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Dietary soy isoflavone attenuated growth performance and intestinal barrier functions in weaned piglets challenged with lipopolysaccharide

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ABSTRACT

This study was conducted to investigate the protective roles of soy isoflavone in weaned pigs challenged with lipopolysaccharide (LPS). A total of 72 weaned piglets (14 days of age) were randomly allotted into either 0 (control group) or 40 mg/kg soy isoflavone (ISO) supplementation group. On days 7 and 14, half of the pigs in each group were challenged with LPS. Soy isoflavone increased average daily gain (ADG) and average daily feed intake (ADFI) of piglets challenged with LPS at days 7–14 ($P < 0.05$). The incidence of diarrhea and plasma concentrations of malondialdehyde (MDA) and endotoxin in piglets from LPS group were higher than those in control group ($P < 0.05$). Soy isoflavone reduced the incidence of diarrhea and plasma concentrations of endotoxin in piglets challenged with LPS ($P < 0.05$). LPS challenge decreased ($P < 0.05$) mRNA abundances of β-defensin 2 (*pBD-2*), mucin (*MUC-4*), zona occludens 1 (*ZO-1*), and *occludin* in jejunal mucosa of piglets, and soy isoflavone upregulated ($P < 0.05$) mRNA abundances of *ZO-1* and *occludin* in jejunal mucosa of piglets challenged with LPS. The present results demonstrated that both p38 and TLR4 pathways in jejunal mucosa of piglets were activated by LPS challenge ($P < 0.05$), and soy isoflavone reduced their activations ($P < 0.05$). Collectively, our results suggested that supplementation of soy isoflavone could partly attenuate the barrier-damaged effects of LPS and improve the intestinal barrier function of weaned piglets, at least partially by inhibiting activations of p38 and TLR4 dependent pathways induced by LPS. This study provides a potential usage of soy isoflavone for alleviating intestinal barrier damages of neonates and piglets.

1. Introduction

The gastrointestinal epithelium is not only a major digestive and absorptive tissue for nutrients, but also functions as important physical barrier between the luminal environment and the body to prevent invasions of pathogens, toxins, and antigens. The intestinal barrier is constituted by enterocyte membranes, tight junctions (TJs), mucous layer, antibacterial peptides, and mucosal immune system [1,2]. It has been documented that immunological challenge would result in damages of intestinal tight junction barriers [3], and finally contribute to the pathogenesis of gut inflammation and disorders. Diet-related components which may be important mediators of inflammations, have gained popularity for disease prevention and treatment in recent years. Isoflavones are a subclass of the flavonoid group, with most abundant presence in soybean, soy products, and legumes. The main components of soy isoflavones, including genistein, daidzein, glycitein and biochanin A [4,5], have been demonstrated to possess various biological properties and exert antiestrogenic [6], anticarcinogenic [7,8], antioxidant [9], anti-inflammatory [10,11] as well as immune-modulatory functions [12]. Increasing evidences have suggested that soy isoflavones may have beneficial effects in the prevention and treatment of inflammatory disorders [5,13] and diarrheal diseases [14,15].

However, little is known about the preventive effects of soy isoflavone on intestinal health in neonates or weanling piglets which are very vulnerable to bacterial and virus infections. The piglet was recognized as the best animal model to study human infant nutrition and intestinal development due to their similarity in anatomy and digestive

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BBI, Bowman–Birk Inhibitors; BCA, bicinchoninic acid; BSA, bovine serum albumin; pBD-2, β-defensin 2; GSH-Px, glutathione peroxidase; F:G, feed to gain ratio; IL-1β, interleukin-1β; IL-8, interleukin-8; ISO, isoflavone; JAM, junctional adhesion molecule; LPS, lipopolysaccharide; MDA, malondialdehyde; PBS, phosphate buffered saline; PVDF, polyvinylidene difluoride; qRT-PCR, quantitative real time PCR; SDS PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PRRS, porcine reproductive and respiratory syndrome; TNF-α, tumor necrosis factor alpha; TJs, tight junctions; TLR4, Toll-like receptor 4; T-SOD, total superoxide dismutase; ZO-1, zona occludens 1.

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physiology [16,17]. Moreover, *in vivo* model of lipopolysaccharide (LPS) immunological challenge has been well demonstrated to mimic the potential bacterial infection on weanling for piglets. Hence, this experiment was carried out to investigate whether soy isoflavone could prevent or suppress the inflammatory development of experimental immunological challenge by LPS in weanling piglets. Our results will provide insight for the future application of using soy isoflavone against intestinal injury in neonates and piglets.

2. Materials and methods

2.1. Animals, diets, and experimental design

The experimental protocols and procedures performed in this study were approved by the Animal Care and Use Committee at Guangdong Academy of Agricultural Sciences.

A total of seventy-two 14-day-old crossbred barrows [*Duroc* × (*Landrace* × *Large white*), initial BW of 5.08 ± 0.46 kg] were used in this study. Piglets were randomly assigned to two dietary treatment groups with 12 replicates (pen) of three piglets per replicate on each group. The piglets in the control group (n = 36) were fed basal diet, while the piglets in soy isoflavone (ISO group) (n = 36) were fed the basal diets supplemented with 40 mg/kg ISO. The basal diets (Table 1) were formulated to meet or exceed nutrient requirements for 5–7 kg piglets recommended by NRC (2012) [18]. The soy isoflavone (ISO) (Patent No. 200810029640, China) with a purity of 98% was synthesized from resorcin and phenylacetic acid as previously described [9], and was provided by Institute of Animal Science, Guangdong Academy of Agricultural Sciences, China.

The piglets were housed in an air-conditioned room with ambient temperature of 32 ± 1 °C and constant humidity. Feed and water were provided to piglets *ad libitum*. Piglets were fed five times per day at 0800, 1100, 1400, 1700, and 2000, and the residual feeds were weighed in the morning of next day.

The experiment lasted 15 days. Piglets were randomly allotted to two treatments, and were fed basal diets either supplemented with or without ISO during days 1 to 7 before LPS challenge. On days 7 and 14, half of pigs (six replicates) in each dietary treatment were injected with either sterile LPS (200 μg/kg BW) or saline as previously described [19]. The LPS (*Escherichia coli* O55:B5, Sigma) were dissolved in sterile 9 g/L NaCl solution (500 mg LPS/L saline). Thus, the four treatment groups were as follows: (1) Control group (piglets fed basal diet and receiving i.p. administration of sterile saline); (2) ISO group (piglets fed the basal diet supplemented with 40 mg/kg ISO and receiving i.p. administration of sterile saline); (3) LPS-challenged group (piglets fed the basal diet and receiving i.p. administration of LPS); (4) ISO + LPS group (piglets fed the basal diet supplemented with 40 mg/kg ISO and receiving i.p. administration of LPS).

Table 1
Composition and nutrient levels of the basal diet (as fed basis).

Ingredient	%	Nutrient level	
Extruded corn	58.08	DE, MJ/kg	14.39
Dehulled soybean meal	20.00	CP, %	20.57
Whey powder	10.00	Calcium, %	0.85
Fishmeal	4.00	Available P, %	0.46
Soybean oil	3.00	Lys, %	1.70
Calcium carbonate	0.85	Met, %	0.66
Dicalcium phosphate	1.00	Met + Cys, %	0.97
Sodium bicarbonate	0.15	Thr, %	1.06
Salt	0.15	Trp, %	0.28
L-Arg. HCl	0.87		
DL-Met	0.22		
Thr	0.30		
Trp	0.08		
Vitamin and mineral mix[1]	1.30		
Total	100.00		

[1] Provided per kilogram of diet: vitamin A, 2200 IU; vitamin D_3, 220 IU; vitamin E, 16 IU; vitamin K_3, 0.5 mg; vitamin C, 200 mg; thiamin, 1.5 mg; riboflavin, 4 mg; pyridoxine, 7 mg; cyanocobalamin, 0.02 mg; pantothenic acid, 12 mg; niacin, 30 mg; folic acid, 0.3 mg; biotin, 0.08 mg; Fe ($FeSO_4.H_2O$), 100 mg; Cu ($CuSO_4 \cdot 5H_2O$), 6 mg; Mn ($MnSO_4 \cdot H_2O$), 4 mg; Zn ($ZnSO_4 \cdot H_2O$), 100 mg; I ($Ca(IO_3)_2$), 0.14 mg; Se (Na_2SeO_3), 0.30 mg; Co ($CoSO_4 \cdot 7H_2O$), 0.15 mg. The carrier was zeolite.

2.2. Sampling

On day 14 of the experiment, blood samples from a total of 24 piglets, with one piglet of each replicate, 6 replicates per group (n = 6/group), were obtained *via* anterior vena cava puncture after 3 h of post-injection of LPS. Blood was collected in 5 mL heparinized vacutainer tubes, and centrifuged at 3500 *g* for 10 min. Plasma samples were stored at −80 °C until analysis for activities of antioxidant enzymes and endotoxin levels. On day 15 after feed deprivation for 12 h and injection of sodium pentobarbital (50 mg/kg BW, Sigma), one piglet with similar BW to average pen weight was chosen from each replicate (totally 24 piglets) for sacrifice. The timeline of current experiment were listed in Fig. 1.

The separation of small intestine was according to the methods as previously described by Yang et al. [20] by a skilled technician with no idea of the experiment design. Briefly, the entire intestinal tract was removed and was divided into three segments: (the proximate 10-cm segment to the pylorus as duodenum; the middle portion as jejunum; and the distal 5-cm section proximal to the ileocecal junction as ileum). The middle portion of jejunum (about 10 cm) were cut longitudinally to expose only mucosa samples and washed three times with ice-cold phosphate buffered saline (PBS) to remove the mucus and digesta. The jejunal mucosa (about 10 g) was gently scraped with a glass slide, and then quickly placed in liquid nitrogen, and stored at −80 °C until use for extractions of total RNA and proteins.

2.3. Measurements

2.3.1. Growth performance

Piglets were weighed individually on days 0, 7, and 14 of the experiment and feed consumption was recorded accordingly. Average daily gain (ADG), average daily feed intake (ADFI), and feed: gain (F:G) ratio after LPS challenge were calculated on pen basis accordingly (n = 6/group). Diarrhea incidence of piglets (n = 6/group) was recorded daily and calculated on pen basis as follows: Incidence of diarrhea (%) = (number of pigs with diarrhea)/(number of pigs × experiment days) × 100%. Piglets that had mushy or watery stools without solid shape for more than 3 times each day were considered to be diarrhea piglets.

2.3.2. Antioxidant capacity analysis

Activities of the total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) in the plasma of piglets (n = 6/group) were determined using commercially available assay kits *via* a multifunctional spectrophotometer (SpectraMax M5, USA). The assay kits (T-SOD, GSH-Px, and MDA) were purchased from Nanjing Jiancheng Insititute of Bioengineering Company (Nanjing, China).

2.3.3. Plasma endotoxin measurement

Plasma concentrations of endotoxin in piglets (n = 6/group) at day 14 of the experiment were determined using a commercial kit of Chromogenic End-point Tachypleus Amebocyte Lysate (Xiamen Houshiji, Ltd., Xiamen, China), according to the manufacturer's instructions.

2.3.4. Relative mRNA expression determination by quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from jejunal mucosa using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocols. Quality and integrity of RNA was checked on 1.5% agarose gel electrophoresis with visualization of complete 28S and 18S bands. Purity and concentration of