PERFORMANCE OF NEWLY-WEANED PIGS FED ALTERNATIVE SOURCES OF PROTEIN

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Soybean meal combined with dried whey and plasma protein are the usual protein sources in diets for newly-weaned pigs. This experiment investigated whether yeast protein with added enzyme can replace plasma protein and dried whey in diets for newly-weaned pigs. A total of 630 newly-weaned piglets (6.8 kg mean BW) were randomly allotted to three dietary treatments in a two-phase feeding program from 0 to 21 days from weaning (Phase1), and 22 to 49 days from weaning (Phase 2). Dietary treatments 1 (control diet) in both phases contained low levels of soybean meal (18%), and high levels of dried whey (15% in phase 1, 10% in phase 2), and plasma protein (5%). Dietary treatments 2 in both phases contained high levels of soybean meal (25%), moderate levels of dried whey (5% in phase 1, 2.5% in phase 2), no plasma protein, and moderate levels of yeast protein (3% in phase 1, 2% in phase

2). Lastly, dietary treatments 3 in both phases contained high levels of soybean meal (25%), no dried whey, no plasma protein, and high levels of yeast protein (4% in phase 1, 3% in phase 2). Treatments 2 and 3 were supplemented with a solid state fermentation (SSF) enzyme complex containing phytase, protease, amylase, cellulase, xylanase, and ß-glucanase as part of the alternative strategy being evaluated in this study. All experimental diets did not contain any antibiotics. Feed intake, total body weight gain, average daily gain (ADG, g/d), feed conversion ratio (FCR, feed/gain), and survival rate were recorded and computed for each phase, as well as for the whole duration of the trial. Fecal consistency was taken at days 7, 14 and 21, and recorded as fecal score. Percent of scouring piglets per pen at day 7 of the trial was also recorded and noted as scouring incidence. Intestinal samples were taken at day 7 and day 28 of the trial to measure duodenal villus height and crypt depth, and to calculate for villus-height-to-crypt-depth ratio (VCR). Feed intake, survival rate and fecal score at day 7 of pigs in treatment 3 during phase 1 were found significantly different ($P < 0.05$), but no difference in the other growth and scouring parameters were observed during phase 2 and over-all duration of the trial. Only the crypt depth at day 7 of treatment 1 was different ($P < 0.05$) among all intestinal morphology data taken. This study demonstrates that the combination of yeast protein and SSF enzyme complex can successfully replace plasma protein and whey powder as protein sources for newly-weaned pigs. This offers opportunities for cost savings when designing diets for newly-weaned pigs.

KEYWORDS: piglets, soybean meal, protein source, yeast protein, SSF enzyme, intestinal morphology

INTRODUCTION

SOYBEAN MEAL IS the most important and widely-used protein source in the animal feed industry (FAO, 2004), but its high concentration in post-weaning diets has a detrimental effect on the newly-weaned pig's small intestine, lowering its digestive capacity and causing post-weaning diarrhea (Dunford et al., 1989). The usual strategy therefore is to combine milk products and plasma protein with soybean meal as protein sources in diets for newly-weaned pigs.

Milk products like dried skimmed milk and dried whey were found to improve growth performance of young pigs (Himmelberg et al., 1985; Lepine et al., 1991). Tokach et al. (1989) suggested that the improved performance of newly-weaned pigs fed a diet containing dried whey probably is the result of the presence of both the carbohydrate (lactose) and protein (lactalbumin) fractions present in whey. Lactalbumin has an excellent amino acid profile, a digestibility of 99%, biological value of 94% and protein efficiency ratio of 3.2 (Robinson, 1986). It was not clear however in the study of Tokach et al. (1989) which of these fractions contributed to the improved performance of newly-weaned pigs. Also, no additive effect was found when the lactose and lactalbumin were added together to the basal diet.

In 1992, Mahan showed that the lactose component of dried whey was the primary component that improved postweaning performance of pigs without negating the contribution of lactalbumin as an amino acid or protein source for the postweaning diet. Recent studies even suggested lactose levels of between 7 to 20% in post weaning diets (Mahan et al., 2004; Cromwell et al., 2008). Aside from being the major carbohydrate source for the newly-weaned pig, these studies suggest that lactose may be important in maintaining a good intestinal environment for the pig by enhancing the growth of *Lactobacillus spp.* present in the stomach and intestinal tract of the pig at weaning.

Spray-dried plasma protein is also consistent in improving growth performance when added to newly-weaned pig diets (Hansen et al., 1993; Kats et al., 1994). Excellent palatability and high nutrient digestibility were among the reasons given in these earlier studies as to why plasma protein is a better protein source than soybean meal in diets of newly-weaned pigs. Recently, however, Pierce et al. (2005) was able to identify that the immunoglobulin G fraction of plasma protein is the one responsible for the enhanced pig performance that occurs when it is fed to newly-weaned pigs.

Intestinal morphology of newly-weaned pigs was used in several studies to explain the differences in their growth performance when fed diets containing different protein sources (Cera et al., 1988; Dunsford et al., 1989; Carlson et al., 2004). These studies showed that although morphological changes in the small intestine of newly-weaned pigs are inevitable consequences of weaning, providing a highly digestible post-weaning diet appears

to minimize these abrupt morphological changes, therefore improving the newly-weaned pig's digestive capacity.

Nevertheless, the more important economic justification of any strategy to provide protein from alternative sources in post-weaning diets should always be investigated (Himmelberg et al., 1985). Faster growth rates and better feed conversion associated with including milk products and plasma protein in post-weaning diets vary in economic value and must be weighed against the additional cost of using these protein sources. Price of milk products, particularly dried whey, has been very unstable in recent years and many are now asking what their optimum levels in diets for newly-weaned pigs should really be (Mavromichalis, 2006). The search for more economical strategies to provide protein in diets for newly-weaned pigs should therefore continue.

It was Carlson et al. (2004) who hinted that yeast protein may appeal as an alternative protein source for animal feed due to the growing restrictions to feeding of animal products and by-products to livestock and poultry. First implemented in the EU (EC, 2002), emerging feed legislation worldwide has put added pressure on the search for alternative digestible protein sources for feed that are safe not only to animals, but to humans and to the environment as well.

A yeast protein, in particular yeast cell extract, was shown to be an effective alternative source of digestible protein for postweaning pig diets in the study by Maribo and Spring (2003). The yeast protein used in their experiment is manufactured from the cell contents of a specific strain of the yeast *Saccharomyces cerevisae* (D'souza & Frio, 2007). This same yeast protein has been found to be an effective substitute to soybean meal (Hunziker and Spring, 2002), fishmeal (Maribo, 2001), and plasma protein (Mahan & Tibbetts, 2000; Maribo & Spring, 2003; Carlson et al., 2004; Halbrook et al., 2004; Maxwell et al., 2004) as a protein source in diets for newly-weaned pigs in several studies.

Aside from being a rich source of digestible amino acids, yeast protein has other components that enhance its functional properties. These include glutamate, which gives it a distinct flavour and improves palatability; inositol, a vitamin that is a fundamental component of cell membranes; and nucleotides, which are important for immunity and maintaining gut integrity and health (D'souza & Frio, 2007).

Objective of the Study

Considering the significant level of functional nutrients found in yeast protein and their positive effect on the gastro-intestinal microflora and feed palatability (Mateo et al., 2004), as well as its high amino acid digestibility (Mateo & Stein, 2007), it is interesting to find out if yeast protein can be used as partial or complete replacement for both plasma protein and dried whey. This study aims to evaluate alternative protein sources in diets for newly-weaned pigs in terms of pig performance (e.g., growth rate, feed conversion, feed intake, survival rate, diarrhea incidence, and fecal score) and intestinal morphology.

Time and Place of the Study

Feeding trial for this study was done at Camille Farm located in General Santos City, Southern Philippines from May to September 2008.

Intestinal histopath slides were done at the Histopathology laboratory of the College of Veternary Medicine at the University of the Philippines Los Banos, Philippines. Digital imaging and villi structure measurements were done at the microscopy lab of the National Institute of Molecular Biology and Biotechnology at the University of the Philippines-Los Banos, Philippines.

MATERIALS AND METHODS

Animals

A total of 630 cross-bred (Large White-Landrace x PD), newly-weaned piglets, with a mean weight of 6.8 kg, were randomly allotted to 18 equal groups. The 18 groups were then randomly allotted to 18 slotted nursery pens of similar sizes, which were also randomly allotted to three dietary treatments, with each treatment having six replicates. Each pen was therefore considered as one experimental unit.

Experimental Diets

Six experimental diets (Table 1) were formulated and fed in two

TABLE 1. **Treatment diet composition with calculated analysis.**

Ingredients	**Phase 1** (0-21 days from weaning)			**Phase 2** (22-49 days from weaning)		
	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 1**	**Treatment 2**	**Treatment 3**
Corn	53.8%	58%	61.6%	60.3%	63.2%	64.5%
Sweet dried whey	15%	5%	—	10%	2.5%	—
Soybean meal 48%	18%	25%	25%	18%	25%	25%
Animal plasma protein	5%	—	—	5%	—	—
Yeast protein	—	3%	4%	—	2%	3%
Coconut oil	3.41%	3.84%	4.02%	2.06%	2.36%	2.49%
Mono-Dicalphosphate	1.52%	1.36%	1.44%	1.66%	1.51%	1.51%
Limestone	1.04%	1.17%	1.33%	1.12%	1.15%	1.25%
Salt	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
L-Lysine HCL	0.38%	0.532%	0.542%	0.225%	0.39%	0.378%
DL-Methionine	0.221%	0.267%	0.262%	0.128%	0.18%	0.173%
L-threonine	0.114%	0.209%	0.215%	0.32%	0.133%	0.127%
L-tryptophan	0.051%	0.082%	0.084%	0.026%	0.058%	0.057%
Acidifier	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Zinc oxide	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Sweetener	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
Vitamin Mineral premix	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Anti-oxidant	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
Choline chloride 50%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
SSF Enzyme product	—	0.02%	0.02%	—	0.02%	0.02%
Mycotoxin binder	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Organic Mineral premix	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
TOTAL	100%	100%	100%	100%	100%	100%

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Ingredients	**Phase 1** (0-21 days from weaning)			**Phase 2** (22-49 days from weaning)		
	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 1**	**Treatment 2**	**Treatment 3**
Calculated Analysis						
Crude Protein %	19.347	19.672	19.884	19.015	19.048	19.338
Crude Fat %	6.018	6.546	6.828	4.900	5.265	5.427
Crude Fiber %	1.805	2.116	2.185	1.931	2.216	2.242
Calcium %	0.951	0.950	0.950	0.931	0.930	0.931
Avail. Phos. %	0.549	0.549	0.551	0.549	0.551	0.551
M.E.Swine kcal/kg	3,450.091	3,450.171	3,449.918	3,399.974	3,399.910	3,399.721
Lysine %	1.450	1.451	1.450	1.300	1.301	1.300
Meth.+Cyst. %	0.870	0.871	0.870	0.780	0.780	0.780
Threonine %	0.942	0.943	0.943	0.845	0.845	0.845
Tryptophan %	0.290	0.290	0.290	0.260	0.260	0.260
Lactose %	10.499	3.502	6.999			

phases. The first three iso-caloric and iso-nitrogenous diets were fed for the first 21 days from weaning—called phase 1—while the next three iso-caloric and iso-nitrogenous diets were fed from 22 to 49 days—referred to as phase 2. The control diet (Treatment 1) in both phases contained a low level of soybean meal, high level of dried whey, and plasma protein. Treatment 2 diets in both phases contained a high level of soybean meal, moderate level of dried whey, no plasma protein, and moderate level of yeast protein. Lastly, treatment 3 diets in both phases contained a high level of soybean meal, no dried whey, no plasma protein, and high level of yeast protein. All diets in both treatments 2 and 3 were supplemented with a solid state fermentation (SSF) produced enzyme complex containing phytase, protease, amylase, cellulase, xylanase, and β-glucanase (Alltech Biotechnology). Addition of this SSF enzyme product to treatments 2 and 3 diets was considered in order to reduce the anti-nutritive effects of higher soybean meal usage in these experimental diets and is part of the alternative strategy to be evaluated in this study. All experimental diets did not contain any antibiotics or in-feed medications.

Feeding and Management

All pigs were fed ad libitum through a line feeder positioned at the front of the pen. Drinking water was provided to all pigs at all times through two drinking nipples in each pen. All vaccination and medication schedules and other management practices were applied similarly to all groups.

Performance Data Collection

Total feed consumption per pen was recorded after each phase. Total initial and final body weights per pen were also recorded for each phase and for the whole trial, and total body weight gain per pen was then computed. Based on the feed consumption and weight gain data, average daily gain (ADG, g/d) and feed conversion ratio (FCR, feed/gain) were computed for each phase, as well as for the whole duration of the trial. The incidence, date and weight of all mortality were also recorded. Pigs that die before the end of each stage were not included in the computation of ADG. Their dead weights, however, were included in the computation of FCR.

Fecal consistency at three randomly selected positions per pen was noted at days 7, 14 and 21, and recorded as fecal score. A fecal score of 1 was given to solid, 2 to soft but well-formed, 3 to soft and no form, and 4 to loose and watery scours. The average fecal score of the three randomly selected positions was considered the scouring score per pen. Percent of scouring piglets per pen at day 7 of the trial was also recorded and noted as scouring incidence. Only pigs showing fecal score of 4 were considered scouring.

Intestinal Data

Intestinal morphology data of one randomly selected pig per pen was taken at day 7 and day 28 of the trial. These pigs were slaughtered and intestinal duodenal samples were collected following the procedure done by Carlson et al. (2004). Duodenal villus height and crypt depth of intestinal samples were measured using light microscopy and ImageJ Microscope Image processing (National Institute of Health, USA). Villus-height-to-crypt-depth ratio (VCR) was then calculated and recorded.

Statistical Analysis

Treatment effects on total body weight, ADG, FCR, feed intake, survival rate, diarrhea incidence at day 7, fecal score at days 7, 14 and 21, and intestinal morphology (duodenal villus height, crypt depth and villus-height-to-crypt-depth ratio) at day 7 and 28 were all determined using Analysis of Variance (ANOVA). Differences among mean performance were considered significant at P values <0.05. When mean performances are found significantly different, a t-test was conducted to determine which particular treatment mean differs from the others.

RESULTS

Total body weight of pigs per pen at the end of phases 1 and 2 did not significantly differ among the three treatments (Table 2). The low P value at the end of phase 1 (0.13) is however noted and could be due to the very low mean performance of pigs in treatment 3 (373.75 kg), which appears to be caused by high mortality among pigs in treatment 3.

TABLE 2. **Performance of newly-weaned pigs fed the three dietary treatments**[a]

Parameters	Dietary Treatment			P value[b]
	Treatment 1	Treatment 2	Treatment 3	
Average body weight per pen, kg				
Initial	240.00	240.00	240.00	
End of Phase 1	396.22	391.33	10.66	0.13
End of Phase 2/Final	790.92	763.00	21.75	0.38
Average daily gain per head, g				
Phase 1	206.82	198.66	172.27	0.08
Phase 2	419.54	398.95	410.40	0.77
Overall	331.90	318.10	311.95	0.60
Total feed intake per pen, kg				
Phase 1	298.33 A	278.33 A	249.50 B	0.02
Phase 2	773.33	744.17	714.00	0.40
Overall	1071.67	1022.50	963.50	0.18
Feed conversion ratio, feed/gain				
Phase 1	1.91	1.84	1.89	0.56
Phase 2	1.97	2.00	1.95	0.74
Overall	1.95	1.95	1.93	0.89
Survival rate, %				
Phase 1	100.00 A	99.03 A	97.13 B	0.03
Phase 2	99.02	97.55	98.00	0.66
Overall	98.92	96.27	94.50	0.14

[a] A total of 630 piglets (35 piglets per replicate and 6 replicates per treatment) were used at the beginning of the 49-day study. Phase 1 (Days 1 to 21) contained an iso-caloric and iso-nitrogenous diets among the 3 treatments, and Phase 2 (Days 21 to 49) also contained an iso-caloric and iso-nitrogenous diets among the treatments. Treatment 1 diets in both phases were based on a low level of soybean meal, high level of dried whey and plasma protein. Treatment 2 diets in both phases were based on a high level of soybean meal, moderate level of dried whey, no plasma protein, and moderate level of yeast protein. Treatment 3 diets in all phases were based on a high level of soybean meal, no dried whey, no plasma protein, and high level of yeast protein.

[b]Data were analyzed using ANOVA with 2 factors, where 1 factor is the replicate (considered as blocking factor) and the other is the treatment.

Similarly, average daily gain (ADG) among treatments did not significantly differ in phases 1, 2 and over-all. As in total body weight however, the P value at the end of phase 1 is notably low at 0.08, and could be again due to the very low mean performance of pigs in treatment 3 (172.27 g). It is interesting to see however, that the P value at the end of phase 2 is very high at 0.77. This indicates that all treatments performed almost similarly in phase 2 in terms of ADG. In fact, pigs in treatment 3 compensated for their poor ADG in phase 1 and even outperformed pigs in treatment 2 during phase 2.

Total feed intake of pigs in phase 1 was found to significantly differ among treatments. T-test showed that treatments 1 and 2 were not significantly different from each other (both were A; i.e. homogeneous groups), but treatment 3 was significantly lower than treatments 1 and 2 (B; i.e. heterogeneous group). However, total feed intake among treatments in phase 2 showed no significant difference. Summing up the feed intake in phases 1 and 2 (overall) did not also show any significant difference.

Feed conversion ratio (FCR) in phases 1, 2 and overall did not show significant differences among treatments. The high P value in all phases is in fact indicative of how similarly all treatments performed in terms of FCR.

Survival rate in phase 1 showed significant difference among treatments. T-test showed that treatments 1 and 2 were not significantly different (A and AB, respectively), while treatment 1 was significantly higher in survival rate than Treatment 3 (A and B, respectively). Treatments 2 and 3 were not significantly different as well (AB and B, respectively). Survival rate in phase 2 and overall showed no significant differences among treatments.

Results of the fecal score and scouring incidence of pigs fed the three dietary treatments and the corresponding P values from the ANOVA (Table 3) showed fecal score at day 7 had significant difference among treatments. T-test showed that treatments 1 and 2 were statistically the same (both were A), but treatment 3 had a significant higher fecal score (B) than treatments 1 and 2. Fecal scores at day 14 and 21 did not significantly differ among treatments.

Scouring incidence at day 7 did not show significant difference. No incidence of scouring was observed at day 14 and 21.

TABLE 3. **Fecal score and scouring incidence of pigs fed different dietary treatments[a], [d]**

	Treatment 1	Treatment 2	Treatment 3	P value[b]
Fecal score[c] at day 7	2.44 A	2.44 A	3.00 B	0.01
Fecal score at day 14	1.72	1.83	1.94	0.19
Fecal score at day 21	1.50	1.78	1.61	0.11
Scouring incidence at day 7, %	3.33	3.33	6.19	0.20

[a] A total of 162 randomly selected sites for fecal score were noted at 7th, 14th and 21st days of the trial (3 sites per replicate, 6 replicates per treatment).
[b] Data were analyzed using ANOVA with 2 factors, where 1 factor is the replicate (considered as blocking factor) and the other is the treatment.
[c] Fecal score: 1 – solid, 2 – soft but well-formed, 3 – soft and no form, 4 – loose and watery
[d] Only pigs showing fecal score of 4 were considered scouring.

The intestinal morphology of pigs fed the three dietary treatments (Table 4) indicated that on day 7, duodenal villus height (VH) for all treatments did not significantly differ. However, crypt depth (CD) showed significant difference. T-test showed that treatment 1 was significantly greater than treatment 2 (A and B, respectively), while treatments 1 and 3 were not significantly different (A and AB, respectively). Treatments 2 and 3 were also not significantly different from each other (B and AB, respectively). No significant difference was observed for duodenal villus height to crypt depth ratio (VH:CD) among all the treatments.

On day 28, no dietary treatment differences were observed for duodenal morphology.

TABLE 4. **Duodenal morphology of pigs at days 7 and 28 fed different dietary treatments[a]**

	Treatment 1	Treatment 2	Treatment 3	P value[b]
Day 7				
Villus height, μ	295.58	284.77	289.99	0.89
Crypt depth, μ	218.94 A	182.14 B	189.13 AB	0.03
Villus height to crypt depth ratio	1.37	1.55	1.54	0.25
Day 28				
Villus height, μ	617.58	595.48	620.86	0.89
Crypt depth, μ	426.58	374.53	451.41	0.51
Villus height to crypt depth ratio	1.51	1.6	1.44	0.60

[a] A total of 36 pigs were randomly euthanized at day 7 and day 28 of the trial (one pig per replicate and 6 replicates per treatment).
[b] Data were analyzed using ANOVA with 2 factors, where 1 factor is the replicate (considered as blocking factor) and the other is the treatment.

DISCUSSION

Pig Performance

The absence of milk products like whey powder in diets for young pigs did not compromise ADG and FCR of newly-weaned pigs in this study. Although pigs in treatment 3, which fed on diets containing no whey powder, had the lowest ADG in phase 1, it was not significantly low and the same pigs recovered in phase 2 and caught up with pigs in treatment 2, which fed on diets with a moderate level of whey powder. The observations of Tokach et al. (1989), Lepine et al. (1991), Mahan (1992) and Mahan et al. (2004) that increasing the level of dried whey in diets of newly-weaned pigs improved weight gain and feed conversion were not observed in this study. Mahan (1992) concluded that the lactose component of dried whey was the primary component that improved postweaning performance of pigs but Nessmith et al. (1997) observed inconsistent response of newly-weaned pigs fed increasing levels of lactose.

The absence of spray-dried plasma protein was also not a limiting factor in the growth and FCR performance of newly-weaned pigs in this study. Pigs in treatment 1, which were the only ones fed a diet containing spray-dried plasma protein, did not grow significantly faster nor had better FCR than pigs fed the other dietary treatments. The improved growth and feed conversion observed by Hansen et al. (1993) and Kats et al. (1994) when spray-dried plasma protein was added to newly-weaned pig diets were not observed in this study.

Total feed intake of pigs in treatment 3 was significantly lowest but only in phase 1. This is probably due to the significantly lower survival rate of pigs in treatment 3 and may not be related to the dietary treatments. Total feed intake per pen was recorded in this trial and was not converted to average feed intake per pig. Feed intake per pen would be reduced when mortality is high in that pen. During phase 2, however, no significant difference in total feed intake was observed among treatments, at which time, no significant difference in survival rate was likewise observed. Furthermore, most of the mortality among pigs on treatment 3 was mainly due to systemic infections, like Pneumonia, and were not due to starvation and diarrhea, which is not in any

way related to feed. The insignificant difference in scouring incidence among treatments at day 7 is proof of this. This study was done in a commercial farrow-to-finish farm which cannot be considered disease-free. The significantly higher fecal score of pigs in treatment 3 taken at day 7 could be bacterial in nature. The insignificant difference in fecal scores taken later at days 14 and 21 of phase 1 is a sign that pigs in the same treatment have already recovered from any infection. All dietary treatments in this study contained no in-feed antibiotic medication in all dietary treatments except for 1 kg of Zinc Oxide per ton.

Dunsford in 1989 demonstrated the detrimental effects of feeding a high concentration of soybean meal in post-weaning diets but this same effect was not observed in this study. Although soybean meal inclusion in the experimental diets used in this study was not as high as what Dunford (1989) used, no negative effects on ADG and FCR was observed when soybean meal inclusion in treatment 1 was increased by almost 40% in treatments 2 and 3. The higher level of soybean meal could not also be related to the significantly lower feed intake and survival rate, and significantly higher fecal score in treatment 3 during day 7 of phase 1 because both treatments 2 and 3 have identical levels of soybean meal. The supplementation of dietary treatments 2 and 3 with a solid state fermentation (SSF) enzyme complex has probably improved the digestibility of these diets by reducing the anti-nutritive effects of soybean meal. This same conclusion was made by Park et al. in 2004 when he and his co-workers observed that the addition of a SSF phytase complex to low P, barley-soybean meal based diets improved energy and nitrogen digestibility by growing pigs. Furthermore, the improved ADG of pigs in treatment 3 during phase 2 supports the observation made by Friesen et al. (1993) that the newly-weaned pig will eventualy develop tolerance to the antigenistic effects of soy protein when fed a high-nutrient-density diet containing more than 20% soybean meal.

The yeast protein included in dietary treatments 2 and 3 appears to be effective in replacing spray-dried plasma protein which was included in dietary treatment 1. This observation supports the conclusions made in earlier studies using the same yeast protein (Mahan & Tibbetts, 2000; Maribo & Spring, 2003; Carlson et al., 2005; Halbrook et al., 2004; Maxwell et al., 2004).

The low level of whey powder in dietary treatment 2 and its absence in dietary treatment 3 also appears to be effectively

replaced by the inclusion of yeast protein in both diets. The significant level of functional nutrients found in yeast protein, as well as its high amino acid digestibility could be promoting growth in the young pig in the same way the protein fraction of whey powder was observed by Lepine et al. (1991) to be stimulating growth in newly-weaned pigs. The phytase, amylase, cellulase, xylanase, and β-glucanase present in the SSF enzyme complex supplemented to treatments 2 and 3 may have also released energy from corresponding substrates found in the diets to compensate for the apparent decrease in energy attributed to the decreasing lactose level. This is possible, according to Rutz and Rigolin (2008), who cited several trials in broilers and at least two trials in swine showing the ability of SSF enzyme complex to release energy as much as 200kcal/kg metabolizable energy in the feed. The growth and feed conversion performance of pigs in this study therefore shows that the combination of yeast protein and SSF enzyme complex is an effective replacement to whey powder in diets for newly-weaned pigs. However, this trial could not identify which particular functional nutrient in yeast protein, and which enzyme activity present in the SSF enzyme complex were responsible for replacing the nutritional contributions of whey powder in diets of newly-weaned pigs.

Intestinal Morphology

The immediate morphological responses in the pig's small intestine brought about by low feed intake immediately after weaning as described by Cera et al. (1988) could not be demonstrated in this study due to the lack of intestinal samples from pigs immediately after weaning. However, the generally shorter VH in the duodenum of pigs at day 7 compared to that of pigs at day 28 is enough to show that the same period of intestinal atrophy characterized by the shortening of the villi has occurred among pigs in this study and supports the observation of other studies done previously (Cera et al., 1988; Dunsford et al., 1989; Marion et al., 2002; Carlson et al., 2005).

The insignificant difference observed in VH of duodenal samples from among the three treatments at both 7 and 28 days suggests that no difference in digestive capacity existed among pigs fed the three dietary treatments. This is further proof that the significantly higher fecal score at day 7 in treatment 3 is not

nutritional in nature and was not brought about by poor nutrient digestion and absorption. This is contrary to the findings of Dunsford et al. (1989) who observed that high levels of soybean meal in the diet of newly-weaned pigs resulted in shorter VH suggesting lower digestive capacity. The presence of the SSF enzyme complex in dietary treatments 2 and 3 could again be responsible for minimizing the detrimental effects (on villi) of the higher soybean meal inclusion in these diets. This could be the same reason for the insignificant difference in ADG and FCR performance observed among treatments.

Increased CD is indicative of less mature enterocytes on the villus, which would be expected to have lower digestive capacity. Dunsford et al. (1989) observed this among pigs fed high levels of soybean meal post-weaning. This study also found a significant difference in duodenal CD among treatments at day 7, but had opposite findings from that of Dunsford et al. (1989). Treatment 1 was found to have significantly greater CD than treatment 2, and while not significant, treatment 1 also had greater CD than treatment 3. This is indicative of better digestive capacity in the duodenum of pigs in treatments 2 and 3. Not only is the detrimental effect (on villi) of higher soybean level in treatments 2 and 3 missing, but also that something common in treatments 2 and 3 (but not in treatment 1) is fueling villus crypt hyperplasia and promoting villus re-growth. Although no significant difference was observed on day 7 for duodenal VH:CD ratio among all the treatments ($P = 0.25$), it is interesting to note that VH:CD ratios in treatments 2 and 3 are almost identical and higher than in treatment 1. A higher VH:CD ratio suggests crypt hyperplasia and increasing villous length which are signs of recovery from atrophy (Pluske et al., 1997). The higher duodenal VH:CD ratio in all treatments and the absence of observed significant differences for total duodenal morphology on day 28 is a sign that by that time all the pigs in this study have recovered from intestinal atrophy brought about by weaning.

Several factors could be contributing to duodenal regeneration after weaning. Glutamine, an abundant free amino acid in the plasma of animals (Wu et al., 1994) and an essential precursor for the synthesis of proteins, purine and pyrimidine nucleotides and amino sugars (Krebs, 1980) was reported to be a major fuel for pig enterocytes (Wu et al., 1995), as well as an essential nutrient for the proliferation of intestinal intraepithelial lymphocytes (Wu,

1996). However, a study by Wu et al. (1996) showed that glutamine supplementation to post-weaning diets did not affect duodenal villus height nor crypt depth at 7 and 14 days post-weaning.

Another factor that could contribute to duodenal growth is the presence of nucleotides. Nucleotides are the building blocks for nucleic acids (i.e., DNA and RNA). Nucleotide requirement is therefore increased among rapidly dividing cells and tissues where increased DNA replication and RNA synthesis occur (Mateo & Stein, 2004). Dietary nucleotide supplementation is therefore associated with enhanced growth and maturation of intestinal epithelial cells. A previous study by Mateo et al. (2005) however, did not show that a diet supplemented with nucleotides in amounts similar to that found in sow's milk could increase duodenal villus height and decrease duodenal crypt depth at day 14 and 28 post-weaning. The study by Carlson et al. (2004) also did not see significant differences in doudenal crypt depths in nucleotide-containing yeast protein supplemented post-weaning diets.

This study contradicts both studies of Mateo et al. (2005) and Carlson et al. (2005) in the aspect of intestinal morphology by showing that nucleotide-containing yeast protein can decrease duodenal crypt depth and promote duodenal regeneration. This study demonstrates that nucleotides can promote regeneration of intestinal epithelial cells in the duodenum thereby improving digestive and absorptive capacity in the proximal small intestine of pigs.

Marion et al. (2002) reported clear evidence that changes in villous height after weaning are largely dependent on the amount of energy intake. This is supported by previous studies made by Kelly et al. (1991) and Pluske et al. (1996) that showed that low feed intake immediately post-weaning was a cause of villous atrophy. Energy released from substrates in SSF enzyme complex supplemented dietary treatments 2 and 3 could have been a source of digestible energy that fueled duodenal regeneration.

IMPLICATIONS

Pig performance in this study demostrated that the combination of yeast protein and SSF enzyme complex succesfully replaced plasma protein and whey powder as digestible protein sources for

newly-weaned pigs, contrary to previous studies. Furthermore, higher levels of soybean meal in post-weaning diets in this study did not limit growth rate and feed conversion efficiency when fed in combination with yeast protein and SSF enzyme complex. This opens up opportunities for cost savings when designing diets for newly-weaned pigs.

The duodenum is the major site for intestinal digestion and absorption. Demonstrating the positive effects of nucleotide-containing yeast protein and SSF enzyme complex in improving the morphology of the proximal intestine of pigs reveals the possibility of directly intervening in the morphological response of the newly-weaned pig's intestine to low feed intake and stress. This can reduce the costly effects of post-weaning lag so common in commercial pig farms worldwide.

REFERENCES

Carlson, M.S., Veum, T.L., & Turk, J.R. (2005). Effects of yeast extract versus animal plasma in weanling pig diets on growth performance and intestinal morphology. J. Swine Health Prod. 13(4), 205-209.

Cera, K.R., Mahan, D.C., Cross, R.F., Reinhart, G.A., & Whitmoyer, R.. (1988). Effect of age, weaning, and post-weaning diet on small intestinal growth and jejunal morphology in young swine. J. Anim. Sci. 66, 574-584.

Cromwell, G.L., Allee, G.L., & Mahan, D.C. (2008). Assessment of lactose level in the mid- to late-nursery phase on performance of weanling pigs. J. Anim. Sci. 86, 127-133.

Dunsford, B.R., Knabe, D.A., & Haensly, W.E. (1989). Effect of dietary soybean meal on the microscopic anatomy of the small intestine in the early-weaned pig. J. Anim. Sci. 67, 1855-1863.

Food and Agriculture Organization of the United Nations (FAO) (2004). Protein sources for the animal feed industry. Proceed. Expert Consultation and Workshop, 142.

Friesen, K.G., Goodband, R.D., Nelssen, J.L., Blecha, F., Reddy, D.N., Reddy, P.G., & Kats, L.J. (1993). The effect of pre- and postweaning exposure to soybean meal on growth performance and on the immune response in the early-weaned pig. J. Anim. Sci. 71, 2089-2098.

Halbrook, A., Maxwell, C.V., Davis, M.E., Johnson, Z.B., Brown, D.C., Dvorak, R., & Musser, R. (n.d.). Efficacy of a vegetable-based peptide product as a replacement for plasma protein in nursery pig diets. (Abstract) J. Anim. Sci., 82, Suppl 1. M92.

Hansen, J.A., Nelssen, J. L., Goodband, R.D., & Weeden, T.L. (1993). Evaluation of animal protein supplements in diets of early-weaned pigs. J. Anim. Sci. 71, 1853-1862.

Himmelberg, L.V., Peo, Jr., E.R., Lewis, A.J., & Crenshaw, J.D. (1985). Weaning weight response of pigs to simple and complex diets. J. Anim. Sci. 61, 18-26.

Kats, L.J., Nelssen, J.L., Tokach, M.D., Goodband, R.D., Hansen, J.A., & LAURIN, J.L. (1994). The effect of spray-dried porcine plasma on growth performance in the early-weaned pig. J. Anim. Sci. 72, 2075-2081.

Kelly, D., Smyth, J. A., & McCracken, K.J. (1991). Digestive development of the early-weaned pig. 2. Effect of level of food intake on digestive enzyme activity during the immediate post-weaing period. Br. J. Nutr. 65, 181-188.

Krebs, H. (1980). Glutamine metabolism in the animal body. In J. Mora & E. Palacios, Eds. Glutamine: Metabolism, enzymology, and regulation, 319-329. New York: Academic Press.

Lepine, A.J., Mahan, D.C., & Chung, Y.K. (1991). Growth performance of weanling pigs fed corn-soybean meal diets with or without dried whey at various L-lysine HCL levels. J. Anim. Sci. 69, 2026-2032.

Mahan, D.C. (1992). Efficacy of dried whey and its lactalbumin and lactose components at two dietary lysine levels on postweaning pig performance and nitrogen balance. J. Anim. Sci. 70, 2182-2187.

Mahan, D.C., Fastinger, N.D. & Peters, J.C. (2004). Effects of diet complexity and dietary lactose levels during three starter phases on postweaning pig performance. J. Anim. Sci. 82, 2790-2797.

Maribo, & Spring, P. (2003). Yeast extract as a protein source for weanling piglets. 9th Symposium on vitamins and additives in nutrition of man and animals, Jena/Thurinigia, Germany.

Marion, J., Biernat, M., Thomas, F., Savary, G., Le Breton, Y., Zabielski, R., Le Huerou-Luron, I., & Le Dividich, J. (2002). Small intestine growth and morphometry in piglets weaned at 7 days of age. Effects of level of energy intake. Reprod. Nutr. Dev. 42, 339-354.

Mateo, C.D., & Stein, H.H. (2004). Nucleotides and young animal health: Can we enhance intestinal tract development and immune function? In Lyons, T. P., & J. K. Jacques, Eds., Nutritional biotechnology in the feed and food industries, 159-168. Proc. Alltech's 20th Annual Symp., Lexington, KY.

Mateo, C.D., D.N. PETERS, R.I. DAVE, A. ROSA, C. PEDERSEN, and H.H. STEIN. 2005. Effects of dietary nucleotides on intestinal microbial activity of newly weaned pigs. Page 51 in Nutritional Biotechnology in the Feed Industry. Proc. Alltech's 21st Annual Symp., Lexington, Kentucky, USA,

May 22-25, 2005, Suppl. 1 (Abstr.)

Mavromichalis, I. (2006, October). How much lactose for piglet diets? Pig International, 23-24.

Maxwell, C.V., Davis, M.E., BROWN, D.C., DVORAK, R., Musser, R. & Johnson, Z.B. (2004). Efficacy of NuPro in nursery diets. Univ. of Ark. Anim. Sci. Dep. Rep. 166-169.

Nessmith, Jr., W.B., Nelssen, J.L., Tokach, M.D., Goodband, R.D., Bergstrom, J.R., Drits, S.S., & Richert, B.T. (1997). Evaluation of the interrelationships among lactose and protein sources in diets for segregated early-weaned pigs. J. Anim. Sci. 75, 3214-3221.

Pluske, J.R., Hampson, D.J., & Williams, I.H. (n.d.). Factors influencing the structure and function of the small intestine in the weaned pig: A review. Livest. Prod. Sci 51, 215-236.

Pluske, J.R., Williams, I.H., & Aherne, F.X. (1996). Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim. Sci 62, 131-144.

Robinson, R.K. (1986). Modern Dairy Technology. Vol. 1. Advances in milk processing. New York: Elsevier Applied Science.

Rutz, F. & Rigolin, P. (2008, April). The quest for improved fiber utilisation: Use of solid state fermentation can help create a positive bottom line for tomorrow's livestock industry. Feed International, 16-19.

Tokach, M.D., Nelssen, J.L., & Allee, G.L. (1989). Effect of protein and (or) carbohydrate fractions of dried whey on performance and nutrient digestibility of early weaned pigs. J. Anim. Sci. 67, 1307-1312.

Wu, G. (1996). Effects of concanavalin A and phorbol myristate acetate on glutamine metabolism and proliferation of porcine intraepithelial lymphocytes. Comp. Biochem. Physiol. 114A, 363-368.

Wu, G., Borbolla, A.G., & Knabe, D.A. (1994). The uptake of glutamine and release of arginine, citrulline and proline by the small intestine of developing pigs. J. Nutr. 124, 2437-2444.

Wu, G., Knabe, D.A., Yan, W., & Flynn, N.E. (1995). Glutamine and glucose metabolism in enterocytes of the neonatal pig. Am. J. Physiol. 268, R334-R342.

Wu, G., Meier, S.A., & Knabe, D.A. (1996). Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. J. Nutr. 126, 2578-2584.