as described above, using 40 µL of diluted homogenate. Standards containing BSA in concentrations from 0-200 µL were prepared with 2 mL Bradford reagent as described previously. The absorbance was read at 595 nm using the spectrophotometer (150-20 spectrophotometer, Hitachi, Japan). Absorbance data were converted to protein concentrations using the Lowry computer software (Elsevier BIOSOFT, Cambridge, UK).

Enzyme activities were expressed as units (EU) per gram of protein, were one unit was defined as the amount of enzyme that hydrolyzed one µmol of the substrate per minute.

Specific activities of disaccharidases (such as lactase and sucrase) can be used as indicators of enterocyte maturity and functional capacity in the small intestine (Tang *et al.*, 1999, Kang *et al.*, 2008), where in rats alkaline phosphatase has been used as an indicator of intestinal maturation (Castillo *et al.*, 1990 cited in Kang *et al.*, 2008). However, comparisons of enzyme activities between studies is complicated by the range of denominators used by researchers to express the enzyme activities (Fan *et al.*, 2002), and further more by the range of units used. The methods used in this study are more comparative than quantitative (pers. comm., P. Iji August 2007).

Table 9. *Enzyme activity (EU) in the duodenum of pigs, 5 days post-weaning, fed semi-moist extruded creep (SMEC) or medicated standard creep (Standard) or a non-medicated standard creep (Non-medicated).*

	Lactase	**Maltase**	**Sucrase**	**AP[#]**	**LAP[#]**
Standard	70 (6)	657 (6)	68 (6)	78 (6)	14.7 (4)
Non-medicated	127 (5)	627 (5)	109 (6)	102 (4)	26.1 (6)
SMEC	128 (6)	692 (4)	90 (4)	86 (6)	30.6 (6)
SEM	22	86	10	10	3.5
P-value	N.S.	N.S.	N.S.	N.S.	N.S.

[#]AP – Alkaline phosphatise, LAP = Leucine amino peptidase, (n) brackets indicates the number of samples in the analysis after outliers were removed, based on the Dixon's test.

Dietary treatment did not affect the duodenal or pancreatic enzyme activity at five days post weaning (Table 9 and 10 respectively). Analysis of pigs fed medicated versus non-medicated creep feeds showed a tendency for pigs fed medicated diets to have lower LAP (14.7 versus 26.1, P=0.051) and sucrase (68 versus 109, P=0.080) activities.

Table 10. *Pancreatic enzyme activity (EU) of pigs, 5 days post-weaning, fed semi-moist extruded creep (SMEC) or medicated standard creep (Standard) or a non-medicated standard creep (Non-medicated).*

	N.	**Chymotrypsin**	**Lipase**
Standard	6	12.89	2,189
Non-medicated	6	12.31	1,702
SMEC	6	10.76	2,418
SED		1.56	563
P-value		N.S.	N.S.

The results in the present study suggest that medicated diets tend to suppress some brush-border enzyme activity, which concurs with the findings of Collington *et al.* (1990) who found that creep diets supplemented with Tylosin lowered lactase and sucrase activity in pigs during the post-weaning phase. Whether the apparent differences between medicated and non-medicated diets in the present study are due to direct or indirect effects (through suppression of feed intake) are unclear. The lack of literature discussing the influence of antibiotics on digestive enzyme activity is surprising considering the wide spread use of antibiotics in diets offered to pigs after weaning.

In line with the finding in the present study, Bikker *et al.* (2006) and Lee *et al.* (2007) found that dietary manipulation did not affect the intestinal activity of maltase, sucrase and amino-peptidase-N (APN) in the proximal small intestine of pigs. In contrast, Lee *et al.* (2007) did report that dietary manipulation significantly reduced the activity of maltase, sucrase and APN ($P<0.05$) and tended to reduce AP activity ($P<0.08$) in the ileum of weaned pigs. Analysis of the enzyme activity in the medial and distal regions of the small intestine may have identified a difference in enzyme activity, particularly in the case of sucrase and maltase whose activity shifts distally along the small intestinally after weaning (Collington *et al.,* 1990) and becomes highest from 50 to 75 % along the small intestine (Pluske *et al.,* 2003).

The lactase and sucrase activity and variation between samples were in line with those reported by Kelly *et al.* (1991), and Pluske *et al.* (2003, on conversion of the units). The lactase, maltase and sucrase activity was greater than the values recorded by Hedemann *et al.* (2006) from the jejunum of pigs sacrificed at nine days post weaning, however, the variation between samples was similar. The difference in lactase activity between the present study and Hedemann *et al.* (2006) are expected considering the differences in sampling age and location along the gastro-intestinal tract. The differences in the maltase and sucrase activity are unexpected for the same reasons. Differences may be attributed to sample preparation (ie use of homogenate or purified vesicles), or may be related to the differences in the duration of creep feeding prior to weaning (20 versus 6 days).

Leucine aminopeptidase (EC 3.4.11.1) is a zinc enzyme which preferentially cleaves leucine, but may also cleave other amino acids (with the exception of arginine and lysine). Most published studies measure the activity of amino peptidase N (EC 3.4.11.2) which is also a zinc enzyme, but it preferentially cleaves alanine, and can cleave other amino acids including proline (IUBMB, online 2009). Both APN and LAP play a role in the final stages of protein digestion in the small intestine (Lehninger, 1978; Shen and Liechty, 2003). LAP is believed to the most abundant peptidase in higher animals (Lehninger, 1978), whilst APN is reported to be the most abundant peptidase in the small intestine of the pig. When comparing the results between studies, it is expected that the values for LAP would be lower than the values for APN for pigs, as is the case with the values reported by Lee *et al.* (2008) and Hedemann *et al.* (2006), which are greater than the values reported in the present study.

The lipase activities reported in the present study are similar to the values reported by Cera *et al.* (1990). Lipase activity has been reported to decline after weaning and stabilise by 7 days post-weaning, independent of weaning age (14 versus 28 days), but was

reported to be affected by weaning weight, with lipase activity being higher in heavier pigs (Pluske *et al.* 2003). A positive relationship between lipase activity and body weight tended to be evident in the present study (P=0.055), suggesting that significant nutrient intake which results in significant body weight gain, are required to beneficially alter lipase activity.

The chymotrypsin activities (and the level of variation between values) are similar to those reported by Jensen *et al.* (1997) for pigs of a similar weight, but not pigs of a similar age. Jensen *et al.* (1997) weaned pigs at 28 days, only weighing 5.96kg, and a temporary decline in the chymotrypsin activity was seen in the week post weaning. Variation between the two studies may also be a result of the different substrates used and the absorbance being read at different frequencies.

Feed intake is a major factor influencing the potential for damage to the gut architecture and function (Pluske *et al.,* 1997), such that feed intake may be more influential than the diet composition. This study found that feed intake and feed conversion did not differ between dietary treatments, which may explain the lack of difference in intestinal enzyme activity.

Histology

Duodenum samples were stored in 10 % buffered formalin solution. Samples were briefly removed and two 5-7 mm cross-section sub-samples were cut from one end of the sample. These sub-samples were then enclosed in labelled cassettes (Bayer Diagnostics Australia Pty Ltd., Pymble, NSW, Australia) and dropped into warm liquid wax. Samples were processed (using a procedure involving serial dehydration of the samples with ethanol, clearing with xylol and impregnating with paraplast wax) over night (for 16 hours) using a Histokinette processor (Leica Instruments GmbH, Bensheim, Germany). After processing samples were embedded in paraffin using the Histo Embedding Centre (Leica EG 1160, Leica Microsystems, Bensheim, Germany).

Each sample was sectioned using a Rotary Microtome (Leitz 1516, Leica Microsystems, Bensheim, Germany) at a thickness of 5-6 µm. Sections was transferred onto microscope slides. These slides were left to dry over night. Slides were inspected using a microscope (Olympus BH, Olympus America Inc. New York, U.S.A.), and two slides from each sample were chosen to stain. Slides were stained using Harris's hematoxylin (Gerorge Gurr Ltd., London, UK), and eosin (Gur Certistain, VWR International Ltd., Poole, UK) before being mounted using Distrene Polystyrene Xylene mountant (BDH Laboratory Supplie, Poole, UK).

Intestinal sections were captured at 4x magnification using a Leica DM LB microscope (Leica Microscope GmbH, Wetzlar, Germany) and morphometric indices were determined as described by Iji *et al.* (2001) using computer aided light microscope image processing analysis systems (SPOT 3.1, Diagnostic Instruments, Inc., Sterlin Heights, MI, USA). Villus height (from the tip of the villi to the villus crypt junction) and Villus + Crypt height (from the tip of the villi to the muscularis mucosae) were measured in twenty well orientated,

intact villi and twenty intake crypts for each intestinal section. Calibration was done using photomicrographs of a stage micrometer recorded at 5x magnification. Villus height and crypt depth and the villus height: crypt depth ratio was calculated.

Histology was performed on the duodenum to assess whether creep diets could affect the gut morphology in weaner pigs. Villus atrophy and its recovery are greatest and occur earliest in the proximal small intestinal compared to the medial or distal small intestines (Marion *et al.* 2002). Dietary treatment did not affect the duodenal villus height, crypt depth or their ratio (VH:CD) in the present study (Table 11). The villus height values are similar to those reported by Verdonk *et al.* (2007), van Beers-Schruers *et al.* (1998), Montagne *et al.* (2007) and Pluske *et al.* (2003).

Table 11. *Histological measures from duodenum samples of pigs, 5 days post-weaning, fed semi-moist extruded creep (SMEC) or medicated standard creep (Standard) or a non-medicated standard creep (Non-medicated).*

	Villus height (VH)	**Crypt depth** (CD)	**VH:CD**
Standard	339 (6)	540 (6)	0.625 (6)
Non-medicated	454 (6)	496 (6)	0.732 (5)
SMEC	284 (4)	405 (4)	0.655 (4)
SEM	91	81	0.048
P-value	N.S.	N.S.	N.S.

(n) Brackets indicate the numbers of samples in the analysis after outliers were removed, based on the Dixon's test.

However, the crypt depths are greater than those reported by Verdonk *et al.* (2007), van Beers-Schruers *et al.* (1998), Montagne *et al.* (2007) and Pluske *et al.* (2003), where both Montagne *et al.* (2007) and Pluske *et al.* (2003) observed crypt elongation over time. Considering that crypt hyperplasia is often secondary to villous atrophy, the degree of cell proliferation in the crypts may suggest villous atrophy has commenced prior to weaning. This assumption is in part supported by the decline in growth rate from the third week (day 14 to 21) to the fourth week (day 22 to 28) of the experiment (see Table 7) where nutrient for growth appear to be a limiting factor.

The exact cause of crypt hyperplasia in newly weaned pigs is unclear, with nutritional, physiological and environmental changes all having an impact (van Beers-Schruers *et al.* 1994). Pigs used in this study were transported in a trailer from the breeder unit to an isolated weaner unit. The severity of crypt hyperplasia observed may be more representative of the gastro-intestinal changes seen in commercially farmed pigs (where transportation between sites is not uncommon), and may also suggest the severity of the intestinal insult was greater and the rate of morphological recovery was relatively slower in the present study.

Miller *et al.* (2007) reported that pre-weaning intestinal maturity, including villus height, was not affected by creep feed intake, even though creep feed intake influenced pre-weaning growth performance. This concurs with the findings of Hedemann *et al.* (2007) who reported that pre-weaning creep feed intake did not alter the intestinal morphology at weaning, nor did it have an effect five days post weaning. Verdonk *et al.* (2007) found

that negative changes to intestinal morphology (but not permeability) which occur post-weaning can be alleviated by high post-weaning feed intake of dry feeds. Miller *et al.* (2007) showed that crypt elongation and villus broadening occurred between four and six weeks of age in unweaned pigs. This suggests that intestinal changes may be driven by age or a decline in milk production (relative to energy requirements of the piglet), and that the process of weaning influences the rate of change rather than being the cause of change.

The variation between pigs for both villus height and crypt depth in the present study is greater than values reported by Verdonk *et al.* (2007) and Gu *et al.* (2004). Given the variation in body weights of the pigs at weaning (5.7 to 11.1 kg), and subsequently in small intestine length, the decision to sample at an absolute distance (40 cm distal of the pyloric junction) as opposed to a relative distance or percentage (in proportion to the length of the small intestine) was unfavourable and would have contributed to the variation. The large variation may also be attributed to the broad range of maternal parities.

The VH:CD in the present study is similar to those reported by Hedemann *et al.* (2006), but considerably lower than those calculated from the studies of Pluske *et al.* (2003) and Verdonk *et al.* (2007). Jung *et al.* (2006) reported that the VH:CD ratio was significantly reduced ($P<0.05$) in pigs inoculated with porcine epidemic diarrhoea virus compared to healthy pigs. The low VH:CD ratio reported in the present study may reflect the increased disease status of weaner pigs housed under commercial conditions in pens, compared to pigs housed individually in research environments.

Microbiology

The microbial analysis was carried out at the Elizabeth MacArthur Agricultural Institute (EMAI), in the NSW Department of Primary Industries Immunology and Molecular Diagnostic Research laboratory.

Preparation of intestinal samples for microbial analysis.

The preparation of intestinal samples was done according to the methods of Patterson *et al.* (2005). On arrival, intestinal sections were cut longitudinally and the contents were scrapped with a sterile slide into labelled centrifuge tubes containing 15 mL of Brain-Heart Infusion Broth (BHIB, Merck, 1.10493.0500) containing 20% Glycerol (Amyl Media RM283) solution. The remaining mucosa was placed in tubes containing 10mL of 0.1% Triton-X 100 solution and shaken for 20 minutes on ice to lyse remaining intestinal cells and release bacteria from the intestinal wall. The samples were centrifuged (Sigma Quantum Scientific 2-16) at 7378 g for 10 minutes. The supernatant was poured off and the bacterial pellet was re-suspended with 5 mL of the previously scrapped digesta, then the contents of the two tubes were combined. Glass beads were added and samples were vortexed to ensure homogeneity. Four sub-samples were then transferred to 2 mL cryotubes and frozen at -80 ^{o}C until further processing.

Enumeration of Gastro-intestinal microflora.

Enumeration of intestinal microflora was done according to the methods of Chapman and Turner (2005). The five intestinal samples (duodenum, jejunum, ileum, caecum and colon) for each pig were thawed at room temperature for 30 minutes. Using a Pasteur pipette, 1.5 mL of sample was transferred to pre-weighed, labelled sterile centrifuge tubes. Tubes were centrifuged (Sigma Quantum Scientific 2-16) at 12,000rpm (10625g) for 10 minutes. The supernatant was discarded and the remaining pellet was weighed. One mL of BHIB-glycerol (20%) solution was aliquoted to each tube. The tubes were vortexed at high speed to re-suspend the pellet. Using a Pasteur pipette the entire sample was transferred to labelled 2 mL cryotubes. Five sterile, small glass beads were added to each tube and the tubes were vortexed at high speed to ensure homogeneity.

Using one plate per pig, serial dilution for each sample in duplicate was performed in a 96 well plate. Dilutions were performed by adding 40 µL of sample into the first row of the 96 well plate and subsequently adding 120 µL of PBS (pH 7.3), and homogenising using the PBS pipette tip. Using new, sterile pipette tip the steps were repeated until all 8 dilutions (including the original sample) were prepared. The drop count dilutions were aliquoted (10 µL in duplicate) on to MacConkey No.3 (MAC3, Amyl Media AM97), DeMan, Rogosa, Sharpe (MRS, Amyl Media AM104), MacConkey (MAC, Amyl Media AM94) and Kanamycin Esculin Azide (KEA, Amyl Media, AM74) and Blood Agar Base No. 2 (BA, Oxoid CM271, + 7% fresh ovine blood) agar plates (which were prepared according to the manufacturer's instructions). All plates except the MRS plates were incubated at 37 ^{o}C overnight. The MRS plates were incubated at 37 ^{o}C + 5 % CO_2 overnight. After incubation, the colonies within each drop were enumerated using a stereo microscope (Kyowa, Japan) and a manual laboratory counter (Clay Adams, U.S.A).

Enumeration of ß-Haemolytic and non-haemolytic ratio.

Using the count data obtained from the drop count plating onto BA agar, an appropriate volume and dilution was determined to grow approximately 100-200 colonies from the same sample on a 90 mm round BA agar plate. The sample was spread using a sterile glass rod and plates were inverted and incubated aerobically at 37 ^{o}C overnight. After incubation plates were scanned and images were enlarged to enumerate the ß-haemolytic and non-haemolytic colonies.

Pathotyping of Gastro-intestinal E.coli.

The methods used for the multiplex Polymerase Chain Reaction (PCR) were adapted from Muller *et al.* (2007). Intestinal samples in BHIB-glycerol (20%) were thawed and vortex thoroughly before 100-200 µL of sample was transferred using a Pasteur pipette to cryotubes containing 800 µL of MacConkey Broth (Merck, 1.05396). Samples were placed on a shaker and incubated at 37 ^{o}C for 18 hours. After this, samples were vortexed and a 50 µL aliquot was transferred to a sterile 1.5 mL centrifuge tube containing 1 mL of sterile water. To preserve the remainder of the inoculated samples, 800 µL of MacConkey Broth containing 20% glycerol was added to the cryotubes. Centrifuge tubes were spun (Sigma Quantum Scientific 2-16) at 10,000 g for 1 minute before the supernatant was poured off and 200 µL of 6% Chelex™ matrix (Biorad USA 142-1253) was added to each 1.5 mL tube and briefly flicked (to ensure the pellet was suspended), before being incubated at 56 ^{o}C

for 20 minutes in a dry heat block. Samples were then vortexed at high speed for ten seconds and incubated at 100 ^{o}C for 8 minutes in a dry heat block and rapidly transferred to ice to cool.

Samples were centrifuged at 9000 g for 10 minutes (to separate the DNA from the Chelex™ matrix). The multiplex PCR was performed in 200 µL 96 well PCR plates with a 25 µL reaction mixture in each well which contained 2 µL of DNA, 2.5 µL of 10x NH_4 reaction buffer, 1.05 µL 50 mM MgCl, 0.4 µL BioTaq (Bioline, BIOTAQ DNA Polymerase BIO-21040) 0.75 µL 10 mM dNTP, 2 µL Orange G (Sigma-Aldrich, O3756-25G), 11.3 µL MilliQ water (MQWATER) and 5 µL of the PCR primers. All plates contained two positive and one negative control. Plates containing 2 µL of DNA template only, were pulse centrifuged to 1000 g (Sorvall Legend T, Rotar 7500649Q, Microplate carrier) briefly before 23 µL of the master mix (described above) was added to each well. Plates were pulse centrifuged again to 1000 g before multiplex PCR was conducted using a thermocycler (Palm Cycler, Corbett Research). The multiplex PCR incorporated the virulence genes escV, stx_1, stx_2, elt, estIa, estIb, invE, astA, aggR, pic, and uidA and these were amplified by colony PCR using the optimized primers in

A positive control was prepared from the EMAI isolate collection to contain the virulence genes. The PCR program had the following thermoconditions; 94 ^{o}C for 5 min, 30 cycles of 94 ^{o}C for 30 sec, 63 ^{o}C for 30 sec, 72 ^{o}C for 90 sec, and 72 ^{o}C for 5 min.

To separate the PCR products, 6 µL of amplified product and 5 µL of GeneRuler™ 100bp DNA Ladder Plus (Fermentas, Canada, SM0321) was loaded onto a 2% agarose gel for electrophoresis (Agarose Multipurpose, Bioline BIO-41025) using 0.5x Tris-Borate-EDTA (TBE, 45 mM Tris Base, 45 mM boric acid, 10 mM EDTA, pH 8)) solution as a running buffer. Gels were placed in electrophoresis tanks (Biomed Analytik Gmbh, Agagel Maxi Biometra) and run at 100 volts for 90 minutes. Gels were then stained in ethidium bromide for 20 minutes before being de-stained in 0.5x TBE for 10 minutes. Gels were imaged using a Gel Doc system (BioRad) and the PCR products were analysed and quantified using Quantity One software (Version 4.6.3., BioRad).

Results and Discussion

The bacterial counts are shown in Table 12. Dietary treatment did not influence the quantity of coliforms, *E.coli* or lactic acid bacteria along the digestive tract of the pigs. Diet influenced the quantity of *Enterococci* bacteria in the jejunum and ileum. Pigs fed the SMEC had higher quantities of *Enterococci* bacteria in the jejunum compared to those fed the standard and non-medicated diets (P=0.021), and had higher quantities of *Enterococci* bacteria in the ileum compared to pigs fed the non-medicated diet (P=0.073). The quantity of all bacterial groups enumerated in this study increased distally along the gastrointestinal tract.

There was no significant difference in the quantity of beta-haemolytic bacteria along the gastro intestinal tract (Table 13). There was no significant difference in the quantity of non-haemolytic bacteria along the gastro intestinal tract except in the colon. Pigs which were fed the SMEC diet had more non-haemolytic bacteria (P=0.081) compared to those fed the non-medicated diet. The quantity of non-haemolytic bacteria in the colon of pigs

fed the medicated diet was not different from the quantity observed in the other two dietary treatments. Diet did not influence the total quantity of bacteria found in the small intestine and caecum of pigs, however it tended (P=0.094) to influence the quantity of total bacteria in the colon, such that pigs fed the SMEC diet had higher quantity of total bacteria compared to pigs fed the non-medicated diet.

Dietary treatment did not affect the ratio of non-haemolytic bacteria to beta-haemolytic bacteria along the gastrointestinal tract, nor did it affect the percentage of beta-haemolytic bacteria along the small intestine and in the caecum (Table 13). In the colon there was a higher percentage (P=0.020) of beta-haemolytic bacteria in pigs fed the medicated diet than in those fed the non-medicated diets.

The results of the multiplex PCR are shown in Table 14. Dietary treatment appears to alter the microbial community along the gastrointestinal tract of the pig. The presence of the ETEC genes estIa and estIb were greatest in pigs fed the non-medicated diet, detection was low in the pigs fed the standard diet and no virulence genes were detected in the SMEC pigs. The ETEC gene eIt gene was not detected in any pigs. There were a higher percentage of EAEC gene astA detected in the SMEC pigs. The presence of EHEC virulence genes stx1 and stx2 were low and did not differ between dietary treatments. The presence of the escV gene (associated with EPEC, ATEC, STEC) was lowest in the SMEC pigs and highest in pigs fed the non-medicated diet.

The limitations and biases of the traditional culture-based techniques used for viable cell counts are well documented, however this method was considered appropriate for the determination of dietary effects on the bacterial groups examined in this study. The apparent dietary influence on *Enterococci* in the jejunum and ileum of pigs in the present study may be related to the dietary texture, as this has been shown to influence the microbiota found in the ileum of grower pigs (Molbak *et al.,* 2008). *Enterococci* are low grade, opportunistic facilitative anaerobes which are normal inhabitants of the gastro-intestinal tract of humans (NHS online, 2008) and pigs. Particular species of *Enterococci* are known to be pathogenic and antibiotic resistant (NHS online, 2008). The *Enterococci* counts observed by Overland *et al.* (2007) in grower finisher pigs are lower than the values observed in the present study. Overland *et al.* (2007) found that bacteria counts (*Enterococci*, coliform and lactic acid bacteria (LAB)) were reduced in pigs fed diets supplemented with organic acids. Guggenbuhl *et al.* (2007) on the other hand reported that dietary supplementation with benzoic acid only reduced *E.coli* and LAB but not *Enterococci.* The differences observed in the present study may also be attributed to dietary additives or processing methods, or may be a reflection of the increased coliform count observed in the jejunum and ileum of pigs fed SMEC. The enumeration data from the present study suggesting that pigs fed the SMEC had more microorganisms than the pigs fed the commercial diets.

The LAB counts in the present study are similar to those observed by Mathew *et al.* (1996) and Wellock *et al.* (2007) whilst the coliform counts are similar to Mathew *et al.* (1996) and greater than those observed by Wellock *et al.* (2007). Mathew *et al.* (1996) found that LAB concentrations declined two days post weaning but rapidly recovered, and coliform counts peaked at 6 days post weaning.

Table 12. *Bacterial counts (log CFU/g) along the gastrointestinal tract of pigs, 5 days post-weaning, fed semi-moist extruded creep (SMEC) or medicated standard creep (Standard) or a non-medicated standard creep (Non-medicated). Values within column groups sharing superscripts are not significantly different.*

Bacterial group	Treatment	N.	Duodenum	Jejunum	Ileum	Caecum	Colon
Coliforms	Standard	6	5.41	6.63	6.84	8.19	7.94
	Non-medicated	6	6.08	6.23	6.40	7.31	7.02
	SMEC	6	6.08	7.04	8.12	8.45	8.42
	SED		0.81	0.69	0.85	0.70	0.79
	P-value		N.S.	N.S.	N.S.	N.S.	N.S.
E.coli	Standard	6	4.72	5.87	5.71	7.76	7.17
	Non-medicated	6	4.96	5.05	4.92	7.10	6.75
	SMEC	6	5.22	5.90	7.28	8.37	8.32
	SED		1.01	0.84	0.88	0.68	0.70
	P-value		N.S.	N.S.	N.S.	N.S.	N.S.
Lactic Acid Bacteria	Standard	6	5.17	6.16	7.07	9.00	8.83
	Non-medicated	6	6.31	7.09	7.21	8.62	8.56
	SMEC	6	5.93	6.54	7.72	8.10	7.79
	SED		0.75	0.71	0.83	0.58	0.78
	P-value		N.S.	N.S.	N.S.	N.S.	N.S.
Enterococci	Standard	6	3.81	4.44	5.21^{ab}	6.50	6.30
	Non-medicated	6	3.96	4.04	3.95^{a}	4.96	4.91
	SMEC	6	4.78	5.60	6.19^{b}	6.46	6.30
	SED		0.48	0.74	0.90	1.07	1.03
	P-value		N.S.	N.S.	0.073	N.S.	N.S.

Table 13. *Counts of B-haemolytic and non-haemolytic bacteria (log CFU/g) along the gastrointestinal tract of pigs, 5 days post-weaning, fed semi-moist extruded creep (SMEC) or medicated standard creep (Standard) or a non-medicated standard creep (Non-medicated). Values within column groups sharing superscripts are not significantly different.*

Bacterial group	Treatment	Duodenum	Jejunum	Ileum	Caecum	Colon
B-haemolytic	Standard	4.36	5.55	5.64	6.52	7.10
	Non-medicated	4.87	4.93	4.66	5.83	5.39
	SMEC	4.88	5.54	6.12	7.07	6.56
	SED	0.68	0.78	0.69	0.79	0.81
	P-value	N.S.	N.S.	N.S.	N.S.	N.S
Non-haemolytic	Standard	5.07	6.57	6.17	7.16	7.49 ab
	Non-medicated	5.41	5.67	5.65	6.45	6.54 a
	SMEC	5.55	6.72	7.52	8.01	8.03 b
	SED	0.93	0.78	0.98	0.68	0.62
	P-value	N.S.	N.S.	N.S.	N.S.	0.081
Total bacterial count	Standard	5.40	6.75	6.45	7.42	7.74 ab
	Non-medicated	5.71	5.80	5.83	6.71	6.61 a
	SMEC	5.78	6.81	7.56	8.17	8.05 b
	SED	0.86	0.76	0.89	0.67	0.64
	P-value	N.S	N.S.	N.S.	N.S.	0.094
Ratio NH: *B*H	Standard	26	39	21	39	12
	Non-medicated	43	9	36	17	35
	SMEC	15	48	75	27	54
	SED	34	30	48	29	30
	P-value	N.S.	N.S.	N.S.	N.S.	N.S.
%-*B*-haemolytic	Standard	30.8	21.5	35.9	34.4	37.6[b]
	Non-medicated	35.6	20.9	24.3	33.1	13.6[a]
	SMEC	26.9	14.6	7.9	21.1	4.5[a]
	SED	21.7	15.6	16.7	19.7	10.7
	P-value	N.S.	N.S.	N.S.	N.S.	0.020

Table 14. *Detection of Virulence genes along the gastrointestinal tract of pigs.*

Virulence genes		Dietary Treatment		
		Non-medicated	Standard	SMEC
ETEC (estIa, estIb)	Duodenum	2/6	0/6	0/6
	Jejunum	3/6	1/6	0/6
	Ileum	3/6	1/6	0/6
	Caecum	1/6	0/6	0/6
	Colon	2/6	0/6	0/6
	Total	11/30 (36.7)[#]	2/30 (6.7)	0/30 (0.0)
EAEC (astA)	Duodenum	1/6	1/6	2/6
	Jejunum	2/6	1/6	3/6
	Ileum	2/6	1/6	3/6
	Caecum	2/6	1/6	4/6
	Colon	2/6	0/6	4/6
	Total	9/30 (30.0)	4/30 (13.3)	16/30 (53.3)
EHEC (stx1 stx2)	Duodenum	0/6	0/6	0/6
	Jejunum	0/6	1/6	1/6
	Ileum	0/6	0/6	0/6
	Caecum	1/6	0/6	0/6
	Colon	0/6	0/6	0/6
	Total	1/30 (3.3)	1/30 (3.3)	1/30 (3.3)
EPEC, ATEC, STEC (escV)	Duodenum	0/6	0/6	0/6
	Jejunum	2/6	1/6	1/6
	Ileum	2/6	2/6	1/6
	Caecum	3/6	2/6	1/6
	Colon	3/6	1/6	0/6
	Total	10/30 (33.3)	6/30 (20.0)	3/30 (10.0)

[#]Percentage of detected virulence genes. Enterotoxigenic *E.coli* (ETEC), enteroaggregative *E.coli* (EAEC), enterohaemorrhagic *E.coli* (EHEC), enteroinvasive *E.coli*, (EPEC), Shiga-toxin producing *E.coli* (STEC), atypical EPEC (ATEC).

E.coli species are listed as the dominate bacteria group cultivated from the small and large intestine of the pig (Jensen, 2001). With the knowledge that the majority of *E.coli* associated with post weaning colibacillosis (PWC) are enterotoxigenic ß-haemolytic strains (Pluske *et al.* 2002), the proportions of ß-haemolytic and non-haemolytic bacteria were assessed to determine whether dietary treatment could change the balance of bacteria along the gastro intestinal tract of newly weaned pigs. The severity of in-appetence in pigs influences the rate of passage of digesta and consequently the opportunity for bacterial attachment to the intestinal wall and subsequent multiplication (Pluske *et al.,* 2002). The corresponding growth performance trial showed that in-feed medication reduced the growth performance of pigs in the pre and acute post weaning phases, and appeared to be related to reduced palatability of medicated feeds. This reduction in palatability may have provided the opportunity (through decreased rate of passage) for

the increased percentage of ß-haemolytic bacteria in the colon of pigs fed the medicated diet. In addition, a complication with in-feed medications is that assumptions of feed intake must be made to determine the appropriate inclusion rate. In the present study the efficiency of the antibiotics may have also been lowered by the impaired feed intake.

The quantity of faecal shed haemolytic *E.coli* is known to increase in the acute post weaning period in pigs with or without diarrhoea, with counts being higher in pigs with diarrhoea (Pluske *et al.,* 2002). PWC is characterised by a massive dominance of haemolytic *E.coli* in the jejunum of pigs with minimal changes to the other co-inhabiting bacterial groups (Pluske *et al.,* 2002). The receptors involved in the adhesion of pathogenic *E.coli* associated with PWC disappear a few weeks after weaning, and not all pigs possess the necessary receptors for the attachment of fimbriae (i.e. K88 also known as F4) for the pathogenic adhesion of *E.coli* (Pluske *et al.,* 2002). Pigs in the present study were not tested for the presence of the receptors; however the piggery where the trial was conducted has experienced outbreaks of PWC.

Dietary treatments which increase the viscosity of digesta along the gastrointestinal tract have been shown to increase the proliferation of ETEC in newly weaned pigs (Hopwood *et al.,* 2004, Pluske *et al.,* 2001, Pluske *et al.*, 2007). Conversely, ETEC infections have been shown to reduce the viscosity of digesta in the ileum of pigs (Pluske *et al.,* 2002). The process of extrusion involves the gelatinization of carbohydrates and is known to improve the digestibility of protein and carbohydrates (Bach Knudsen and Jorgensen, 2001; Mariscal-Landin *et al.,* 2002; Sun *et al.,* 2006) which theoretically could reduce the viscosity of the digesta, and alter the rate of passage. These benefits could limit the proliferation of pathogenic bacteria in the small intestine, and limit unfavourable fermentation in the large intestine. Future research to clarify the influence extrusion has on nutrient digestibility, viscosity and rate of passage it required and nutritional strategies to reduce the viscosity of digesta (in relation to nutrient digestibility) should be employed to limit the opportunities for ETEC proliferation.

Entertoxigenic *E.coli* associated with post weaning colibacillosis are generally identified by the detection of virulence genes which encode for heat labile enterotoxin (LT) or heat stable enterotoxins (STa and STb) or various fimbriae (Do *et al.,* 2006; Muller *et al.,* 2007). The presence and severity of ETEC have been shown to be related to the crude protein (CP) content of the post weaning diet (Gu *et al.,* 2004; Pluske *et al.,* 2002), where high proportion of undigested CP enter the large intestine and promote proliferation of pathogenic bacteria. The lack of detection of ETEC virulence genes (estIa, estIb and elt) in pigs fed the SMEC may indicate that protein digestion in the small intestine was improved in those pigs fed the extruded diets, but further research is required to confirm this. The present study suggests that extruded SMEC may have an antimicrobial like effect against ETEC along the gastro-intestinal tract, similar to that of in-feed antibiotics. A similar trend was observed for the virulence gene escV which is associated with enteropathogenic *E.coli* (EPEC), shiga-toxin producing *E.coli* (STEC) and atypical EPEC (ATEC). These *E.coli* groups are also diarrheagenic (Muller *et al.,* 2007) but less commonly associated with post weaning colibacillous in pigs.

The astA virulence gene is associated (but not exclusively) with the enteroaggregative *E.coli* (EAEC), which can cause diarrhoea and even death in pigs due to a heat stable enterotoxin (Menard and Dubreuil, 2002). *E.coli* carrying the astA virulence gene does not

always possess the potential to express enterotoxin (Menard and Dubreuil, 2002); therefore astA is not a direct measure of pathogenicity. The multiples PCR methods used in the current study are not quantitative and therefore limit the interpretation of dietary influence on *E.coli* along the gastro-intestinal tract. Further studies using methodologies which allow quantification of pathogenic *E.coli* along the gastrointestinal tract are desired.

Discussion

The ability to significantly influence the pre-weaning performance of the piglet by the use of a semi-moist extruded creep feed appears to be limited. Whilst minor improvements in performance were seen in some studies, the influence of feeding a creep diet over not feeding any feed during lactation was greater than the type of feed offered.

Where semi-moist extruded creep feed showed significant promise was in the performance of piglets immediately post-weaning, pigs fed semi-moist extruded creep appeared to handle the post-weaning growth check better than the other treatments. Improvement in the growth rate of piglets post-weaning was seen, significantly in one study, when they received semi-moist extruded creep compared with the standard regime. However, the studies were conflicted as to whether this was a result of higher feed intake or an improved conversion of feed to gain.

The form or composition of the diet didn't significantly affect the enzyme activity within the gastrointestinal tract, although the natural variance in the level of activity makes assessment of any differences difficult. Other studies tend to suggest that it is feed intake rather than the diet's composition or presentation that influences enzyme activity.

Gut histology was not significantly influenced by dietary treatment, although there was a tendency for those pigs fed the higher-quality semi-moist extruded creep to have both lower villus height and smaller crypt depth. This is somewhat expected with the increased digestibility of the ingredients in the semi-moist extruded creep, and our understanding of the role of poorer quality ingredients in stimulating gut activity.

Diet didn't have a significant effect on the gut flora present throughout the intestinal tract, although pigs fed the semi-moist extruded creep tended to have a higher bacterial count. The colon was the site of biggest difference, with increased total bacterial count, reflecting an increased count of non-haemolytic bacteria. This result is difficult to explain, though a faster gut transition time is expected with the lower viscosity of the semi-moist extruded creep diet.

Whilst there was this increase in total bacterial count, there was a lack of enterotoxigenic *E.coli* (ETEC) virulence genes identified in pigs fed the semi-moist extruded creep, which suggests enhanced protein digestion within the small intestine. Semi-moist extruded creep may have an "anti-microbial" effect, with the lack of detection of virulence genes associated with diarrheagenic *E.coli* groups.

Experiment 3 *- Influence of semi-moist creep feeding on lifetime performance of pigs in commercial production systems.*

Hypothesis

Enhanced gut development and increased weaning weights resulting from the use of semi-moist creep feeding regimes will be reflected through a significant improvement in lifetime performance and a reduction in disease incidence.

Methodology

As part of the study on pre-weaning performance of suckers at Farm C, a whole of life study was conducted to assess the effect of pre-weaning feed treatment on subsequent performance. Piglets received semi-moist extruded creep (SMEC), a medicated standard creep or a non-medicated creep from seven to 35 days of age, at 35 days of age all piglets received the standard feeding regime of the farm.

Results

There were no significant differences in the weight of pigs at or prior to weaning, however, pigs fed the SMEC diet were heavier than the standard treatment at the end of the week immediately post-weaning (Table 15), with the non-medicated group intermediate. This weight advantage for pigs fed the SMEC declined as the animals grew, such that there was no difference in live weight at any of the subsequent weighings.

Table 15. *Whole of life performance (means) for pigs receiving semi-moist extruded creep (SMEC), a medicated standard creep (Standard) or a non-medicated standard creep (Non-medicated) during days 7 to 35. Values within column groups sharing superscripts are not significantly different.*

	Day 7 Weight (kg)	Day 28 Weight (kg)	Day 35 Weight (kg)	Day 42 Weight (kg)	Day 69 Weight (kg)	Day 140 Weight (kg)
Standard	2.71	7.32	7.97[a]	9.94	22.39	80.31
Non-medicated	2.59	7.44	8.14[ab]	10.25	21.69	77.79
SMEC	2.64	7.68	8.43[b]	10.42	22.45	79.24
SED	0.07	0.19	0.20	0.24	0.48	1.24
P-value	N.S.	N.S.	0.058	N.S.	N.S.	N.S.

Mortality was monitored throughout the growth period (Table 16). Prior to weaning mortalities were similar between the medicated standard creep and the semi-moist extruded creep treatments, however, the non-medicated standard creep group had only half the number of deaths. The data suggests that there may be a reduction in post-weaning mortality when SMEC is offered (from day 7 to 35), with the advantage being greatest in the nursery phase (days 29 to 68), however, a much larger trial would need to be conducted to confirm this.

Table 16. *Whole of life mortality for pigs receiving semi-moist extruded creep (SMEC), a medicated standard creep (Standard) or a non-medicated standard creep (Non-medicated) during days 7 to 35.*

	Day 7 to 28	Day 29 to 68	Day 69 to 140	Total Deaths	Day 29 to 140	Post-weaning Mortality (%)
Standard	13	13	2	28	15	6.91
Non-medicated	6	10	4	20	14	6.25
SMEC	14	9	2	25	11	5.09

Discussion

Semi-moist extruded creep feeds allowed pigs to avoid some of the effects of the post-weaning growth check, however this small improvement in growth was not maintained throughout the whole of life. The nature of a commercial operation and the many variables present affecting pig performance may not have allowed this reduced growth-check to be expressed in later growth performance, although the effects may be reflected in the lower post-weaning mortality observed compared to both the medicated and non-medicated treatment groups.

Experiment 4 *- Capacity of semi-moist creep feeds to improve the performance of lightweight piglets and runts*

Hypothesis

Semi-moist extruded creep feeds will support improved performance in segregated lightweight piglets or runts.

Methodology

The lightest eight per cent of piglets at weaning (approximately 21 days of age) were segregated from the remaining weaners, housed in groups of 20 piglets and kept in a temperature controlled facility. Piglets were offered one of two diets, semi-moist extruded creep or a slurry of water and standard creep feed (15.5MJ DE/kg, 0.90g AvL/MJ DE) supplemented with 17% skim-milk powder for 21 days.

Feed intake and FCR results are reported on a dry feed basis.

Results

Average piglet weaning weight was low with the average of this set approximately 70% of the normal weaning weight for this farm (6.0kg). Whilst there were no significant differences between treatments, piglets consumed more semi-moist extruded creep than the standard slurry (table 10), however, they did not convert feed to gain as efficiently as those on the standard treatment. There was no significant difference in growth rates between treatments, though, those on the semi-moist extruded creep tended to grow slower than those on the standard treatment.

Table 10. *Improving the performance of lightweight weaners (mean ± s.e.) through the use of semi-moist extruded creep (SMEC) versus a standard creep feeding regime (Standard).*

	No. of pens / weaners	Start Weight (kg)	End Weight (kg)	Weight Gain (kg)	ADFI (kg)	FCR
Standard	6 / 120	4.27 ± 0.06	8.06 ± 0.17	3.79 ± 0.14	0.363	2.17
SMEC	6 / 120	4.20 ± 0.06	7.71 ± 0.13	3.51 ± 0.12	0.397	2.20
SED		0.09	0.22	0.18		
P-value		N.S.	N.S.	N.S.		

Discussion

Whilst there was no improvement in performance through the use of semi-moist extruded creep it was the preferred feed by the operators. As semi-moist extruded creep is a clean, solid pellet that encompasses a comparative amount of skim milk powder and can be fed dry it makes for a more hygienic environment. On a similar basis, it was also labour-saving, as it did not need to be made fresh for each feed event.

3. Outcomes

This project has seen the successful development of a process of high-temperature cooking that delivers a highly-palatable, semi-moist (80% dry matter) extruded creep feed that incorporates high-quality ingredients such as whole egg and milk into a shelf-stable product.

Whilst we were able to develop a high-quality product, its ability to significantly influence the pre-weaning performance of the piglet appears to be limited. Whilst minor improvements in performance were seen in some studies, the influence of feeding a creep diet over not feeding any feed in lactation was greater than the type of feed offered.

Semi-moist extruded creep feeds allowed pigs to avoid some of the effects of the post-weaning growth check; however this small improvement in growth was not maintained throughout the whole of life. The ability of the pig to express these small differences is likely to be confounded by the many variables that exist in the commercial environment.

Diet form or composition didn't significantly influence the enzyme activity within the gut, or the structure within the intestines, nor did it have a significant effect on gut flora present throughout most of the intestines, although there was an increased total bacterial count in the colon of pigs fed the semi-moist extruded diet. The use of the semi-moist extruded feed did however influence the type of bacteria present, with a lack of detection of virulence genes associated with diarrheagenic *E.coli* groups.

Whilst the semi-moist extruded feed did not improve performance of runt pigs post-weaning, the form of the diet – a clean solid pellet, and its ease of handling resulted in it being the preferred feed, by management, over a slurry gruel mix of standard creep feed, milk powder and water. This product has the ability to replace the requirement for using slurry feeds resulting in improved hygiene.

4. Application of Research

- This research has established a set of production parameters for the manufacture of shelf-stable semi-moist feeds.
- The product developed during this research has the ability to replace liquid gruels as a feed for runt piglets at weaning, resulting in reduced labour requirements and increased hygiene.
- The product developed appears to have a beneficial effect on gut flora which may be of importance to particular production systems experiencing gut health issues.
- The production parameters established may allow us to exploit the current hypothesis on the stimulation of gut development by poorer quality ingredients. The increased palatability of the product is likely to result in enhanced intake of these poorer quality ingredients. Similarly the increased palatability of this product makes it suitable as a delivery device for less palatable medications and supplements.
- The production parameters established are likely to be applicable to other feeds, for example, a highly palatable lactation diet for low appetite sows.

5. Conclusion

This project has delivered:

- A set of production parameters for the manufacture of shelf-stable semi-moist feeds.
- A highly-palatable product that is suitable as a delivery device for less palatable ingredients, whether they be medications, supplements or poorer quality ingredients that may play a greater role in gut development.

6. Limitations/Risks

- The substantial 'noise' that exists within a commercial pig operation appears to limit the expression of the benefits of a reduced post-weaning check.

7. Recommendations

- There is a need to assess the benefits available from using semi-moist extruded diets to deliver poorer quality ingredients in an aid to developing gut health.
- Establish whether this technology is applicable in increasing the nutrient intake of poor appetite sows during lactation.

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