Reducing the risk of post-weaning *E. coli* diarrhoea using different sources of fibre in diets

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By

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Executive Summary

This project comprised three studies aimed at determining the impacts that feeding non-starch polysaccharides (NSP) have on aspects of gastrointestinal tract (GIT) structure and function, production, and circulating measures of physiology and immune function. Experiment 1, 'Virulence testing and serotyping of an enterotoxigenic *E. coli* (ETEC) isolate', was directed at confirming the nature of the *E. coli* isolate used at Murdoch University in controlled infection studies. Experiment 2, 'Determining the insoluble fibre requirement of weaner pigs when fed a diet containing either a low or high level of soluble fibre', used the same *E. coli* isolate to infect pigs after weaning and examine the effects of soluble and insoluble fibre on post-weaning diarrhea (PWD), production, and physiological responses. Experiment 3, 'Reducing the risk of *E. coli* by establishing a fibre recommendation after weaning', was a commercial validation study conducted at CHM Westbrook based on the findings from Experiment 2.

The results of Experiment 1 clearly demonstrated that the toxin and serotype profile of the ETEC isolate used at Murdoch University for controlled infection studies is representative of those found in field cases of PWD. Experiment 2 examined different ratios of insoluble NSP (iNSP) (as Opticell®) and soluble NSP (sNSP; as purified or semi-purified sources) in the diets under conditions of ETEC infection. The results suggested that using an iNSP source for weaner pigs in antimicrobial-free diets with lower sNSP levels had some beneficial effects on measures related to GIT structure and function, expression of PWD, and production in the 2 weeks after weaning, however in the third week after weaning, beneficial effects of higher sNSP levels were noted. Furthermore, and as measured by expression of tight-junction protein gene expression in the small intestinal epithelium, increasing the dietary iNSP content when there was minimum inclusion of sNSP enhanced intestinal barrier function. Diminished barrier function is a key aspect associated with weaning, so even in the absence of clinical disease/inclusion of antimicrobial compounds in feed/water, higher levels of dietary insoluble NSP could be considered to assist in the restoration of barrier function in the post-weaning period.

Nevertheless and in contrast to Experiment 2, Experiment 3, which examined the inclusion of Opticell® (albeit at lower levels than used in Experiment 2) or sugar beet pulp (SBP), failed to improve the performance of pigs in the post-weaning period. Pigs in this study received medications that most likely contributed to the lack of effects, suggesting that the concomitant use of medications is likely to reduce any beneficial effects of feeding iNSP

In conclusion, the data derived from the three experiments conducted in this project suggest that the type and level of NSP, in corollary to the ratio between sNSP: iNSP, play important roles for pig growth, GIT structure and function, expression of PWD, and aspects of physiology of weaner pigs. Under conditions of higher pathogenic bacterial load and antimicrobial-free production, increasing the iNSP content in the diet and trying to minimise sNSP levels for weaner pigs immediately after weaning should be considered. Data from the commercial validation study suggest, however, that the effects of including antimicrobial compounds need to be considered when evaluating the potential efficacy of manipulation of fibre types and content of the diet to reduce PWD and improve production indices.

Table of Contents

1. Introduction

A number of different dietary and (or) feeding strategies are being examined around the world, including in Australia, to ameliorate the post-weaning malaise given increased scrutiny of the use of antimicrobial agents in diets. The extent to which dietary fiber, and the balance between soluble and insoluble fiber, might modify gastrointestinal tract (GIT) structure and function to influence performance and health and reduce post-weaning diarrhea (PWD) in the absence of dietary antimicrobials has received a lot of research effort in recent years, with some positive results, however the precise relationships have still not been fully established. In this regard, associations between dietary fibre (as non-starch polysaccharides, or NSP) and pathogen proliferation (e.g., enterotoxigenic *Escherichia coli*; ETEC) in the GIT of weaner pigs have been extensively studied, and published evidence from research conducted in weanling pigs suggests that soluble NSP increases the expression of PWD (e.g., McDonald et al., 2001; Pluske et al., 2002; Hopwood et al., 2006) whilst feeding insoluble NSP decreases expression of PWD (e.g., Kim et al., 2008; Molist et al., 2010). Insoluble NSP reduce digesta retention time and hence the time available for proliferation of toxin-producing *E. coli* in the GIT. Also, mechanical contact between mucosa-bound *E. coli* and insoluble NSP has been suggested as a mechanism associated with reduced *E. coli* adhesion in the ileal mucosa of pigs fed increased dietary insoluble NSP (Molist et al., 2010). However, the presence of soluble NSP can increase digesta retention time and hence hinder the effect of insoluble NSP on reducing the time for pathogen proliferation in the GIT (Kim et al., 2012).

Traditionally, in-feed antimicrobials have been used to ameliorate pathogen infection-associated diseases such as PWD. However, and due largely to the development of resistance to antibiotics, other solutions for mitigating PWD for the Australian pork industry are required. There are, of course, many feed additives in the marketplace available for use, however these additives (1) will act to ameliorate GIT dysfunction after weaning in different ways and by different mechanisms, (2) often show variable responses in the field, and (or) (3) may be withdrawn from diets when margin over feed cost is decreased. In contrast, manipulating the use of endogenous dietary nutrients, in this case carbohydrates (through dietary fibre), through diet formulation to mitigate PWD offers an alternative (or complementary) approach to feed additives. In this regard, the nutritional strategy proposed in this project was to alter the ratio of dietary fibre to try and establish the 'optimum' level of insoluble fibre that can have beneficial effects on decreasing PWD. Even though previously we have demonstrated, in dietary protein studies, that feeding a lower protein diet (<180 g/kg) immediately after weaning decreases PWD via reducing production of toxic protein fermentation by-products such as ammonia, phenol and amines that irritating enterocytes (Heo et al., 2008, 2009, 2010), PWD is recognized as a multi-factorial disease. Therefore, reducing dietary protein content alone does not sufficiently eliminate PWD, hence our focus on the dietary fibre content in diets.

The involvement of dietary NSP on the development of enteric diseases has been demonstrated, as mentioned previously, however a number of key questions have been raised with respect to fully understanding both the background and the putative mechanisms for the association. These questions include: (1) does both soluble and insoluble NSP encourage pathogen proliferation; (2) does solubility or the viscous forming ability of NSP attribute to the increased pathogen proliferation in the GIT; (3) does the interaction between dietary protein and NSP, which will eventually determine fermentable carbohydrates: fermentable protein ratio in the lower small intestine, affect substrates for pathogens in the lower intestine; and (4) how much NSP (balance between soluble and insoluble) is optimum for prevention of enteric disease at immediate post-weaning period? (Kim et al., 2012).

Given that diets fed to pigs after weaning invariably contain a mixture of soluble and insoluble NSP, a question that had to be addressed in this project was how much dietary insoluble NSP is 'optimum', in the presence of a low or a high soluble NSP content, for the control of PWD. The soluble NSP content in commercial diets for weaner pigs in Australia can vary considerably depending on the ingredients used, between 5 g/kg in a sorghum and animal protein-based diet to 30 g/kg in a wheat, barley, soybean meal and animal protein-based diet. Therefore, the optimum insoluble NSP requirement to enhance GIT health and function and mitigate PWD needs to be determined without and with the presence of soluble NSP.

This project comprised three studies aimed at addressing this overall objective. Experiment 1, involved 'Virulence testing and serotyping of an ETEC isolate' and was aimed at confirming the nature of the *E. coli* isolate we have at Murdoch University and use for controlled infection studies. Experiment 2, 'Determining the insoluble fibre requirement of weaner pigs when fed a diet containing either a low or high level of soluble fibre', used the *E. coli* isolate to experimentally infect pigs after weaning and examine the effects of soluble and insoluble fibre on PWD and production and physiological responses. Experiment 3, 'Reducing the risk of *E. coli* by establishing a fibre recommendation after weaning', was the commercial validation done at CHM Westbrook of the major findings from Experiment 2.

2. Methodology

Experiment 1

Virulence testing and serotyping of an ETEC isolate

This part of the methodology, "Virulence gene testing of enterotoxigenic *E. coli* isolates", was completed in advance of Experiment 2 of the project to ensure that the serotype used at Murdoch University for inolculation experiments was representative of a major serotype found in field cases of post-weaning diarrhoea (PWD) in Australia at the time. This was because the *E. coli* isolate used in infection studies at Murdoch University has been questioned previously for its virulence gene profile, e.g., the isolate may not be representative of isolates causing disease and mortaiulity in the field.

To test this proposition, the *E. coli* isolate used at Murdoch University was sent to ACE Laboratory Services (Bendigo, Victoria) for virulence testing and for serotyping (DPI Bendigo). The results of these analyses are shown in Appendix 1. The data clearly show that the toxin and serotype profile of the isolate are representative of those found in field cases of PWD, and provided the necessary support for the infection study proposed for Experiment 2 to continue.

Experiment 2

Determining the insoluble fibre requirement of weaner pigs when fed a diet containing either a low or high level of soluble fibre

Aim

To determine the optimum insoluble non-starch polysaccharide (NSP) level in a weaner diet with low and high soluble NSP under conditions of enterotoxigenic *E. coli* infection.

Hypotheses

3. Increasing insoluble NSP in a diet for weaner pigs will reduce the incidence of post-weaning diarrhoea (PWD) and improve indices of GIT health.
4. A greater level of insoluble NSP will be required to reduce the incidence of PWD when a weaner diet contains higher levels of soluble NSP.

Materials and Methods

This experiment was conducted in August 2013 at the (base-funded) Medina Research Station. A completely randomised block experiment was conducted to explore the effects of insoluble NSP contents, with low and high soluble NSP, on indices of gut structure and function and the incidence of PWD. The experimental design was a 2 x 4 factorial experiment using 96 male pigs individually housed with the factors in the study being two soluble NSP levels (approximately 7 g/kg vs. 28 g/kg; **low** versus **high**) and four levels of added insoluble fibre (0, 30, 60, and 90 g/kg added Opticell®, to create insoluble NSP levels of approximately 5.5, 19, 34.5 and 51 g/kg insoluble NSP). The soluble NSP contents were achieved by adding a mixture of soluble arabinoxylan [as purified NSP wheat, AXRF; a gift from Dr. Barbara Williams, The University of Queensland. Analysis conducted at The University of Queensland showed 35.9% arabinoxylan in the AXRF (air-dried material) with a A/X ratio of 0.61], soluble ß-glucan (purified from barley and added as Glucagel®; approximately 600 g/kg soluble ß-glucan), and pectins [purified from citrus; added as GENU Pectin Type 115 with approximately 650 g/kg galacturonic acid (CP Kelco)] in ratios that were "typical" of those found in an Australian weaner diet based on common ingredients. Ground white rice was used as the base cereal given it has a very low NSP content, hence any effects of the soluble and insoluble NSP in the diet could be attributed to those added. These diets were fed for only two weeks after weaning, after which time all surviving pigs were fed a commercial weaner diet. Diets were devoid of antimicrobial compounds.

All pigs were orally infected with an ETEC strain (0149:K91:F4; Experiment 1) on d 5, 6 and 7 after arrival from a commercial farm 2 hours east of the Medina Research Station, where they were weaned at approximately 28 d of age. Faecal consistency (FS) score, diarrhoea index (DI), and the number of therapeutic antibiotic treatments were recorded for all pigs, as described previously (Heo et al., 2009). Faecal ß-haemolytic *E. coli* shedding was measured on d 0, 5, 7, 9, 11, and 13. Faeces were collected for subsequent determination of total tract apparent digestibility and volatile fatty acid (VFA) levels. On d 5 after arrival, 6 pigs (randomly selected from each treatment) were bled to determine baseline contents of haptoglobin, plasma urea and white blood cell counts. On d 8/9 after weaning, blood samples were collected from the same 6 pigs selected from each treatment group for bleeding on d 5. The blood samples were analysed for haptoglobin, plasma urea and white blood cell counts to compare with pre-infection levels.

On d 8/9, the same six pigs selected from each treatment group for bleeding on d 5 and 8 were euthanised with intestinal tissue, digesta and mesenteric lymph nodes (MLN) samples collected. The haemolytic *E. coli* colonies were counted in the ileal digesta and ileal mucosa samples by plating serially diluted samples on 5% sheep blood agar plate (McDonald et al., 2001). Samples of small intestine were taken and placed in 10% phosphate-buffered formalin for subsequent measurement of villous height and crypt. To measure paracellular translocation of haemolytic *E. coli* into the system, tight junction protein mRNA gene expression in the jejunal epithelium and migrated haemolytic *E. coli* into the mesenteric lymph nodes were measured. The viscosity and dry matter (DM) content of the ileal digesta were measured.

The remaining 6 pigs from each treatment were used for measurement of performance indices during a 21-d feeding experiment (weekly). Data would therefore provide an approximate indication whether increasing soluble or insoluble NSP levels would influence performance of pigs. Following completion of the experiment after 21 d, the remaining pigs were transported to an Eco shelter and grown out as per commercial practice.

Chemical, plasma and gene analysis

Dry matter content was determined using AOAC official method (930.15, AOAC, 1997). The N content was determined using combustion method (990.03, AOAC 1997). The NDF, ADF and lignin contents were determined using the AOAC official methods 925.10 (AOAC, 1997). Gross energy content was determined using a ballistic bomb calorimeter (SANYO Gallenkamp, Loughborough, UK). Crude protein content was calculated as N content × 6.25. The TiO_2 contents of diet and faecal samples were determined using the method described by Short et al. (1996). The insoluble and soluble **NSP** contents of the diet samples and individual ingredient samples were determined as alditol acetates by GLC using the method of Theander and Westerlund (1993).

Within 30 min of collection of the digesta samples, viscosity was determined. An aliquot of the digesta from the ileum was placed in an Eppindorf tube, mixed on a vortex, and centrifuged at 12,000 *g* for 8 min (Quantum Scientific Pty. Ltd., Milton, Qld, Australia). The supernatant fraction (0.5 ml) was placed in a Brookfield LVDV-II + cone-plate rotational viscometer (CP40; Brookfield Engineering Laboratories Inc., Stoughton, MA, USA), and the viscosity of all samples was measured. The viscosity was measured in mPas. The pH of the digesta was measured by inserting the electrode of a calibrated portable pH meter (Schindengen pH Boy-2; Schindengen Electric MFG, Tokyo, Japan) into the collected sample.

To determine VFA concentrations, thawed digesta samples were diluted 1:1 (w/v) with distilled water, mixed, centrifuged and the supernatant fraction was analysed chromatographically. The supernatant fraction (0·1 ml) was added to a 1 ml internal standard solution containing valeric acid before processing with capillary GC. A working standard and a control (distilled water) were included in each run of the analysis, with the working standard containing acetic acid (60 mM), propionic acid (20 mM), isobutyric acid (6·67 mM), butyric acid (20 mM), isovaleric acid (10 mM), valeric acid (10 mM) and caproic acid (4 mM). The Hewlett Packard 5890A capillary GC (Agilent Technologies, Forrest Hill, Vic., Australia) was maintained at injector and flame ionisation detector settings of 260 and 265˚C, respectively, and an initial and final oven temperature of 120 and 240˚C, respectively. The carrier gas flow rate was 5 ml/min and the split-flow rate was 70 ml/min. The Hewlett Packard Chemstation integration system was used to calculate the VFA concentrations from the area of the peaks.

Haptoglobin content in the plasma sample was determined using a modified method of Makimura and Suzuki (1982). Modifications were a higher concentration of sodium dihydrogen phosphate dihydrate (30 mM in reaction mix) and the use of a commercial supply of haemoglobin (Sigma-Aldrich, H2625) to produce the haemoglobin reagent (30 g/L in normal saline). The method was adapted onto an Olympus Au400 Autoanalyser (Olympus, Tokyo, Japan). Whole blood count was done using an automatic haematology analyzer (ADIVA 2120, Bayer Healthcare, Siemens, Germany). Plasma urea content was measured using a urease kinetic method with an automatic analyser (Randox Daytona, Crumlin Co., Antrim, UK).

For mucosal histology examination, ring-shaped sections of the intestine from the jejunum and ileum were excised, dehydrated, and embedded in paraffin wax, after fixation for several days in 10% phosphate-buffered formalin. From each of these, six transverse sections (4–6 mm) were cut, stained with haematoxylin and eosin, and mounted on glass slides. The height of 10 well oriented villi, their associated crypts, and thickness of the muscular layer were measured with a light microscope (OLYMPUS CX31, Tokyo, Japan) using a calibrated eyepiece graticule.

Expressions of the mRNA encoding tight junction proteins, Zonula Occludin-1 (ZO-1), occludin, and claudin, were determined in jejunal mucosal scrapings by a reverse transcription-polymerase chain reaction (RT-PCR). For RNA extraction, approximately

100 mg of mucosal tissue scraping from the jejunum was placed into 1mL of TRIzol Reagent (Invitrogen, VIC, Australia). This was then homogenised using a tissue homogenizer for 45 seconds. Total RNA was extracted using the PureLink RNA mini kit (Invitrogen, VIC, Australia) according to the manufacturer's instructions. Any possible contamination of genomic DNA was eliminated using PureLink DNase treatment (Invitrogen, VIC, Australia). Primers for the target genes occludin, ZO-1 and claudin were designed using Primer Quest (www.idtdna.com/scitools/applications/primerquest) and then further tested using Amplify 3X. Sequences were searched and selected from Genbank (http://www.ncbi.nlm.nih.gov/genbank/) and then primers were designed using the Primer Quest option once the relevant sequence was found. Primer sequences used were: occludin (Forward 5'-GCAGCAGTGGTAACTTGGA-3'; Reverse 5'-GTCGTGTAGTCTGTCTCGTAATG-3'), ZO-1 (Forward 5'-CGGCGAAGGTAATTCAGTGT-3', Reverse 5'-CGGTTTGGTGGTCTGTAAGT-3'), and claudin (Forward 5'-GCCCGTCCATCGTCCACCG-3', Reverse 5'-CAGGAGGCTGGCATGAGGTGTG-3'). Primers were ordered from Sigma.

Expression of occluding, ZO-1 and claudin were normalised to an endogenous control gene (Actin, ß) to give a ΔCt value. This accounted for variability in the initial starting amount of cDNA. An aliquot of a previously run sample from a standard curve with a known Ct value was also placed in every run, to compare run-to-run variance and to determine the amount of the gene. Cycling conditions for RT-PCR consisted of two holds of 50 °C for 2 min, 95 °C for 10 min and then cycling for 40 cycles for 95 °C 15 sec, 60 °C for 1 min and 60 °C for 60 sec. Each sample was run in triplicate.

The ΔCt has been traditionally used in Real-Time PCR data analysis. This does not take into account the efficiency of the primers that can vary from run to run. Thus we used both methods of data analysis. The actin beta Ct values were subtracted from the Ct value for each gene to give ΔCt values. These values were used to carry out statistical comparisons between and within genes.

For graphical representation, the fold variation was then determined using the $2^{-(\Delta\Delta Ct)}$ method according to publisher's protocols and the manufacture's recommendations. Fold variation was calculated by determining the difference in ΔCt values between a chosen reference and test sample ($\Delta\Delta$Ct value), and application of the $2^{-(\Delta\Delta Ct)}$ formula. When comparing individual genes between animals, the reference used was the expression levels of each gene in the control animals. The Pfaffl method is the relative expression of a target gene that is calculated based on the efficiency (E) of the primers used and the Ct deviation of the unknown gene versus a control, and expressed in comparison to a reference gene (Pfaffl, 2001).

$$\text{Pfaffl ratio} = \frac{(E_{\text{target}})^{\Delta Ct \text{ target (control - sample)}}}{(E_{\text{ref}})^{\Delta Ct \text{ ref (control - sample)}}}$$

Statistical analysis

Two-way analysis of variance with randomised block design was used for statistical evaluation of data using the statistical package Genstat (version 16, VSN International Ltd., Hemel Hempstead, UK). The pig was the experimental unit. Polynomial comparison was used to examine effect of increasing insoluble NSP content on measured variables when ANOVA result showed significant effect of insoluble NSP. Blood indices data, which were measured before and after ETEC infection, were analysed using repeated-measures ANOVA. Simple linear regression analysis was conducted to establish relationship between insoluble NSP concentration in the diet and measured variables such as performance of pigs and total tract gross energy digestibility.

Results

Diets

Details of the diets formulated for Experiment 2 are shown in Table 1, and the analysed contents of the soluble and insoluble NSP contents of the diets are shown in Table 2. The full NSP profile of the diets and ingredients used in the study are shown in Appendix 2.

The Opticell® contained: 558 g/kg crude fibre, 642 g/kg ADF and 737 g/kg NDF. The Glucagel® contained: 2.8 g/kg and 0.8 g/kg ADF and NDF respectively (no crude fibre was detected). The wheat soluble NSP product contained: 44 g/kg crude fibre, 73 g/kg ADF and 268 g/kg NDF.

For comparison, the soluble and insoluble NSP contents and Van Soest analytes' contents are shown in Table 3.

Table 1- Composition (%) and calculated analyses of the 8 experimental diets used in Experiment 2.

Product: FR NSNI - Low soluble no insoluble NSP
Product: FR LSLI - Low soluble low insoluble NSP
Product: FR LSMI - Low soluble medium insoluble NSP
Product: FR NSHI - Low soluble high insoluble NSP
Product: FR HSNI - High soluble no insoluble NSP
Product: FR HSLI - High soluble low insoluble NSP
Product: FR HSMI - High soluble medium insoluble NSP
Product: FR HSHI - High soluble high insoluble NSP

IngrCode	Ingredient Name	Ver:	Product FR NSNI 10	Product FR LSLI 8	Product FR LSMI 8	Product FR NSHI 8	Product FR HSNI 8	Product FR HSLI 8	Product FR HSMI 8	Product FR HSHI 7
44	Broken rice		63.8233	58.5904	52.9786	47.3668	58.8268	53.2151	47.6033	42.9142
401	Bloodmeal 85% Ring dried		2.0000	2.0000	2.0000	2.0000	2.0000	2.0000	2.0000	2.0000
412	Meat Meal 50%		4.2546	4.2075	4.1361	4.0647	4.2105	4.1391	4.0678	2.5443
423	Fishmeal 60%		10.0000	10.0000	10.0000	10.0000	10.0000	10.0000	10.0000	10.0000
441	Skim milk powder		3.1938	3.7481	4.5044	5.2607	3.7163	4.4725	5.2288	6.7475
445	Whey powder, acid		10.0000	10.0000	10.0000	10.0000	10.0000	10.0000	10.0000	10.0000
447	Milk Casein		5.0000	5.0000	5.0000	5.0000	5.0000	5.0000	5.0000	5.0000
492	Canola Oil		.0000	1.7213	3.6406	5.5599	0.4910	2.4103	4.3296	6.0000
501	Lysine		0.0991	0.0938	0.0899	0.0860	0.0940	0.0900	0.0861	0.0949
502	Methionine		0.1765	0.1826	0.1905	0.1983	0.1823	0.1901	0.1980	0.2086
504	Threonine		0.0950	0.0941	0.0938	0.0935	0.0941	0.0938	0.0935	0.0979
505	Tryptophan		0.0764	0.0760	0.0758	0.0755	0.0760	0.0758	0.0755	0.0732
544	BJ Grower Premix		0.1000	0.1000	0.1000	0.1000	0.1000	0.1000	0.1000	0.1000
573	Salt		0.2000	0.2000	0.2000	0.2000	0.2000	0.2000	0.2000	0.2000
575	Choline Chloride 60%		0.0274	0.0322	0.0364	0.0405	0.0321	0.0362	0.0403	0.0424
701	Titanium		0.1500	0.1500	0.1500	0.1500	0.1500	0.1500	0.1500	0.1500
801	soluble arabinoxylan		0.2230	0.2230	0.2230	0.2230	1.3370	1.3370	1.3370	1.3370
802	Soluble betaglucan		0.4580	0.4580	0.4580	0.4580	2.7500	2.7500	2.7500	2.7500
803	Pectin		0.1230	0.1230	0.1230	0.1230	0.7400	0.7400	0.7400	0.7400
OPTICELL	Opticell		.0000	3.0000	6.0000	9.0000	.0000	3.0000	6.0000	9.0000
	Ingredient Total:		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	Formula Cost ($/Ton):		525.83	573.25	629.42	685.60	547.89	604.07	660.24	720.89

Nutr	Nutrient Name	Units	Product FR NSNI	Product FR LSLI	Product FR LSMI	Product FR NSHI	Product FR HSNI	Product FR HSLI	Product FR HSMI	Product FR HSHI
2	GE	MJ/kg	16.399	16.331	16.313	16.295	16.528	16.510	16.491	16.425
3	DE	MJ/kg	14.845	14.800	14.800	14.800	14.800	14.800	14.800	14.800
7	NE	MJ/kg	10.456	10.416	10.407	10.398	10.316	10.307	10.298	10.289
10	Dry Matter	%	89.382	89.681	90.007	90.334	89.558	89.884	90.211	90.448
11	Protein	%	21.566	21.314	21.088	20.862	21.633	21.407	21.181	20.558
12	Fat	%	2.199	3.856	5.706	7.557	2.681	4.532	6.383	7.870
13	Cude Fibre	%	0.942	2.654	4.360	6.066	0.886	2.592	4.299	5.974
14	Ash	%	3.749	3.688	3.616	3.544	3.691	3.619	3.547	3.052
17	Av Lysine	%	1.351	1.347	1.347	1.347	1.347	1.347	1.347	1.347
18	Lysine	%	1.419	1.413	1.411	1.410	1.413	1.412	1.410	1.402
19	Av Methionine	%	0.602	0.605	0.610	0.616	0.605	0.610	0.616	0.623
20	Av Cystine	%	0.182	0.176	0.171	0.165	0.177	0.171	0.165	0.158
21	Av Meth + Cys	%	0.783	0.781	0.781	0.781	0.781	0.781	0.781	0.781
22	Av Threonine	%	0.851	0.848	0.848	0.848	0.848	0.848	0.848	0.848
23	Av Tryptophan	%	0.297	0.296	0.296	0.296	0.296	0.296	0.296	0.296
24	Av Isoleucine	%	0.770	0.768	0.768	0.768	0.768	0.768	0.768	0.768
25	Av Leucine	%	1.571	1.565	1.564	1.562	1.565	1.564	1.562	1.557
29	Av Phos	%	0.615	0.614	0.614	0.614	0.614	0.614	0.614	0.560
30	Calcium	%	1.100	1.100	1.100	1.100	1.100	1.100	1.100	0.954
31	Phosphorus	%	0.764	0.757	0.750	0.743	0.757	0.750	0.744	0.677
32	Phytate P	%	0.074	0.068	0.061	0.055	0.068	0.061	0.055	0.050
35	ND Fibre	%	3.621	3.348	3.054	2.760	3.583	3.289	2.995	2.705
36	AD Fibre	%	1.039	2.473	3.903	5.332	0.976	2.406	3.835	5.202
37	AD Lignin	%	0.383	1.252	2.118	2.984	0.353	1.219	2.086	2.957

Nutrient Ratios: (Template WEANER 1)

Nutr Name	Per	Nutr Name	Product FR NSNI	Product FR LSLI	Product FR LSMI	Product FR NSHI	Product FR HSNI	Product FR HSLI	Product FR HSMI	Product FR HSHI
17 Av Lysin	1.00	3 DE	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091
21 Av Meth	1.00	17 Av Lysin	0.580	0.580	0.580	0.580	0.580	0.580	0.580	0.580
22 Av Threo	1.00	17 Av Lysin	0.630	0.630	0.630	0.630	0.630	0.630	0.630	0.630
23 Av Trypt	1.00	17 Av Lysin	0.220	0.220	0.220	0.220	0.220	0.220	0.220	0.220
24 Av Isole	1.00	17 Av Lysin	0.570	0.570	0.570	0.570	0.570	0.570	0.570	0.570
25 Av Leuci	1.00	17 Av Lysin	1.163	1.162	1.161	1.160	1.162	1.161	1.160	1.156
94 Av Val	1.00	17 Av Lysin	0.808	0.805	0.800	0.796	0.805	0.801	0.796	0.787

Table 2 - The analyzed non-starch polysaccharide (NSP) contents (analysis conducted at The University of New England) of the 8 experimental diets used in Experiment 2.

Diets 1-4: "Low" soluble NSP content

Diets 5-8: "High" soluble NSP content

Diet	Soluble NSP (g/kg)	Insoluble NSP (g/kg)	Total NSP (g/kg)
1	6.8	5.4	10.1
2	7.3	18.1	25.3
3	7.5	33.2	40.7
4	7.5	49.2	56.8
5	27.9	6.5	34.3
6	26.9	19.7	46.2
7	28.4	35.9	64.2
8	32.4	53.3	85.7

Table 3 - A comparison of the NSP and acid detergent fibre (ADF), neutral detergent fibre (NDF), crude fibre (CF) and hemicellulose (HC) contents of the 8 experimental diets.

Diet	Soluble NSP (g/kg)	Insoluble NSP (g/kg)	NDF (g/kg)	ADF (g/kg)	CF (g/kg)	HC (g/kg)
1	6.8	5.4	23.6	4.4	ND	19.2
2	7.3	18.1	46.9	15.3	11.3	31.6
3	7.5	33.2	77.0	43.3	29.3	33.7
4	7.5	49.2	97.1	46.9	43.0	50.2
5	27.9	6.5	29.1	4.1	1.0	25.0
6	26.9	19.7	45.8	27.6	13.0	18.2
7	28.4	35.9	79.8	33.7	26.6	46.1
8	32.4	53.3	102.3	50.6	49.7	51.7

Post-weaning diarrhoea and the faecal scores of E. coli

Figure 1 shows that the ETEC infection procedures worked successfully in this experiment, supporting use of the Murdoch University *E. coli* isolate to successfully induce diarrhoea.

There were no clear dietary differences in the faecal scores (Table 4) and the scores indicated, in accordance with the Standard Operating Procedure used in the study (mandated by DAFWA AEC), that the infection did not cause persistent clinical diarrhea (Table 4), which was generally low regardless. Nevertheless and on d 9, there was a trend (P=0.074) for feeding a high level of soluble NSP to increase the faecal score (more diarrhoea), although this was determined by an interaction (P=0.007) with the insoluble NSP level. In the euthanised pigs, a trend was observed on d 9 (P=0.069) after weaning for a linear effect (P=0.0101) of increasing insoluble NSP content to decrease the excretion of ß-haemolytic *E. coli* in the faeces (Table 4).

The ß-haemolytic *E. coli* counts in the ileum and MLN are shown in Table 5. There were neither main effects nor an interaction (P>0.05) of the soluble and insoluble NSP contents of the diets on the colony forming units count in the ileal mucosa or the MLN. The overall counts were lower in the MLN than the ileal mucosa.

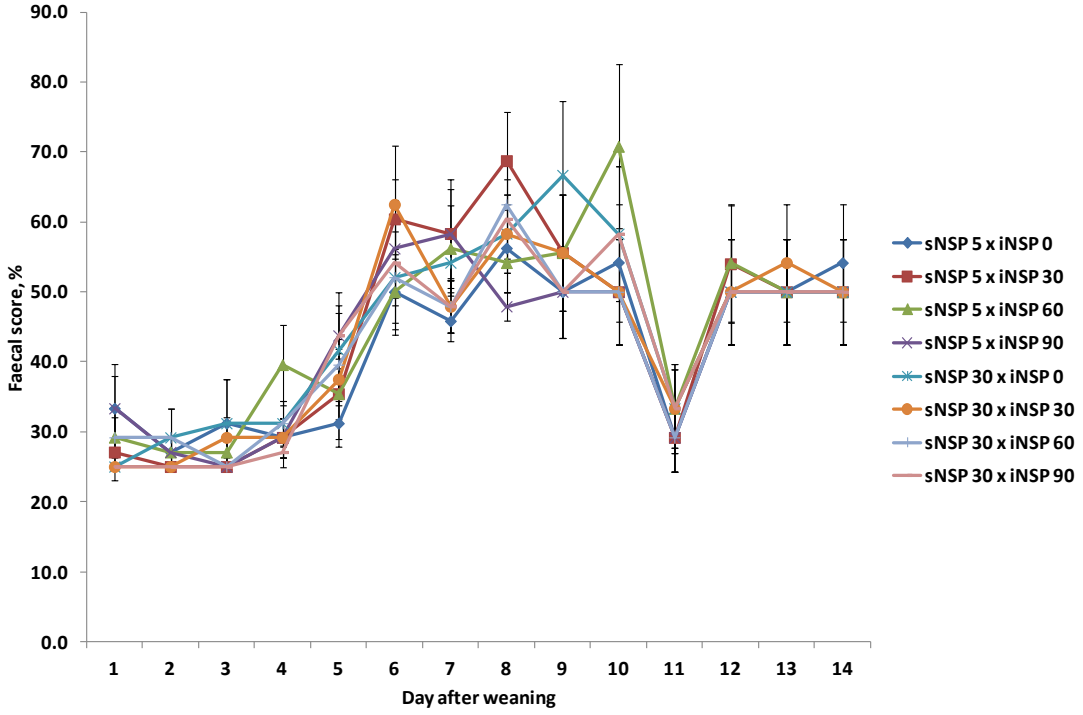

Figure 1 - Expression of faecal score (scored daily from 1, hard faeces, to 4, watery diarrhea, and then converted to percentages) before and after experimental infection with *E. coli* (infection occurred on d 5, 6 and 7 after weaning) in pigs fed diets differing in soluble and insoluble NSP contents for 14 d after weaning.

Production results and GIT indices

Table 6 presents the production data in the first 3 weeks after weaning. There was a trend for a main effect (P=0.107) of insoluble NSP content on an increase in bodyweight at d 14, and a linear (P=0.046) effect on bodyweight. At d 21 after weaning, the main effect of insoluble NSP was significant (P=0.046) and the linear effect stronger (P=0.019) for bodyweight. Figure 2 shows the relationships between fibre contents and production indices. In the first 14 d after weaning, each 10 g/kg increase in the analysed dietary NDF content (to ≈ 100 g/kg) increased average daily gain (ADG) and

average daily feed intake (ADFI) by 6.4 and 8.1 g/day (R^2=0.84, *P* = 0.085; R^2=0.89, *P* = 0.056, respectively), whilst each 10 g/kg increase in the analysed dietary ADF content (to ≈ 50 g/kg) increased ADG and ADFI by 10.6 and 13.3 g/day (R^2=0.89, *P* = 0.058; R^2=0.95, *P* = 0.027, respectively).

Table 6 also shows that the effects on bodyweight of insoluble NSP were reflected in changes in ADG, although the presence of an ADG interaction (P=0.049) between soluble and insoluble NSP contents during week 3 suggests a change in growth pattern. In this regard, surviving pigs were all fed a commercial pelleted weaner diet in the third week after weaning, and performance was then monitored until pigs were all moved to an eco-shelter at d 21. Data presented in Figure 3 and Figure 4 suggests that a minimum (30-40 g/kg) level of insoluble NSP was required in the diet in the first 14 d after weaning to maximise feed conversion efficiency in the subsequent week (d 15-21). Moreover, Figure 5 suggests that feeding a high level of soluble NSP in the third week after weaning, when all pigs were on the common diet, was beneficial to ADG.

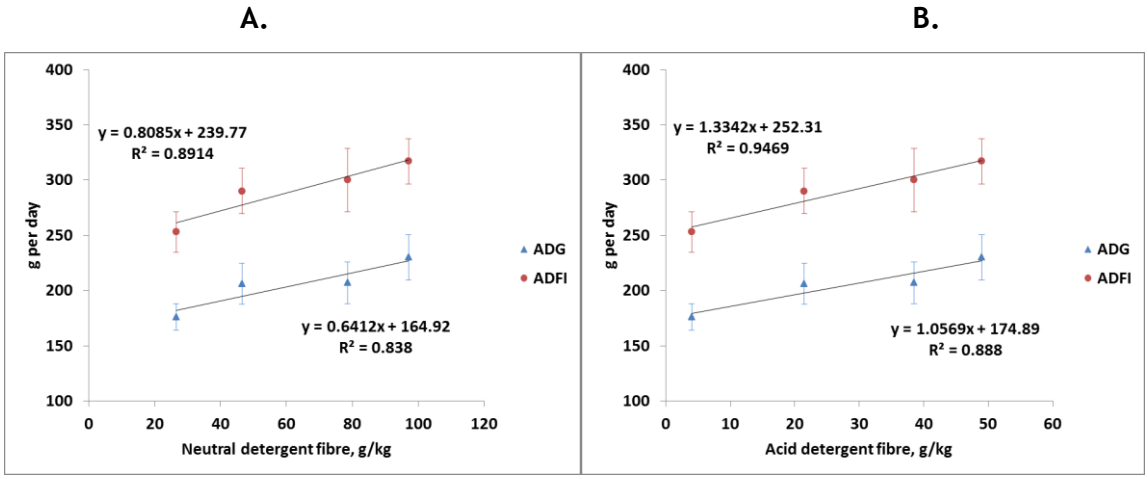

Figure 2 - The influence of increasing neutral detergent fibre content (A) and acid detergent fibre (B) (supplied as Opticell®) on average daily gain (ADG) and average daily feed intake (ADFI) in the first 2 weeks after weaning.

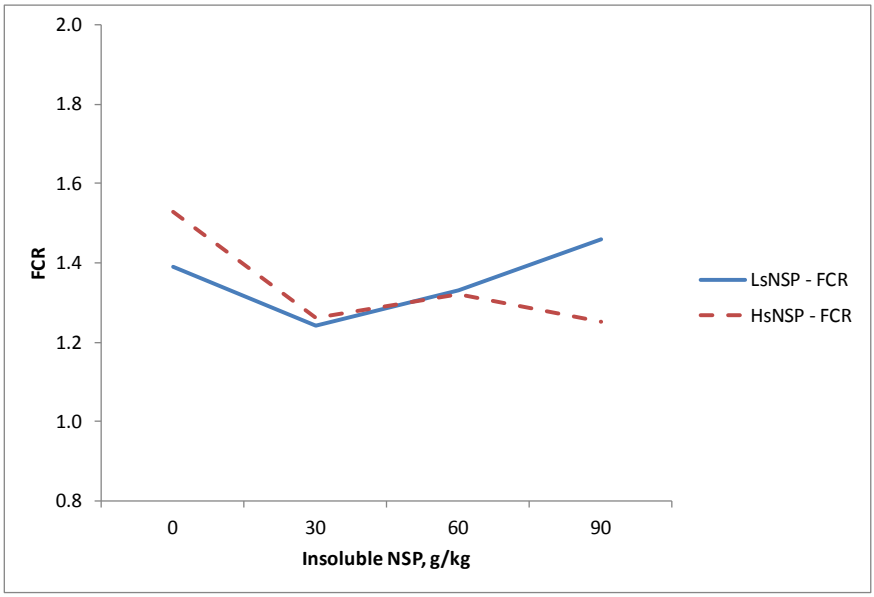

Figure 3 - The effects of feeding either a low soluble NSP diet (LsNSP) or a high soluble NSP diet (HsNSP) in the first two weeks after weaning on the FCR in the third week after weaning, where all pigs were fed a common diet. Insoluble NSP content refers to the level of added Opticell®.

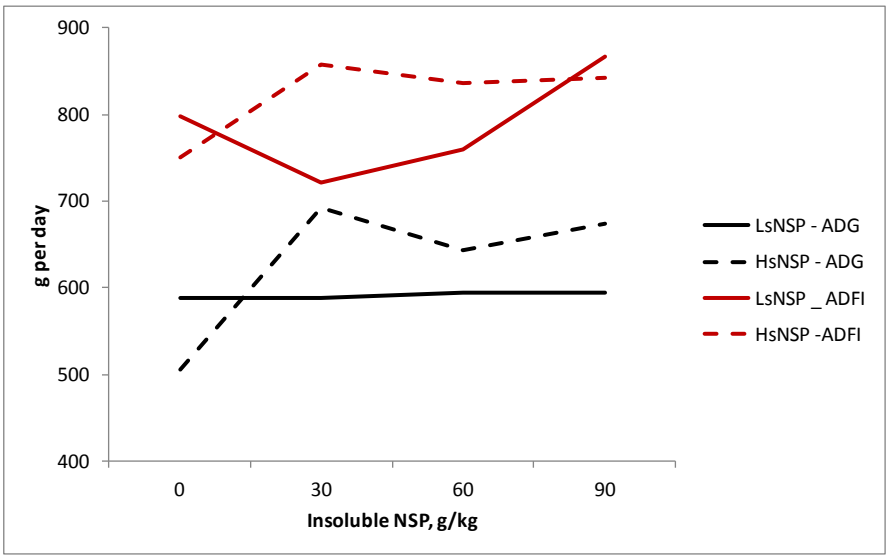

Figure 4 - The effects of feeding either a low soluble NSP diet (LsNSP) or a high soluble NSP diet (HsNSP) in the first two weeks after weaning on the ADG and ADFI in the third week after weaning, where all pigs were fed a common diet. Insoluble NSP content refers to the level of added Opticell®.

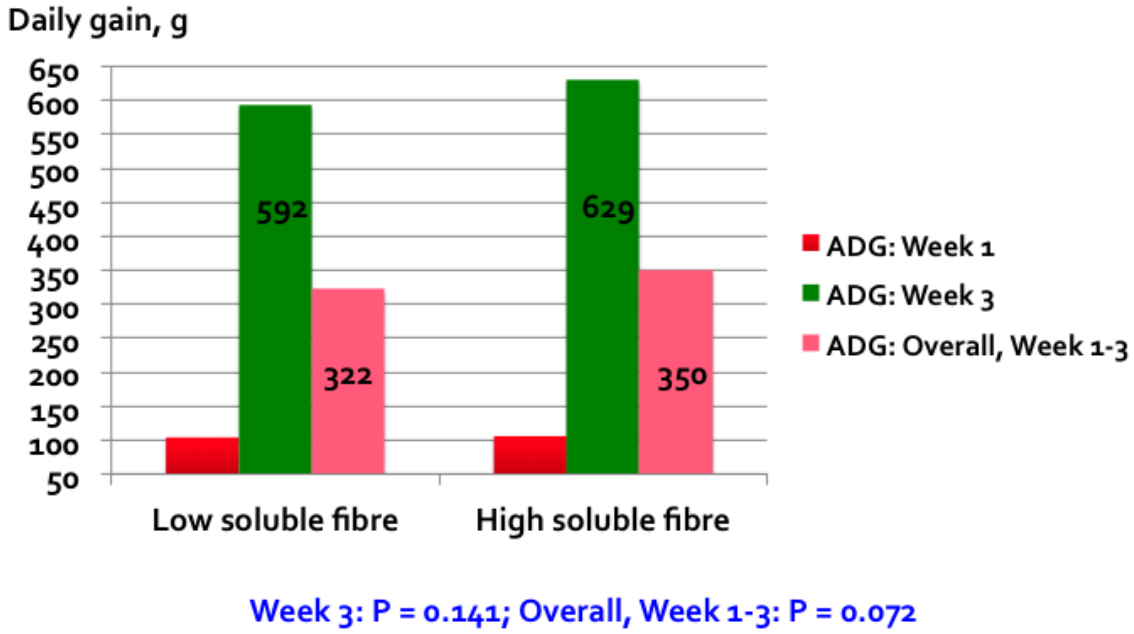

Figure 5 - Average daily gain in different periods after weaning in pigs fed a low or high soluble fibre content.

Dietary effects on ADFI showed positive effects of both soluble and insoluble NSP, with strong linear effects of the insoluble NSP content on feed intake (Table6). Similarly, there was a main effect of insoluble NSP content on the FCR during the experimental period, although in week 3 (P=0.035) and overall (P=0.038), the interaction between soluble and insoluble NSP contents determined the FCR. Higher levels of soluble NSP in combination with higher levels of Opticell® generally improved the FCR (Table6).

In the sub-sample of euthanized pigs, there was a main effect trend (P=0.104) for pigs fed more soluble NSP to have a heavier GIT. Feeding a greater content of soluble NSP in the diet decreased (P=0.002) the pH in the contents of the caecum only. The viscosity

of the contents of the terminal ileum showed a weak tendency (P=0.154) to increase with the feeding of more soluble NSP (Table7).

There was a significant main effect of soluble NSP on the total concentration of VFA in the caecum (61.6 vs. 76.0 mM, P=0.037, for low versus high soluble NSP content respectively). There was a significant main effect of soluble NSP content (P=0.007) and insoluble NSP content (P=0.037) on the molar ratio of acetic acid, with more acetic acid being produced in pigs fed a lower soluble NSP content or the highest level of Opticell®. This resulted in less propionic acid (P=0.036) being produced in the pigs fed more Opticell®. Significant main effects of soluble NSP content were found for the molar ratio of butyric acid (P=0.002; more soluble NSP increased the concentration) and for isovaleric acid (P=0.001; more soluble NSP decreased the concentration) (Table7).

Blood indices and apparent digestibility coefficients

Blood indices were analysed using repeated-measures ANOVA to take into account the effect of ETEC infection and the interaction between infection and dietary treatment. There were very few differences between treatments in this study in the concentrations of plasma urea and haptoglobin. Plasma urea content was increased by infection (2.08 vs. 2.44 mmol/L, respectively; P=0.040), however, no interactions were significant between infection and either soluble or insoluble NSP (Table 8).

There was no (P>0.05) interaction nor were there any main effects (P>0.05) of feeding soluble NSP or insoluble NSP on the apparent ileal digestibility (AID) and apparent total-tract digestibility (ATTD) of N in pigs at d 9 after weaning. On d 14 after weaning, the ATTD of gross energy (GE) displayed a main effect for insoluble NSP, with increasing levels of insoluble NSP in the diet causing a decline in the ATTD of GE (P<0.001). There was a trend for an interaction (P=0.095) however between the soluble and insoluble NSP contents, with the combination of low soluble NSP and zero added Opticell® having the highest ATTD of GE (90.9%) (Table 9).

Figure 6 displays the relationships between increasing amounts of insoluble NSP and the ATTD of GE for pigs fed a low (≈ 5 g/kg) or high (≈ 30 g/kg) soluble NSP diet. The figure demonstrates clearly that beyond approximately 45 g/kg added Opticell® (equivalent to ≈ 27 g/kg total insoluble NSP in the diet), feeding a greater amount of soluble NSP in the diet mitigates the decline in the ATTD of GE observed when a low soluble NSP content is fed in the diet.

Table 10 shows the effects of feeding soluble and insoluble NSP on selected blood parameters associated with blood cell counts. The data indicate that the presence of sNSP and iNSP in the diet significantly altered blood cell numbers. Infection with ETEC increased the number of red blood cells (RBC, P<0.05), white blood cells (WBC, P<0.001) and platelets (P<0.001). The number of RBC was affected by an infection (I) x sNSP interaction such that infection increased RBC in the pigs fed the low sNSP diet while the pigs fed high soluble NSP diets maintained the number of RBC. In contrast, the number of WBC was affected by an I x iNSP interaction such that increasing the iNSP content in the diet increased the number of WBC before infection while iNSP decreased the number of WBC after infection (P<0.05).

Table 11 shows the effects of feeding soluble and insoluble NSP on relative expression of tight junction protein genes in the jejunal epithelium. Expression of occludin tended to be influenced by a sNSP x iNSP interaction, such that increasing iNSP with low sNSP increased occludin expression, while increasing iNSP with high sNSP decreased occludin expression (P=0.072). Expression of ZO-1 tended to be influenced by the iNSP content in the diet (P=0.082).

Table 12 shows the effects of feeding soluble and insoluble NSP on villous height and crypt depth in the jejunum of the small intestine. The data indicate that crypt depth in

the ileum was increased in the pigs fed a high sNSP diets (P<0.05). There was a tendency for a sNSP x iNSP interaction, such that crypt depth was lowest in pigs fed a diet with low sNSP without inclusion of iNSP, while it was deepest in pigs fed a diet with high sNSP without supplementation of iNSP (P=0.056). There were no effects on villous height.

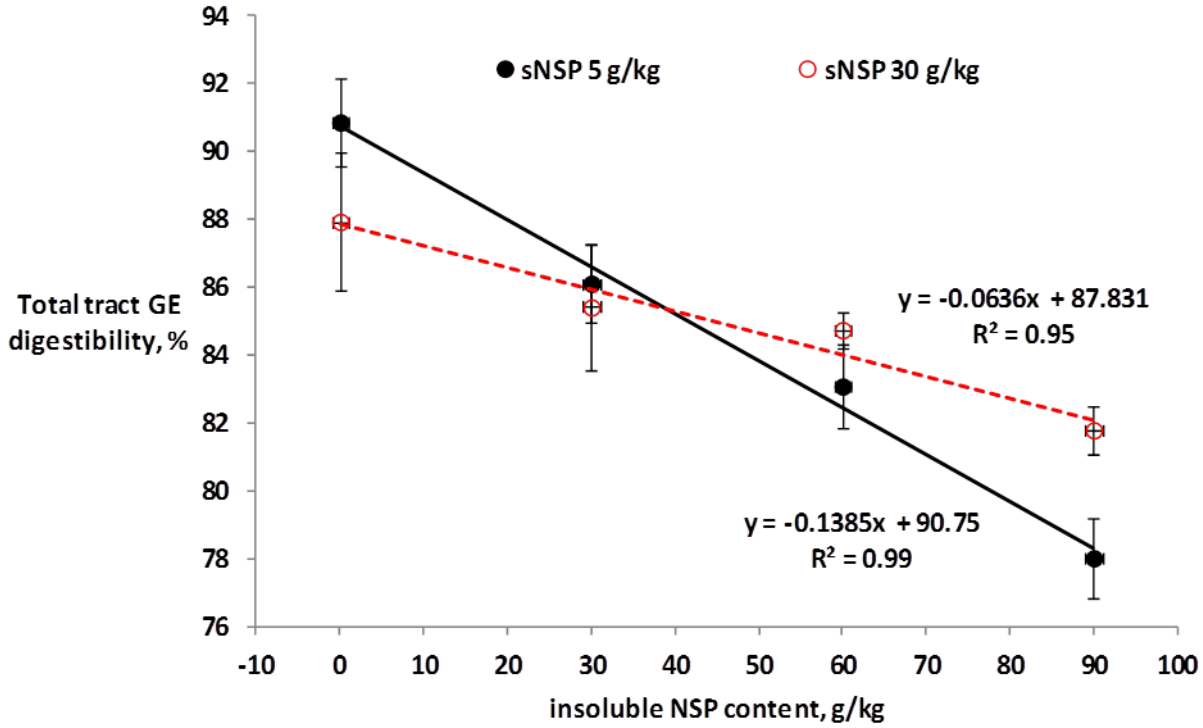

Figure 6 - The relationships between increasing amounts of insoluble NSP (as Opticell®) and total tract apparent digestibility of gross energy (GE) for pigs fed a low (≈ 5 g/kg) or high (≈ 30 g/kg) soluble NSP in the diet.

Table 4 - The effects of feeding either low or high soluble NSP and increasing levels of insoluble NSP (as Opticell®; g/kg of diet) on the faecal ß-haemolytic *E. coli* score[1] assessed at various time points after weaning.

sNSP	Low				High					P=			iNSP		
iNSP (as Opticell®)	0	30	60	90	0	30	60	90	SEM	sNSP	iNSP	sNSPxiNSP	Lin	Qud	Cub
Day 0	0.7	0.8	0.0	0.4	0.3	1.1	0.3	0.3	0.32	NS	NS	NS			
Day 5	0.1	0.8	1.6	0.7	0.8	1.4	0.5	0.6	0.36	NS	NS	0.053			
Day 9	0.9	1.7	1.4	0.8	2.8	0.8	1.7	1.6	0.39	0.074	NS	0.007			
Day 7	1.5	1.8	1.8	1.1	1.1	1.3	0.9	1.4	0.39	NS	NS	NS			
Day 11	0.7	0.2	0.7	0.3	0.7	0.2	0.2	0.2	0.23	NS	0.062	0.046	NS	0.014	NS
Day 13	0.2	0.2	0.7	0.3	0.2	0.0	0.0	0.7	0.21	NS	NS	NS			
Mean	0.8	1.1	1.2	0.7	1.0	1.0	0.6	1.0	0.19	NS	NS	0.069			
Cumulative	3.3	4.8	5.1	3.1	4.7	4.4	3.0	4.4	0.78	NS	NS	0.095			
Day 9 *E. coli* score in euthanized pigs	1.3	0.2	0.0	0.0	0.5	1.2	0.3	0.0	0.38	NS	0.069	NS	0.010	NS	NS
DI[2], %	4.5	7.4	6.7	4.4	7.6	5.5	2.2	3.2	2.36	NS	NS	NS			
Mean No. days of diarrhoea	0.42	0.75	0.67	0.42	0.83	0.58	0.25	0.33	0.236	NS	NS	NS			
FS[3], %															
Day 1-5	30.4	28.3	31.7	31.7	31.7	29.2	30.8	29.2	1.95	NS	NS	NS			
Day 6-10	51.9	60.8	56.0	53.5	57.0	56.3	52.8	54.0	3.23	NS	NS	NS			
Day 11-14	45.8	45.8	46.9	44.8	45.8	46.9	44.8	45.8	0.83	NS	NS	NS			
Total antibiotic treatments/pig	0.9	1.0	1.1	0.8	1.6	1.5	0.8	0.6	0.43	NS	NS	NS			
Mean antibiotic treatments/pig/day	0.07	0.07	0.08	0.05	0.12	0.11	0.06	0.04	0.031	NS	NS	NS			

[1]*E. coli* score assessed from 1-5 ; [2]DI, diarrhea index (calculated); [3]FS, faecal score (consistency).

Table 5 - The effects of feeding either low or high soluble NSP (sNSP) and increasing levels of insoluble NSP (iNSP; as Opticell®; g/kg of diet) on ß-haemolytic *E. coli* counts (log_{10} colony forming units/g) in the ileal mucosa and mesenteric lymph nodes (MLN) of pigs killed at d 8/9 after weaning.

sNSP	Low				High					P=			iNSP		
iNSP	0	30	60	90	0	30	60	90	SEM	sNSP	iNSP	sNSPxiNSP	Lin	Qud	Cub
Ileal mucosa	6.30	6.67	6.41	5.39	6.70	6.69	4.81	5.50	0.996	0.707	0.513	0.749			
MLN	2.54	3.57	3.50	1.40	2.25	3.49	3.09	2.72	0.850	0.821	0.267	0.722			

Table 6 - The effects of feeding either low or high soluble NSP (sNSP) and increasing levels of insoluble NSP (iNSP; as Opticell®; g/kg of diet) on production indices in the first 21 d after weaning.

sNSP	Low				High					P=			iNSP		
iNSP	0	30	60	90	0	30	60	90	SEM	sNSP	iNSP	sNSPxiNSP	Lin	Qud	Cub
Body weight, kg															
Day 0	8.8	8.8	8.9	8.9	8.9	8.8	8.8	8.9	0.05	NS	NS	NS	NS	NS	NS
Day 7	9.4	9.4	9.2	9.4	9.5	9.5	9.6	9.3	0.17	NS	NS	NS	NS	NS	NS
Day 14	12.3	12.4	12.0	13.0	12.3	12.8	12.8	12.9	0.29	NS	0.107	NS	0.046	NS	NS
Day 21	16.4	16.6	16.2	17.2	15.8	17.6	17.3	17.7	0.47	0.126	0.046	NS	0.019	NS	NS
ADG[1], g															
Week 1	106	111	97	100	93	123	116	93	15.4	NS	NS	NS	NS	NS	NS
Week 2	286	298	267	343	279	312	310	371	24.7	NS	0.018	NS	0.012	NS	NS
Week 1-2	176	195	194	228	175	217	220	232	19.0	NS	0.059	NS	0.011	NS	NS
Week 3	588	588	595	595	505	693	643	674	34.7	0.141	0.038	0.049	0.034	NS	NS
Week 1-3	313	326	298	351	285	376	361	379	21.3	0.072	0.023	NS	0.014	NS	0.062
ADFI[2], g															
Week 1	159	161	179	175	161	174	206	156	19.7	NS	NS	NS	NS	NS	NS
Week 2	337	365	368	441	356	430	434	453	28.1	0.048	0.012	NS	0.002	NS	NS
Week 1-2	243	265	264	320	263	315	336	315	22.6	0.039	0.050	NS	0.008	NS	NS
Week 3	798	722	760	867	751	858	837	843	36.9	NS	NS	0.059	0.039	NS	NS
Week 1-3	428	417	429	502	426	496	503	491	23.5	0.045	0.044	NS	0.006	NS	NS
FCR[3], g:g															
Week 1	1.76	2.09	2.48	2.32	2.26	1.74	1.84	2.36	0.274	NS	NS	NS	NS	NS	NS
Week 2	1.20	1.23	1.44	1.29	1.28	1.41	1.42	1.24	0.061	NS	0.018	NS	NS	0.007	NS
Week 1-2	1.41	1.39	1.60	1.45	1.49	1.49	1.54	1.40	0.052	NS	0.028	NS	NS	0.073	0.014
Week 3	1.39	1.24	1.33	1.46	1.53	1.26	1.32	1.25	0.057	NS	0.008	0.035	NS	0.005	0.075
Week 1-3	1.40	1.29	1.47	1.44	1.50	1.35	1.40	1.30	0.044	NS	0.020	0.038	NS	NS	0.004

[1]ADG, average daily gain; [2]ADFI, average daily feed intake; [3]FCR, feed conversion ratio.

Table 7 - The effects of feeding either low or high soluble NSP (sNSP) and increasing levels of insoluble NSP (iNSP; as Opticell®; g/kg of diet) on live weight, empty bodyweight, intestinal pH, ileal viscosity and volatile fatty acid (VFA) concentration and molar ratios in the caecum of the (sub-sample of) euthanised pigs (pigs euthanised at d 8/9 after weaning, at approximately 36/37 d of age).

sNSP	Low				High					P=			iNSP		
iNSP	0	30	60	90	0	30	60	90	SEM	sNSP	iNSP	sNSPxiNSP	Lin	Qud	Cub
Liveweight, kg	8.7	8.6	8.6	8.5	8.7	8.8	7.6	8.6	0.57	NS	NS	NS			
Empty weight, kg	7.6	7.4	7.4	7.5	7.4	7.6	7.5	7.4	0.21	NS	NS	NS			
GIT, kg	1.1	1.2	1.2	1.1	1.3	1.2	1.4	1.2	0.09	0.104	NS	NS			
Ileal pH	6.8	7.0	7.0	6.9	6.7	6.9	6.8	6.9	0.14	NS	NS	NS			
Caecal pH	6.0	6.2	6.2	6.2	5.6	5.7	5.8	6.0	0.17	0.002	NS	NS			
Ileal digesta viscosity, mPas	2.43	1.38	2.58	1.93	2.34	3.35	2.56	2.56	0.608	0.154	NS	NS			
Total VFA, mmol/L	72.9	48.5	64.2	61.0	68.6	86.3	82.6	66.6	9.37	0.037					
Molar ratios, %															
Acetic	53.5	56.4	53.4	60.4	47.6	48.9	49.5	55.9	2.72	0.007	0.037		0.017		
Propionic	32.4	35.4	34.9	28.1	38.1	34.1	37.4	31.7	2.25		0.036		0.041		
Butyric	9.8	7.1	8.2	8.0	13.0	13.0	11.2	10.9	1.57	0.002					
Isobutyric	0.07	0.10	0.13	0.15	0.0	0.35	0.0	0.0	0.123						
Isovaleric	1.59	0.51	1.44	1.93	0.27	0.75	0.65	0.80	0.307	0.001		0.070			
Caproic	2.36	0.50	1.93	1.34	1.07	2.86	1.34	0.78	0.608			0.024			

Table 8 - The effects of feeding either low or high soluble NSP (sNSP) and increasing levels of insoluble NSP (iNSP; as Opticell®; g/kg of diet) on the concentrations of plasma urea and haptoglobin measured at d 5 (before infection) and d 8/9 (after infection) after weaning.

sNSP	Infection (I)	Low				High					P=			
iNSP		0	30	60	90	0	30	60	90	SEM	I	I x sNSP	I x iNSP	I x sNSP x iNSP
Plasma urea, mmol/L	Before	1.9	2.5	2.7	1.9	1.6	2.0	2.1	2.0	0.39	0.040	0.539	0.174	0.454
	After	2.6	2.7	3.0	2.5	2.9	2.0	2.2	1.7					
Plasma haptoglobin, mg/mL	Before	2.4	2.8	3.0	2.8	2.3	2.2	2.2	2.4	0.28	0.193	0.403	0.581	0.680
	After	2.5	3.0	2.6	3.2	2.6	2.4	2.4	2.6					

Table 9. The effects of feeding either low or high soluble NSP (sNSP) and increasing levels of insoluble NSP (iNSP; as Opticell®; g/kg of diet) on apparent digestibility coefficients at d 9 and d 14 after weaning.

sNSP	Low				High					P=			Regression
iNSP	0	30	60	90	0	30	60	90	SEM	sNSP	iNSP	sNSP x iNSP	Lin
Day 9													
AID[1] of N	79.6	75.5	81.1	71.8	75.0	65.3	76.2	73.6	4.22	0.144	0.190	0.579	
ATTD[2] of N	81.5	77.1	79.8	77.6	78.9	73.3	76.3	75.1	3.07	0.167	0.407	0.999	
Day 14													
ATTD of GE	90.9	86.1	83.1	78.0	87.9	85.4	84.7	81.8	1.4	0.637	0.001	0.095	0.001

[1]AID: Apparent ileal digestibility; [2]ATTD: Apparent total tract digestibility.

Table 10 - The effects of feeding either low or high soluble NSP (sNSP) and increasing levels of insoluble NSP (iNSP; as Opticell®; g/kg of diet) on blood cell counts before and after ETEC infection (d 5 and 8 after weaning, respectively).

	Infection (I)	sNSP		iNSP				SEM	P=			
		5	30	0	30	60	90		I	I x sNSP	I x iNSP	
RBC ($x10^{12}/L$)[1]	Before	6.54^{ab}	6.52^{ab}	6.45^{a}	6.62^{ab}	6.52^{ab}	6.52^{ab}	0.099	0.046	0.044	0.678	
	After	6.80^{b}	6.52^{ab}	6.63^{ab}	6.77^{b}	6.52^{ab}	6.73^{b}					
WBC ($x10^{9}/L$)[2]	Before	11.7^{ab}	11.6^{ab}	11.1^{a}	11.3^{a}	11.8^{ab}	12.4^{ab}	0.99	0.001	0.589	0.030	
	After	14.2^{bc}	14.5^{bc}	14.5^{bc}	13.3^{b}	16.6^{c}	13.0^{ab}					
Lymphocyte, %	Before	54^{ab}	57^{b}	57^{b}	55^{ab}	58^{b}	51^{a}	2.9	0.587	0.245	0.002	
	After	55^{ab}	54^{ab}	52^{a}	55^{ab}	51^{a}	60^{b}					
Neutrophil, %	Before	40^{b}	37^{ab}	37^{ab}	39^{b}	36^{ab}	43^{bc}	2.7	0.414	0.167	0.001	
	After	40^{b}	40^{b}	42^{b}	40^{b}	44^{bc}	34^{a}					
Monocyte, %	Before	3.6^{ab}	4.0^{bc}	4.2^{c}	3.7^{bc}	3.5^{ab}	3.8^{bc}	0.35	0.045	0.579	0.756	
	After	3.4^{ab}	3.5^{ab}	3.6^{ab}	3.4^{ab}	3.1^{a}	3.7^{b}					
Platelet, ($x10^{9}/L$)	Before	587^{ab}	533^{a}	582^{ab}	541^{a}	553^{a}	564^{ab}	68.9	0.001	0.599	0.729	
	After	696^{bc}	661^{b}	717^{bc}	637^{b}	651^{b}	708^{bc}					

[abc]Means with different superscript within a variable are significantly different (P<0.05). There was no significant I x sNSP x iNSP interaction.
[1]RBC: red blood cell
[2]WBC: white blood cell

Table 11 - The effects of feeding either low or high soluble NSP (sNSP) and increasing levels of insoluble NSP (iNSP; as Opticell®; g/kg of diet) on tight junction protein mRNA expression[1] in the jejunal epithelium measured after ETEC infection.

sNSP	Low				High					P=			iNSP		
iNSP	0	30	60	90	0	30	60	90	SEM	sNSP	iNSP	sNSPxiNSP	Lin	Qud	Cub
Occludin	1.0	2.1	1.5	2.4	3.1	0.7	0.9	1.2	0.72	0.618	0.658	0.072			
ZO-1[2]	1.0	0.8	0.9	1.4	2.1	0.7	0.6	1.2	0.38	0.701	0.082	0.214		0.013	
Claudin	1.0	0.9	2.2	1.6	3.2	0.6	0.9	1.7	0.70	0.786	0.319	0.223			

[1]Expressed as a fold difference in relation to the low sNSP and no addition of iNSP treatment.
[2]Zonula Occludin-1

Table 12 - The effects of feeding either low or high soluble NSP (sNSP) and increasing levels of insoluble NSP (iNSP; as Opticell®; g/kg of diet) on villous height and crypt depth after weaning.

sNSP	Low				High					P=			iNSP		
iNSP	0	30	60	90	0	30	60	90	SEM	sNSP	iNSP	sNSPxiNSP	Lin	Qud	Cub
Jejunum															
Villous height, μm	391	432	398	348	424	374	437	390	26.6	0.460	0.350	0.190			
Crypt depth, μm	252	262	263	251	255	243	257	262	10.5	0.726	0.891	0.528			
VH:CD ratio[1]	1.57	1.66	1.53	1.39	1.67	1.54	1.71	1.49	0.112	0.409	0.315	0.555			
Ileum															
Villous height, μm	203	239	223	209	238	233	221	216	11.2	0.300	0.211	0.270			
Crypt depth, μm	343	401	411	344	422	411	386	408	20.3	0.032	0.434	0.056			
VH:CD ratio[1]	1.69	1.72	1.83	1.66	1.79	1.78	1.77	1.89	0.116	0.344	0.955	0.660			

[1]Villous height:Crypt depth ratio

Experiment 3

Reducing the risk of E. coli by establishing a fibre recommendation after weaning

Introduction

Data from Experiment 2 conducted under controlled infection conditions at Medina Research Station indicated that the addition of insoluble fibre, as Opticell®, ameliorated some of the indices associated with PWD and improved some of the production indices. Given these data, an experiment was designed to examine the effects of Opticell® and a soluble (fermentable) source of NSP, as sugar beet pulp (SBP), under commercial conditions at CHM Westbrook, Queensland. The overall objective of the study was to determine the effect of level and source of the two different fibre sources (as Opticell® and SBP) on the performance of nursery pigs in terms of feed intake, growth rate, feed efficiency, water intake and survival.

Materials and Methods

This experiment was conducted at the CHM Westbrook base-funded facility in Queensland (AEC Approval No: CHM PP 52/13). The methodology for this experiment is described in the following pages, however the study consisted of six experimental pelleted diets as follows:

- Diet 1: Creep 5-15 kg Control – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE
- Diet 2: Creep 5-15 kg + 3 % SBP – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE
- Diet 3: Creep 5-15 kg + 5 % SBP – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE
- Diet 4: Creep 5-15 kg + 3 % Opticell – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE
- Diet 5: Creep 5-15 kg + 5 % Opticell – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE
- Diet 6: Creep 5-15 kg + 3 % SBP + 3 % Opticell – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE

These diets were fed for the first 2 weeks after weaning and then all pigs transitioned to the Control treatment in Weeks 3 and 4.

The diet formulations are shown on the accompanying pages. The Opticell® was added at both 30 g/kg (3%) and 50 g/kg (5%) to the diet. Higher levels, which seemed to show promise in Experiment 2, were not considered in this study because of the high additional cost to the overall diet (Table 3.1). The SBP was similarly added at 30 g/kg (3%) and 50 g/kg (5%) to the diet, although it is noteworthy that previous studies in weaner pigs have included up to 120 g/kg (12%) in the diet (e.g., Lizardo et al., 1997; Schiavon et al., 2004). The cost of the diets was as follows (as of April/May 2014):

Table 13 - Diet costs.

Diets	Cost $/T
Base (Control)	$ 848.55
3% SBP	$ 885.07
5% SBP	$ 948.98
3% Opticell	$ 910.22
5% Opticell	$ 994.70
3% SBP/3% Opticell	$1,022.95

Data were analyzed using GenStat 16th Edition.

Results

Diet formulations are shown in Table 14. For most of the diet variables, the formulated amount was in reasonably good agreement to the analysed level expect for CP, in which the formulated level was always in excess of that tested in the laboratory. As commented below, Treatment 3 (50 g/kg SBP) had to be withdrawn from the

experiment in the third week (i.e., following two weeks of pigs being fed the diet) due to high morbidity and a number of deaths (Table 3.7) caused by proliferation of ß-haemolytic *E. coli* O139 K88, which was resistant to all antibiotics but neomycin and ceftiofur.

Table 15 displays the weekly performance data. In general, there were significant treatment and block (date of entry) main effects for all indices in all weeks of the study, although in Week 3 and Week 4 when all pigs were receiving the Control diet, the main effect of treatment had disappeared. However and in general, there appeared to be a disadvantage in offering 30 g/kg (3%) Opticell® as these pigs grew slower. With regard to pig live weight, pigs fed 30 g/kg (3%) Opticell® had a lower weight (11.4 kg) compared to the pigs fed the Control diet or the combination diet (12.4 and 12.6 kg, respectively).

For the cumulative data (Table 16), similar trends are shown as in Table 3.3. In Weeks 1-3 in particular (or d 1-7 and d 1-21 respectively), feeding all diets other than the combination [30 g/kg (3%) Opticell® and 50 g/kg (5%) SBP] appeared to be a disadvantage to the production indices relative to the Control diet. In Week 4 there were still significant treatment effects with the combination diet performing similarly to the Control diet. Overall and as shown in Table 3.4, the ADG and FCR were comparable between the combination diet and the Control diet.

Table 3.5 ('Treatment Stage Data') shows results of the 14-d diet treatment period (from d 1 to 14) and then the ensuing period of 14 d (from d 15 to 28) when all pigs were fed the Control diet. For the first 14 d after weaning, pigs fed treatments other than the Control diet or the diet with 30 g/kg (3%) Opticell® and 50 g/kg (5%) SBP diet performed inferiorly ($P<0.001$ to $P=0.005$). In the subsequent 14-d period, there were no treatment differences ($P>0.05$) in production indices.

There were differences in water usage in Weeks 1 and 2 of the study, with pigs eating 50 g/kg (5%) Opticell® drinking less water (Table 3.6). The morbidities and mortalities in the experiment are displayed in Table 3.7, and show that the rates of morbidity and mortality were highest in pigs fed the two SBP diets alone.

Table 3.8 shows the results from the blood sample taken at ≈ d 15 of the study. The data show that plasma urea content was decreased in pigs fed the diet with 50 g/kg (5%) Opticell® and the combination of 3% SBP+5% Opticell® compared with the control group ($P=0.002$). There was no other treatment effect on blood indices.

Table 14 - Analysis of diets used in Experiment 3 for crude protein (CP), crude fat (Fat), dry matter (DM), ash (Ash), crude fibre (CF), digestible energy (DE), acid detergent fibre (ADF) and neutral detergent fibre (NDF).

	Diet 1: Control			Diet 2: 3% SBP			Diet 3: 5% SBP			Diet 4: 3% Opticell			Diet 5: 5% Opticell			Diet 6: 3 % SBP/ 3 % Opticell		
	Test [1]	Form[2]	Diff[3]	Test	Form	Diff	Test	Form	Diff	Test	Form	Diff	Test	Form	Diff	Test	Form	Diff
CP, %	20.3	22.3	91	18.1	22.0	82	18.9	22.1	85	18.1	21.9	83	19.3	22.0	88	19.5	21.9	89
Fat, %	5.2	7.1	73	7.1	8.2	87	6.9	8.8	78	12.5	8.2	153	8.3	8.9	94	6.8	9.5	72
DM, %	90.9	90.8	100	90.8	90.8	100	91.4	91.0	100	92.2	91.1	101	91.2	91.6	100	91.6	91.6	100
Ash, %	5.5			5.5			4.9			5.6			4.8			5.0		
CF, %	2.1	2.2	97	3.0	2.6	117	2.6	2.8	92	5.2	5.1	103	3.9	7.0	56	3.4	5.4	63
DE, MJ/kg	15.5	15.0	103	15.4	15.0	103	15.5	15.0	104	15.7	15.0	105	15.5	15.0	103	15.6	15.0	104
ADF, %	2.5			3.3			4.8			6.2			5.1			4.5		
NDF, %	7.8			9.3			10.4			10.8			11.2			10.3		

[1]Results reported on an "as received" basis (Test = as tested); [2]Results reported as calculated on the formulation sheet (Form = formulated); [3]Tested value expressed as a percentage of formulated value (Diff = percentage difference).

Table 15 - Weekly Data (including negative FCRs)

		Control	3% SBP	3% OPT	5% OPT	SBP/ OPT	SED	Trtmt	Block	T x B
Live weight	Entry	6.1	5.8	6.1	6.0	6.1	0.36	0.971	0.562	0.775
	Week 1	6.6^{c}	6.4^{bc}	6.3^{ab}	6.2^{a}	6.4^{bc}	0.08	0.002	<0.001	0.696
	Week 2	7.9^{c}	7.5^{b}	7.1^{a}	7.4^{b}	7.9^{c}	0.14	<0.001	<0.001	0.224
	Week 3	9.8^{b}	9.3^{a}	8.9^{a}	9.2^{a}	9.8^{b}	0.21	<0.001	0.005	0.246
	Week 4	12.4^{bc}	11.8^{ac}	11.4^{a}	11.8^{ac}	12.6^{b}	0.31	0.005	0.004	0.345
Week 1	ADG	0.071^{c}	0.056^{bc}	0.036^{ab}	0.032^{a}	0.056^{bc}	0.010	0.002	0.483	0.630
	ADFI	0.18^{c}	0.17^{bc}	0.14^{a}	0.14^{ab}	0.17^{c}	0.014	0.012	0.657	0.710
	FCR	2.73^{a}	3.73^{a}	4.05^{a}	6.51^{b}	3.76^{a}	0.869	0.005	0.181	0.114
Week 2	ADG	0.213^{cd}	0.168^{b}	0.125^{a}	0.185^{bc}	0.227^{d}	0.017	<0.001	<0.001	0.441
	ADFI	0.32^{c}	0.31^{bc}	0.26^{a}	0.28^{ab}	0.33^{c}	0.020	0.006	0.337	0.253
	FCR	1.53^{a}	2.13^{b}	2.14^{b}	1.60^{a}	1.49^{a}	0.126	<0.001	<0.001	0.024
Week 3	ADG	0.271	0.261	0.264	0.263	0.282	0.017	0.775	0.051	0.749

		Control	3% SBP	3% OPT	5% OPT	SBP/ OPT	SED	Trtmt	Block	T x B
	ADFI	0.45	0.43	0.42	0.42	0.45	0.022	0.604	0.263	0.469
	FCR	1.68	1.65	1.64	1.63	1.62	0.101	0.953	0.006	0.958
Week 4	ADG	0.376	0.366	0.365	0.377	0.406	0.021	0.320	0.010	0.367
	ADFI	0.61	0.59	0.58	0.58	0.63	0.022	0.096	0.004	0.178
	FCR	1.63	1.62	1.62	1.55	1.57	0.086	0.841	0.192	0.880

Table 16 - Cumulative Data

		Control	3% SBP	3% OPT	5% OPT	SBP/ OPT	SED	Trtmt	Block	T x B
Week 1	ADG	0.071^{c}	0.056^{bc}	0.036^{ab}	0.032^{a}	0.056^{bc}	0.010	0.002	0.483	0.630
(d1-7)	ADFI	0.18^{c}	0.17^{bc}	0.14^{a}	0.14^{ab}	0.17^{c}	0.014	0.012	0.657	0.710
	FCR	2.73^{a}	3.73^{a}	4.05^{a}	6.51^{b}	3.76^{a}	0.869	0.005	0.181	0.114
Week 2	ADG	0.136^{c}	0.105^{b}	0.077^{a}	0.100^{b}	0.133^{c}	0.010	<0.001	0.234	0.224
(d 1-14)	ADFI	0.25^{c}	0.24^{bc}	0.20^{a}	0.21^{ab}	0.25^{c}	0.016	0.005	0.485	0.394
	FCR	1.85^{a}	2.54^{c}	2.60^{c}	2.15^{b}	1.89^{a}	0.099	<0.001	<0.001	<0.001
Week 3	ADG	0.180^{b}	0.156^{a}	0.138^{a}	0.154^{a}	0.181^{b}	0.010	<0.001	0.473	0.246
(d 1-21)	ADFI	0.32	0.30	0.27	0.28	0.32	0.017	0.057	0.468	0.480
	FCR	1.75^{a}	1.93^{bc}	1.97^{c}	1.83^{ab}	1.74^{a}	0.046	<0.001	0.898	0.010
Week 4	ADG	0.227^{bc}	0.207^{ab}	0.193^{a}	0.208^{ab}	0.236^{c}	0.011	0.005	0.175	0.345
(d 1-28)	ADFI	0.39^{b}	0.37^{ab}	0.35^{a}	0.35^{a}	0.39^{b}	0.017	0.044	0.134	0.308
	FCR	1.71^{ab}	1.79^{b}	1.80^{b}	1.71^{ab}	1.67^{a}	0.045	0.028	0.743	0.569

[a,b]Means in a row with different superscripts differ significantly (P<0.05); ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of difference of the means.

Table 17 - Treatment Stage Data

		Control	3% SBP	3% OPT	5% OPT	SBP/ OPT	SED	Trtmt	Block	T x B
Wk 1 & 2	ADG	0.136^{c}	0.105^{b}	0.077^{a}	0.100^{b}	0.133^{c}	0.010	<0.001	0.234	0.224
	ADFI	0.25^{c}	0.24^{bc}	0.20^{a}	0.21^{ab}	0.25^{c}	0.016	0.005	0.485	0.394
	FCR	1.85^{a}	2.54^{c}	2.60^{c}	2.15^{b}	1.89^{a}	0.099	<0.001	<0.001	<0.001
Wk 3 & 4	ADG	0.319	0.309	0.309	0.315	0.338	0.014	0.198	0.186	0.417
	ADFI	0.53	0.51	0.50	0.50	0.54	0.019	0.166	0.030	0.242
	FCR	1.67	1.64	1.63	1.60	1.60	0.047	0.490	0.902	0.440

Table 18 - Water Usage Data

		Control	3% SBP	3% OPT	5% OPT	SBP/ OPT	SED	Trtmt	Block	T x B
Water intake	Week 1	5.6^{c}	5.6^{c}	3.5^{ab}	2.7^{a}	4.6^{bc}	0.83	0.009	0.004	0.627
	Week 2	7.7^{b}	6.8^{b}	6.7^{b}	4.6^{a}	6.8^{b}	1.01	0.031	<0.001	0.313
	Week 3	10.5	11.2	10.8	9.5	10.4	1.52	0.895	0.237	0.434
	Week 4	12.5	13.0	12.6	10.2	16.4	2.48	0.162	0.080	0.536

Table 19 - Mortality/Morbidity

	Control	3% SBP	5% SBP	3% OPT	5% OPT	SBP/ OPT
Mortality	0	8	6	2	1	0
Morbidity	4	3	5	3	2	5
TOTAL	4	11	11	5	3	5

Table 20 - The effects of feeding different diets after weaning on selected blood measurements.

Diet	Control	3% SBP	3% Opticell®	5% Opticell®	5% SBP	3% SBP + 3% Opticell®	SEM	P value
Analyte								
Triglycerides, mM	0.45	0.38	0.50	0.38	0.41	0.36	0.050	0.732
Urea, mM	4.32^{bc}	3.61^{ab}	4.98^{c}	3.44^{a}	4.80^{c}	3.65^{ab}	0.252	0.002
Glucose, mM	5.12	4.62	4.40	4.72	5.22	4.36	0.253	0.149
C-reactive peptide, µg/L	0.32	0.32	0.25	0.26	0.26	0.29	0.030	0.598

^{abc}Means with different superscript within a variable are significantly different ($P<0.05$).

Reducing the Risk of *E. coli* by Establishing a Fibre Recommendation After Weaning.	
Experiment No: 28-2014	AEC Approval No: CHM PP 52/13
Authors: Robert van Barneveld and Robert Hewitt	**Start date:** March 20, 2014 **Finish date:** May 22, 2014

Objectives:

To determine the effect of level and source of insoluble fibre on the performance of nursery pigs in terms of feed intake, growth rate, feed efficiency and survivability.

Null Hypothesis:

Inclusion of fibre in the diet of weaner pigs will not enhance growth performance.

Experimental Design:

Randomised block, blocked by sex and entry weight.

Treatments:

Treatment 1: Control	Treatment 2: Sugarbeet Pulp 3 %
Treatment 3: Sugarbeet Pulp 5 %	Treatment 4: Opticell 3 %
Treatment 5: Opticell 5 %	Treatment 6: SBP 3 % + Opticell 3 %

Method:

A total of 140 newly-weaned pigs (23 d) entered the facility each week, with 6 weeks of entries being used in this experiment (11 pens per treatment). Upon entry pigs were sexed and graded into large, small and medium pigs and assigned a pen (n=14). Pens were weighed (average entry weight 6.1±0.11 kg) and allocated to treatment using a randomised block design with diet as the treatment with sex, weight and entry date as blocking factors. Pens were weighed weekly.

An individual block within the weaner facility consisted of 10 pens of identical configuration (1 m x 2.8 m). Penning was open galvanised panelling with fully-slatted plastic floor tiles. Water was supplied *ad libitum* via two nipple drinkers per pen and supplementary radiant heat provided via a bar heater.

Feed was offered to each individual pen via a round multi-space adjustable plastic transit feeder. Diets were offered *ad libitum* throughout. Weekly feed disappearance was calculated from feed deliveries and weighed refusal on the final day of the week. Water usage was measured via individual water meters on each pen. Medications used in the all treatments were administered via the drinking water.

Treatment 3 was withdrawn after 3 weeks of experimentation due to high morbidity. Data were analysed via an unbalanced (ANOVA) with week of entry as a blocking factor and entry weight as a covariate, and differences between treatments were determined by LSD ($P<0.05$).

WEANER RESEARCH PROGRAM

RESULTS SUMMARY

Diets (attached):

Diet 1: Creep 5-15 kg Control – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE

Diet 2: Creep 5-15 kg + 3 % SBP – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE

Diet 3: Creep 5-15 kg + 5 % SBP – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE

Diet 4: Creep 5-15 kg + 3 % Opticell – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE

Diet 5: Creep 5-15 kg + 5 % Opticell – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE

Diet 6: Creep 5-15 kg + 3 % SBP + 3 % Opticell – 15.0 MJ DE/kg, 0.8 g AvL/MJ DE

Medication Regime:

All treatments:

- Draxxin (*Tulathromycin* 100 mg/ml) - 0.25 ml intramuscularly per pig upon entry.
- Sol-U-Mox Powder (*Amoxycillin trihydrate* 870 mg/g) – 65.7 g/ 1,000 kg of live weight in water for first 14 days and then from day 19-21 and 26-28.
- Neopharm (*Neomycin sulfate* 600 mg/g) – 60.0 g/1,000 kg of live weight in water from day 15-18 and 22-25.

A blood sample from two pigs per pen was obtained on d 15 of experiment.

Formula basic data

Name : CREEP 5-15KG CONTROL

Raw material	%	[Kg]
14240 WHEAT 13.0	64.066667	961.0
34600 SOYBEAN MEAL 46.0	8.2	123.0
35160 F/F.SOYABEAN 38.0	1.333333	20.0
40100 BLOOD MEAL 90.0	1.2	18.0
40150 DRIED PORCINE PLASMA	1.5	22.5
40680 MEAT MEAL 51.0	7.133333	107.0
41483 PERFECT DIGEST FPI SD	2.0	30.0
43170 CHOCOLATE MILK POWDER	10.0	150.0
45581 STOCKFEED BLENDED OIL	3.0	45.0
51610 ZINC OXIDE	0.3	4.5
52810 CHOLINE CHLORIDE 60%	0.093333	1.4
52950 BETAINE (BETAFIN)	0.1	1.5
53020 M.H.A.Calcium	0.1	1.5
53100 LYSINE HCL	0.3	4.5
53200 L-THREONINE	0.1	1.5
53800 ROVABIO XYLAN	0.053333	0.8
58705 BIOTRONIC TOP3	0.1	1.5
59060 PIGORTEK 6661	0.02	0.3
61750 BIOFIX/MYCOFIX PLUS (BIOMIN)	0.2	3.0
90100 BN STARTER PREMIX	0.2	3.0
	100.0	1500.0

Analysis

[VOLUME]	%	:	100.0	PHENYL	%	:	0.830955	W3_FA	%	:	0.150133
DRYMATTER	%	:	90.746933	THREONINE	%	:	0.885871	W6_FA	%	:	2.041587
MOISTURE	%	:	9.151533	TRYPTOPHAN	%	:	0.243942	W3+W6_FA	%	:	0.84172
PROTEIN	%	:	22.318933	M+C	%	:	0.796614	#FAW6:W3		:	13.59849
C_FIBRE	%	:	2.158467	CALCIUM	%	:	0.931687	C18:2W6LIN	%	:	0.689587
DE_PIG_MJ	MJ/KG	:	15.033133	PHOSPHORUS	%	:	0.77701	#AILYS/DEP		:	0.080324
DE_PIG_MC	MCAL/KG	:	2.764372	AV_PHOS	%	:	0.554943	#MET/LYS		:	0.298127
LEUCINE	%	:	1.552599	#CAL/PHO		:	1.199067	#M+C/LYS		:	0.558747
ISOLEUCINE	%	:	0.828589	#CAL/AVPHO		:	1.678886	#TRY/LYS		:	0.171102
LYSINE	%	:	1.425715	CHOLINE	MG/KG	:	1508.0	#THR/LYS		:	0.621352
METHION	%	:	0.425045	FAT/EE	%	:	7.083267	#ISO/LYS		:	0.581174

Formula basic data

Name : CREEP 5-15KG CONTROL+3% SBP

Raw material	%	[Kg]
14240 WHEAT 13.0	60.266667	904.0
18013 SUGARBEET PULP (UNMOLLASSED)	3.0	45.0
34600 SOYBEAN MEAL 46.0	1.733333	26.0
34750 SOYCOMIL R (ADM)	0.733333	11.0
35160 F/F.SOYABEAN 38.0	8.266667	124.0
40100 BLOOD MEAL 90.0	1.133333	17.0
40150 DRIED PORCINE PLASMA	1.5	22.5
40680 MEAT MEAL 51.0	6.8	102.0
41483 PERFECT DIGEST FPI SD	2.0	30.0
43170 CHOCOLATE MILK POWDER	10.0	150.0
45581 STOCKFEED BLENDED OIL	3.0	45.0
51610 ZINC OXIDE	0.3	4.5
52810 CHOLINE CHLORIDE 60%	0.086667	1.3
52950 BETAINE (BETAFIN)	0.1	1.5
53020 M.H.A.Calcium	0.106667	1.6
53100 LYSINE HCL	0.3	4.5
53200 L-THREONINE	0.1	1.5
53800 ROVABIO XYLAN	0.053333	0.8
58705 BIOTRONIC TOP3	0.1	1.5
59060 PIGORTEK 6661	0.02	0.3
61750 BIOFIX/MYCOFIX PLUS (BIOMIN)	0.2	3.0
90100 BN STARTER PREMIX	0.2	3.0
	100.0	1500.0

Analysis

[VOLUME]	%	:	100.0	PHENYL	%	:	0.808329	W3_FA	%	:	0.24822
DRYMATTER	%	:	90.748333	THREONINE	%	:	0.887481	W6_FA	%	:	2.612733
MOISTURE	%	:	9.150133	TRYPTOPHAN	%	:	0.24046	W3+W6_FA	%	:	1.51088
PROTEIN	%	:	22.005473	M+C	%	:	0.794948	#FAW6:W3		:	10.525878
C_FIBRE	%	:	2.5602	CALCIUM	%	:	0.92882	C18:2W6LIN	%	:	1.263087
DE_PIG_MJ	MJ/KG	:	15.007	PHOSPHORUS	%	:	0.75883	#AILYS/DEP		:	0.079907
DE_PIG_MC	MCAL/KG	:	2.72704	AV_PHOS	%	:	0.538943	#MET/LYS		:	0.299459
LEUCINE	%	:	1.535602	#CAL/PHO		:	1.224016	#M+C/LYS		:	0.55601
ISOLEUCINE	%	:	0.829733	#CAL/AVPHO		:	1.723409	#TRY/LYS		:	0.168185
LYSINE	%	:	1.429738	CHOLINE	MG/KG	:	1512.633333	#THR/LYS		:	0.62073
METHION	%	:	0.428148	FAT/EE	%	:	8.1788	#ISO/LYS		:	0.580339

Formula basic data

Name : CREEP 5-15KG CONTROL+5% SBP

Raw material	%	[Kg]
14240 WHEAT 13.0	54.993333	824.9
18013 SUGARBEET PULP (UNMOLLASSED)	5.0	75.0
34750 SOYCOMIL R (ADM)	3.066667	46.0
35160 F/F.SOYABEAN 38.0	10.0	150.0
40150 DRIED PORCINE PLASMA	1.5	22.5
40680 MEAT MEAL 51.0	6.466667	97.0
41483 PERFECT DIGEST FPI SD	2.0	30.0
43170 CHOCOLATE MILK POWDER	12.466667	187.0
45581 STOCKFEED BLENDED OIL	3.0	45.0
51610 ZINC OXIDE	0.3	4.5
52810 CHOLINE CHLORIDE 60%	0.086667	1.3
52950 BETAINE (BETAFIN)	0.1	1.5
53020 M.H.A.Calcium	0.1	1.5
53100 LYSINE HCL	0.266667	4.0
53200 L-THREONINE	0.08	1.2
53800 ROVABIO XYLAN	0.053333	0.8
58705 BIOTRONIC TOP3	0.1	1.5
59060 PIGORTEK 6661	0.02	0.3
61750 BIOFIX/MYCOFIX PLUS (BIOMIN)	0.2	3.0
90100 BN STARTER PREMIX	0.2	3.0
	100.0	1500.0

Analysis

[VOLUME]	%	:	100.0	PHENYL	%	:	0.77472	W3_FA	%	:	0.266875
DRYMATTER	%	:	91.031667	THREONINE	%	:	0.885543	W6_FA	%	:	2.73344
MOISTURE	%	:	8.8668	TRYPTOPHAN	%	:	0.243358	W3+W6_FA	%	:	1.650008
PROTEIN	%	:	22.1315	M+C	%	:	0.797577	#FAW6:W3		:	10.242411
C_FIBRE	%	:	2.828853	CALCIUM	%	:	0.920129	C18:2W6LIN	%	:	1.373433
DE_PIG_MJ	MJ/KG	:	15.005887	PHOSPHORUS	%	:	0.762061	#AILYS/DEP		:	0.079841
DE_PIG_MC	MCAL/KG	:	2.521029	AV_PHOS	%	:	0.540699	#MET/LYS		:	0.302035
LEUCINE	%	:	1.528039	#CAL/PHO		:	1.207422	#M+C/LYS		:	0.557849
ISOLEUCINE	%	:	0.89418	#CAL/AVPHO		:	1.701739	#TRY/LYS		:	0.170212
LYSINE	%	:	1.429737	CHOLINE	MG/KG	:	1504.946667	#THR/LYS		:	0.619375
METHION	%	:	0.43183	FAT/EE	%	:	8.808273	#ISO/LYS		:	0.625416

Formula basic data

Name : CREEP 5-15KG CONTROL+3% OPTICELL

Raw material	%	[Kg]
14240 WHEAT 13.0	59.866667	898.0
19925 OPTICELL	3.0	45.0
34600 SOYBEAN MEAL 46.0	1.733333	26.0
34750 SOYCOMIL R (ADM)	0.866667	13.0
35160 F/F.SOYABEAN 38.0	8.266667	124.0
40100 BLOOD MEAL 90.0	1.133333	17.0
40150 DRIED PORCINE PLASMA	1.5	22.5
40680 MEAT MEAL 51.0	7.066667	106.0
41483 PERFECT DIGEST FPI SD	2.0	30.0
43170 CHOCOLATE MILK POWDER	10.0	150.0
45581 STOCKFEED BLENDED OIL	3.0	45.0
51610 ZINC OXIDE	0.3	4.5
52810 CHOLINE CHLORIDE 60%	0.086667	1.3
52950 BETAINE (BETAFIN)	0.1	1.5
53020 M.H.A.Calcium	0.106667	1.6
53100 LYSINE HCL	0.3	4.5
53200 L-THREONINE	0.1	1.5
53800 ROVABIO XYLAN	0.053333	0.8
58705 BIOTRONIC TOP3	0.1	1.5
59060 PIGORTEK 6661	0.02	0.3
61750 BIOFIX/MYCOFIX PLUS (BIOMIN)	0.2	3.0
90100 BN STARTER PREMIX	0.2	3.0
	100.0	1500.0

Analysis

[VOLUME]	%	:	100.0	PHENYL	%	:	0.815224	W3_FA	%	:	0.24778
DRYMATTER	%	:	91.069667	THREONINE	%	:	0.880713	W6_FA	%	:	2.611267
MOISTURE	%	:	8.7688	TRYPTOPHAN	%	:	0.239344	W3+W6_FA	%	:	1.50896
PROTEIN	%	:	21.90614	M+C	%	:	0.790175	#FAW6:W3		:	10.53865
C_FIBRE	%	:	5.052733	CALCIUM	%	:	0.923713	C18:2W6LIN	%	:	1.253893
DE_PIG_MJ	MJ/KG	:	15.0062	PHOSPHORUS	%	:	0.769543	#AILYS/DEP		:	0.0799
DE_PIG_MC	MCAL/KG	:	2.72124	AV_PHOS	%	:	0.54809	#MET/LYS		:	0.299755
LEUCINE	%	:	1.528311	#CAL/PHO		:	1.20034	#M+C/LYS		:	0.556611
ISOLEUCINE	%	:	0.824655	#CAL/AVPHO		:	1.685331	#TRY/LYS		:	0.168598
LYSINE	%	:	1.419617	CHOLINE	MG/KG	:	1513.166667	#THR/LYS		:	0.620388
METHION	%	:	0.425537	FAT/EE	%	:	8.152	#ISO/LYS		:	0.5809

Formula basic data

Name : CREEP 5-15KG CONTROL+5% OPTICELL

Raw material	%	[Kg]
14240 WHEAT 13.0	53.726667	805.9
19925 OPTICELL	5.0	75.0
34750 SOYCOMIL R (ADM)	3.0	45.0
35160 F/F.SOYABEAN 38.0	10.0	150.0
40150 DRIED PORCINE PLASMA	1.5	22.5
40680 MEAT MEAL 51.0	7.333333	110.0
41483 PERFECT DIGEST FPI SD	2.0	30.0
43170 CHOCOLATE MILK POWDER	12.933333	194.0
45581 STOCKFEED BLENDED OIL	3.0	45.0
51610 ZINC OXIDE	0.3	4.5
52810 CHOLINE CHLORIDE 60%	0.086667	1.3
52950 BETAINE (BETAFIN)	0.1	1.5
53020 M.H.A.Calcium	0.093333	1.4
53100 LYSINE HCL	0.266667	4.0
53200 L-THREONINE	0.086667	1.3
53800 ROVABIO XYLAN	0.053333	0.8
58705 BIOTRONIC TOP3	0.1	1.5
59060 PIGORTEK 6661	0.02	0.3
61750 BIOFIX/MYCOFIX PLUS (BIOMIN)	0.2	3.0
90100 BN STARTER PREMIX	0.2	3.0
	100.0	1500.0

Analysis

[VOLUME]	%	:	100.0	PHENYL	%	:	0.780071	W3_FA	%	:	0.265101
DRYMATTER	%	:	91.608333	THREONINE	%	:	0.881474	W6_FA	%	:	2.725933
MOISTURE	%	:	8.190133	TRYPTOPHAN	%	:	0.240939	W3+W6_FA	%	:	1.640735
PROTEIN	%	:	22.00976	M+C	%	:	0.783634	#FAW6:W3		:	10.282609
C_FIBRE	%	:	6.97032	CALCIUM	%	:	0.958969	C18:2W6LIN	%	:	1.354747
DE_PIG_MJ	MJ/KG	:	15.005353	PHOSPHORUS	%	:	0.800048	#AILYS/DEP		:	0.079986
DE_PIG_MC	MCAL/KG	:	2.503311	AV_PHOS	%	:	0.574913	#MET/LYS		:	0.298507
LEUCINE	%	:	1.518359	#CAL/PHO		:	1.19864	#M+C/LYS		:	0.552927
ISOLEUCINE	%	:	0.885176	#CAL/AVPHO		:	1.668026	#TRY/LYS		:	0.170005
LYSINE	%	:	1.417247	CHOLINE	MG/KG	:	1513.013333	#THR/LYS		:	0.621962
METHION	%	:	0.423058	FAT/EE	%	:	8.878807	#ISO/LYS		:	0.624574

Formula basic data

Name : CREEP 5-15KG CONTROL+3% SBP/OPTI

Raw material	%	[Kg]
14240 WHEAT 13.0	50.106667	751.6
18013 SUGARBEET PULP (UNMOLLASSED)	3.0	45.0
19925 OPTICELL	3.0	45.0
34750 SOYCOMIL R (ADM)	2.4	36.0
35160 F/F.SOYABEAN 38.0	10.0	150.0
40150 DRIED PORCINE PLASMA	1.5	22.5
40680 MEAT MEAL 51.0	7.0	105.0
41483 PERFECT DIGEST FPI SD	2.0	30.0
43170 CHOCOLATE MILK POWDER	16.533333	248.0
45581 STOCKFEED BLENDED OIL	3.0	45.0
51610 ZINC OXIDE	0.3	4.5
52810 CHOLINE CHLORIDE 60%	0.08	1.2
52950 BETAINE (BETAFIN)	0.1	1.5
53020 M.H.A.Calcium	0.093333	1.4
53100 LYSINE HCL	0.233333	3.5
53200 L-THREONINE	0.08	1.2
53800 ROVABIO XYLAN	0.053333	0.8
58705 BIOTRONIC TOP3	0.1	1.5
59060 PIGORTEK 6661	0.02	0.3
61750 BIOFIX/MYCOFIX PLUS (BIOMIN)	0.2	3.0
90100 BN STARTER PREMIX	0.2	3.0
	100.0	1500.0

Analysis

[VOLUME]	%	:	100.0	PHENYL	%	:	0.732648	W3_FA	%	:	0.258205
DRYMATTER	%	:	91.629067	THREONINE	%	:	0.883163	W6_FA	%	:	2.69284
MOISTURE	%	:	8.2094	TRYPTOPHAN	%	:	0.242183	W3+W6_FA	%	:	1.600805
PROTEIN	%	:	21.93286	M+C	%	:	0.782516	#FAW6:W3		:	10.429064
C_FIBRE	%	:	5.371347	CALCIUM	%	:	0.959564	C18:2W6LIN	%	:	1.331553
DE_PIG_MJ	MJ/KG	:	15.00588	PHOSPHORUS	%	:	0.797869	#AILYS/DEP		:	0.079883
DE_PIG_MC	MCAL/KG	:	2.370733	AV_PHOS	%	:	0.584347	#MET/LYS		:	0.301725
LEUCINE	%	:	1.539929	#CAL/PHO		:	1.202659	#M+C/LYS		:	0.549683
ISOLEUCINE	%	:	0.896605	#CAL/AVPHO		:	1.642112	#TRY/LYS		:	0.170123
LYSINE	%	:	1.423576	CHOLINE	MG/KG	:	1491.386667	#THR/LYS		:	0.620383
METHION	%	:	0.429529	FAT/EE	%	:	9.467427	#ISO/LYS		:	0.629826

3. Outcomes

Traditionally, feeding highly digestible ingredients and including in-feed antimicrobials as growth promoters has been recommended in diets for weanling pigs, with or without the presence of *E. coli* disease. However, the combination of bans/restrictions of use of in-feed antimicrobials in many countries, antibiotic resistance, consumer concerns and (or) increases in price of compounds, has led to a reexamination of the types of diets fed in the post-weaning period. The inclusion of specific types of dietary fibre as a means to overcome issues in the post-weaning period such as PWD has received considerable attention, particularly in Europe. In weanling pig diets, functional characteristics of fibrous-containing ingredients are likely more important than the chemical composition of the fibrous ingredients *per se* (Molist et al., 2014). With this in mind, the overall aims of Experiments 2 and 3 in this project were to evaluate (1) increasing levels of dietary insoluble NSP in combination with a 'low' and 'high' level of soluble NSP in the diet using (mostly) purified sources of insoluble and soluble NSP and under conditions of experimental ETEC infection, and (2) further evaluate this 'proof of concept' study under commercial conditions, based on the outcomes of Experiment 2. For Experiment 2 it was reasoned that, and as discussed by Pluske et al. (2001), since commercial weaner diets used in Australia endogenously contain a mixture of soluble and insoluble NSP in the cereals, legumes and/or oilseed meals included in the diet, specifically separating the effects of insoluble or soluble NSP on PWD, production indices and aspects of GIT structure and function are impossible unless (semi) purified diets are fed. We attempted to separate the effects of insoluble or soluble NSP on these measures by calculating 'typical' levels of insoluble and soluble NSP in Australian commercial diets and then adding back (semi) purified sources of the insoluble and soluble NSP to the diets, from wheat, barley and legumes/oilseeds (as pectins), to mimic feeding typical' commercial diets. Ground white rice was used as the base cereal, given it is inherently low in NSP (Pluske et al., 2002).

In Experiment 2, the major findings could be summarised as follows:

- Higher levels of the insoluble fibre source (as Opticell®) had positive effects on reducing faecal *E. coli* excretion following experimental infection (albeit from low base level of infection) *and* improving ADG and ADFI in the first 14 d after weaning, and body weight after 21 d.
- The inclusion of soluble fibre sources in the diet for the first 14 d after weaning was contraindicative, e.g., greater *E. coli* shedding, but no negative impact on performance.
- The apparent total tract digestibility (ATTD) of GE decreased with increasing fibre contents, however feeding more soluble fibre reduced the extent of that reduction. There were no significant effects on the apparent ileal digestibility (AID) or ATTD of nitrogen, even though there was a noticeable decline in AID with increasing insoluble NSP concentration.
- From d 14 after weaning (and possibly before), higher amounts of soluble fibre appeared to bestow a benefit on performance in the overall 21 d after weaning possibly attributable to the (a) greater weight of the GIT and (or) a greater hindgut production of butyrate.

These data are in general agreement to sentiments expressed in a review by Molist et al. (2014), who reported that 'moderate levels' (not stated) of insoluble fibre sources, but preferably as coarse particle size and when pigs have a compromised health status, might have positive effects promoting gut health during the first 2 weeks after weaning. These positive effects might be associated with enhanced maturation of the GIT as well as with the physical effect of dietary fibre on the growth of intestinal microbiota and the blocking of the adhesion of pathogenic bacteria to the GIT mucosa. On the other

hand, inclusion of soluble and rapid fermentable fibre sources in the diet for the first 2 weeks after weaning, especially with early weaning in farms with poor health status, might be contraindicative due to the limited digestive capacity of the piglets. Once the pigs adapt to solid feed, higher amounts of soluble and fermentable fibre sources can be gradually included in the diet to promote healthy fermentation of undigested nutrients and better absorption of SCFA by the colon mucosa. Under poor hygiene conditions, the level of fermentable fibre and crude protein content of the post-weaning diets should be limited to avoid intestinal dysbiosis, which might increase the risk of PWD (Molist et al., 2014). In general, our findings in Experiment 2 support this notion.

In accordance, much of the evidence presented to date suggests that the amount of soluble and viscosity-elevating NSP should be restricted and insoluble NSP should be included in the practical formulation of diets for weaner pigs to minimise pathogen-originated enteric disease such as PWD. Feeding insoluble NSP has long been recognised as a dietary component for decreasing expression of PWD in weaned pigs. Early evidence reported by Smith and Halls (1968) suggested that incorporation of barley hulls, which is mostly insoluble fibre, prevented expression of PWD, while barley meal that contained soluble ß-glucan increased the expression of PWD in ETEC-challenged pigs. The role of insoluble fibre on enteric pathogen proliferation has been highlighted more recently. For example, supplementation of 20 g oat hulls/kg in diets for weaner pigs significantly decreased expression of PWD in pigs fed cooked rice- (Mateos et al., 2006) or extruded rice- (Kim et al., 2008) based diets. Addition of 20 g pure cellulose/kg in a wheat-, barley-, maize- and soybean meal-based diet decreased the incidence of PWD from 50% to 16% (Hanczakowska et al., 2008) and from 23% to 8% (Swiatkiewicz and Hanczakowska (2006). Molist et al. (2010) found in an *E. coli* K88 infection study that incorporation of both coarse or finely ground wheat bran (40 g/kg) decreased the *E. coli* K88 population in the ileal digesta and ileal mucosa, reduced microbial density in the ileal digesta, and decreased production of valeric acids, which is produced from proline fermentation and irritants for the epithelium like other BCFA. These effects were more profound in the pigs fed coarsely prepared wheat bran, implicating a role for particle size in the amelioration of PWD. In the present study, the Opticell® used was not as coarse as that used in the study by Molist et al. (2010), and may help to explain some of the differences found in the magnitude of the control of PWD after infection. Moreover, Gonzalez-Ortiz et al. (2014) reported *in vitro* that soluble extracts obtained from wheat bran and locust bean gum exhibited the highest anti-adhesive properties against ETEC K88.

Moreover, a study by Gerritsen et al. (2012) examined the effect of insoluble NSP (iNSP) in weaned diets on post-weaning piglet performance, intestinal activity, and microbial composition. In their Exp. 1, 180 piglets were weaned at 28 d of age and divided over 3 treatments: positive control (PC; highly digestible protein), negative control (NC; standard cereal-based diet), and an experimental diet {iNSP; standard + 15% iNSP [wheat straw and oat (*Avena sativa*) hull]}. Diets were fed during 14 d after weaning. The inclusion of iNSP increased (P<0.001) ADFI and G:F during d 0 to 14 after weaning and increased the ADG (P = 0.008) during d 0 to 7 after weaning compared with the NC diet. In their Exp. 2, 36 piglets were fed the same diets as in Exp. 1. On d 5 and 14 after weaning, 18 piglets were euthanised to determine enzyme activity, intestinal morphology, and microbial population in the ileum and colon and organ weight. The iNSP diet reduced the concentration of *E. coli* bacteria in the ileum (P=0.021) and in the colon (P=0.002) digesta and tended to increase (P=0.060) the amylase activity. The iNSP diet stimulated the physical adaptation of the gastrointestinal tract because stomach weight as percentage of BW was heavier (P=0.004) than for the NC diet. The authors summarised by saying that the consumption of diets with higher iNSP content in the

early weaning period affected the microbial colonization without reducing enzyme activity or animal performance compared with a standard weaned pig diet.

In terms of more practical recommendations, Bolduan et al. (1988) recommended feeding 50 g crude fibre/kg weaner diet for optimum hindgut development, Mateos et al. (2006) suggested 60 g NDF/kg for amelioration of PWD, and the BSAS (2003) recommended 70–130 g NDF/kg in a weaner diet. By way of comparison, the diets in Experiment 2 providing for a decrease in PWD contained 75-100 g NDF/kg of diet. Based on these data and data from Experiment 2, it can be theorised that a weaner diet for mitigation of PWD should contain an amount of soluble NSP, at least immediately after weaning, of 30–50 g insoluble NSP (e.g., from sources such as oat hulls, pure cellulose/lignocellulose, barley hulls, wheat bran), to minimize the risk of PWD. However, as discussed by Pluske et al. (2001), practical weaner diets contain cereals, legumes and/or oilseed meals that contain considerable amounts of both soluble and insoluble NSP. It is estimated that a typical wheat-based weaner diet contains between 70–110 g NDF and 30–50 g ADF/kg. Further difficulties in recommending NSP levels also originate from the diversity of fibre structure and the generic lack of information about NSP fermentability and their ability to increase intestinal viscosity. Published studies used diverse types and levels of NSP, not all of which are "practical" ingredients (e.g., semi-synthetic diet or highly digestible cereal sources), which make comparisons difficult. Moreover, and from a feed formulation perspective, iNSP and sNSP levels are not found in ingredient matrices.

As mentioned above, it would appear that the particle size of insoluble fibre is a *key determinant* of its effects in the GIT, as using (mostly) insoluble NSP in the form of large particle sizes showed promising results to minimise the expression of PWD in commercial weaner diets containing considerable amounts of soluble NSP. Molist et al. (2010) used a diet containing maize (320 g/kg), wheat (200 g/kg), barley (170 g/kg) and soybean meal (140 g/kg). If the NSP contents of this basal diet are calculated using typical NSP content reported by Bach Knudsen (1997), the total and soluble NSP contents of the diet are 167 g/kg and 45 g/kg. In this "commercial" weaner diet the supplementation of 40 g coarsely prepared wheat bran significantly decreased PWD at 48 and 72 h (faecal score 1.5 vs. 0.5, $P<0.05$) post-infection with *E. coli* K88, through decreasing *E. coli* K88 population in the ileal digesta and ileal mucosa. This result may indicate that even though this "commercial" weaner diet contained considerable amounts of soluble NSP, their potentially deleterious effects can be mitigated by addition of coarsely prepared insoluble NSP, which may disperse the viscous network formed by the interaction between soluble NSP and water (Choct, 1997) in the digesta and hence increase digesta flow. The practical feeding challenge presented, however, relates to how best incorporate insoluble NSP as a coarser particle size (e.g., wheat bran) into pelleted nursery diets.

Despite these initially promising results in Experiment 2 with insoluble NSP (as Opticell®), the translation of 'best bet' dietary approaches (within the confines of commercial practice and reality, e.g., diet cost and diet specifications) to Experiment 3, the commercial validation study failed to substantiate the results from Experiment 2. The Control diet generally performed better than diets with 30 and 50 g/kg Opticell® or 30 and 50 g/kg SBP, but similarly to the combination diet of 30 g/kg Opticell® and 30 g/kg SBP. However the cost of the combination diet was ≈ $175/tonne more than the Control diet and failed to provide any benefits in terms of morbidity or mortality.

A surprising aspect of Experiment 3 was the poorer performance of the pigs fed SBP, and especially the higher mortality in pigs fed 3% or 5% SBP given that dietary ZnO and water medication (Sol-u-mox for the first 14 d after weaning) was available. These data are in contrast to the study by Schiavon et al. (2004), who examined the effects of SBP on growth performance and on some health parameters in antibiotic-free diets for

weaned piglets on a commercial farm. In the study by Schiavon et al. (2004), a conventional diet (C) and one containing 120 g/kg of SBP (in replacement of maize and wheat) were fed to 668 weaned piglets from 21 to 64±3 days of age. The dietary treatment did not affect faecal consistency. In comparison to C, SBP-fed pigs showed (slightly) reduced ADG over 36 to 49 d of age (528 vs. 498 g/d; P<0.05) and 50 to 64 d of age (677 vs. 631 g/d, respectively; P<0.01). Digestibility of NDF of diet C increased with age from 441 to 526 g/kg whereas that of diet SBP increased from 465 to 638 g/kg. The differences between diets became significant after 36 d of age (P<0.01). From 29 to 35 d of age higher contents of water (793 vs. 713 g/kg; P<0.01), acetic (322 vs. 206 µmol/g dm; P<0.01) propionic (108 vs. 81 µmol/kg DM; P<0.01) acids and lower counts of faecal coliforms such as *E. coli* (6.9 vs. 8.2 $\log_{10}$/g; P<0.01), clostridia (1.3 vs. 2.3 log10/g; P<0.01) and *Staphylococcus* spp. (6.7 vs. 8.1 log10/g; P<0.01) were found in the feces of the SBP-fed piglets compared to those of C. These differences progressively disappeared with time.

Schiavon et al. (2004) reported that some piglets showed clinical signs of purulent arthritis and meningitis, but no signs of diarrhea were observed. The SBP group showed, with respect to C, a significantly lower number of piglet deaths caused by meningitis (15 vs. 30%, respectively; P<0.05), and a significantly lower number of piglets removed because of lack of growth (33 vs. 76%, respectively; P<0.01). Schiavon et al. (2004) concluded that no clear evidence to explain this result was found, however they concluded that the inclusion of 12% SBP in antibiotic-free diets could improve the health status of piglets with little effect on growth performance.

Reasons for the completely contrasting results are hard to reconcile considering the studies were done on different farms in different countries with different diets and with different background health statuses. The data do articulate the difficulties encountered in trying to draw parallels between experiments. One possibility for the worse performance and higher morbidity/mortality of pigs fed 3% and 5% SBP in Experiment 3 may relate to the diets being pelleted, which might have solubilized some of the less fermentable fibre in the SBP (Kocher and Choct, 2001). Another possibility is simply variation in the quality of the SBP and (or) the percentage of sugar/molasses in the SBP that was fed to pigs.

Nevertheless, the fact that feeding the SBP deteriorated performance and increased the morbidity/mortality of pigs demonstrates not only the ambiguity in the literature but also raises the issue of solubility (that can be measured as viscosity) versus fermentability of a feed ingredient, in this case SBP. Fonseca et al. (2012) reported that the inclusion of 15 g cellulose/kg diet in a post-weaning diet reduced the incidence of PWD as compared with a control diet or a diet that included 30 g soybean hulls or 90 g citrus pulp/kg (11, 14, 25 and 22% PWD incidence, respectively). The authors concluded that there was a positive relationship between the presence of highly fermentable ingredients, such as citrus pulp and soybean hulls, and the incidence of PWD. The SBP used in Experiment 3 was also most likely quite fermentable. Also, Montagne et al. (2012) studied the interaction between fibre content of the diet and sanitary conditions of the farm on the growth and health status of weaned pigs. In this research, pigs fed a combination of 60 g SBP and 20 g soybean hulls/kg diet and allocated in rooms with a high infection pressure showed a lower feed intake for the first 2 weeks after weaning and a higher incidence of PWD than pigs from the other experimental groups. The authors concluded that the inclusion of ingredients with highly fermentable fibre immediately after weaning represent an additional risk factor for pig health and growth, especially under poor sanitary conditions.

Nevertheless, there is evidence (Wellock et al., 2008) in ETEC-challenged weaner pigs suggesting that non-viscous but 'soluble' NSP does not encourage pathogen proliferation and hence expression of PWD, as increasing the NSP content in that study without an

increase in intestinal viscosity did not increase PWD and increased the colonic *Lactobacillus*: coliform ratio. The same principle may extend to the understanding that *fermentable*, but not *viscosity-increasing*, NSP such as fructooligosaccharides and SBP encourage proliferation of microbes such as *Lactobacillus acidophilus* and *Bifidobacteria* and reduce the expression of PWD (Halas et al., 2009; Hermes et al., 2009). Also, a study showed that feeding 70-g/kg type 2 resistant starch (resistant granules) as a raw potato starch, which is not digested by the host's enzyme system but fermented by intestinal microbes, significantly reduced faecal score in the first week after weaning (Bhandari et al., 2009). However and again, it was evident that the SBP used in Experiment 3 acted contrarily to these studies that report a beneficial effect of feeding SBP.

Unlike viscous forming soluble NSP, insoluble NSP is known to decrease digesta retention time and decrease small intestinal pathogen proliferation (Kim et al., 2012). Although feeding insoluble NSP is known to increase endogenous nitrogen flow as well, proliferation of nitrogen utilisers might be limited due to the shorter retention time of digesta and the presence of fibre, which may attract saccharolytic microbes. The concentration of insoluble NSP progressively increases as the digesta moves posteriorly because other digestible nutrients will be progressively digested and absorbed while insoluble NSP remain intact in the small and large intestine (Kim et al., 2012). However and as noted in the present study (Table 8 and Figure 5), feeding more insoluble NSP (as Opticell®) decreased the ATTD of GE, but without a significant effect on the AID of nitrogen (although there was a noticeable indication of decreased AID of nitrogen with more insoluble fibre in the diet) or actual performance. Recently, Gutierrez et al. (2013), formulating diets to net energy (NE) rather than DE or ME, reported similar findings. Traditionally, high levels of insoluble NSP have been reported to decrease nutrient digestibility (Lenis et al., 1996) and hence growth of pigs (Degen et al., 2009), but evidently the appropriate formulation of the diet (Gutierrez et al., 2013) can counteract these influences. Diets in Experiment 3 were calculated on the basis of DE due to the absence of reliable data on NE for SBP and Opticell®, however such an influence may go some way in explaining the inferior growth performance seen in Experiment 3 in pigs fed these products.

Non-starch polysaccharides and intestinal barrier function

The presence of insoluble fibre in the diet means continuous mechanical interaction between digesta and the epithelial mucus layer. This mechanical contact may cause a 'wash-out' of mucins and mucous-bound microbes, which could be a part of the reason why mucous-bound *E. coli* was significantly decreased in the pigs' ileal epithelium when they were fed a diet containing wheat bran, especially coarsely prepared wheat bran (Molist et al., 2010). Decreased microbial density in the ileal digesta only in pigs fed a coarsely-prepared wheat bran diet but not in pigs fed a finely-ground wheat bran in the study by Molist et al. (2010) was most likely due to the increased digesta transit time, and suggests that particle size of insoluble NSP is an important issue which needs to be considered in GIT health. Nevertheless, inclusion of insoluble NSP in the form of cellulose or wheat bran increases mucin production and increases enterocyte and goblet cell turnover in rats (Vahoney et al., 1985). Evidence also indicates that insoluble NSP could reduce intestinal permeability and bacterial translocation in the GIT. For example, Mariadason et al. (1999) measured intestinal permeability using conductance and Cr-EDTA flux method and reported a 20% reduction in intestinal permeability in the distal colon of rats fed a diet containing 100 g wheat bran/kg compared with rats fed a diet without wheat bran. Another rat study conducted by Spaeth et al. (1990) examined the incidence of bacterial translocation into the mesenteric lymph node (MLN) in rats orally fed either control total parental nutrition solution, control+cellulose powder, control+coarsely ground maize cobs or control+citrus pectin. The incidence of bacterial

translocation into the MLN was significantly decreased in rats fed cellulose (15%) and coarsely ground maize cobs (30%) compared to rats fed the control (70%) or citrus pectin (65%) diets. This particular study demonstrated that insoluble fibre in the forms of cellulose or maize cobs could reduce bacterial translocation in the GIT, while the viscosity increasing soluble fibres did not demonstrate such protective effect.

Based on this literature, it was hypothesised that when pigs were infected with ETEC, supplementation of increasing levels of iNSP will decrease the number of mucosa-bound β-haemolytic *E. coli* in the ileum, and reduce the translocation of β-haemolytic *E. coli* into the MLN. This failed to occur in the present study ((Experiment 2; Table 5). Contradictory to this finding, the blood cell count data showed that infection significantly increased the number of white blood cells and hence effects of iNSP on bacterial attachment and translocation through the epithelial barrier was observed under considerable ETEC infection pressure. Further study is required to test the proposed mechanisms of iNSP on attachment of ETEC to the mucosal layer and translocation into the MNL before drawing a firm conclusion.

More recently in weaner pigs, Chen et al. (2013) evaluated the effects of fibre source on intestinal mucosal barrier function. A total of 125 piglets were randomly allotted on the basis of their body weight and litters to one of five experimental diets, i.e., a control diet without fibre source (CT), and diets in which expanded maize was replaced by 10% maize fibre (MF), 10% soybean fibre (SF), 10% wheat bran fibre (WBF) or 10% pea fibre (PF). Piglets fed on the WBF and PF diets had a lower diarrhoea incidence compared with the MF- and SF-fed animals. A higher ratio of villous height: crypt depth in the ileum of WBF-fed piglets and higher colonic goblet cells in WBF- and PF-fed piglets were observed compared with CT-, MF- and SF-fed piglets. In the intestinal digesta, feeding WBF and PF resulted in increased *Lactobacillus* spp. counts in the ileum and *Bifidobacterium* spp. counts in the colon. Lower *E. coli* counts occurred in the ileum and colon of WBF-fed piglets than in SF-fed piglets. Tight junction protein (zonula occludens 1; ZO-1) and Toll-like receptor 2 (TLR2) gene mRNA levels were up-regulated in the ileum and colon of pigs fed WBF; however, feeding MF and SF raised IL-1α and TNF-α mRNA levels. Furthermore, higher diamine oxidase activities, transforming growth factor-α, trefoil factor family and MHC-II concentration occurred when feeding WBF and PF. These authors concluded that the various fibre sources had different effects on ileal and colonic barrier function, but that WBF and PF improved intestinal barrier function, probably mediated by changes in microbiota composition, and concomitant changes in TLR-2 gene expression.

In the present study (Experiment 2), small intestinal histology and tight junction protein gene expression data suggested that the interaction between sNSP and iNSP plays a significant role in intestinal epithelium barrier function. Data in Table 11 demonstrated that increasing iNSP in the diet statistically tended (there was large variation in the data) to up-regulate occludin expression when dietary sNSP content was minimal, while increasing iNSP tended to down-regulate occludin when dietary sNSP content was high. Therefore, the source, type and level of fibre along with the ratio of sNSP: iNSP in the diet can affect measures of intestinal structure and function during the immediate post-weaning period, especially under conditions of high bacterial infection load sometimes observed in commercial facilities.

4. Application of Research

Results from Experiment 2 of this project suggest that supplementing appropriate iNSP sources, whilst trying to minimise the level of sNSP, in antimicrobial-free diets for weaner pigs has beneficial effects on aspects of intestinal structure and function, growth, and selected measures of physiology in the circulation in the 2 weeks after weaning. Increasing the amount of dietary sNSP thereafter appeared to be beneficial to

production. However many other factors such as type, source, and level of fibre along with the sNSP: iNSP ratio also plays interactive roles. Generally, increasing iNSP content in the diet for weaner pigs can be recommended only when there is considerable bacterial infection load likely to cause PWD and critical antimicrobial compounds are not used. In this regard, the concomitant use of medication seemingly reduces any potential beneficial effects of iNSP, perhaps due to alteration of the microbiota in the GIT.

Nevertheless, an interesting outcome of this study was the finding that small intestinal epithelium barrier function, measured as the expression of several tight junction protein genes, was enhanced with increasing dietary iNSP content and minimal inclusion of sNSP. Diminished barrier function is a key negative aspect associated with the stressors of weaning, so perhaps even in the absence of clinical disease/inclusion of antimicrobial compounds in feed/water, higher levels of dietary insoluble NSP should be considered to assist in the restoration of barrier function in the post-weaning period.

5. Conclusion

The data derived from the three experiments conducted in this project suggest that the type and level of NSP, in corollary to the ratio between sNSP: iNSP, play important roles for pig growth, GIT structure and function, expression of PWD, and aspects of physiology of weaner pigs. Under conditions of higher pathogenic bacterial load and antimicrobial-free production, increasing the iNSP content in the diet and trying to minimise sNSP levels for weaner pigs immediately after weaning should be considered. Data from the commercial validation study suggests, however, that the effects of including antimicrobial compounds need to be considered when evaluating the potential efficacy of manipulation of fibre types and content of the diet to reduce PWD and improve production indices. Further investigation in relation to this possible interaction is warranted experimentally.

6. Limitations and Risks

Experiments 2 and 3 were conducted in different conditions such as different infection loads, different diets, different weaning ages/genotypes and varied use of medications. Therefore, the interpretation of results from the two experiments should be carried out with some caution.

7. Acknowledgements

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9. Appendix 1

Laboratory Results of E. coli toxin PCR Analysis

Laboratory Results

ACE Laboratory Services
12 Gildea Lane, Bendigo East, VIC 3550
Phone: (03) 5443 9665 **Fax:** (03) 5443 9669
Email: info@acelabservices.com.au

To: John Pluske
Director, Animal Research Institute
Murdoch University
90 South Street
Murdoch WA 6150

Report Date: 13/08/12
Date samples received: 09/08/12

PCR Report

Laboratory Number: 4767/12 No Samples Submitted: 4

E.coli Toxin PCR Results

Sample No. / Description	1 Original Broth	2 Original Plate	3 Passaged Broth	4 Passaged Plate
Toxin	**Result**	**Result**	**Result**	**Result**
LT1	**Positive**	**Positive**	**Positive**	**Positive**
ST1	**Positive**	**Positive**	**Positive**	**Positive**
ST2	**Positive**	**Positive**	**Positive**	**Positive**
EaeA	Not Detected	Not Detected	Not Detected	Not Detected
EAST	**Positive**	**Positive**	**Positive**	**Positive**
Stx2e	Not Detected	Not Detected	Not Detected	Not Detected
F18	Not Detected	Not Detected	Not Detected	Not Detected
F4 K88	**Positive**	**Positive**	**Positive**	**Positive**
F5 K99	Not Detected	Not Detected	Not Detected	Not Detected
F6	Not Detected	Not Detected	Not Detected	Not Detected

Comments: The samples will be sent for E.coli serotyping.

Page 1 of 1 Report prepared by: date Report checked by date
This report may not be reproduced except in full. This analysis relates to the samples submitted and it is the submitters responsibility to ensure that the sample is representative of the material tested.

10. Appendix 2

Analyzed Non-starch polysaccharide data for diets and ingredients (Experiment 2) - g/Kg

Sample ID		Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total
Low sNSP 0% Opticell	Free Sugars	0.00	0.24	0.00	0.00	0.00	0.20	41.20	40.85	82.38
	SNSP	0.00	0.00	0.10	0.31	0.18	2.26	0.64	4.03	6.75
	INSP	0.00	0.00	0.00	0.63	0.45	0.54	0.51	2.06	3.39
	Total NSP	0.00	0.00	0.10	0.94	0.63	2.80	1.15	6.09	10.14
Low sNSP 3% Opticell	Free Sugars	0.00	0.23	0.00	0.15	0.00	0.20	38.59	38.48	77.58
	SNSP	0.00	0.00	0.09	0.33	0.20	2.38	0.77	4.30	7.26
	INSP	0.00	0.00	0.00	0.98	1.41	2.88	1.14	14.13	18.08
	Total NSP	0.00	0.00	0.09	1.31	1.61	5.26	1.91	18.43	25.34
Low sNSP 6% Opticell	Free Sugars	0.00	0.23	0.00	0.00	0.00	0.00	39.15	39.00	78.38
	SNSP	0.00	0.00	0.08	0.35	0.21	2.46	0.94	4.33	7.52
	INSP	0.00	0.00	0.00	1.46	2.48	5.35	1.58	26.50	33.21
	Total NSP	0.00	0.00	0.08	1.81	2.69	7.81	2.52	30.83	40.73
Low sNSP 9% Opticell	Free Sugars	0.00	0.24	0.00	0.00	0.00	0.19	42.10	41.64	84.08
	SNSP	0.00	0.05	0.10	0.49	0.26	2.40	1.14	4.13	7.56
	INSP	0.00	0.36	0.38	2.03	3.31	8.11	2.30	39.11	49.25
	Total NSP	0.00	0.41	0.48	2.52	3.57	10.51	3.44	43.24	56.81
High sNSP 0% Opticell	Free Sugars	0.00	0.26	0.00	0.00	0.00	0.36	41.55	43.21	85.38
	SNSP	0.05	0.00	0.09	1.48	1.56	2.45	0.90	24.54	27.87
	INSP	0.00	0.00	0.00	1.59	1.29	0.74	0.57	3.48	6.49
	Total NSP	0.05	0.00	0.09	3.07	2.85	3.19	1.47	28.02	34.36
High sNSP 3% Opticell	Free Sugars	0.00	0.24	0.00	0.00	0.00	0.36	39.09	41.32	81.01
	SNSP	0.06	0.00	0.09	1.52	1.59	2.68	0.96	22.55	26.43
	INSP	0.00	0.00	0.38	1.65	2.26	2.89	1.04	14.38	19.74
	Total NSP	0.06	0.00	0.47	3.17	3.85	5.57	2.00	36.93	46.17
	Free Sugars	0.00	0.26	0.00	0.13	0.00	0.41	42.77	45.01	88.51

Sample ID		Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total
High sNSP 6% Opticell	SNSP	0.06	0.00	0.08	1.60	1.59	2.68	1.16	24.49	28.43
	INSP	0.00	0.00	0.00	2.71	3.32	5.42	1.72	27.20	35.86
	Total NSP	0.06	0.00	0.08	4.31	4.91	8.10	2.88	51.69	64.29
High sNSP 9% Opticell	Free Sugars	0.00	0.31	0.00	0.37	0.00	0.51	48.98	50.85	100.83
	SNSP	0.07	0.00	0.09	1.88	1.73	2.63	1.40	28.29	32.37
	INSP	0.00	0.00	0.00	2.99	4.44	8.44	2.59	41.32	53.30
	Total NSP	0.07	0.00	0.09	4.87	6.17	11.07	3.99	69.61	85.67
B-glucal (Glucagel)	Free Sugars	0.00	0.00	0.00	0.29	0.00	2.03	0.65	43.03	45.85
	SNSP	0.00	0.00	0.19	10.01	9.99	3.28	0.93	539.09	506.74
	INSP	0.00	0.00	0.00	2.12	0.72	0.40	0.55	339.45	308.51
	Total NSP	0.00	0.00	0.19	12.13	10.71	3.68	1.48	878.54	815.25
Wheat NSP (AX Product)	Free Sugars	0.00	0.00	0.00	3.05	1.94	3.54	1.85	5.32	15.70
	SNSP	0.14	0.06	0.13	80.42	140.35	1.17	3.67	10.64	208.47
	INSP	0.00	0.00	0.32	71.99	105.42	10.11	5.99	14.67	183.60
	Total NSP	0.14	0.06	0.45	152.41	245.77	11.28	9.66	25.31	392.07
White rice	Free Sugars	0.00	0.00	0.00	0.00	0.00	0.16	0.14	1.81	1.95
	SNSP	0.00	0.00	0.00	0.17	0.05	0.66	0.09	0.32	1.16
	INSP	0.00	0.00	0.00	0.81	0.50	2.52	0.60	2.59	5.94
	Total NSP	0.00	0.00	0.00	0.98	0.55	3.18	0.69	2.91	7.10
Pectin (GENU Pectin)	Free Sugars	0.00	0.00	0.00	2.32	0.00	25.55	0.26	175.82	203.95
	SNSP									
	INSP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total NSP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Opticell	Free Sugars	0.00	0.00	0.00	0.80	0.00	0.17	0.73	0.89	2.50
	SNSP	0.10	0.06	0.00	0.85	0.05	1.02	5.30	0.44	6.99
	INSP	0.80	0.48	0.00	12.82	35.63	59.03	18.88	32.79	143.05
	Total NSP	0.90	0.54	0.00	13.67	35.68	60.05	24.18	33.23	150.04