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Dietary *Astragalus* polysaccharide alleviated immunological stress in broilers exposed to lipopolysaccharide

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ABSTRACT

This study was conducted to investigate whether dietary *Astragalus* polysaccharide (APS) could alleviate immunological stress response of chickens after challenge with lipopolysaccharide (LPS). A total of 360 one-day-old commercial Arbor Acres broilers were randomly assigned in a 2 × 2 factorial design. The main factors were immunological stress (LPS or saline) and dietary APS (0 or 3 g APS/kg feed). At 12, 14, 33 and 35 days of age, chickens were injected intramuscularly with either 500 μg/kg body weight of LPS or sterile saline. The results showed that the decreased daily feed intake and daily weight gain caused by immunological stress were dramatically attenuated by APS supplementation. The LPS challenge led to an increased mRNA abundance of TLR4, NF-κB, IL-1β, IL-6, avian uncoupling protein, α1-acid glycoprotein, hemopexin and y^+LAT2. However, these negative effects of the LPS administration were ameliorated by APS supplementation. Moreover, dietary APS inhibited the LPS-induced depression of amino acid digestibilities. In conclusion, APS is able to alleviate LPS-induced immunological stress response in chickens. The beneficial effect may be attributed to suppressing the expression of pro-inflammatory cytokines through reducing the TLR4 and NF-κB genes transcription, and therewith improving energy and protein metabolism.

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1. Introduction

Immunological stress is known as exogenous immunostimulation. Many factors such as pathogenic microorganisms, heavy vaccinations, drug abuse, contamination, and other external forces involved in rearing will attack animals, cause the loss of immune homeostasis, and trigger immunological stress response. Under stress, body protein and fat anabolism will be weakened, while catabolism will be promoted in order to satisfy the requirement of nutrients to synthesize immune effector molecules. There is a shift in the partitioning of dietary nutrients away from skeletal muscle accretion toward metabolic responses that support the immune system [1]. This may affect growth performance and enteric diseases, causing great economic losses. Therefore, it is important to set a balance between nutrition and immunity of broiler chickens. Minimizing immunological stress and its impact is an important strategy to realize the full potential of genetic and nutritional advancements for efficient production in the broiler industry. Thus, exploring an effective nutritional additive to modulate the immune system is of great significance to protect broilers from immunological stress.

Astragalus polysaccharide (APS) is a polysaccharide isolated from the radix of *Astragalus membranaceus*, a traditional Chinese medicinal herb with a wide range of anti-bacterial [2], anti-tumor [3], anti-oxidant [4,5], anti-inflammatory [6,7], and immune modulating [8] properties. In recent years, APS has won more and more attention in the field of animal nutrition because of its low side effect and drug resistance. To our knowledge, although many studies have been conducted to characterize the effects of APS on the performance and immunity of broiler chickens, most of them were conducted with healthy broilers without challenging conditions. Several studies have demonstrated the inhibitory effect of APS on lipopolysaccharide (LPS) induced pro-inflammatory cytokines production *in vitro*, however, the underlying mechanisms are largely unknown, and the metabolic regulation is rarely reported.

In the present study, we used a well-documented model to induce immunological stress in broiler chickens by injecting LPS, the major active component of the outer membrane of Gram-negative bacteria. We hypothesized that dietary supplementation with APS may alleviate immunological stress response and ameliorate the metabolic disorder of nutrients in broiler chickens exposed to LPS. This hypothesis was tested by determining the expression of Toll-like receptor 4 (TLR4) pathway-associated factors,

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Table 1
Ingredients and nutrient composition of broiler diets on fed basis.

Item	1–21 days	22–42 days	Nitrogen-free diet
Ingredient (%)			
Maize	58.00	62.70	–
Corn starch	–	–	62.70
Sucrose	–	–	25.10
Cellulose	–	–	4.40
Soyabean meal (43% CP)	34.40	29.80	–
Soyabean oil	3.00	4.00	3.00
Limestone	1.30	0.60	1.00
Dicalcium phosphate	1.60	1.30	1.90
Salt	0.40	0.40	0.40
DL-Met	0.20	0.10	–
Chromic oxide	–	–	0.30
L-Lys	0.10	0.10	–
Premix[a]	1.00	1.00	1.20
Calculation of nutrients (%)			
ME (MJ/kg)	12.30	12.92	13.04
CP	20.70	19.00	–
Ca	1.00	0.85	0.96
Available P	0.44	0.40	0.44
Total Lys	1.20	1.05	–
Total Met	0.50	0.38	–
Total Met + Cys	0.84	0.68	–

ME, metabolizable energy; CP, crude protein.
[a] Premix provided per kg of feed: vitamin A, 8000 IU; vitamin D3, 2500 IU; vitamin K3, 2.65 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; vitamin B12, 0.025 mg; vitamin B12, 0.025 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; niacin, 50 mg; Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0.15 mg; I, 0.35 mg.

inflammatory cytokines and nutrient transporters, and ileal digestibilities of amino acids (AA).

2. Materials and methods

2.1. Animals and management

The animal protocol for the present study was approved by the Animal Care and Use Committee of the College of Animal Science and Technology of the Northwest A&F University (Shaanxi, China). A total of 360 one-day-old commercial Arbor Acres broiler chicks were randomly assigned to 4 treatments with 6 replicate pens per treatment and 15 birds per pen. The initial body weight (BW) of chicks did not differ among treatments ($P > 0.05$). All chicks were housed in an environmentally controlled henhouse with double-floor metabolism cages. Maize-soybean-based basal diets without antibiotics were formulated to meet the nutrient requirements as recommended by the National Research Council (1994) during the starter (days 1–21) and finisher (days 22–42) periods (Table 1). The initial temperature inside the house was 34 °C and was decreased by 2 °C each consecutive week until 24 °C through to slaughter. Relative humidity was set at 50% throughout the study. The lighting program was 23 h of light for the first 2 weeks, and 20 h afterwards. All broiler chickens had *ad libitum* access to feed and water.

2.2. Experimental design

The chicks were assigned on a study design based on a 2 × 2 factorial arrangement of main factors. The main factors were immune stress (injection with LPS at a dosage of 500 μg/kg body weight or saline) and dietary APS (0 or 3 g APS/kg feed). The LPS (*Escherischia coli* serotype O55:B5; Sigma-Aldrich Inc., St. Louis, MO, USA) was dissolved in sterile saline at 0.5 mg/ml and chickens were injected with the solution at 0.5 ml/kg body weight or an equivalent amount of sterile saline at 12, 14, 33, and 35 days of age. All the four injections were given on the left pectoralis. APS used in the experiment was provided by North China Pharmaceutical Company Limited (NCPC; Shijiazhuang, Hebei, China) with a purity of 70% (does not contain protein and nucleic acid) and a molecular weight of 20–60 kDa. The monosaccharides of APS were D-galactose, D-xylose, and D-glucose, in a molar ratio of 1:1.2:12.2. APS backbone consists of α-(1,4)glucopyranose. Galactofuranose is connected at C-6 of glucose with β-(1,6)glycosidic bond, and xylopyranose at C-3 of glucose with β-(1,3)glycosidic bond, which constitute the branches. The dose of 3 g APS/kg feed was chosen according to our pilot trial results.

2.3. Performance parameters

Birds were group weighed by cage at 1, 21, and 42 days of age. Feed intake was monitored by cage at 21 and 42 days of age. Average daily weight gain (DWG), daily feed intake (DFI), and feed conversion ratio (FCR) were calculated for each period and for the overall experiment. These performance parameters were corrected according to mortality.

2.4. Body temperature

At day 12 of age, body temperature was measured by inserting 3 cm of a digital thermometer for livestock (MC-347, Omron, Co., Ltd., China) into the cloaca every 2 h after injection, and the change of body temperature was monitored for 24 h continuously.

2.5. Relative weights of lymphoid organs

Two birds from each replicate group were randomly weighed and killed within 4 h post-injection at days 14 and 35 of age, respectively. The thymus, spleen, and bursa were removed from each bird. The organ weights were immediately measured following dissection and were expressed relative to BW (g of organ/kg of BW).

2.6. Determination of blood parameters

At days 14 and 35 of age, 4 h after LPS or saline administration, about 5 ml of blood were collected from the brachial vein of six broilers per treatment (one bird/replicate) and withdrawn into heparinized tubes. Plasma samples were separated by centrifugation at 350 × *g* for 15 min at 4 °C. The supernatant was dispensed into 1.5 ml centrifuge tubes and stored at −80 °C. Plasma glucose was measured according to the glucose oxidase-peroxidase (GOD-POD) method [9]. The level of hormones including cortisol, insulin, and thyroid hormone (T3 and T4) were determined using ^{125}I-radioimmunoassay (RIA) kits (Jiancheng Bioengineering Institute, Nanjing, China).

2.7. Collection of liver, skeletal muscle, and intestinal mucosa samples

After the birds were killed and dissected, the left lobe of the livers were collected without the cholecyst and 1 cm^3 segments were cut off from the chest muscle with a scalpel. The samples were rinsed thoroughly with ice-cold phosphate-buffered saline (PBS; pH 7.4) to remove blood contamination. The small intestine was dissected free of the mesentery and placed on a chilled stainless steel tray. 10 cm segments were cut at distal duodenum, mid-jejunum, and mid-ileum. The intestinal segments were opened longitudinally and the contents were flushed gently with PBS. Mucosa was collected by scraping using a sterile glass microscope slide at 4 °C. All samples were rapidly frozen in liquid nitrogen, and stored at −80 °C until analysis. These steps were completed within 15 min after killing.

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