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Original research article

Effect of dietary supplementation with protease on growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression of weaned piglets

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ABSTRACT

This study was conducted to investigate the effect of dietary protease supplementation on the growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression in weaned piglets. A total of 300 weaned piglets (21 days of age Duroc × Large White × Landrace; initial BW = 6.27 ± 0.45 kg) were randomly divided into 5 groups. The 5 diets were: 1) positive control diet (PC), 2) negative control diet (NC), and 3) protease supplementations, which were 100, 200, and 300 mg per kg NC diet. Results indicated that final BW, ADG, ADFI, crude protein digestibility, enzyme activities of stomach pepsin, pancreatic amylase and trypsin, plasma total protein, and intestinal villus height were higher for the PC diet and the supplementations of 200 and 300 mg protease per kg NC diet than for the NC diet ($P < 0.05$). Supplementations of 200 and 300 mg protease per kg NC diet significantly increased the ratio of villus height to crypt depth (VH:CD) of duodenum, jejunum and ileum compared with NC diet ($P < 0.05$). Feed to gain ratio, diarrhea index, blood urea nitrogen, and diamine oxidase were lower for the PC diet and supplementations of 200 and 300 mg protease per kg NC diet than for the NC diet ($P < 0.05$). Piglets fed the PC diet had a higher peptide transporter 1 (PepT1) mRNA abundance in duodenum than piglets fed the NC diet ($P < 0.05$), and supplementations of 100, 200 and 300 mg protease per kg NC diet increased the PepT1 mRNA abundance in duodenum ($P < 0.05$) comparing with the NC diet. Piglets fed the PC diet had a higher b0,+AT mRNA abundance in jejunum than piglets fed the NC diet ($P < 0.05$), and supplementations of 200 and 300 mg protease per kg NC diet increased the b0,+AT mRNA abundance in jejunum and ileum comparing with the NC diet ($P < 0.05$). In summary, dietary protease supplementation increases growth performance in weaned piglets, which may contribute to the improvement of intestinal development, protein digestibility, nutrient transport efficiency, and health status of piglets when fed low digestible protein sources.

1. Introduction

The cost of pork production mainly comes from the feed, and the significant increases of feed cost during the last decade have reduced profit margins of pork production (Schmit et al., 2009). The use of exogenous feed enzymes has been one of the most widely used strategies to improve nutrient utilization efficacy and reduce the feed cost in the animal industry (Adeola and Cowieson, 2011). Proteases have been routinely included to swine diets for many

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years as part of enzyme cocktails containing xylanases, celluase, amlyase and glucanases (Yin et al., 2001, 2004; Omogbenigun et al., 2004; Ji et al., 2008; Jo et al., 2012). An enzyme cocktail (β-glucanase, xylanase and protease) improved the digestibilities of crude protein and energy at ileal and the total tract levels of the hulless barley based diets for young piglets (Yin et al., 2001). Similarly, an enzyme cocktail (arabinoxylanase and protease) improved the nutritional value of diets containing wheat bran or rice bran for growing pig (Yin et al., 2004). Dietary supplementation with enzyme cocktails including proteases improved nutrient utilization and growth performance in weaned pigs (Omogbenigun et al., 2004). A beta-glucanase-protease enzyme blend product improved the ileal digestibility of crude protein and other nutrients (Ji et al., 2008). Supplementation of 0.05% of enzyme cocktails (α-amylase, β-mannanase, and protease) to a corn and soybean meal (SBM) diet or a complex diet improved the performance of growing pigs (Jo et al., 2012). Although the above positive effects have been reported, the contribution of protease on these improvements is still not clear. Recently, proteases have been used alone in the pig diets with the availability of several commercial stand-alone proteases, and new mechanisms of action have been proposed (O'Doherty and Forde, 1999; McAlpine et al., 2012a, 2012b; Guggenbuhl et al., 2012). However, efficacy of protease in weaned piglets and its mechanisms behind are still not clear especially when low digestible protein sources are used. Therefore, this study was conducted to investigate the effect of dietary supplementation with protease on the growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression in weaned piglets.

2. Materials, methods and management

2.1. Animals and diets

A total of 300 weaned piglets (21 days of age Duroc × Large White × Landrace; initial BW = 6.27 ± 0.45 kg) were provided by Wens Group (Guangzhou, Guangdong). They were randomly divided into 5 groups (60 piglets per group and 10 piglets per pen). All piglets were housed in environmentally controlled rooms equipped with water nipples and stainless-steel feeders. Diets and water were offered ad libitum throughout the duration of the experiment. Room temperature and air humidity were maintained at 25°C and 50%, respectively. All procedures were approved by the Animal Care Committee at the South China Agricultural University. The animals used in this experiment were cared for in accordance with the guidelines established by University Council of Animal Care.

As shown in Table 1, the basal diets used were formulated to meet the nutrient requirement of pigs (NRC, 2012). For 14 d, piglets were fed the following diets: 1) a standard commercial diet, named as a positive control (PC) diet (22.21% soybean meal, 5% whey protein and 3% fish meal), 2) a negative control (NC) diet (30.06% soybean protein without whey protein and fish meal), 3) 100 mg protease per kg NC diet, 4) 200 mg protease per kg NC diet, and 5) 300 mg protease per kg NC diet. The protease used is a commercial protease (Jefo, Saint-Hyacinthe, Canada). The five diets were isonitrogenous and iso-caloric, and pelleted at a condition of 0.4 MPa and 75°C. The major source of protein in the PC diet was from fishmeal and concentrated whey protein, which were substituted with soybean meal in the NC diet.

2.2. Data recording and sample collection

Health status was monitored daily, and BW and feed intake was registered throughout the study. Average daily gain (ADG), average daily feed intake (ADFI), feed to gain ratio, and diarrhea index were calculated.

Table 1
Ingredients and nutrient composition of the basal diet (as fed basis).

Item	PC	NC
Ingredient, g/kg		
Corn, 8% CP	45.10	45.10
Soybean	142.10	160.60
Whey power, 12% CP	120.00	120.00
Soybean meal, 43% CP	50.00	100.00
Wheat flour	50.00	50.00
Concentrated whey protein, 34% CP	50.00	0.00
Concentrated soybean protein, 64% CP	30.00	40.00
Spray-dry plasma	30.00	30.00
Fishmeal	30.00	0.00
Glucose	25.00	25.00
Sucrose	20.00	20.00
Calcium hydrogen phosphate	7.00	11.50
Soy oil	7.60	5.20
L-Lysine	3.10	4.00
Limestone	3.60	4.00
ZnO	2.50	2.50
L-Threonine	1.20	1.80
DL-Methionine	1.30	1.70
Choline	1.00	1.00
L-Tryptophan	0.50	0.60
Premix[1]	10.00	10.00
Nutrient composition, % [2]		
DE, MJ/kg	14.52	14.52
CP	21.00	21.00
Ca	0.6	0.6
P	0.45	0.45
NaCl	0.55	0.55
Lysine	1.53	1.54
Met + Cys	0.86	0.87
Threonine	1.06	1.05
Tryptophan	0.31	0.32
Arginine	0.65	0.64
Valine	1.08	1.09
Leucine	1.53	1.55
Isoleucine	0.86	0.87
Histidine	0.50	0.48
Phenylalanine	0.94	0.94

PC = positive control diet; NC = negative control diet.
[1] Premix provided per kg of diet: vitamin A,19,200 IU; vitamin D_3, 4,800 IU; vitamin E, 60 IU; vitamin K_3, 6 mg; vitamin B_1, 6 mg; vitamin B_2, 12 mg; vitamin B_6, 7.2 mg; vitamin B_{12}, 0.05 mg; niacin, 60 mg; calcium pantothenate, 30 mg; nicotinic acid, 15 mg; folic acid, 3.60 mg; biotin, 0.60 mg; Fe, 305 mg; Cu, 250 mg; Zn, 1,910 mg; Mn, 51 mg; I, 0.50 mg; Se, 0.50 mg; Co, 0.50 mg.
[2] Nutrient levels were calculated.

Blood samples were collected at 0800 via the jugular vein into 10 mL heparinized vacuum and then centrifuged at 3,000 × *g* for 10 min to collect plasma. Plasma was frozen at −80°C until analysis. All blood samples were analyzed in duplicate.

Pigs were euthanized with an overdose injection of 10% sodium pentobarbital before sampling. The entire intestine was then removed and dissected free of mesenteric attachments and placed on a smooth and cold surface. The duodenum, jejunum and ileum were separated. The isolated intestinal segments were immediately opened lengthwise following the mesentery line and flushed with ice-cold saline (154 mmol/L NaCl, 0.1 mmol/L phenylmethylsulfonyl fluoride [PMSF], pH 7.4) and divided into 15-cm segments. Each tube, which contained approximately 15 g of tissue, was tightly capped and stored at −80°C. For morphology measurement, samples were flushed and fixed in 10% buffered formalin at least 48 h before histology process.

At day 14, feces samples were collected and added with 10% HCl, and then stored at−80°C before analysis. Before analysis, samples were dried at 65°C and grounded into fine powder and then apparent total tract digestibility was measured.