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Effects of grape seed procyanidins on growth performance, immune function and antioxidant capacity in weaned piglets

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ABSTRACT

The trial was conducted to investigate effects of grape seed procyanidins (GSP) on growth performance, immune function and antioxidant capacity in weaned piglets. A total of 160 Duroc × Landrace × Yorkshine piglets weaned at 21 days of age (body weight, 6.99 ± 0.11 kg) were randomly assigned to one of the five treatment groups with 4 replicate pens per group and 8 piglets per pen. The treatments included control group (fed a basal corn–soybean meal diet, BD), antibiotics group (BD + 20 mg/kg of flavomycin + 100 mg/kg of aureomycin), and three different GSP level groups (BD + 50, 100, 150 mg/kg GSP respectively). The trial lasted 28 days. Diarrhea scores were recorded daily, and the average daily gain (ADG), average daily feed intake (ADFI) and feed/gain ratio (F:G) were calculated. Blood samples were collected on d 14 and 28 for the measurement of serum immune parameters and antioxidant indices. Dietary GSP or antibiotics supplementation did not exert significant effects on ADG, ADFI and F/G during overall experimental period. As compared with the control group, supplementation with antibiotics or different levels of GSP decreased the diarrhea incidences in piglets significantly ($P < 0.05$). Pigs offered diets supplemented with GSP at doses of 100 or 150 mg/kg generated higher ($P < 0.05$) serum immunoglobulin (Ig) G, IgM, complement 4 (C_4) and interleukin-2 (IL-2) concentrations, produced stronger ($P < 0.05$) serum total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities and lower serum malondialdehyde (MDA) content than those fed basal diet or antibiotics diet. In conclusion, administration of GSP in weaned piglets diet had no effect on the growth performance during the whole experiment. However, the beneficial effects on diarrhea incidences, immune responses and antioxidant abilities suggested that GSP has a positive role in weaned piglets.

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1. Introduction

It is well known that early weaning is an effective way to maximize the whole herd production, and is becoming increasingly popular in modern intensive pork production system. However, early weaning is often associated with a range of disorders in piglets including low feed intake, depressed growth rate, poor immunocompetence and increased susceptibility to enteric pathogens to cause diseases (Che et al., 2012; Lauridsen, 2010; Williams, 2003). Antibiotics have been widely used to prevent infectious diseases and enhance growth performance (Frydendahl, 2002; Li et al., 2011). However, the misuse of antibiotics has led to bacterial resistance in livestock and humans, as well as antibiotics-residue in animal products (Fàbrega, et al., 2008; Monroe and Polk, 2000). Thus, the use of antibiotics as growth promoters for livestock will be completely banned. This situation spawned investigations into alternatives of feed additives with high efficiency and low toxicity.

Piglet grows rapidly and produces large amounts of free radicals (Rollo, 2002). The ingestion of dietary antioxidants may help to counter negative effects of oxidative stress associated with fast growth rate (Catoni et al., 2008). It is therefore predicted that polyphenols with powerful antioxidant capacity and abundant source would play an important role during growth (Catoni et al., 2008).

Procyanidins (PCs) are polyphenolic compounds from the flavonoids group that are rich in cereals, vegetables, fruits like grapes, berries and apples, as well as beverages such as cacao and tea. Grape seed procyanidins (GSP) extracted from grape seeds function as powerful antioxidants and possess anti-inflammatory and immunomodulatory activities in vitro and in vivo (Terra et al., 2007, 2009, 2011). Scientific studies have shown that the antioxidant power of procyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C (Shi et al., 2003). Extensive

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researches suggest that GSP are beneficial in many areas of health because of its antioxidant effect (Aldini et al., 2003; Faria et al., 2006; Janisch et al., 2006; Lu et al., 2004; Simonetti et al., 2002). Although the various biological functions of GSP have received extensive attention, there is little information available in the literature about application of GSP in weaned piglets.

We hypothesized that dietary GSP supplementation may enhance antioxidant capacity, stimulate immune system, reduce diarrhea incidence and therefore improve growth performance in weaned piglets. Therefore, this study was conducted to test this hypothesis. Furthermore, using antibiotics treatment as a positive control, the present study was also conducted to compare the effects of GSP with antibiotics in weaned piglets diet to reduce episodes of diarrhea.

2. Materials and methods

The experiments were carried out in accordance with the Chinese guidelines for animal welfare and experimental protocol.

2.1. Animal and experimental design

A total of 160 crossbred piglets (Duroc × Landrac × Yorkshire) weaned at 21 days of age with an average initial body weight (BW) of 6.99 ± 0.11 kg were allocated to 5 treatment groups with 4 replicate pens per treatment and 8 piglets per pen in a randomized complete block design according to the BW and sex. Treatments consisted of control group (basal diet), antibiotics group (basal diet + 20 mg/kg of flavomycin + 100 mg/kg of aureomycin), 50 mg/kg GSP group (basal diet + 50 mg/kg GSP), 100 mg/kg GSP group (basal diet + 100 mg/kg GSP) and 150 mg/kg GSP group (basal diet + 150 mg/kg GSP). Piglets were given ad libitum access to water and the assigned diet during the 28-d experimental period.

Experimental diets were formulated to meet the nutrient requirements for pigs proposed by NRC (2012). The ingredient compositions and nutrient levels of the basal diets were presented in Table 1.

Table 1
Ingredient composition and nutrient content of the basal diets.

Item	Content (%)
Ingredients	
Corn	57.17
Soybean meal (44% CP)	20.00
Expanded soybean	6.00
Fish meal (63.5% CP)	6.00
Whey powder	5.00
Soybean oil	3.00
Limestone	0.56
Dicalcium phosphate	0.85
Salt	0.20
L-Lys HCl (98%)	0.20
DL-Met (98%)	0.02
Vitamin and mineral premix[a]	1.00
Calculated chemical composition (% DM)	
Crude protein	19.5
Digestible energy (MJ/kg)	14.8
Calcium	0.80
Available phosphorus	0.48
Arginine	1.24
Histidine	0.45
Isoleucine	0.73
Leucine	1.30
Lysine	1.36
Methionine	0.53
Threonine	0.85
Tryptophan	0.25
Valine	0.90

[a] Vitamin and mineral premix supplied per kilogram of diet: retinyl acetate, 1.6 mg; cholecalciferol, 0.05 mg; DL-α-tocopheryl acetate, 40 mg; menadione sodium bisulfite (62.5% menadione), 2.2 mg; vitamin B_1, 2.5 mg, vitamin B_2, 6.5 mg; vitamin B_6, 5 mg; niacin, 40 mg; vitamin B_{12}, 50 μg; Zn ($ZnSO_4$), 120 mg; Fe ($FeSO_4$), 120 mg; Cu ($CuSO_4$), 20 mg; Mn ($MnSO_4$), 30 mg; Se (Na_2SeO_3), 0.3 mg; I (CaI_2), 0.3 mg.

2.2. Growth performance and diarrhea incidence

The body weight of pigs was recorded 1 h prior to feeding in the morning at d 1, d 14 and d 28 of the experiment, and the feed intake of each pen was recorded daily. The average daily gain (ADG), average daily feed intake (ADFI) and feed/gain ratio (F:G) were then calculated.

The diarrhea incidence in piglets was evaluated according to a previous study (Li et al., 2011) as following: diarrhea incidence (%) = the total number of pigs with diarrhea during the trial/(number of pigs × total experimental days) × 100.

2.3. Sampling and assay

On day 14 and day 28 of the experiment, blood samples from two piglets per pen were collected 1 h prior to feeding in the morning. Blood was collected (10 ml) from vena cava into a 10-ml anticoagulant-free vacutainer tube, centrifuged at 3000 × *g* for 10 min to obtain the serum, and stored at −70 °C for later analysis.

The serum were used for the determination of serum antioxidant indices, including total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA), as well as serum immune indexes, including immunoglobulin (Ig) G, IgA, IgM, interleukin-2 (IL-2), complement 3 (C_3)and complement 4 (C_4). Serum concentrations of IgG, IgA, IgM, IL-2, C_3 and C_4 were measured by enzyme linked immunosorbent assay (ELISA). T-AOC, GSH-Px, MDA, SOD and CAT detection kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The T-AOC was measured by the method of ferric reducing-antioxidant power assay. Activity of GSH-Px was detected by colorimetric method of 5,5′-dithiobis-*p*-nitrobenzoic acid. Activity of SOD was measured by the xanthine oxidase method. Activity of CAT was mensurated by ammonium molybdate colorimetry. Enzyme activity was expressed as units per milliliter for serum. The MDA level was tested as an indicator of lipid peroxidation via 2-thiobarbituric acid color reaction.

2.4. Statistical analysis

Data were analyzed by one-way ANOVA in SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) followed by Duncan's multiple range tests. Diarrhea incidence was subjected to arcsine transformation before statistical analysis to ensure homogeneity of variance. Results were expressed as means and the standard error of the mean (SEM). A *P*-value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Growth performance

The effects of GSP on growth performance of weaned piglets are presented in Table 2. From day 1 to 14, day 14 to 28, or day 1 to 28, no significant differences in ADG, ADFI and F/G were observed among treatments. Dietary supplementation with GSP or antibiotics did not exert significant effects on growth performance (ADG, ADFI and F/G) during overall experimental period.

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