

Effects of Eco-friendly Multi-enzyme on Diarrhea and Immune Response of weaned Pigs^{*}

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친환경 복합효소제 첨가가 이유자돈의 설사 및 면역반응에 미치는 영향

민예진 · 김준수 · 김신아 · 장기범 · 문다예 · 김병현 · 최지환 · 송민호

This study was designed to investigate the effects of multi-enzyme on diarrhea and immune responses of weaned pigs. A total 36 weaned pigs (5.92 ± 0.48 kg BW; 28 d old) were randomly allotted to 2 dietary treatments (3 pigs/pen, 6 replicates/treatment) in a randomized complete block design. The dietary treatments were a typical diet based on corn and soybean meal (CON) and CON with 0.1% multi-enzyme (Multi; mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase). Pigs were fed their respective diets for 6 wk. Frequency of diarrhea, levels of packed cell volume (PCV), white blood cells (WBC), immunoglobulins, cortisol, tumor necrosis factor- α (TNF- α), transforming growth factor- β (TGF- β), and C-reactive protein (CRP) were measured. Multi group tended to decrease ($p < 0.1$) diarrhea frequency than CON group during 2 wk after weaning. Lower values of PCV on d 3 ($p < 0.05$) and d 7 ($p < 0.1$) were found in Multi group compared with CON group. There were no significant differences on WBC number and immunoglobulin (Ig) M and A between Multi and CON groups. However, Multi group tended to increase ($p < 0.1$) Ig G on d 7 than CON group. Moreover, Multi group showed modulated immune responses, indicated by decreased levels of cortisol ($p < 0.05$) on d 7 and 14, TNF- α on d 3 ($p < 0.05$) and d 7 ($p < 0.10$), TGF- β on d 2 ($p < 0.05$) and d 7 ($p < 0.10$), and CRP ($p < 0.10$) on d 3 and 7 after weaning compared with CON group. Consequently, inclusion of multi-enzyme in

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diets for weaned pigs improved gut health and modulated immune responses of weaned pigs.

Key words : *diarrhea, immune responses, multi-enzyme, weaned pigs*

I . Introduction

Most of feed ingredients contain non-starch polysaccharides (NSP) that is considered as anti-nutritional factor (Choct, 1997). Soybean meal and corn are the most widely used feed ingredients in the world (FAO, 2004). Soybean meal includes approximately 22.7% NSP, such as β -galactomannan and α -galactosides and corn also contains about 10% NSP, mainly arabinoxylan and β -galactomannan (CVB, 1998). The NSP in diets for non-ruminant animals may have anti-nutritional effects due to its viscosity, and they may affect modification of gut physiology, and interaction with gut microflora (Choct, 1997). Pigs cannot break down NSP using endogenous enzymes secreted from their digestive tract. Thus, a strategy for facilitating digestion of NSP has been required. Supplementation of exogenous enzymes has been considered one of solutions to break NSP and increase absorption and utilization of nutrients (Barrera et al., 2004; Olukosi et al., 2007; Cho and Kim., 2013).

Weaning is the most important event for nursery pigs because tremendous changes in gastrointestinal physiology, microbiology, and immunology can cause extreme stress to weaning pigs (Kim et al., 2017). Especially at the early period of post-weaning, weaning stress may have detrimental effects on gut health and immune responses, resulting in post-weaning diarrhea and severe inflammation and leading to the death in the worst case (Park et al., 2016; Song et al., 2015). Moreover, digestive tract and immune system of weaned pigs is not fully developed and thus they are more sensitive to NSP and/or diseases (Park et al., 2016; Song et al., 2015). The pig production industry has been paying attention to the management of nursery pigs after weaning (Park et al., 2016; Song et al., 2015). We hypothesized that supplementation of dietary enzyme mixture (multi-enzyme; a mixture of carbohydrases and proteases) facilitate the hydroxylation of NSP in a typical diet based on corn and soybean meal, thereby improve gut health and immune status of weaned pigs. Therefore, the present study was designed to examine the effects of multi-enzyme on diarrhea and immune responses of weaned pigs.

II . Materials and Methods

The Chungnam National University Institutional Animal Care and Use approved all experimental protocols used in this study (approval code: CNU-00611).

1. Experimental design, animals, and diets

A total of 36 weaned pigs [Duroc × (Landrace × Yorkshire); 5.92 ± 0.48 kg of average BW; 28 d old] were used in this study. Pigs were moved to nursery pens equipped with a feeder and waterer in an environmentally controlled room and randomly assigned to 2 dietary treatments with 3 pigs per pen and 6 replicated pens per treatment in a randomized complete block design. The dietary treatments were a normal diet based on corn and soybean meal (CON) and CON added with 0.1% multi-enzyme based on the feed (as-fed basis) [Multi; mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase] which were commercially purchased. Pigs were fed respective dietary treatment for 6 wk using a 2-phase feeding program with declining diet complexity and each phase lasted 3 wk. The diets did not include spray-dried plasma, antibiotics, or zinc oxide to avoid their antibacterial or physiological effects (Table 1). Pigs were allowed free access to diets and water at all times.

Table 1. Composition of experimental diets for weaned pigs (as-fed basis)

Items	Phase 1 ¹⁾	Phase 2 ²⁾
Ingredient (%)		
Corn	31.57	51.56
Soybean meal, 44%	18.00	26.56
Soy protein concentrate	16.96	8.00
Dried whey	24.00	10.00
Lactose	4.00	-
Soybean oil	3.00	1.35
Limestone	1.00	1.00
Monocalcium phosphate	0.90	0.90
Vitamin premix ³⁾	0.20	0.20
Mineral premix ⁴⁾	0.20	0.20

Items	Phase 1 ¹⁾	Phase 2 ²⁾
L-lysine-HCl	0.08	0.17
DL-methionine	0.09	0.07
Total	100	100
Calculated energy and nutrient content		
ME, Mcal/kg	3.53	3.42
Crude protein, %	24.49	22.51
Calcium, %	0.81	0.73
Phosphorus, %	0.69	0.63
Lysine, %	1.54	1.41

¹⁾ Phase 1 = wk 1 to 3 (21 days).

²⁾ Phase 2 = wk 4 to 6 (21 days).

³⁾ Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D₃, 2,500 IU; vitamin E, 30 IU; vitamin K₃, 3 mg; D-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline, 400 mg; vitamin B₁₂, 12 µg.

⁴⁾ Provided per kilogram of diet: Fe, 90 mg from iron sulfate; Cu, 8.8 mg from copper sulfate; Zn, 100 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.35 mg from potassium iodide; Se, 0.30 mg from sodium selenite.

2. Sample collection and analyses

1) Frequency of diarrhea

The behavior of pigs was recorded to figure out clinical evidence of diarrhea. A scoring system by the method of Pierce et al. (2005) was used to show not only the incidence but also the levels of diarrhea. The diarrhea score for each pen were recorded daily in the morning from d 1 to d 14 by three specialists who did not have any information about the experiment. Fresh fecal matter was scored using the following range: 1 = normal hard feces; 2 = slightly soft feces; 3 = soft, partially formed feces; 4 = loose, semi-liquid feces; and 5 = watery, mucous-like feces (1 - 3: feces, 4 - 5: diarrhea). The frequency of diarrhea was expressed as the number of pen days with diarrhea score greater than 4 in total recorded days.

2) Blood collection

Blood samples for physiological parameters measurement were collected from the jugular vein of 2 randomly selected pigs from each pen using EDTA tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) on d 1, 2, 3, 7, and 14 after weaning. Serum samples were

collected by centrifugation at $3,000 \times g$ at 4°C for 15 min and stored -80°C until subsequent analyses.

3) Determination of white blood cell and packed cell volume

Whole blood samples were analyzed for total white blood cell (WBC) and packed cell volume (PCV) using a multiparameter automated hematology analyzer calibrated for porcine blood (scil Vet abc hematology analyzer, scil animal care company, F-67120 Altorf, France).

4) Analyses of cortisol, cytokines, and C-reactive protein

Cortisol, cytokines, and C-reactive protein (CRP) were measured in the serum collected on d 1, 2, 3, 7 and 14 after weaning using porcine ELISA kits following the manufacturer's procedure (cortisol [Cusabio Biotech Co., Ltd., Wuhan, P.R. China]; tumor necrosis factor- α [TNF- α ; Genorise Scientific, Inc., Berwyn, PA]; transforming growth factor- β 1 [TGF- β 1; Genorise Scientific, Inc., Berwyn, PA]; CRP [Genorise Scientific, Inc., Berwyn, PA]). All samples were analyzed in duplicate. A standard curve was included in each assay plate for cortisol, cytokines, and CRP. Results of ELISA analyses were measured using a microplate reader at 450 nm (Epoch microplate spectrophotometer, BioTek instruments Inc., Winooski, VT). The intra-assay coefficients of variation for cortisol, TNF- α , TGF- β 1, and CRP were 8, 6, 6, and 6%, respectively. The inter-assay coefficients of variation for cortisol, TNF- α , TGF- β 1, and CRP were 10, 8, 11, and 9%, respectively.

5) Quantitation of immunoglobulins

Immunoglobulins were also measured in the serum on d 7 after weaning using porcine ELISA kits following the manufacturer's procedure [Immunoglobulin G, M, and A (Ig G, Ig M, and Ig A, BlueGene Scientific Co., Ltd., Shanghai, China)]. All samples were analyzed in duplicate. A standard curve was included in each assay plate for immunoglobulins. Results of ELISA analyses were measured using a microplate at 450 nm (Epoch microplate spectrophotometer, BioTek instruments Inc., Winooski, VT). The intra-assay coefficients of variation for Ig G, Ig M, and Ig A were 4.4%. The inter-assay coefficients of variation for Ig G, Ig M, and Ig A were 6.6%.

6) Statistical analyses

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA) in a randomized complete block design. The pen was used as the experimental unit for PCV,

WBC count, cortisol, $\text{TNF-}\alpha$, $\text{TGF-}\beta$, CRP, and immunoglobulin content. The chi-square test was used for the frequency of diarrhea. Statistical significance and tendency were considered at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

III. Results and Discussion

Multi group tended to decrease ($p < 0.1$) frequency of diarrhea than CON group (Fig. 1). Similar with the result of diarrhea frequency, PCV of weaned pigs fed diets with multi-enzyme decreased ($p < 0.05$) on day 3 and had tendency to decrease on d 7 compared with those fed control diets (Fig. 2). PCV is used an indicator of diarrhea or dehydration, as a higher level of PCV means that severe dehydration has progressed due to diarrhea (Pare et al., 1993; Kang et al., 2017). There were no effects of multi-enzyme supplementation on number of WBC (Fig. 3) and contents of serum immunoglobulin M, and A (Table 2). However, Multi group showed the increased tendency ($p < 0.1$) of IgG than CON group on d 7 after weaning. Multi group reduced ($p < 0.05$) cortisol level on d 7 and 14 compared with CON group (Fig. 4). The serum cortisol is usually used an indicator of stress and cortisol level of weaned pigs is significantly elevated at the weaning (Moesser et al., 2007). Level of $\text{TNF-}\alpha$ on d 3 ($p < 0.05$) and d 7 ($p < 0.1$) was

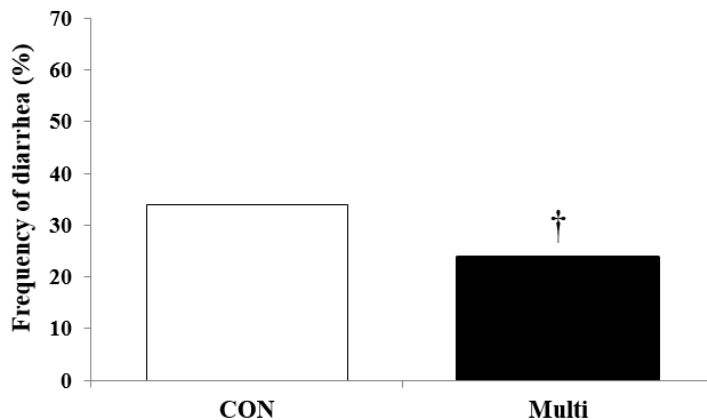


Fig. 1. Frequency of diarrhea in weaned pigs for the first 2 weeks after weaning.

CON = control diet included corn and soybean meal, Multi = CON with 0.1% multi-enzyme (mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase). Frequency of diarrhea (%) = (number of pen days with diarrhea score greater than 4 / total number of recorded days) \times 100. Data was analyzed by the χ^2 test. Multi-enzyme supplementation tended to decrease ($p = 0.08$) the frequency of diarrhea than CON.

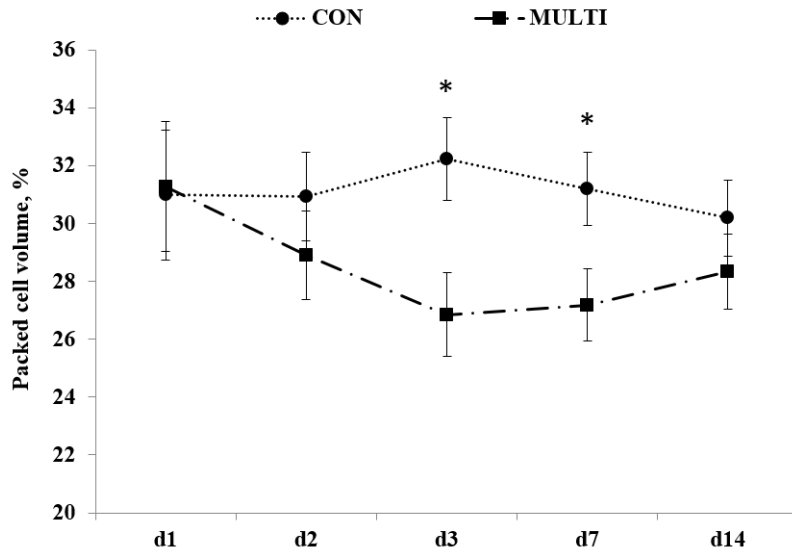


Fig. 2. Packed cell volume of weaned pigs fed dietary treatments.

Values are means \pm SEM. CON = control diet included corn and soybean meal, Multi = CON with 0.1% multi-enzyme (mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase). Multi enzyme supplementation decreased ($p < 0.05$) the packed cell volume on d 3 and 7 than control diet.

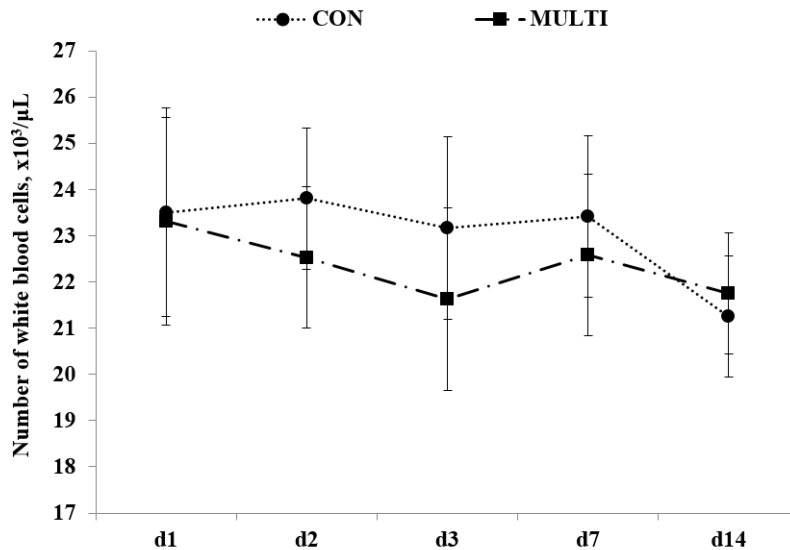


Fig. 3. Number of white blood cell of weaned pigs fed dietary treatments.

Values are means \pm SEM. CON = control diet included corn and soybean meal, Multi = CON with 0.1% multi-enzyme (mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase). No statistical differences were observed between CON and Multi.

influenced by supplementation of multi-enzyme in diets for weaned pigs (Fig. 5). Supplementation of multi-enzyme had effects on another immune-related cytokine level, TGF- β , and CRP (Fig. 6 and 7). Multi group showed lower levels of TGF- β on d 2 ($p < 0.05$) and d 7 ($p < 0.1$) and also showed decreased level ($p < 0.1$) of CRP on d 3 and 7 compared with CON group.

Table 2. Immunoglobulin of weaned pigs fed dietary treatments

Items	CON ¹⁾	Multi ²⁾	SEM ³⁾	p-value
Ig G, mg/mL	16.27	26.65	3.678	0.063
Ig M, mg/mL	1.226	1.229	0.004	0.679
Ig A, mg/mL	0.127	0.130	0.001	0.173

¹⁾ CON = control diet based on corn and soybean meal.

²⁾ Multi = CON with 0.1% multi-enzyme (mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase).

³⁾ SEM = standard error of mean.

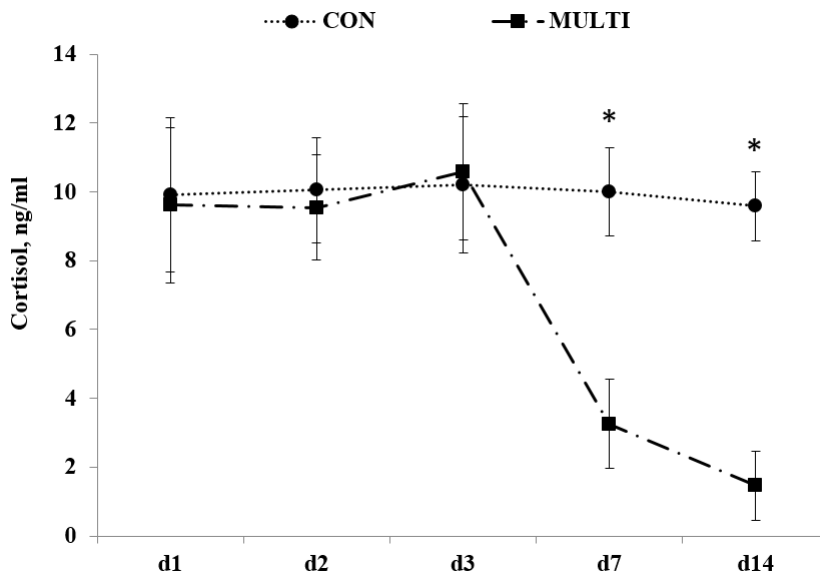


Fig. 4. Cortisol of weaned pigs fed dietary treatments.

Values are means \pm SEM. CON = control diet included corn and soybean meal, Multi = CON with 0.1% multi-enzyme (mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase). Multi enzyme supplementation decreased ($p < 0.05$) the cortisol on d 7 and 14.

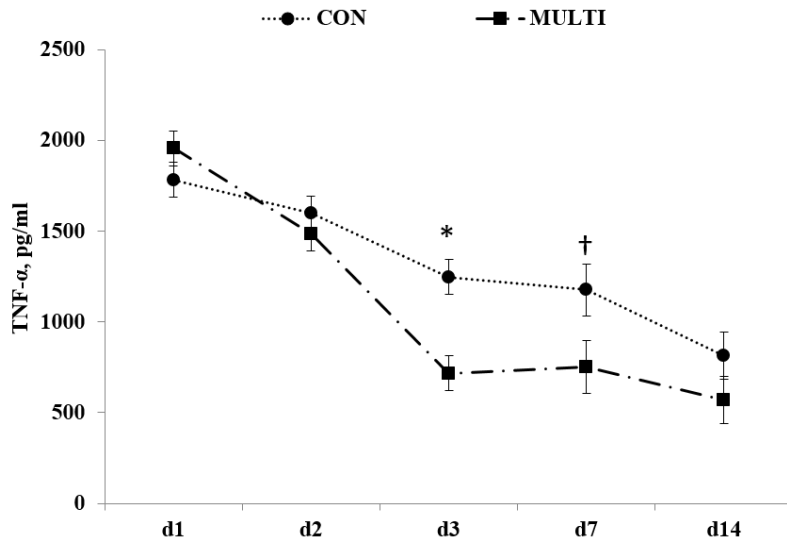


Fig. 5. Tumor necrosis factors- α of weaned pigs fed dietary treatments.

Values are means \pm SEM. CON = control diet included corn and soybean meal, Multi = CON with 0.1% multi enzyme (mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase). Multi enzyme supplementation decreased ($p < 0.05$) the TNF- α on d 3 and tended to decrease ($p < 0.10$) TNF- α on d 7.

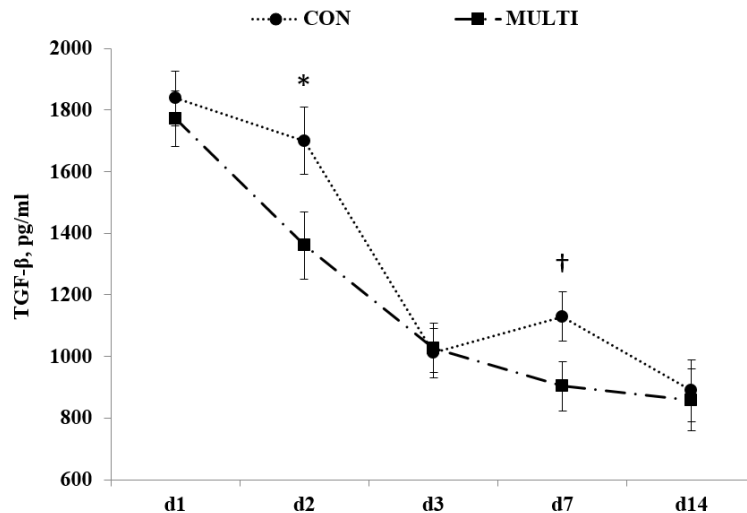


Fig. 6. Transforming growth factors- β of weaned pigs fed dietary treatments.

Values are means \pm SEM. CON = control diet included corn and soybean meal, Multi = CON with 0.1% multi enzyme (mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase). Multi enzyme supplementation decreased ($p < 0.05$) the TGF- β on d 2 and tended to decrease ($p < 0.10$) on d 7.

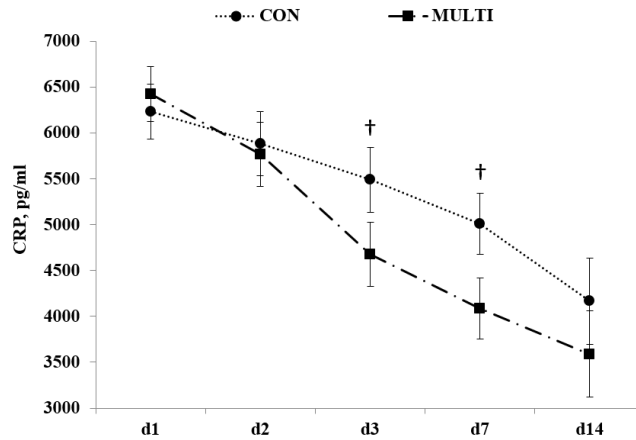


Fig. 7. C-reactive proteins of weaned pigs fed dietary treatments.

Values are means \pm SEM. CON = control diet included corn and soybean meal, Multi = CON with 0.1% multi enzyme (mixture of β -mannanase, xylanase, α -amylase, protease, β -glucanase, and pectinase). Multi enzyme supplementation tended ($p < 0.10$) to decrease the CRP on d 3 and 7.

Previous research reported that NSP has detrimental effects on gut health and immune system due to its viscosity, physiological and morphological effects on digestive tract, and interaction with the microflora of the gut (Choct, 1997; Kang et al., 2017). In the present study, supplementation of multi-enzyme, a mixture of carbohydrases and proteases, showed improvement of gut health and modulation of immune responses, indicated by decreased diarrhea frequency and lower levels of cortisol, immune-related cytokines, and CRP. It may be attributed to hydrolysis of NSP in corn and soybean meal, main ingredients of experiment diet, by the action of multi-enzyme. There were some literatures that investigate effects of multi-enzyme supplementation on productive performance and nutrient digestibility (Barrera et al., 2004; Olukosi et al., 2007; Cho and Kim, 2013) but it is difficult to find research on positive effects of multi-enzyme on gut health and immune system. Thus, a lot of studies are required to establish beneficial effects of multi-enzyme supplementation on gut health and immune system of weaned pigs. The present study indicated that supplementation of multi-enzyme including various carbohydrases and proteases improved gut health and modulated immune responses of weaned pigs.

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