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Effects of oridonin on immune cells, Th1/Th2 balance and the expression of BLys in the spleens of broiler chickens challenged with *Salmonella pullorum*

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ABSTRACT

The aim of this study was to investigate the effects of oridonin (ORI) on the immune cells, Th_1/Th_2 balance and the expression of B lymphocyte stimulator (BLys) in the spleens of broilers infected with *Salmonella pullorum*. In a completely randomized design, 300 one-day-old AA male broilers were divided to 5 treatments. The groups included a noninfection control (CON) group receiveed a basal diet; a *S. pullorum* infect control group received the basal diet; and *S. pullorum* infect group receiveed the basal diet plus 50, 80, and 100 mg/kg ORI, respectively. The results showed that *Salmonella* challenge increased the relative weights of the spleen, white blood cell counts, lymphocyte and heterophil percentage, H/L ratio, the concentration and mRNA levels of spleen proinflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, and IL-10, as well as the anti-inflammatory target Blys ($P < .05$), and modulated the Th1/Th2 balance ($P < .05$). ORI pretreatment decreased the relative weight of the spleen and inhibited the release and expression of these proinflammatory mediators and the anti-inflammatory target BLys. The results suggested that ORI supplementation may have immunosuppressive and multiple modulation effects on activated microglia through modulation of the Th1/Th2 balance and BLys expression.

1. Introduction

Salmonella pullorum is a fowl-specific pathogen that causes considerable economic losses through mortality and increased morbidity in broiler chickens (Shivaprasad, 2000; Wigley et al., 2001). The bacteria, when delivered through the oral, intraperitoneal, or subcutaneous routes, can enter the chicken immune system and then be taken up by macrophages and dendritic cells at local sites, which causes mononuclear phagocytes (MPs) and endothelial cells to produce immune regulators, such as TNF-α and members of the IL family, and causes clinical signs of hepatosplenomegaly accompanied by characteristic anemia, leukocytosis and hemorrhage (Loessner et al., 2007). Accordingly, it is very important to reduce chicken infection by this bacterium to ensure the safety of poultry food.

Antibiotic therapy has been widely utilized as a strategy to prevent *Salmonella* infection in poultry production industry (Zhang-Barber et al., 1999). But many countries in the European Union and North America have forbidded the use of antibacterials drugs as feed supplements due to the feasibility of antibiotic resistantance and to the residue effects of frequent antibiotic use in animal growth promoters. There is increasing pressure for forbidding the use of antimicrobials at the growth promotion and treating disease in food animal production (Hashemi and Davoodi, 2011). Alternative management and dietary strategies to control the incidence and severity of *S. pullorum* and to enhance host body resistance have become a topic of great interest. Due to the consumer preference for herbal plant extracts, the application of oridonin (ORI) has been increasing in appeal. The antibacterial property of ORI has been well recognized and widely tested *in vitro* against a large range of pathogenic bacteria, which includes both gram-positive and negative bacteria (Kadota et al., 1997).

Oridonin is an ent-kaurene diterpene compound isolated from the traditional Chinese herbs Rabdosia rubescens, also known as dong ling cao. As notes, ORI has complimentary biological and multiple pharmacological activities, for example, an antitumor, antioxidant, anti-inflammatory, antibacterial, and antiviral activities (Tian and Chen, 2013; Owona and Schluesener, 2015). Among the various biological activities of ORI, immune-regulatory and anti-inflammatory activity are very important. These could be ascribed to the immunoregulatory activity of oridonin, for example, T cell depletion in the peripheral immune system (Guo et al., 2013) and suppression of immunological

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cytokines (TNF-α, IL-1 β, IL-2, IL-6 and IL-10) productions (Hu et al., 2008; Xu et al., 2009). But so far there have been no available reports about the immunosuppressive effects of ORI on an animal model. So the purpose of present study is to investigate its preventive effect on the relative organ weight, immune cells, Th1/Th2 balance and expression of B lymphocyte stimulator (BLys) in the spleens of broiler chickens challenged with *Salmonella pullorum* to examine the potential immunoregulatory mechanism.

2. Materials and methods

2.1. Oridonin

ORI used in the experiment was purchased from Laieryin Biological Technology Company Limited (Luoyang, Henan province, P. R. China) with a purity of 98%.

2.2. S. pullorum

S. pullorum (CVCC 3377) serotype Enteritidis was used in this study and was obtained from the China Veterinary Culture Collection Center (Beijing, China). The selected *S. pullorum* was cultured in just trypticase soy broth at 41 °C for 12 h, washed, and then resuspended in 0.5 mL PBS (phosphate buffered saline, pH 7.2) at a final concentration of 10^8 CFU mL $^{-1}$. The *S. pullorum* concentrations were detected using traditional colony counting methods on a standard plate.

2.3. Broiler and experimental diets

During the feeding study, were willing to comply with all of the specific regulation and reguirements, which were approved by the Institutional Animal Care and Use Committee of Henan University of Science and Technology. The study was carried out in a commercial broiler chicken farm in Jianxi District, Luoyang city, China.

1 day-old 300 commerical male broiler chickens (Arbor Acres) were purchased from Xinan hatchery in Luoyang city, China, and were placed in separated cages of comparable size (1.3 m × 1.2 m) in a controlled environment remained at 32–34 °C during the first week, and then gradually reduced from 32 to 22 ± 1 °C on d 7 to 21 d. From d 1 to d 7, 23 h of lighting was offered, subsequently, 18 h lighting time every day at d 8 to d 21. There has no difference for the average initial body weight among the tests group. During the study period, a basal diet (corn and soybean meal, as a mash) with meeting or exceeding the recommendations of the National Research Council (NRC, 1994) and clean water were offered *ad libitum*. The basal diets (starter) were as shown in Table 1. The ORI was finally added and mixed on the basis of the balanced unmedicated diet. Prepared feed was placed in a sealed bottles at 4 °C.

2.4. Experimental design

The chickens were randomly allocated into 5 treatments, with 6 replicates of 10 broiler chickens per pen for 21 d. The 5 treatments were as follows: noninfection control (CON) group receiveed a basal diet; a *S. pullorum* challenge control (SCC) group received the basal diet; and *S. pullorum* control group receiveed the basal diet plus 50 (O1), 80 (O2), and 100 (O3) mg/kg ORI, respectively. Birds in the SCC, O1, O2, and O3 treatment groups were primed with 0.5 mL of *S. pullorum* Enteritidis suspension (1 × 10^9 CFU each bird) on d 3 post-hatch, and the CON chicks were primed the same amount of sterile buffered peptone water.

2.5. Sample collection and procedures

Four, eleven and eighteen days (at 7, 14 and 21 days of age) after the *S. pullorum* challenge, six bird per treatment group were randomly chooseded and were euthanized with cervical dislocation. 3.0 mL samples of blood were collected into microtube containing 100 μL of sodium citrate solution, mixed, and were immediately sent to the Veterinary Microbiology Laboratory for elevating the total and specific differential white blood cell (WBC) counts by the Wright's stain method. The carcasses of broiler were aseptically opened, and the sample of spleen and bursa were surgically removed to an ice-cold plate, and weighed to calculated the immune organ index. Then, these organ were rinsed with a physiological saline solution and splitted into two pieces, then stored at −80 °C with respect to the RNA quality and the concentration of immune stress indexes, respectively. In 2.5 mL of the ascorbate-EDTA solution, about 5 g spleen were homogenised by a mechanical homogenizer, centrifuged at 3000 × *g* for 15 min at 4 °C, and then collected and stored the supernatant of sample at −80 °C for the further quotation analysing. Each sample's protein concentration was quantitative analyzed by the Bradford method.

Table 1
Ingredients and nutrient level of the experimental diet (g/kg diet as fed basis).

Feed Ingredients (g/kg)	1-21d
Corn silage	575
Soybean meal	327
Corn gluten meal	30
Soybean oil	28
Limestone	9.5
Dicalcium phosphate	17.5
Salt	3
Choline chloride	3.0
Premix[a]	3.0
L-Lysine	2.5
DL-Methionine	1.5
Total	1000
Calculated nutrients levels (g/kg)	
AME (MJ/kg)	14.5
CP	232.0
Ca	10.7
Available Phosphorus	4.2
Lys	11.8
Met	4.8
Met + cys	9.1

[a] Each kg of premix contained: Fe (from ferrous sulfate), 80 mg; Cu (from copper sulfate), 8 mg; Mn (from manganese sulfate), 110 mg; Zn (Bacitracin Zn), 65 mg; iodine (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg. Vitamin A (transretinyl acetate), 10,000 IU; Vitamin D_3 (cholecalciferol), 3000 IU; Vitamin E (all-*rac*-α-tocopherolacetate), 30 IU; menadione, 1.3 mg; thiamine 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; calcium pantothenate, 10 mg; pyridoxine·HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B_{12} (cobalamine), 0.013 mg.

2.6. Detection of the splenic inflammatory cytokines levels

Enzyme-linked immunosorbent assay (ELISA) levels of TNF-α, IL-2, IL-4, IL-6, IL-10 and BLys were analyzed by the ELISA kits (monoclonal antibody against chicken for TNF-α, IL-2, Il-4, IL-6 and IL-10: Adlitteram Diagnostic Laboratories, San Diego, CA, USA; monoclonal antibody against mouse for BLys, R & D Systems, USA) in line with the producer's protocols. The measurement values were expressed as pg/mg protein.

2.7. Design of primers and detection of splenic TNF-α, IL-2, IL-4, IL-6, IL-10, BLys and qPCR

According to the instructions provided by the manufacturer, total RNA of spleen tissue was separated with TRIzol reagent (Invitrogen Invitrogen Trading (Shanghai) Co., Ltd., China). The RNA pellets were

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