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Effects of Eco-friendly Multi-enzyme on Growth Performance, Intestinal Morphology, and Nutrient Digestibility of weaned Pigs

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친환경 복합효소제 첨가가 이유자돈의 성장, 장내 형태학, 영양소 소화율에 미치는 영향

김성기 · 조명우 · 김준수 · 장기범 · 김신아 · 문다예 · 김병현 · 김영화 · 박준철 · 최지환 · 송민호

This experiment was conducted to investigate the effects of multi-enzyme on growth performance, intestinal morphology, and nutrient digestibility 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. Measurements were growth performance, morphology of ileum, apparent ileal digestibility and apparent total tract digestibility of

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dry matter, crude protein, and energy of weaned pigs. There were no significant differences on growth performance during overall experimental period. No differences were found for the morphology of ileum and nutrient digestibility between CON and Multi groups. Therefore, the results in the current study indicated that multi-enzyme supplementation in diets had no effects on growth performance, intestinal morphology, and nutrient digestibility of weaned pigs.

Key words : *growth performance, intestinal morphology, multi-enzyme, nutrient digestibility, weaned pigs*

Ⅰ. Introduction

Supplementation of exogenous enzymes in swine diets has been attracted much attention in recent years to increase nutrient digestibility and utilization of non-starch polysaccharides (NSP) (Ayoade et al., 2012; Kang et al., 2017; Kim et al., 2017). Non-starch polysaccharides cannot be digested by endogenous digestive enzymes of pigs. The high NSP in diets decrease utilization of nutrients and thus decrease the growth performance of pigs and chickens. Soybean meal is a main ingredient as a protein source in diets for livestock in the world (FAO, 2004). However, it includes not only abundant amounts of proteins but also plenty of carbohydrates that are mainly composed of NSP and oligosaccharides (Choct, 1997). Soybean meal is composed of about 22.7% NSP such as β-galactomannan and α-galactosides and corn, another main ingredient in livestock diets, also contains 10% NSP, mainly arabinoxylan and β-galactomannan (CVB, 1998).

Weaned pigs have immature digestive tracts and cannot produce enough endogenous digestive enzyme compared with adult pigs (Park et al., 2016; Song et al., 2015). Especially, when the nursery pigs face to weaning, they would be subjected to extreme stress which affects gastrointestinal physiology, microbiology, and immunology (Hampson, 1986; Pluske et al., 1997; Brooks et al., 2001). Thus, weaned pigs have difficulty to digest corn and soybean meal based diets that contain high amount of NSP by their endogenous enzymes. Most of studies focused on the effects of exogenous enzymes when they were supplemented in diets containing high fiber ingredients, such as rice, barley, wheat, and palm kernel expellers, while there few studies were performed on typical corn and soybean meal based diets with exogenous enzymes (Shim et al., 2003; Fan et al., 2009). Therefore, the purpose of this study was to evaluate the effects of multi-enzyme supplementation in a corn and soybean meal based diets on growth performance, intestinal morphology, and nutrition digestibility of weaning 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 experiment. Pigs were moved to nursery pens equipped with a feeder and waterer in an environmentally controlled room and randomly assigned to 2 dietary treatments (3 pigs/pen, 6 replicated pens/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] which were commercially purchased. Pigs were fed respective dietary treatments for 6 wk using a 2-phase feeding program with declining diet complexity and each phase was 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. For measurements of intestinal morphology, apparent total tract digestibility (ATTD), and apparent ileal digestibility (AID), 12 pigs were randomly selected (2 pigs from each pen).

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

Items	Phase 1[1)]	Phase 2[2)]
Mineral premix[4)]	0.20	0.20
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 D_3, 2,500 IU; vitamin E, 30 IU; vitamin K_3, 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) Growth performance

The pigs were individually weighed at the start and 42 of the experiment and the average body weight gain (ADG) was calculated. Amounts of feed supplied per pen were recorded and the rest of feed was weighed at the end of experiment. Average daily feed intake (ADFI) was calculated the difference between the amount of supplied and residual feed. The gain-to-feed ratio (G:F) was the ratio between ADG and ADFI.

2) Intestinal morphology

After the 6 wk experimental period, the 12 pigs selected as previously mentioned were euthanized using CO_2 after injection of zoletil. Tissue samples from the small intestine were directly collected. Segments of ileum (about 5 cm in length) were taken and flushed with distilled water to take out any excess blood and digesta. Then, the tissues were immersed in a 6% formaldehyde phosphate buffer and kept at 4℃. The fixed intestinal tissues were embedded in paraffin, sectioned at a 5 μm thickness, and stained with hematoxylin and eosin. The slides were imaged using a digital slide scanner (NanoZoomer Digital Pathology System; Hamamatsu Co., Bridgewater, NJ). All measurements were conducted in the associated slide-viewing software (NDP. view; Hamamatsu Co.). The intestinal morphological measurements included villus height, width, and area, crypt depth, and goblet cell number.

3) Apparent total tract digestibility

For the measurement of ATTD, chrome oxide, an indigestible marker, was added at 0.25% of the respective dietary treatments. The 12 pigs selected as previously mentioned were fed each dietary treatment with 0.25% chromic oxide between d 36 and 42 after weaning. Fecal samples were collected for 3 days post the 4-d adjustment period. Fecal samples were immediately stored at -20℃. Fecal samples were dried in a forced-air drying oven at 60°C and ground through a cyclone mill (Foss Tecator Sycltec 1093, HillerØd, Denmark) before analysis. Fecal samples were analyzed for dry matter (method 930.15; AOAC, 2000), nitrogen (method 988.05; AOAC, 2000), and gross energy using a bomb calorimeter (Parr 1281 Bomb Calorimeter, Parr Instrument Co., Moline, IL, USA).

4) Apparent ileal digestibility

After the 6 wk experimental period, the 12 pigs selected as previously mentioned were euthanized using CO_2 after injection of zoletil. Tissue samples from the small intestine were directly collected. Digesta samples were immediately stored at -20℃. Digesta samples were dried in a forced-air drying oven at 60°C and ground through a cyclone mill (Foss Tecator Sycltec 1093, HillerØd, Denmark) before analysis. Digesta samples were analyzed for dry matter (method 930.15; AOAC, 2000), nitrogen (method 988.05; AOAC, 2000), and gross energy using a bomb calorimeter (Parr 1281 Bomb Calorimeter, Parr Instrument Co., Moline, IL, USA).

5) Statistical analysis

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 the growth performance, intestinal morphology, and nutrient digestibility. Statistical significance and tendency were considered at $p<0.05$ and $0.05 \leq p<0.10$, respectively.

Ⅲ. Results and Discussion

No difference was found for ADG, ADFI, and G:F ratio between treatments during the overall experimental period (Table 2). There were also no significant differences on intestinal morphology including villus height, crypt depth, and ratio of villus height to crypt depth in ileum between treatments (Table 3). In terms of nutrient digestibility, no significant differences were observed (Fig. 1 and 2) for both apparent total tract digestibility and apparent ileal digestibility of DM, CP, and energy between treatments.

Table 2. Growth performance of weaned pigs fed dietary treatments during overall experimental period

Items	CON[1)]	Multi[2)]	SEM[3)]	p-value
ADG, g/d	451	525	25	0.105
ADFI, g/d	789	811	23	0.495
G:F, g/g	0.571	0.645	0.030	0.136

[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.

Table 3. Morphology of ileum of weaning pigs fed dietary treatments

Items	CON[1)]	Multi[2)]	SEM[3)]	p-value
Villus Height, μm	348	384	17.79	0.612
Villus Width, μm	177	171	10.59	0.902
Crypt depth, μm	174	172	15.80	0.937
Villus:crypt, μm:μm	2.16	2.28	0.20	0.926
Villus area, μm^2	60,482	60,092	3,514	0.901
Goblet cell	10.09	14.25	2.10	0.315

[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.

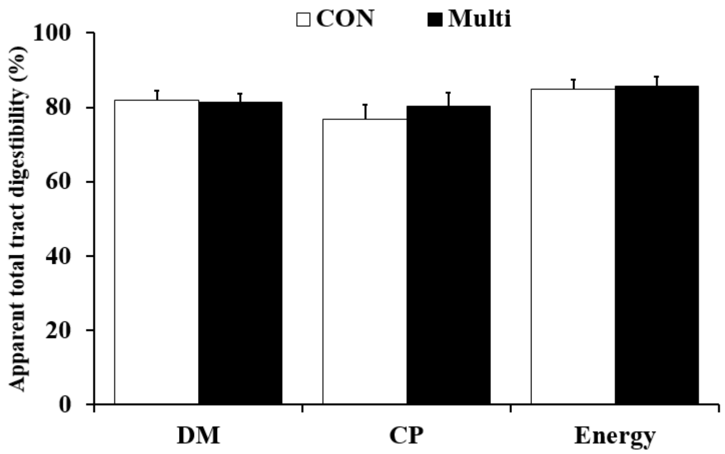

Fig. 1. Apparent total tract digestibility of weaned pigs fed dietary treatments.

Values are means ± SEM. CON = control diet based on 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.

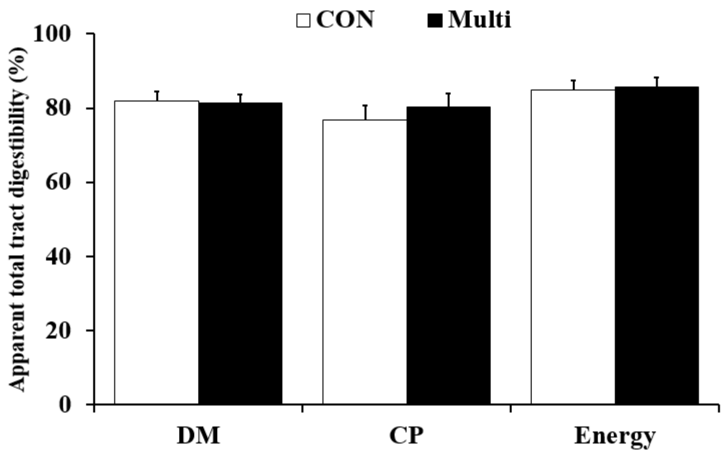

Fig. 2. Apparent ileal digestibility of weaned pigs fed dietary treatments.

Values are means ± SEM. CON = control diet based on 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.

The present experiment did not observe any positive influence of multi-enzyme composed of β-mannanase, xylanase, α-amylase, protease, β-glucanase, and pectinase on growth performance, gut morphology, and nutrient digestibility compared with CON. Some published papers also showed consistent results with this research (Officer, 1995; Barrera et al., 2004; Olukosi et al., 2007). This variation in previous and current studies might be due to the variation in composition of diets, types of enzymes, and the mode of action of exogenous enzymes in the gastrointestinal tract.

Consequently, this research indicated that supplementation of multi-enzyme in corn and soybean meal based diets for weaned pigs have no influence growth performance, intestinal morphology, and nutrient digestibility.

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