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Cytokine mRNA Expression in the Small Intestine of Weanling Pigs Fed Diets Supplemented with Specialized Protein or Peptide Sources

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ABSTRACT : Cytokines play a central role in the mucosal immune response and are involved in regulation of nutrient absorption, metabolism and animal growth. This study investigated the effect of diet manipulation with specialized protein or peptide sources on expression of cytokine (IL-1, IL-6, IL-10, and TNF-α) mRNA abundance in different intestinal regions and at different ages post-weaning in piglets. A total of 48 (17 days of age, 6.16±0.34 kg BW) weanling pigs were fed either a corn-soy/whey protein basal diet, the basal diet supplemented with spray-dried plasma protein (SDPP), or the basal diet supplemented with Peptiva®, a hydrolyzed marine plant protein. A fourth treatment group was fed the SDPP diet, but the feed intake level was limited (SDPP-LF). Pigs were killed at 3 and 10 d, and intestinal cytokine mRNA was measured by real-time PCR using the relative quantification method. The SDPP-LF group exhibited an increased TNF-α mRNA abundance compared with the *ad libitum* SDPP group ($p<0.05$). The TNF-α and IL-10 mRNA abundance increased from the proximal to distal part of the intestine, and the mRNA abundance was greater ($p<0.01$) in the distal intestine as compared with the proximal and middle intestine. The cytokines IL-1-β, IL-10 and TNF-α mRNA abundance also increased from d3 to d10 postweaning ($p<0.01$). In summary, restricted feeding increased the TNF-α mRNA abundance in the small intestine, however neither SDPP nor peptide supplementation affected cytokine mRNA expression. Abundance of mRNA for most cytokines examined in this study increased with age post-weaning, suggesting that during 10 d after weaning the mucosal immune system is still under development. (**Key Words :** Cytokine, Peptide, Pig, Real-time PCR, Spray-dried Plasma Protein)

INTRODUCTION

Cytokines are a group of factors that exert a profound influence on the functional state of immune cell populations. Results from previous research suggest that a major component of the growth inhibition observed in immunologically challenged animals is mediated by pro-inflammatory cytokines (Johnson, 1997). The detrimental effect of immunological stress on the nutritional status of animals is of considerable economic importance, because nutrients are diverted away from growth to support immune-related processes (Spurlock, 1997). Interleukin 1 (IL-1, Ling et al., 1994), IL-6, and tumor necrosis factor (TNF, Lang et al., 1992) are known to have functions beyond immune processes and have profound metabolic effects. Both TNF and IL-1 were linked to depressed protein synthesis and increased degradation rates in rodents as a model of immune challenge (Zamir et al., 1992). For comprehensive reviews of cytokine impacts on animal growth see Johnson (1997) and Spurlock (1997).

Intestinal epithelial cells are capable of expressing a number of cytokines, thereby possessing the ability to affect the local immune response (Wittig and Zeitz, 2003). Since the development of pro- and prebiotics, manipulation of luminal immune function has proven to be a potential way of maintaining gut health and may serve as therapy for intestinal-related diseases. Dietary nucleotides (Gil, 2000) and oligosaccharide (Yin et al., 2008) enhanced gene expression of IL-1, IL-6 and IL-8 in fetal small intestine explants. The improved weight gain of pigs fed a high quality protein, spray-dried plasma protein (SDPP) is partially due to improved feed intake and reduced TNF-α, IL-1-β, and IL-6 expression in the liver, spleen, and pituitary (Touchette et al., 2002).

Certain dietary proteins can be antigenic and elicit an immune reaction in the gut of young animals. For example Li et al. (1990) demonstrated antigenic hypersensitivity to dietary soy protein in early-weaned pigs, while soy protein that reduces antigenic response is beneficial compared to regular soybean meal (Kim et al., 2007; Yang et al, 2007).

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Table 1. Ingredient composition and calculated analysis of experimental diets[a]

Item	Control	6% SDPP	0.5% Peptiva®
Ingredients			
Ground corn	37.50	37.50	37.50
Soybean meal, dehulled	15.00	15.00	15.00
Dried whey	20.00	20.00	20.00
Lactose	5.00	5.00	5.00
Fish meal, menhaden	2.50	2.50	2.50
SDPP (AP920)[b]	-	6.00	-
Peptiva®[c]	-	-	0.50
Soy protein concentrate	12.18	4.87	11.44
Soybean oil	2.53	1.60	2.58
Dicalcium phosphate	1.47	1.24	1.49
Calcium carbonate	0.50	0.67	0.52
Salt	0.25	0.25	0.25
Synthetic lysine	0.177	0.044	0.20
D-L-methionine	0.14	0.076	0.14
Vitamin premix[d]	0.25	0.25	0.25
Trace mineral premix[e]	0.15	0.15	0.15
Corn starch	2.35	4.85	2.48
Totals	100.00	100.00	100.00
Calculated analysis[f]			
Crude protein (%)	22.00	22.00	22.00
Total lysine (%)	1.50	1.50	1.50
Methionine+cystine (%)	0.86	0.86	0.86
Calcium (%)	0.90	0.90	0.90
Total phosphorus (%)	0.80	0.80	0.80
ME (Mcal/kg)	3.415	3.415	3.415

[a] Diets were fed for the whole experimental period (d 0 to d 10 d after weaning).

[b] SDPP = Spray-dried plasma protein, a high quality protein containing high immunoglobulin content, AP920®, provided by American Protein Corp, Ames, IA.

[c] Peptiva®: a hydrolyzed marine plant product, provided by Vitech Biochem, San Fernando, CA.

[d] Vitamin premix provided per kg of diet: 12,400 IU of vitamin A as acetate, 2,067 IU of stabilized vitamin D3, 82 IU of vitamin E as DL-tocopheryl acetate, 5.5 mg of vitamin K as menadione sodium bisulfate, 11 mg of riboflavin, 36 mg of D-Pantothenic acid as calcium pantothenate, 63 mg of niacin as nicotinamide, 0.28 mg of D-biotin, 1.65 mg folic acid, 2.07 mg pyridine, 1.25 mg thiamine, and 0.05 mg of vitamin B12.

[e] Trace mineral premix provided per kg of final diet: 20 mg of copper as copper sulfate, 150 mg of iron as ferrous sulfate, 40 mg of manganese as manganese sulfate, 150 mg of zinc as zinc sulfate, 0.5 mg of iodine as calcium iodine, 0.3 mg of selenium as sodium selenite.

[f] Values were calculated using NRC (1998) data, and data supplied by the manufacturer of SDPP and Peptiva®.

Conversely SDPP can promote growth and maintain gut health in weanling pigs, most likely by supplying dietary immunoglobulins (Pierce et al., 2005). Only limited data are available on the impact of specialty protein supplements on cytokine expression in the small intestine. Considering the morphological and functional differences in different regions of the small intestine, the objectives of this study were to investigate the effects of functional protein or peptide supplementation and feed limitation on spatial and temporal expression of cytokine mRNA in the small intestine of weanling pigs. The functional protein source used was a commercially available source of SDPP (AP-920®, American Proteins Corp., Ames, IA). The peptide supplement was a commercially available hydrolyzed marine plant protein (Peptiva®, Vitech Biochem, San Fernando, CA) which has shown potential to alter immunity and improve performance in weanling pigs at 0.3% of supplementation (Wang et al., 2000).

MATERIALS AND METHODS

Animals and diets

All animal procedures were approved by the Virginia Tech Animal Care and Use Committee. A total of 48 crossbred (Yorkshire×Landrace×NPD Hamline®) male weanling pigs (17±2 d, 6.16±0.34 kg BW) were randomly distributed among four dietary treatments based on BW at the day of weaning. Intestinal tissue was collected at two sampling times (3 d and 10 d after weaning). Four complex nursery diets (Table 1) were formulated to meet the NRC 1998 requirements and to have similar nutrient and metabolizable energy concentrations for all treatments. The four dietary treatments were: i) a corn-soy-whey protein based basal diet; ii) basal diet supplemented with 6% SDPP (AP920®, American Proteins Corp., Ames, IA), iii) basal diet supplemented with 0.5% Peptiva®, and iv) a SDPP limited fed group (SDPP-LF). The latter group of pigs was fed the 6% SDPP diet, but the feed intake was restricted to the same quantity consumed by the control group on the previous day. Pigs were individually placed in double-decker nursery pens (0.6×0.9 m) with plastic coated, expanded metal floors and a baffle between decks. Each pen was equipped with a nipple waterer and a stainless steel feeder. Pens were located in two environmentally controlled rooms with continuous lighting and a temperature of 27°C with recommended air ventilation rates (Murphy et al., 1990). Twenty-four pigs were killed 3 d after weaning and 10 d after weaning. Feed consumption for all pigs was recorded daily and BW was obtained at the beginning and the end of the trial.

Sampling and tissue preparation

Pigs were euthanized with an overdose injection of 10% sodium pentobarbital before sampling. The entire small intestine was then removed and dissected free of mesenteric attachments and placed on an ice-cold stainless steel surface. The intestinal lumen was opened lengthwise following the mesentery line and washed with ice-cold PBS (NaH_2PO_4, 1.47 mM; Na_2HPO_4, 8.09 mM, NaCl, 0.145 M) and divided into three sections of equal lengths (proximal, middle and distal sections). The epithelia were scraped from the surface

Table 2. Real-time PCR primer sequences and amplification efficiencies

Target	Acc. no.		Primer sequences[a]	Size	Slope	Efficiency[b]	R^2
GAPDH[c]	AF017079	Forward	TGATGCCCCCATGTTTGTG	77	-3.19	106%	0.99
		Reverse	CAGGAGGCATTGCTGATGATC				
TNF-α	X57321	Forward	CTGGCCCCTTGAGCATCA	69	-3.02	110%	0.98
		Reverse	ACGGGCTTATCTGAGGTTTGAG				
IL-1	X52731	Forward	ATCGACCATCTCTCTCTGAATCAGA	73	-2.94	118%	0.99
		Reverse	ATGCCGTCCCCAGGAAGT				
IL-6	M80258	Forward	CGCAGCCTTGAGGATTTCC	80	-3.20	105%	0.99
		Reverse	GGACGGCATCAATCTCAGGT				
IL-10	L20001	Forward	TCAGGAGCCAACTGCAGCTT	80	-3.33	100%	0.97
		Reverse	AGTGGGAGGCAGTCAGGACA				

[a] All the primers were designed using Primer Express software.

[b] The efficiency was measured by plotting the log10 of the input template versus the ΔC_t; a slope of -3.33 demonstrates that the efficiency is 100%.

[c] GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as a housekeeping gene.

using glass slides; approximately 0.25 g of sample was wrapped in aluminum foil and immediately frozen in liquid nitrogen and stored at -80°C for later analysis.

Reverse transcription and real-time PCR SYBR green detection

The mRNA abundance of two pro-inflammatory factors, interleukin-1-β (IL-1-β) and tumor necrosis factor alpha (TNF-α), and two anti-inflammatory factors (IL-6 and IL-10) was measured by real-time PCR. All primers were designed based on GenBank gene sequences using Primer Express software (Applied Biosystems, Foster City, CA) and synthesized by MWG-Biotech (High Point, NC). The primer sequences and gene accession numbers are listed in Table 2. Standard housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a calibrator.

Total RNA was extracted using Tri-Reagent (Molecular Research Center, Inc, Cincinnati, OH) following the procedures provided by the company with minor modifications (RNA precipitation was at -20°C for 2 h instead of at room temperature for 10 min). Absorbance at 260 and 280 nm (Model U-2000, Hitachi Instrument Inc, Tokyo, Japan) was used to quantify and determine the quality of total RNA. Integrity of total RNA was verified by denaturing agarose gel electrophoresis. Reverse transcription (RT) was performed using TaqMan Reverse Transcription reagents (Roche Molecular System Inc., Branchburg, NJ). The reverse transcription mixture consisted of 1× TaqMan RT buffer, 5.5 mM manganese chloride, 500 μM each dNTP, 2.5 μM random hexamers, 0.4 U/μl RNAse inhibitor, 1.25 U/μl MultiScribe Reverse Transcriptase, and 200 ng total RNA in a 10 μl reaction. The RT was performed using a thermal cycler (PCT^{TM}-200, MJ Research, Ramsey, MN) with the following temperature profile: 10 min at 25°C, 60 min at 45°C, 5 min at 95°C.

Real time PCR was performed using an Applied Biosystems 7300 Real-Time PCR system (Foster City, CA) using SYBR Green PCR master mix (Applied Biosystems) and the relative quantification method. Each reaction contained: 12.5 μl 2X SYBR Green PCR master mix, 0.5 μl of both forward and reverse primers (5 μM), 400 ng cDNA, and water to a final volume of 25 μl. Amplification was performed in MicroAmp® optical 96-well reaction plates. The PCR parameters were as follows: 50°C for 2 min, 95°C for 10 min to activate AmpliTaq Gold DNA polymerase followed by 40 cycles of 15 s at 95°C, 1 min at 60°C. Following the final cycle, a melting curve analysis (95°C for 15 s, 60°C for 30 s, and 95°C for 15 s) was performed to examine the specificity in each reaction well for all tested samples in a given run. A single melting peak for each reaction confirmed the presence of a single PCR product.

Comparative ΔΔCt method of real-time PCR data analysis

The Comparative $\Delta\Delta C_t$ method is based on the principle that the difference in threshold cycles (ΔC_t) between the target gene and a housekeeping gene is proportional to the relative expression level of the target gene (Livak and Schmittgen, 2001; Pfaffl, 2001). To ensure the accuracy of the obtained data, the reaction efficiencies of the two assays should be similar and close to 100%. The amplification efficiency was determined by serial dilutions (4 ng/μl, 0.8 ng/μl, 0.4 ng/μl, 0.08 ng/μl, and 0.008 ng/μl) of positive control cDNA and amplification by real time PCR using gene specific primers. The amplification efficiency was calculated by plotting the log of the template concentration versus the ΔCt of each reaction curve. According to the equation of $E = 10^{(-1/slope)}-1$, a slope of -3.33 represents 100% efficiency of amplification. The amplification efficiency for each gene is shown in Table 2 and all are within the acceptable range of the manufacturer's recommendation. Linear regression coefficients (R^2) of greater than 0.99 indicated acceptable standard curves. For each RNA sample tested, triplicate C_t values were obtained and averaged. The threshold was adjusted for each plate in a

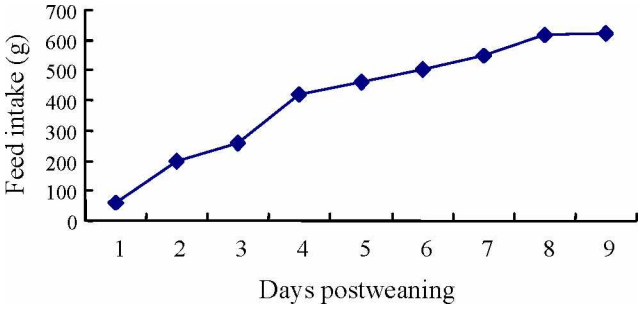

Figure 1. Daily feed intake curve of the control group. Data were obtained from 12 individually penned pigs.

PCR run and was close to 0.20.

The fold change of each target gene was normalized to the housekeeping gene, and expressed relative to the proximal segment of pigs fed the control diet at d 10 of age (calibrator) using the following formula:

$$\text{Fold change} = 2^{-\Delta\Delta Ct}$$

Where, $\Delta\Delta C_t = (C_{t\ target}-C_{t\ housekeeping})_{treatment}-(C_{t\ target}-C_{t\ housekeeping})_{calibrator}$.

Using this analysis, if the level of the target gene was not affected by diet or age, the values of the mean fold change for each diet and time point should be very close to 1 (since $2^0 = 1$).

Statistical analysis

Before the statistical analysis was performed, all $2^{-\Delta\Delta Ct}$ data were logarithmically transformed (base 10) to obtain normally distributed data then analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC). For the mRNA abundance, the model included the main effects of dietary protein sources, intestinal segments, age, and all appropriate two-way interactions. The three-way interaction was removed from the model once it was determined to be non-significant ($p>0.05$). The changes in mRNA expression were tested using ANOVA followed by the Tukey test for multiple comparisons with unequal sample sizes.

RESULTS

Growth performance

Daily feed intake increased dramatically during the first four days post-weaning, and the increased trend was diminished around 8 to 9 days post-weaning (Figure 1). Pigs fed SDPP-LF had lower feed intake compared to SDPP *ad libitum* group (440 vs. 367 g/d, $p<0.10$), and also numerically lower intake than the control group due to the rapid increase in daily feed intake post-weaning. No differences in ADG and G:F were observed among treatments at 10 d post-weaning ($p>0.05$, Table 3).

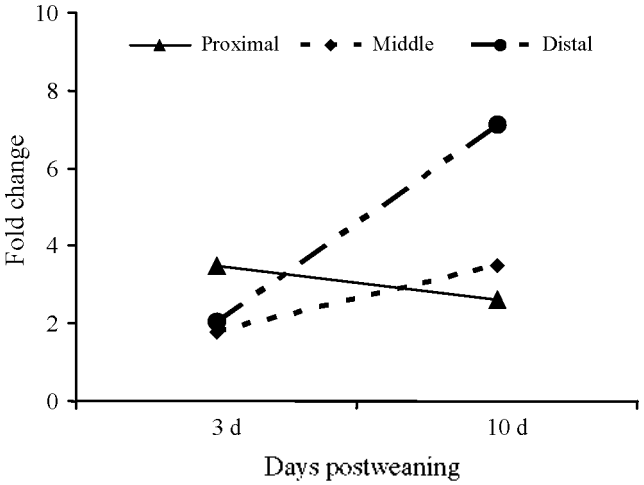

Figure 2. Time×segment interaction of IL-1β mRNA expression along the small intestine ($p = 0.01$). Total of 48 pigs were used.

Cytokines

A two-way interaction of age x intestinal segment was observed for IL-1-β ($p<0.01$, Table 4). The IL-1-β mRNA abundance increased two- to three fold in the middle intestine and distal intestine from 3 to 10 d after weaning, while the mRNA abundance decreased slightly in the proximal intestine (Figure 2). The IL-1-β mRNA abundance increased with age and the levels on d 10 were almost twice as much as that on d 3 postweaning ($p<0.05$).

The pigs fed the SDPP diet had a lower relative abundance of TNF-α than the control and Peptiva® groups ($p<0.10$ by contrast) and lower abundance than the SDPP-LF group ($p<0.05$; Table 4). The abundance of TNF-α mRNA for the SDPP-LF and SDPP *ad libitum* group was 1.88 and 0.82, respectively. The TNF-α mRNA abundance increased from the proximal to the distal small intestine ($p<0.005$). The mRNA abundance in the proximal intestine,

Table 3. Effects of dietary supplementation with spray-dried plasma protein or Peptiva® on growth performance of early-weanling pigs[a]

Item	Experimental dietary treatments				SEM
	Control	6% SDPP	0.5% Peptiva®	SDPP-limit fed[b]	
ADG (g)	282	309	273	270	30
ADFI (g)	401	440	439	367	26
G:F	0.69	0.70	0.62	0.74	0.05

[a] A total of 48 weanling pigs were used. There were six observations per treatment. Pigs were weighed at d 10 post-weaning.

[b] The SDPP-LF group pigs were fed a 6% spray-dried plasma protein diet, and feed intake level was restricted.

Table 4. Dietary protein and developmental regulation of cytokine mRNA abundance in the small intestine of young pigs [a, b, c]

Item		IL-1-β	TNF-α	IL-6	IL-10
Dietary treatments [d]	Control	4.02	1.07 [x, y]	2.09	3.46
	SDPP	1.61	0.82 [x]	4.12	4.06
	Peptiva®	3.46	1.47 [x, y]	2.23	6.53
	SDPP-LF	3.08	1.88 [y]	3.65	7.93
	p-value	0.29	0.10	0.44	0.46
Segments	Proximal	3.02	0.96 [x]	2.29	2.82 [x]
	Middle	2.47	1.14 [x]	2.41	4.97 [x]
	Distal	3.83	1.78 [y]	4.41	10.00 [y]
	p-value	0.16	0.005	0.16	0.0002
Sampling time	3 d	2.33 [x]	0.93 [x]	2.58	3.26 [x]
	10 d	4.01 [y]	1.67 [y]	3.25	8.28 [y]
	p-value	0.04	0.02	0.52	0.03
Confidence limits [e]	Lower	2.43	0.98	2.06	3.72
	Upper	4.05	1.61	4.15	8.17
	---------- Interaction p-value, p<0.05 significant ----------				
Interaction [f]	D*S	0.43	0.46	0.46	0.38
	D*T	0.30	0.53	0.27	0.64
	S*T	0.01	0.21	0.62	0.62

[a] Six pigs from each diet were sampled on d 3 and d 10 postweaning.

[b] All the data were acquired using real-time PCR. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was the housekeeping gene, and proximal segments of the pigs that were fed the control diet and killed on 3 d postweaning served as the calibrator sample.

[c] The data were transformed to log10 to obtain a normal distribution prior to statistical analysis. The fold change of gene expression is log 10 of each mean presented in the table.

[d] Control = Soy protein diet, SDPP = 6% spray-dried plasma protein diet, Peptiva = 0.5% Peptiva diet, SDPP-LF = Pigs were fed the SDPP diet, but feed intake level was restricted.

[e] 95% upper and lower confidence limits.

[f] Two-way interactions, D = Diet; S = Intestinal segment; T = Sampling time.

[x, y] Within each main designed factors, means without same superscripts differ (p<0.05).

middle intestine, and distal intestine was 0.96, 1.14, and 1.78, respectively. Similar to IL-1-β, TNF-α mRNA abundance increased (p<0.05) from d3 to d10.

No dietary effect was observed for IL-10 mRNA abundance. The IL-10 mRNA abundance increased dramatically from the proximal intestine and middle intestine to the distal intestine (p<0.05). The mRNA abundance in the distal intestine was 3.5 times higher than in the proximal intestine, and 2.0 times higher than in the middle intestine. IL-10 mRNA abundance was more than two-fold greater at 10 d than 3 d postweaning (3.26 vs. 8.28, p<0.05). Interleukin 6 mRNA abundance was not influenced by diet, intestinal segment or age.

DISCUSSION

Our previous study (Zhao et al., 2007) suggested that improved growth performance with SDPP supplementation was environment-dependent and was accentuated when pigs were raised under a sub-optimal environment. Thus, it is not unexpected that growth performance was not different in pigs fed diets supplemented with SDPP compared with other treatment groups in this study, given that the study was conducted in an experimental facility where husbandry conditions were more fastidious than at a normal commercial farm. The benefits of SDPP on growth performance are partially due to high immunoglobulin concentrations and their impact on immune function (Pierce et al., 2005). Touchette et al. (2002) showed that 7% SDPP reduced mRNA expression of TNF-α, IL-1-β, and IL-6 expression in the liver, spleen, and pituitary glands in pigs. Both IL-1-β and TNF-α are the principal cytokines that mediate acute inflammation and act on endothelial cells to stimulate inflammation and the coagulation pathway. It is well known that activation of the immune system is usually associated with reduced feed intake and growth (Coffey et al., 1995). Thus reduced levels of the proinflammatory cytokines by SDPP supplementation may as a result provide more nutrients available for growth. In line with Touchette (2002), our result indicated that SDPP at adequate feed intake reduced TNF-α mRNA abundance in the small intestine.

Since the identification of peptide transport in the intestinal epithelial cells (Klang et al., 2005), numerous studies have shown that absorption of digested protein products in the small intestine occurs primarily in the form of small peptides rather than AA (Ganapathy et al., 1994). Theoretically, it is possible that incorporation of small peptides into the diet would be beneficial for animal growth. Wang et al. (2000) indicated that 0.3% Peptiva® improved growth performance and reduced diarrhea in weanling pigs. Wang's data along with field reports suggested a therapeutic

effect of adding Peptiva® at low level (0.3-1.0%) in pigs and chickens. However, the cytokine mRNA responses observed in our study do not support an intestinal anti-inflammatory effect with this level of the supplemental peptide source. Most previous studies are inconsistent regarding benefits of peptides on performance and supplementation levels (Zambonino et al., 1997; Bregendahl et al., 1998; Lindemann et al., 1998). More research is needed to understand the mode of action of peptides on digestion and absorption along with the concept of functional protein supplements.

Due to rapid initiation of feed consumption in the first several days post-weaning, ADFI of the SDPP-LF group was only 80% of the SDPP group and was slightly lower than the control group. Much of the intake limitation happened during the first few days post-weaning. In our study, feed intake was dramatically increased with four days post-weaning and then peaked around day 9. Piglets usually spend several days adjusting to weaning stress and fully recover around 10 days. Pie et al. (2004) reported increased inflammatory cytokine (IL-1β, IL-6, and TNF-α) expression in the small intestine two days post-weaning with a rapid return to pre-weaning levels on 3 to 8 days post-weaning. The authors suggested that increased inflammatory cytokines were indicators of weaning stress. Limited feed intake is one of the most important factors affecting weanling pig performance and intestinal morphology (Li et al., 1990; Kelly et al., 1991). Consuming enough feed usually solves most of the problems that occur during the weaning period. A strong relationship (R = 0.82) between total DM intake and villus height was established in pigs fed cow's milk for 5 d after weaning (Pluske et al., 1996). The increased TNF-α expression, which was threefold higher in the intestine than the *ad libitum* group, may suggest that limit fed pigs were in a higher stress condition due to inadequate supply of nutrients for normal function. In support of this theory, a previous study suggested that insufficient feed intake post-weaning initiates adverse intestinal morphological changes and leaves the intestinal lining more penetrable to luminal antigens (Pluske et al., 1997). On the other hand, Pie et al. (2007) reported that fasting piglets for 48 h postweaning did not influence cytokine mRNA content 4 and 10 d post-weaning in the ileum and colon. This may be due to a difference between fasting for 48 h, which is an acute stress, and limited feeding in our study, which is a chronic stress. The intestinal epithelium is the first barrier to the external environment within the gut lumen. The intestinal mucosal immune system must balance two opposing functions: mounting an immune response to pathogens, while maintaining tolerance to antigens derived from commensal bacteria and food (Lindsay and Hodgson, 2001). In order to carry out both functions, the intestine is morphologically differentiated from the proximal to the distal site. Limited information is available regarding the distribution pattern of cytokines in the small intestine. In this study, the distal intestine had the highest level of cytokine levels while the proximal section had the lowest, which may indicate the presence of more immunogenic microorganisms in the distal part than the proximal part of the small intestine. By using germ-free pigs, Shirkey et al. (2006) reported that relative expression of cytokines interleukin-1β (IL-1β) and IL-6 generally increased distally in the small intestine and that different bacterial species differentially affect intestinal morphology and expression of proinflammatory cytokines. The authors concluded that neonatal bacterial colonization patterns may have long-term effects on intestinal health and development. Impact of bacterial population on host gut health and immune reaction is well known. Bacterial colonization patterns in the gut impact cytokine expression patterns in the intestine, and cytokines could be indicators of bacterial population dynamics.

In summary, adequate feed consumption is critical for growth and intestinal immune function development in weanling pigs. Benefits of SDPP on performance may partially result from regulating pro-inflammatory cytokine, TNF-α, in the small intestine of weanling pigs. However, feed intake seems to play a more critical role than dietary composition, in that pigs limited fed with SDPP could not overcome the stress of reduced feed intake. Most of the cytokines were expressed higher in the distal part of the small intestine and increased from d 3 to d 10 post-weaning. These results suggest that weanling pigs at 20 d of age may not have a mature mucosal immune system and the distal part of the small intestine may play a more important role in gut immunity.

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