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Short communication

Effect of dietary inulin supplements on growth performance and intestinal immunological parameters of broiler chickens

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ABSTRACT

The objective of this study was to assess the effects of dietary inulin supplementation on growth performance and intestinal immune parameters of broilers. A total of 280 one-day-old Cobb 500 male broilers were randomly allocated into four groups of seven replicate pens and given a maize-soybean basal diet supplemented with 0, 5, 10 and 15 g/kg of inulin during the 42 days of the experiment. Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were determined from d 1 to 21 (starter), and from d 22 to 42 (grower). Intestinal T lymphocyte subpopulations, the production of immunoglobulin A (IgA) and cytokines as well as mucin mRNA expression were measured at 21 d and 42 d. Feed intake was increased quadratically ($P=0.001$) as the dietary inulin level increasing during starter period only. However BWG and FCR of broilers were not affected by inulin supplementation in either period. At d 21 and as the dietary inulin concentration increasing, proportion of T $CD4^+$ T lymphocyte and $CD4^+/CD8^+$ ratio in ileum tissue tended ($P=0.05$–0.087) to be linearly increased, IgA concentration in cecal content and mucin mRNA expression in jejunum tissue were linearly increased ($P=0.006$–0.01), whereas concentrations of interleukin-6 and interferon-γ in ileum tissue quadratically ($P<0.05$) decreased. The effects of dietary inulin on these intestinal immunological parameters were minimal at the 42-d age of broilers. These results indicated that dietary inulin at the levels of 5–10 g/kg may have the beneficial effects on enhancing intestinal immune function of broiler chicken at younger age when the intestinal function is not fully developed.

1. Introduction

Use of prebiotics and probiotics as alternatives to antibiotics in poultry industry has become an increasing practice due to the global trend of banning on the use of in-feed antibiotics as growth promoters. Inulin-type fructans have attracted considerable attention from producers mainly because studies have showed various beneficial effects on animal performance when they are used as prebiotics (Flickinger and Fahey, 2002; Verdonk et al., 2005). Moreover, the inulin can selectively stimulate the growth and/or activity of the beneficial flora thereby benefit host wellbeing and health (Roberfroid, 2007). However, variations in the effects of inulin on growth performance (Ortiz et al., 2009; Rebolé et al., 2010) in poultry have been reported, possibly due to variations in the products used, inclusion levels in the diet, diet composition, animal characteristics and husbandry hygiene. Avian intestinal immune function has received increasing attention because it is closely associated with intestinal infectious diseases, such as coccidiosis and salmonellosis, which causes enormous losses in poultry industry (Lillehoj and Trout, 1996). It is regarded that avian gut-associated lymphoid tissue plays an important role in host defense against pathogenic invasion (Janardhana et al., 2009). Inulin and fructooligosccharides (FOS) have been shown to modulate immunological processes of animals and humans (Seifert and Watzl, 2007). However, there is little information in this area for birds. Janardhana et al. (2009) reported that FOS had an immunosuppressant effect at both functional and phenotypic levels in the cecal tonsil of broilers, but for inulin this effect is unknown. Therefore, the objective of the current study was to assess the effects of dietary supplementation of inulin on growth performance and on intestinal immune function of broiler chickens.

2. Materials and methods

2.1. Animal, experimental design and feeding management

A total of 280 one-d old Cobb 500 male broiler chickens (average body weight 40.74 ± 0.3 g) obtained from a local hatchery plant were used in a 42-d feeding experiment. The sex of the chicken was identified by an expert in the hatchery plant. All

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experimental protocols were approved by the Northwest A&F University Animal Care and Use Committee. The treatments were a maize-soybean basal diet supplemented with 0, 5.5, 11.0 and 16.5 g/kg of an inulin product (Orafti® GR, Tienen, Belgium). The product was derived from chicory and contained 92% inulin with an average degree of polymerization (DP) ≥ 10 and 8% glucose/fructose/sucrose. The actual inulin content in the diets were 0, 5, 10 and 15 g/kg. The treatments were arranged as completely randomized design. The broilers were first randomly allocated into 28 pens (10 birds per pen) that were then randomly divided into 4 groups (7 pens per group) and assigned to the treatments described above. The pens with plastic mesh floors (0.54 m^2 floor area/pen) were located in an environmentally controlled room. The temperature of the room was set at 34 °C for the initial 3 d and was gradually reduced by 2 °C per week. Birds received 24 h constant light during the first 3 weeks and then reduced to 16 h per day for the last 3 weeks. The broilers were fed starter diet from d 1 to d 21 (starter period) and grower diet from d 22 to d 42 (grower period) of the experiment. The basal diets (Table 1) for both periods were free of antibiotics and coccidiostats and were formulated according to the nutrient requirements of NRC (1994). Inulin was incorporated into the basal diet weekly by mixing it into respective diet to achieve the designated concentration.

Diets were offered twice daily (8:00 am and 6:00 pm) for *ad libitum* intake and birds had free access to water for the entire experimental period. Residual feed and birds were weighed weekly to determine the feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR).

2.2. Sample collection

On d 21 and d 42, two birds from each pen were randomly picked out and the jejunum (from the entrance of bile ducts to Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileo-cecal junction) were immediately removed and dissected after the birds were killed by cervical dislocation. A 3 cm long ileum section before the ileo-cecal junction was separated and kept at 4 °C for cell isolation. The remaining ileum and the whole jejunum were immediately placed into liquid nitrogen after being washed with ice-cold phosphate buffered saline (PBS; 0.01 M, pH = 7.4) and stored at −80 °C until assayed. In addition, the cecum with its content was also removed after being ligated at its both ends and stored at −80 °C.

Table 1
Composition (%, as-fed basis) of the starter (d 1–21) and grower (d 22–42) basal diets.

Item	Starter	Grower
Ingredients		
Maize	56.8	62.1
Soybean meal	36.6	31.8
Soybean oil	3.00	3.00
Limestone	1.00	1.00
Dicalcium phosphate	1.70	1.50
L-Lys · HCl	0.10	0.05
DL-Met	0.15	0.05
Salt	0.30	0.30
Choline chloride	0.15	0.05
Mineral premix[a]	0.10	0.10
Vitamin premix[b]	0.10	0.05
Nutrient levels (Calculated)		
Metabolizable energy (MJ/kg)	12.7	13.0
Crude protein (%)	21.3	19.59
Ca (%)	0.96	0.89
Available P (%)	0.43	0.39
Lysine (%)	1.22	1.05
Methionine (%)	0.52	0.41

[a] Mineral premix supplied the following per kg of diet: Mn, 120 mg; Zn, 80 mg; Fe, 80 mg; Cu, 8 mg; I, 0.35 mg, Se, 0.15 mg.

[b] Vitamin mixture supplied the following per kg of diet: retinyl acetate, 103.2 mg; cholecalciferol, 1.5 mg; menadione, 2 mg; thiamine, 2 mg; riboflavin, 7 mg; cyanocobalamin, 0.015 mg; DL-α-tocopheryl acetate, 7 mg; pyridoxine, 2.5 mg; niacin, 45 mg; pantothenic acid, 12 mg; folic acid, 0.1 mg; biotin, 0.11 mg.

2.3. Determination of intestinal lymphocyte subpopulations

The procedure used to isolate intestinal mucosal lymphocyte was modified from published approaches (Davies and Parrott, 1981; Chai and Lillehoj, 1988). Briefly, the mesentery-, fat- and blood vessels-free ileum segments were cut into < 0.5 cm pieces, washed with PBS containing 100 IU/ml penicillin and 100 μg/ml streptomycin and incubated at 37 °C with 20 ml digestive culture medium (Yang and Guo, 2006) for 90 min. The suspensions were filtered through 200 μm nylon cloth and the filtrates were centrifuged with an Allegra™ 64R Centrifuge (Beckman Coulter, Inc., CA) at 500*g* for 10 min. The resultant cell pellet was re-suspended in 3.0-ml 40% (v/v) Percoll medium (Pharmacia, Piscataway, NJ), which was then placed on 4.0 ml 70% (v/v) Percoll medium, and the cells were recovered from the interface of the two solutions after centrifuging at 800*g* for 30 min. The recovered cell fraction was washed twice with PBS and then re-suspended in 1.0 ml of RPMI-1640 culture medium to obtain a single lymphocyte cell suspension.

The lymphocyte subpopulations ($CD3^+$, $CD4^+$ and $CD8^+$ T cells) in the lymphocyte cell suspension prepared above were determined using the flow cytometric method (Holt et al., 2010; Yang and Guo, 2006). Viable cell counts were determined by trypan blue exclusion and the cell concentrations were adjusted to 2×10^6 viable cells/ml. The cell suspension (100 μl) was then incubated with mouse anti-chicken CD3-FITC, mouse anti-chicken CD4-PE and mouse anti-chicken CD8α-SPRD antibodies cocktails (Southern Biotech, Birmingham, AL) at 4 °C in the dark for 30 min. After incubation, the cells were washed twice with PBS and then re-suspended in 500 μl PBS and enumerated by a flow cytometer (Beckman).

2.4. Determination of intestinal IgA and cytokines

The cecal content was thawed at room temperature, thoroughly mixed and 1.0 ml subsample was mixed with 2.0 ml of PBS. The mixture was subsequently centrifuged at 5000*g* for 30 min and the supernatant was collected for measuring the IgA concentration with a commercial ELISA kit (KYM, Beijing, China) following the manufacturer' instructions.

The cytokines were determined in ileum tissue by first grinding 1.0 g of sample with 2.0 ml of PBS in mortar on ice, followed by centrifuging the homogenized suspension at 10,000*g* for 15 min to obtain the supernatant that was then used to determine the levels of interleukin (IL)-2, IL-6, IL-10 and interferon-γ (IFN-γ) by commercial ELISA kits (KYM, Beijing, China).

2.5. Quantitative real-time polymer chain reaction (RT-qPCR)

Total RNA was extracted from jejunum tissue using the TRIzol® Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The integrity and concentration of total RNA were determined by Epoch Microplate Spectrophotometer (BioTek Instruments, Inc., VT) at optical density (OD) 260/280. Reverse transcription was performed by a PrimeScript RT reagent Kit with gDNA Eraser (Takara, Dalian, China) following the manufacturer's protocol. The primers for mucin 2 gene (MUC2, *Gallus gallus*, GI423101) were 5′-AGGCCAGTTCTATGGAGCACAGTT-3′ (forward), and 5′-TTGAGTGCCCAGAGGGACATTTCA-3′ (reverse), and for β-actin (Beta-actin, *Gallus gallus*, GI: 396526) were 5′-