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ANIMAL

Effects of dietary enzyme cocktail on diarrhea and immune responses of weaned pigs

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Abstract

Weaning is the most stressful event for nursery pigs because they are moved from familiar to unfamiliar environments. In addition, weaned pigs have immature digestive and immune systems. This situation makes weaned pigs susceptible to diseases and makes the absorption of nutrients from diets difficult. A feed approach, such as dietary enzyme supplementation, can be considered a solution. This study investigated the effects of dietary enzyme cocktail 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 0.05% enzyme cocktail (Cocktail; combination of xylanase, α-amylase, protease, β-glucanase, and pectinase). Pigs were fed their respective diets for 6 wk. Incidence of diarrhea, packed cell volume (PCV), white blood cells (WBC) count, and immunoglobulin content were measured. A significantly lower incidence of diarrhea ($p < 0.05$) was observed in the Cocktail group as compared with the CON group. The Cocktail group also showed a decreased PCV ($p < 0.1$) on d 3 after weaning than the CON group. However, no differences were observed for number of WBC and contents of immunoglobulin G, M, and A between the Cocktail and CON groups. Consequently, inclusion of an enzyme cocktail in diets for weaned pigs had a positive influence on gut health by reducing the incidence of diarrhea in the present study.

Keywords: enzyme cocktail, diarrhea, immune responses, weaned pigs

Introduction

At weaning, piglets are separated from sows and moved from their familiar environment to unknown surroundings with other piglets (Song et al., 2015). Weaning induces excessive stress to nursery piglets (Song et al., 2015; Park et al., 2016). During lactation, sows provide their litter colostrum and milk which contain lots of immune-related components to protect nursery piglets from diseases (Song et al., 2015). However, after weaning, diets for weaned pigs are switched

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from sow milk to solid type feed that cannot provide immune-related components. Moreover, the immune and digestive systems of weaned pigs are relatively immature (Song et al., 2015; Park et al., 2016). Thus, death of weaned pigs caused by infections and diarrhea is still a problem in the pig production industry (Song et al., 2015; Park et al., 2016).

Most feed ingredients include anti-nutritional factors (ANFs), such as non-starch polysaccharides (NSP) (Choct, 1997). Soybeans consist of approximately 20% NSP, representing 3% soluble and 17% insoluble NSP (Choct, 1997). Non-starch polysaccharides in diets for monogastric animals may have anti-nutritive effects due to its viscosity, modification of gut physiology, and interaction with gut microflora (Choct, 1997). The anti-nutritive effects of NSP may be especially detrimental to weaned pigs because of their immature digestive and immune systems.

Nutrition and feed approaches, like modified feed ingredients, feed additives, or feeding methods can be possible solutions (Park et al., 2016). One feed approach is the supplementation of exogenous enzymes, such as carbohydrases and proteases. Breaking down ANFs may be achieved by adding exogenous digestive enzymes in diets, thus reducing their detrimental influence. This study hypothesized that supplementation of dietary enzyme cocktail, a mixture of carbohydrases and proteases, helped hydrolyse NSP in soybean meal, thereby improving gut health and immune status of weaned pigs. Therefore, this research was designed to investigate the effects of dietary enzyme cocktail (a mixture of carbohydrases and proteases) on diarrhea and immune responses of weaned pigs.

Materials and Methods

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

Experimental design, animals, and diets

A total of 36 weaned pigs [Duroc × (Landrace × Yorkshire); 5.92 ± 0.48 kg of average body weight (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 with 3 pigs per pen and 6 replicated pens per treatment in a randomized complete block design. The experimental treatments were as follows: a normal corn and soybean meal based diet (CON) and CON with 0.05% enzyme cocktail [Cocktail; mixture of xylanase (4,000,000 U/kg), α-amylase (1,000,000 U/kg), protease (500,000 U/kg), β-glucanase (150,000 U/kg), and pectinase (25,000 U/kg)], which were commercially purchased. Pigs were fed for 6 wk using a 2-phase feeding program with declining diet complexity and each phase was 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[w]	Phase 2[x]
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

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

Items	Phase 1[w]	Phase 2[x]
Lactose	4.00	-
Soybean oil	3.00	1.35
Limestone	1.00	1.00
Monocalcium phosphate	0.90	0.90
Vitamin premix[y]	0.20	0.20
Mineral premix[z]	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
Crud protein (%)	24.49	22.51
Calcium (%)	0.81	0.73
Phosphorus (%)	0.69	0.63
Lysine (%)	1.54	1.41

[w]Phase 1 = wk 1 to 3 (21 days).
[x]Phase 2 = wk 4 to 6 (21 days).
[y]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 B_{12}, 12 μg.
[z]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.

Sample collection and analyses

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 existence but also the levels of diarrhea. The diarrhea score for each pen was recorded daily in the morning from d 1 to d 14 by three specialists who had no information about dietary treatments. Fresh fecal matter was recorded 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 presence of diarrhea in total days.

Blood and immunoglobulin

Blood samples 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) with anticoagulant at weaning and on d 1, 3, 7 and 14 after weaning. Serum samples were collected by centrifugation at 3,000 × g at 4℃ for 15 min and stored at -80℃ until analysis for immunoglobulin G, M, and A. Whole blood samples were analyzed for total white blood cell (WBC) and red blood cell (RBC) counts as well as 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).

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 PCV, WBC count, and immunoglobulin content. The chi-square test was used for the incidence of diarrhea. The variability of the data was shown as the standard error and a probability level of $p < 0.05$ was considered as significant difference.

Results and Discussion

Weaned pigs in the Cocktail group showed significantly lower ($p < 0.05$) frequency of diarrhea than weaned pigs in CON group (Fig. 1). Consistently with the result of diarrhea incidence, PCV of Cocktail group tended to decrease ($p < 0.1$) on d 3 compared with that of CON group (Fig. 2). Usually, PCV is used an indicator of diarrhea or dehydration, as a higher level of PCV means that dehydration has progressed due to diarrhea (Pare et al., 1993). However, no significant differences were observed for immune response indices, such as number of WBC and contents of serum immunoglobulin G, M, and A (Fig. 3 and Table 2).

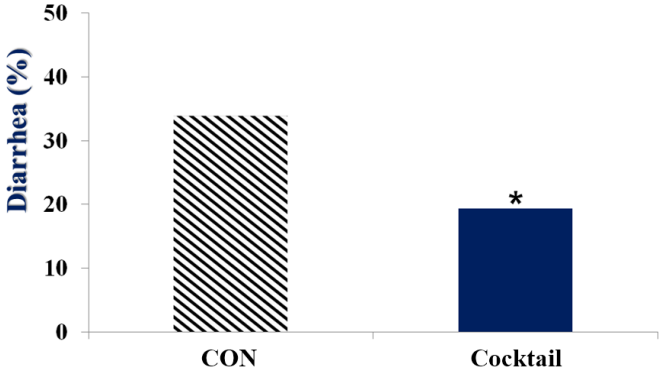

Fig. 1. Frequency of diarrhea in weaned pigs fed dietary treatments. CON = control diet included corn and soybean meal, Cocktail = CON with 0.05% enzyme cocktail (mixture of xylanase, α-amylase, protease, β-glucanase, and pectinase). Significant difference ($p < 0.05$) was observed between CON and Cocktail.

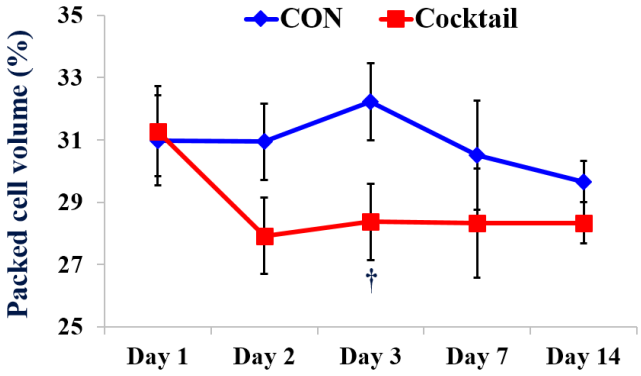

Fig. 2. Packed cell volume of weaned pigs fed dietary treatments. Values are means ± SEM. CON = control diet included corn and soybean meal, Cocktail = CON with 0.05% enzyme cocktail (mixture of xylanase, α-amylase, protease, β-glucanase, and pectinase). Cocktail supplementation tended ($p < 0.10$) to decrease the packed cell volume on d 3.

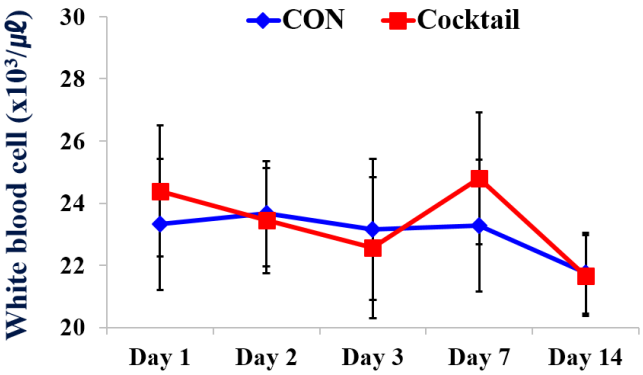

Fig. 3. Number of white blood cell of weaned pigs fed dietary treatments. Values are means ± SEM. CON = control diet included corn and soybean meal, Cocktail = CON with 0.05% enzyme cocktail (mixture of xylanase, α-amylase, protease, β-glucanase, and pectinase). No statistical differences were observed between CON and Cocktail.

Table 2. Immunoglobulin of weaned pigs fed dietary treatments.

Items	CON[x]	Cocktail[y]	SEM[z]	p-value
Ig G (mg/mL)	16.24	20.70	4.33	0.494
Ig M (mg/mL)	1.225	1.228	0.004	0.639
Ig A (mg/mL)	0.126	0.128	0.001	0.172

[x]CON = control diet based on corn and soybean meal.
[y]Cocktail = CON with 0.05% enzyme cocktail (mixture of xylanase, α-amylase, protease, β-glucanase, and pectinase).
[z]SEM = standard error of mean.

It is well documented that the major detrimental effects of NSP are caused by its viscosity, physiological and morphological effects on digestive system, and interaction with the microflora of the gut (Choct, 1997). Especially, its viscose nature is considered a major factor for the nutritional effects of NSP and they alter intestinal transit time and modification of the intestinal mucosa (Choct, 1997). Generally, soluble NSP increase gut viscosity, and high gut viscosity decreases the rate of diffusion of substrates and digestive enzymes, resulting in low interaction of NSP at the mucosal surface (Choct, 1997). In addition, soluble NSP interact with the glycocalyx of the intestinal brush border and increases the thickness of the rate-limiting unstirred water layer of the mucosa, thereby the efficiency of nutrient absorption via the intestinal wall is reduced (Choct, 1997). On the other hand, an increased level of insoluble NSP decreases the residence time of digesta, and it may provide insufficient time for digestion and absorption of nutrients (Choct, 1997). However, shortened transit time of digesta do not allow the proliferation of fermentative anaerobic organisms in the small intestine, which can cause detrimental effects (Choct, 1997; Smits et al., 1998; Langhout et al., 1999). Cho and Kim (2013) have reported that exogenous enzymes, such as β-mannanase and xylanase, in a low nutrient density diet had positive effects on performance and nutrient digestibility of finishing pigs. Choct (1997) also said that ANFs are effectively eliminated by adding xylanases and β-glucanases, which cause a partial depolymerization of NSP. In this research, supplementation of an enzyme cocktail, a mixture of carbohydrases and protease, in diets improved diarrhea incidence. However, further research is required to investigate the specific mechanisms underlying these positive effects of enzyme cocktail on gut health.

Therefore, the results of this study indicated that supplementation of an enzyme cocktail in diets for weaned pigs improved gut health, resulting in reduced diarrhea incidence and PCV.

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