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Abstract

This experiment was conducted to evaluate various levels of milk by-products in weaned pig's diet on growth performance, blood profiles, diarrhea incidence, and intestinal morphology of weaned pigs. A total of 160 weaned pigs ([Yorkshire × Landrace] × Duroc), average 5.97 ± 1.53 kg body weight (BW), were allotted to 1 of 4 treatments in 5 replications with 8 pigs per pen by BW. Pigs were fed each treatment diet with various levels of milk by-products (phase 1: 5, 10, 20 or 30%; phase 2: 0, 5, 10 or 15%) for 5 weeks. Linear increase of BW, average daily gain, average daily feed intake, and gain to feed ratio with increasing milk by-products was observed in whole experimental period (linear response, $p < 0.05$). In blood profiles, however, there were no differences in blood urea nitrogen, insulin like growth factor 1, and immunoglobulin A and G among the treatments. In addition, no differences were found in diarrhea incidence and intestinal morphology. In conclusion Increasing milk by-product content in the diets of weaned pigs improved growth performance but high or low level of milk by-product content did not negatively affect blood profiles, diarrhea incidence, and gut health of nursery pigs.

Keywords

Milk by-products, Weaned pigs, Growth performance, Blood profiles, Intestinal morphology

Introduction

The nutritional management for nursery pigs is important for successful swine production [1]. The process of weaning is one of the most stressful events, thereby reducing the growth rate, feed intake, and gastrointestinal health of weaned pigs due to the digestive and immune system dysfunctions [2]. During this period, piglet malnutrition caused by sudden change in the feed offered need to be controlled [2]. Sow's milk is a fluid, highly palatable and digestible and it is replaced with milk by-products (lactose or whey powder) [1, 3].

Lactose is a disaccharide present in milk, and it is the main energy source for weaned pigs. Supplementation of milk by-products in weaned pig's diets helps to improve feed intake, diet palatability, and growth performance of weaned pigs [4, 5, 6]. Moreover, some studies reported that inclusion of lactose in diets for weaned pigs could improve gastrointestinal health (prebiotic effects) [3]. This is because lactose could be fermented by microbiota to produce lactic acid and volatile fatty acid (VFA), resulting in reduced stomach pH and suppression of the growth of pathogenic bacteria [2, 6, 7, 8].

However, the use of milk by-products may be limited due to the increased feed cost rates. The international price trends of dairy products are highly unstable, and prices are relatively expensive compared to cereal grains [1]. Weaned pig feed contains high levels of milk by-products, which increase feed costs and reduce the efficiency of pig production.

It is well known that the activity of lactase, an enzyme in involved in the digestion of milk carbohydrates, is high at birth and then rapidly decreases after 2 weeks [9]. Undigested carbohydrates can move to the hindgut and increase over-fermentation in the hind gut (increase the incidence of diarrhea) [10]. Therefore, finding optimal levels of dietary milk by-products for weaned pigs is important to reduce swine production cost and any detrimental effects on weaned pigs.

Therefore, this study was conducted to investigate the effect of various levels of milk by-products in weaning pig diet on growth performance, blood profile, diarrhea incidence, and intestinal morphology of weaned pigs.

Materials and Methods

Animal management and housing

A total of 160 crossbred ([Landrace x Yorkshire] x Duroc) piglets were used in this experiment. Weaned pigs (averaging 5.79 ± 1.527 kg initial BW) were assigned to 4 treatments based on sex and initial BW with 5 replicates of 8 per pen according to randomized complete block design. The experimental pigs were housed in 1.54 x 1.96m^2 plastic floor. Feed and water were provided ad-libitum through feeder and nipple during whole experimental periods. The diets were formulated to meet or exceed the nutrient requirements of pigs in accordance with the NRC [11]. The ambient temperature in the nursery facility was maintained at 31 °C at the first, and then gradually fallen to 27 °C at the end of the experiment. The BW and feed intake were recorded at 0, 2 and 5 weeks to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F ratio).

Experimental Diets

The experimental diets were provided to weaned pigs following 2 phases (0 to 2 weeks, 3 to 5 weeks). Treatments included: NM [corn, soybean meal-based diet (basal diet) + levels of milk product, phase 1: 5%, phase 2: 0%], LM (basal diet + levels of milk product, phase 1: 10%, phase 2: 5%), %], MM (basal diet + levels of milk product, phase 1: 20%, phase 2: 10, HM (basal diet + levels of milk product, phase 1: 30%, phase 2: 15%). Whey powder and lactose were added to each diet, and all nutrients of experimental diets met or exceeded the nutrient requirement of NRC [11]. Experimental diets contained 20.56%, 18.88% of CP and 1.35%, 1.15% of total lysine during phase 1, phase 2 respectively. The formula and chemical composition of experimental diets were presented in Tables 1 and 2.

Blood sampling and analysis

Blood samples were taken from the jugular vein of 6 pigs per treatment for measuring blood urea nitrogen (BUN), insulin like growth factor 1 (IGF-1), and immunoglobulin A and G (IgA and IgG). After the blood sample was collected in disposable culture tubes, the samples were centrifuged for 15 min by 3,000 rpm at 4°C (Eppendorf centrifuge 5810R, Germany). Sera samples were aspirated by pipette and stored at –20°C until later analysis. Total BUN concentration was analyzed using a blood analyzer (Ciba-Corning model, Express Plus Ciba Corning Diagnostics Co., Medfield, MA, USA.) and IGF-1 concentration was analyzed with hormone analyzer (DPC Immulite 2000, Siemens Healthineers, Germany). Serum IgG and IgA concentrations were analyzed by ELISA assay by the manufacture's protocols (ELISA Starter Accessory Package, Pig IgG ELISA Quantitation Kit, Pig IgA ELISA Quanti-tation Kit; Bethyl, Montgomery, AL, USA). All samples were assayed in duplicates with 1:20,000 (IgG) or 1:10,000 (IgA) fold dilution.

Diarrhea incidence

Observation of diarrhea incidence was conducted every 8:00 am for 35 days. Data was recorded by each pen for 2 phases (phase 1 and phase 2). Score of diarrhea incidence was given into 5 numbers by counting pigs with evidence of watery diarrhea (0=No evidence of watery diarrhea, 1=2 pigs, 2=4 pigs, 3=6 pigs, and 4=8 pigs show the evidence of watery diarrhea in the pen) [12].

Morphology of small intestine

Three pigs from each treatment were selected on days 14 and 35 of the experiment. The measurements of intestinal morphology included villus height, crypt depth, villus:crypt. Small intestine samples were taken (≒ 3 cm in length) at the proximal, middle, and distal ends of the small intestine. These were fixed in neutral buffered formalin and processed by the standard paraffin method. Sections (2~3 cm) were stained with hematoxylin and eosin and examined under a light microscope (Leica DM500 microscope with Leica DFC425; Leica Microsystems Inc., Morrisville, NC, USA).

Chemical analyses

The diets were grounded by a Cyclotec 1093 Sample Mill (Foss Tecator, Hillerod, Denmark) and grounded diets were analyzed. All analyses were performed in duplicate samples and analyses were repeated if results from duplicate samples varied more than 5% from the mean. Experimental diet was analyzed for contents of dry matter by oven drying at 135°C for 2 h (method 930.15; AOAC) [13] and crude ash (method 942.05; AOAC) [13]. Crude fat was hydrolized in HCl solution to release bound fat and then extracted with diethyl ether and petroleum ether (method 954.02; AOAC) [13]. The nitrogen content was analyzed by using the Kjeldahl procedure with Kjeltec (KjeltecTM 2200; Foss Tecator, Sweden) and calculating the CP content (nitrogen×6.25; procedure 981.10; AOAC) [13].

Statistical analysis

The experimental data were analyzed with the general linear model (GLM) procedure of the SAS (SAS Inst. Inc., Cary, NC). The model included diet as the fixed effect and block as the random effect. Orthogonal polynomial contrasts were used to determine linear and quadratic effects by increasing the milk-by products supplementation levels. In phase 1 (0 to 2 weeks), Proc IML was used to generate coefficients for unequally spaced contrasts because treatments spacing was unequal. For data on growth performance analysis, a pen was considered the experimental unit, while individual pig was used as the unit for data on blood profiles, diarrhea incidence, and intestinal morphology. To test hypotheses, $p<0.05$ was considered significant, if pertinent, trends ($0.05<p<0.10$) are also reported.

Results & Discussion

The effects of various levels of milk by-products on growth performance of weaned pigs was presented in Table 3. There were linear increases in BW, ADG, ADFI, and G:F ratio when weaned pigs fed the diets with increased milk by-products during whole experimental period. However, no differences were observed in BW, ADG, ADFI, and G:F ratio among the treatments in the phase 2 (0 to 2 weeks). These results agree with previous study reported by O'Doherty [7]. They tested diets containing with lactose in different levels (0, 18, or 35%) for weaned pigs. In that study, increased ADG and G:F ratio were observed in pigs fed diets with medium and high level of lactose (18% and 35%) compared to pigs fed diet with no lactose level (0%) from day 0 to 38. However, feed intake from day 0 to 17 had no difference among the treatments. Similarly, Pierce et al. [14] suggested that weaned pigs fed the diet supplemented with high lactose (30%) had a higher ADG during days 14 to 21 compared to weaned pigs fed the diet with low lactose (18%), whereas there were no differences in feed intake and feed conversion ratio during days 14 to 21. Recently, Jang et al. [15] showed that increasing the amount of whey permeate (0 to 19%) in weaned pig's diets during days 0 to 11 resulted in a linear increase in BW, ADG, and G:F ratio. The improvement of growth performance of weaned pigs fed the diet containing with milk by-products reported above could be associated with its sweetness, leading to improve diet palatability [10]. In the current study, beneficial effect of milk by-products on growth performance of weaned pigs were observed in mainly in the phase 2 (3 to 5 weeks). We hypothesized that feeding high levels of milk by-products for weaned pigs could increase growth performance in phase 1 (0 to 3 weeks) because endogenous lactase activity could decline after 2 weeks of post weaning period [16].

The effects of various levels of milk by-products on the blood profiles of weaned pigs were presented in Tables 4 and 5. As a result of the analysis, there were no differences in blood profiles such as BUN, IGF-1, IgA, and IgG during the whole experimental period. However, IGF-1 was the lowest in the NM treatment diet compared to LM, MM and HM treatment diets and showed a quadratic tendency at 5 weeks (p=0.082). The concentration of BUN is associated with utilization of amino acids and the ability to retain dietary nitrogen in the body [17]. Serum IgG and IgA are an indicator to determine the chronic infection or inflammation [18]. In the current study, no differences were observed in IgA, and IgG among the treatments. Yu et al. [16] reported that weaned pigs fed the diets with 4 or 6% of lactose had a lower serum urea nitrogen compared to weaned pig fed control diet (0% of lactose). This study showed that supplementing diets with lactose tended to improve growth performance and reduce BUN in weaned pigs. BUN is known to be a potential indicator of nitrogen (especially protein and amino acid) utilization in weaned piglets [16]. These results demonstrate that increased lactose content is associated with increased digestion, absorption, and utilization of nutrients [16]. Zhao et al. [10] reported that increasing milk by-products in the diet of weaned piglets can increase the production of lactic acid and VFAs in the gastrointestinal tract due to lactose fermentation, which can decrease gastric pH and thereby help improve digestion of proteins (increasing pepsin activity or reducing pathogen infection). IGF-1, as a polypeptide growth hormone, plays an important role in growth and differentiation for body tissue, activating both the mitogen activated protein kinase and PI3K signaling pathways which promote tissue growth and maturation. In this study, there was no difference in IGF-1 concentration when weaned pig fed the diet with different levels of milk by-products.

The effects of various levels of milk by-products supplementation on diarrhea incidence of weaned pig was shown in Table 6. In this study, diarrhea incidence had no difference among the treatments. However, MM treatment diet tended to be lower than those in NM, LM and HM treatment diets. It was also numerically highest in the HH treatment. Zhao et al. [10] reported that lactose content that is high compared to the lactase activity of weaned piglets may cause excessive fermentation, which may lead to intestinal osmotic imbalance and cause diarrhea. Additionally, it was recommended that the dose be set to 20%, 15%, and 0 for piglets aged 0 to 7 days, 7 to 14 days, and 14 to 35 days after weaning, respectively. Weaning stress also can cause gastric changes in which the number of E. coli increases, resulting in severe diarrhea [19]. However, many researchers have pointed out that supplementation of fiber sources or moderate of milk by-products could increase the gastrointestinal health of weaned pigs [20, 21, 22]. This is because fiber sources or moderate level of milk by-products (prebiotic effect) could be utilized by bacteria to produce lactic acid and VFA in the hindgut of pigs [2, 6, 7, 8, 14]. In the current study, barley usage was 15% in phase 1 and phase 2 experimental diets. Barley has relatively high soluble fiber source which decreases passage rate, thus reducing diarrhea occurrence [23, 24]. Therefore, diarrhea incidence of weaned pigs was not affected by the diets with various levels of milk by-products because of barley content in all experimental diets.

The effects of various levels of milk by-products supplementation on the villus height and crypt depth of weaned pigs were presented in Table 7. There were no differences in the villus height and crypt depth of the proximal, mid, and distal part of small intestine of weaned pigs fed the diet with various levels of milk by-products. However, villus:crypt ratio showed linear tendency at 5 weeks. Similar results were found from the study conducted by Pierce et al. [14]. They fed the diets with 15% or 33% of lactose to weaned pigs for 6 days. In that study, no differences were observed in villus height, crypt depth and villus:crypt ratio of weaned pigs. The differences of results in previous research works and current study may be due to the variation of BW and age of animals, diet composition, or inclusion levels of milk by-products. Further studies should be conducted to investigate the potential mechanism action of milk by-products fed to weaned pigs.

Conclusion

Beneficial effect of various levels of milk by-products fed to weaned pigs are found in growth performance during whole experimental period. However, there were no differences in blood profiles and gut health of weaned pigs. To our knowledge, inclusion of low level of milk by-products (10 to 5%) in weaned pig’s diet had no negative effects on blood profiles, diarrhea incidence, and gut health compared to pigs fed diet with high level of milk by-products (30 to 15%). However, linear increase in growth performance of weaned pigs was observed in this study. Future studies should evaluate diets with various levels of milk by-products with different sources such as whey permeate or skim milk powder so that optimal inclusion level may be identified for weaned pigs.

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Not applicable.

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Tables and Figures

Table 1. Formula and chemical composition of the experimental diets (phase 1, 0 to 2 weeks)

	Treatments[1]			
Ingredients	**NM**	**LM**	**MM**	**HM**
Corn	41.83	36.72	26.48	16.22
Soybean meal	31.51	31.69	32.10	32.50
Barley	15.00	15.00	15.00	15.00
Whey powder	2.00	4.00	8.00	12.00
Lactose	3.00	6.00	12.00	18.00
Soypeptide	1.37	1.46	1.64	1.81
Soy-oil	1.72	1.61	1.38	1.17
Mono dicalcium phosphate	1.36	1.35	1.31	1.30
Limestone	1.06	1.04	1.01	0.97
L-lysine-HCl, 78%	0.30	0.29	0.24	0.20
DL-methionine, 80%	0.08	0.09	0.09	0.10
L-threonine, 99%	0.07	0.06	0.05	0.04
Vitamin premix[2]	0.10	0.10	0.10	0.10
Mineral premix[3]	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Choline-Cl, 50%	0.10	0.10	0.10	0.10
Zinc oxide	0.10	0.10	0.10	0.10
Sum	100.00	100.00	100.00	100.00
Calculated composition[4]				
ME, kcal/kg	3265.02	3265.00	3265.00	3265.03
Crude protein, % [5]	21.19	21.02	20.43	19.21
Crude fat, % [5]	4.81	5.01	2.82	3.44
Crude ash, % [5]	7.08	5.89	7.19	6.71
Lysine, %[6]	1.35	1.35	1.35	1.35
Methionine, %[6]	0.35	0.35	0.35	0.35
Methionine + Cystine, %[6]	0.76	0.76	0.76	0.76
Calcium, %	0.80	0.80	0.80	0.80
Total phosphorus, %	0.65	0.65	0.65	0.65
Available phosphorus, %	0.40	0.40	0.40	0.40

[1]NM (Almost no milk by-products, 5%/0%), LM (Lower milk by-products, 10%/5%), MM (Middle milk by-products, 20%/10%), HM (High milk by-products, 30%/15%).
[2]Provided the following per kilogram of diet: vitamin A, 8,000IU; vitamin D_3, 1,800IU; vitamin E, 60IU; vitamin K_3, 2mg; vitamin B_1, 2mg; vitamin B_2, 7mg; vitamin B_5, 25mg; vitamin B_3, 27mg; vitamin B_6, 3mg; biotin, 0.20g; folic acid, 1.00mg; vitamin B_{12}, 0.03g.
[3]Provided the following per kilogram of diet: Se, 0.30mg; I, 1.00mg; Mn, 51.60mg; Cu, 26.25mg; Fe, 150.00mg; Zn, 72.00mg; Co, 0.50mg.
[4]Calculated value.
[5]Analyzed value.
[6]Calculated SID value: Lysine, 88.15%; methionine 91.43%; methionine + cystine, 89.47%.

Table 2. Formula and chemical composition of the experimental diets (phase 2, 3 to 5 weeks)

	Treatments[1]			
Ingredients	**NM**	**LM**	**MM**	**HM**
Corn	51.96	46.85	41.70	36.60
Soybean meal	26.22	26.40	26.59	26.79
Barley	15.00	15.00	15.00	15.00
Whey powder	0.00	2.00	4.00	6.00
Lactose	0.00	3.00	6.00	9.00
Soypeptide	2.22	2.31	2.40	2.50
Soy-oil	1.55	1.44	1.34	1.22
Monodicalcium phosphate	1.19	1.17	1.17	1.15
Limestone	0.91	0.90	0.88	0.86
L-lysine-HCl, 78%	0.20	0.18	0.16	0.14
DL-methionine, 80%	0.04	0.04	0.04	0.05
L-threonine, 99%	0.01	0.01	0.02	0.00
Vitamin premix[2]	0.10	0.10	0.10	0.10
Mineral premix[3]	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Choline-Cl, 50%	0.10	0.10	0.10	0.10
Zinc oxide	0.10	0.10	0.10	0.10
Sum	100.00	100.00	100.00	100.00
Calculated composition[4]				
ME, kcal/kg	3265.02	3265.00	3265.03	3265.00
Crude protein, % [5]	19.62	18.86	18.99	19.92
Crude fat, % [5]	1.87	1.71	1.52	1.93
Crude ash, % [5]	5.18	7.97	5.67	6.33
Lysine, %[6]	1.15	1.15	1.15	1.15
Methionine, %[6]	0.30	0.30	0.30	0.30
Methionine + Cystine, %[6]	0.65	0.65	0.65	0.65
Calcium, %	0.70	0.70	0.70	0.70
Total phosphorus, %	0.60	0.60	0.60	0.60
Available phosphorus, %	0.32	0.32	0.32	0.32

[1]NM (Almost no milk by-products, 5%0%), LM (Lower milk by-products, 10%/5%), MM (Middle milk by-products, 20%/10%), HM (High milk by-products, 30%/15%).
[2]Provided the following per kilogram of diet: vitamin A, 8,000IU; vitamin D_3, 1,800IU; vitamin E, 60IU; vitamin K_3, 2mg; vitamin B_1, 2mg; vitamin B_2, 7mg; vitamin B_5, 25mg; vitamin B_3, 27mg; vitamin B_6, 3mg; biotin, 0.20g; folic acid, 1.00mg; vitamin B_{12}, 0.03g.
[3]Provided the following per kilogram of diet: Se, 0.30mg; I, 1.00mg; Mn, 51.60mg; Cu, 26.74mg; Fe, 150.00mg; Zn, 72.00mg; Co, 0.50mg.
[4]Calculated value.
[5]Analyzed value.
[6] Calculated SID value: Lysine, 81.83%; methionine 90.00%; methionine + cystine, 89.23%.

Table 3. Influence of various milk by-products levels in weaned pig diet on growth performance of weaned pigs[1]

Criteria	Treatment[2]				SEM[3]	p-value	
	NM	LM	MM	HM		Linear	Quadratic
BW[4], kg							
Initial	5.97	5.97	5.97	5.97	0.313	0.639	0.543
2 weeks	7.09	7.26	7.42	7.56	0.383	0.237	0.911
5 weeks	10.46	11.53	12.94	12.75	0.653	<0.01	0.294
ADG[5], g/d							
0 to 2 weeks	80	92	103	114	9.222	0.237	0.926
3 to 5 weeks	160	203	263	247	15.432	<0.01	0.142
ADFI[6], g							
0 to 2 weeks	171	187	192	201	6.907	0.161	0.916
3 to 5 weeks	451	517	553	539	22.339	0.031	0.166
G:F[7] ratio							
0 to 2 weeks	0.447	0.486	0.519	0.551	0.035	0.344	0.956
3 to 5 weeks	0.341	0.385	0.479	0.470	0.022	0.016	0.502

[1]A total of 160 crossbred pigs were fed from average initial body weight 5.97 ± 1.527 kg.
[2]NM (Almost no milk by-products, 5%/0%), LM (Lower milk by-products, 10%/5%), MM (Middle milk by-products, 20%/10%), HM (High milk by-products, 30%/15%).
[3]Standard error of mean.
[4, 5, 6, 7]BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

Table 4. Influence of various milk by-products levels in weaned pig diet on BUN and IGF-1 of weaned pigs[1]

Criteria	Treatments[2]				SEM[3]	p-value	
	NM	LM	MM	HM		Linear	Quadratic
BUN[4] (mg/dL)							
2 weeks	16.63	15.01	9.33	13.49	1.158	0.103	0.586
5 weeks	16.20	16.17	13.37	13.27	0.722	0.105	0.983
IGF-1[5] (ng/mL)							
2 weeks	62.03	73.02	80.47	72.16	5.381	0.437	0.434
5 weeks	47.23	69.20	67.03	57.66	5.451	0.951	0.082

[1]Least squares means of 6 observations per treatment.
[2]NM (Almost no milk by-products, 5%/0%), LM (Lower milk by-products, 10%/5%), MM (Middle milk by-products, 20%/10%), HM (High milk by-products, 30%/15%).
[3]Standard error of mean.
[4]BUN: blood urea nitrogen.
[5]IGF-1: insulin like growth factor 1.

Table 5. Influence of various milk by-products levels in weaned pig diet on IgA and IgG of weaned pigs[1]

Criteria	Treatments[2]				SEM[3]	p-value	
	NM	LM	MM	HM		Linear	Quadratic
IgA[4] (mg/mL)							
2 weeks	0.32	0.25	0.27	0.17	0.025	0.127	0.799
5 weeks	0.61	0.89	0.73	0.79	0.084	0.681	0.327
IgG[5] (mg/mL)							
2 weeks	1.87	2.00	1.95	1.78	0.052	0.622	0.220
5 weeks	3.06	3.24	3.36	3.40	0.080	0.109	0.661

[1]Least squares means of 6 observations per treatment.
[2]NM (Almost no milk by-products, 5%/0%), LM (Lower milk by-products, 10%/5%), MM (Middle milk by-products, 20%/10%), HM (High milk by-products, 30%/15%).
[3]Standard error of mean.
[4]IgA: immunoglobulin A.
[5]IgG: immunoglobulin G.

Table 6. Influence of various milk by-products levels in weaned pig diet on diarrhea incidence of weaned pigs[1]

Criteria	Treatment[2]				SEM[3]	p-value	
	NM	LM	MM	HM		Linear	Quadratic
Diarrhea scores[4]							
0 to 2 weeks	1.20	1.32	1.26	1.29	0.055	0.571	0.555
3 to 5 weeks	1.00	0.92	0.84	1.06	0.054	0.773	0.109

[1]A total of 160 crossbred pigs were fed from average initial body weight 5.97 ± 1.527 kg.
[2]NM (Almost no milk by-products, 5%/0%), LM (Lower milk by-products, 10%/5%), MM (Middle milk by-products, 20%/10%), HM (High milk by-products, 30%/15%).
[3]Standard error of mean.
[4]Diarrhea incidence: 0(no occurrence) to 4(all pigs diarrhea); Data were measured by average total diarrhea incidence during each phase.

Table 7. Influence of various milk by-products levels in weaning pig diet on morphology of small intestine diameter in weaning pigs[1]

Criteria	Treatments[2]				SEM[3]	p-value	
	NM	LM	MM	HM		Linear	Quadratic
Weaning phase 1 (2 weeks), μm							
Proximal of small intestine							
Villus height	225.21	220.61	242.93	184.00	18.012	0.699	0.623
Crypt depth	281.38	283.90	280.45	270.84	20.019	0.901	0.905
Villus:Crypt	0.83	0.77	0.87	0.70	0.043	0.649	0.745
Medium of small intestine							
Villus height	252.43	258.28	245.76	261.85	10.810	0.937	0.928
Crypt depth	251.30	237.72	212.67	281.86	19.440	0.864	0.463
Villus:Crypt	1.04	1.10	1.17	1.30	0.068	0.885	0.643
Distal of small intestine							
Villus height	224.96	273.43	256.27	282.67	14.209	0.339	0.708
Crypt depth	229.45	258.29	251.29	259.30	20.197	0.721	0.841
Villus:Crypt	1.07	1.14	1.04	1.08	0.066	0.902	0.842
Weaning phase 2 (5 weeks), μm							
Proximal of small intestine							
Villus height	337.66	292.49	321.31	304.75	8.917	0.432	0.351
Crypt depth	512.10	488.36	441.12	446.20	13.901	0.091	0.910
Villus:Crypt	0.67	0.60	0.73	0.68	0.025	0.452	0.570
Medium of small intestine							
Villus height	310.27	328.73	324.22	317.85	12.365	0.871	0.703
Crypt depth	379.89	389.46	350.52	344.15	10.717	0.201	0.513
Villus:Crypt	0.83	0.85	0.83	0.93	0.035	0.203	0.942
Distal of small intestine							
Villus height	283.34	281.46	307.78	290.05	6.556	0.411	0.876
Crypt depth	375.13	375.93	349.90	338.92	8.704	0.109	0.467
Villus:Crypt	0.75	0.75	0.88	0.87	0.027	0.085	0.651

[1]Least squares means of 3 observations per treatment.
[2]NM (Almost no milk by-products, 5%/0%), LM (Lower milk by-products, 10%/5%), MM (Middle milk by-products, 20%/10%), HM (High milk by-products, 30%/15%).
[3]Standard error of mean.