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Effects of synbiotic on the intestinal morphology and humoral immune response in broiler chickens

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ABSTRACT

The objective of this study was to investigate the effects of synbiotic on humoral response to Newcastle disease vaccine and intestinal morphology. A total of 108 one-d-old fast-growing broiler chickens (Ross 308) were allocated to pens, then, pens were assigned to 1 control and 2 treatments with 3 pens per treatment and 12 chickens per pen. Chickens were reared at standard condition for 6 week and provided a standard basal diet and diets supplemented with 0.1 or 0.2% synbiotic. On d 8, 22, 32, and 42, 12 chickens from each treatment were randomly selected and blood samples were collected. Antibody response was measured by the hemagglutination inhibition technique. On d 22, and 42, 12 chickens from each treatment were killed and 3 segments of intestine were dissected for evaluation of intestinal morphology. Antibody titers were increased in chickens fed the diet supplemented with synbiotic ($P<0.05$). Antibody level in chickens fed the diet supplemented with synbiotic increased at d 22, 32, and 42 compared to d 8. Amount of antibody was increased at d 32 compared to other day in chickens fed 0.2% synbiotic supplement ($P<0.05$). Antibody level was progressively reduced in the control group at d 22, 32, and 42. Duodenal villus height was greater in both treatments than their controls ($P<0.05$). Duodenal villus surface area was also greater in chickens fed 0.1% synbiotic supplement than controls at d 22, 32, and 42 ($P<0.05$). Jejunal villus width and surface area were lower in chickens fed 0.2% synbiotic supplement than controls on d 22 and 42 ($P<0.05$). Ileal villus height was lower ($P<0.05$) in chickens fed 0.2% synbiotic supplement than controls on d 42, while the villus width and surface area were only greater ($P<0.05$) in chickens fed the diet supplemented with 0.1% synbiotic. The sum of measured villus surface area in 3 intestinal parts was greater only in chickens fed 0.1% synbiotic supplement on d 42 ($P<0.05$). Villus types changed from leaf and tongue to convoluted and ridge shapes in both treatments on d 22 and 42 (ileum, 0.1 and 0.2% synbiotic) and 32 (jejunum, 0.2% synbiotic) compared to their controls ($P<0.05$). It is concluded that synbiotic had beneficial effects on antibody production, and antibody levels in chickens fed synbiotic supplement were maintained or increased during rearing. In intestine, only 0.1% synbiotic had positive effect on intestinal morphology.

1. Introduction

Synbiotics are defined as the combination of probiotics and prebiotics (de Vrese and Schrezenmeir, 2008). Probiotics are live microorganisms, which may have

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beneficial effects on health of the host when administered in adequate amounts. Probiotic bacteria have been used to improve animal performance, perhaps, by maintaining the normal microflora of host animals. The main action of probiotics is a reinforcement of the intestinal mucosal barrier against adverse agents (Fioramonti et al., 2003). Probiotic bacteria also stimulate antigen-specific and nonspecific immune responses. The ingestion of probiotics results in the reduction of some fecal enzymes, which are capable of converting pro-carcinogens to carcinogens in the gastrointestinal system (Shah, 2007). Prebiotics are defined as "non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth or activity or both of limited number of bacteria in the colon, which can improve host health" (Gibson and Roberfroid, 1995).

The main reason for using a synbiotic is that a probiotic, without its prebiotic 'feed source,' does not survive well in the digestive system. Without such a necessary feed source for the probiotic, it will have less intolerance for oxygen, low pH, and temperature (Sekhon and Jairath, 2010). Synbiotics encourage the growth of the probiotic organism by providing the specific substrate to the probiotic organism for its fermentation (Farnworth, 2001). In addition, the probiotic will have to compete against other bacteria that will take over if its specific feed source is not available. Synbiotics have been reported to provide different health benefits such as antimicrobial, anticarcinogenic, immunomodulatory, antidiarrhoeal, antiallergenic, hypolipidaemic, antitoxic, and hypoglycaemic activities. They also help in improving mineral absorption and balance and may have anti-osteoporotic activity (Zubillaga et al., 2001; Holzapfel and Schillinger, 2002; Slizewska et al., 2010)

Recent research of synbiotic has been focused on functional benefits such as resistance to gastrointestinal bacterial infection, antibacterial and immune activity in broiler chickens. It has been reported that dietary inclusion of synbiotic increased the growth performance and improved intestinal morphology and nutrient absorption (Awad et al., 2008). This study was designed to investigate whether high concentration of synbiotic (twice the amount used in previous studies) could increase humoral response to Newcastle disease vaccine and compensate the effect of weak vaccination route (i.e., drinking water) used in this experiment without any adverse effects on performance and intestinal morphology of broiler chickens.

2. Materials and methods

2.1. Animals, management and treatments

One hundred and eight, 1-d-old fast-growing broiler chickens (Ross 308) were assigned to 1 control and 2 treatments with 3 replicate pens per treatment and 12 chickens per pen. Chickens were housed in pens of identical size (1×1 m) in a deep litter system with wood shaving. Chicks were reared at standard condition for 6 week and provided ad libitum access to water and a standard basal diet. The basal diets were in mash form and formulated for starter (1–10 d), grower (11–24 d), and finisher (25–42 d) growth periods and the composition is shown in Table 1 (NRC, 1994). There was no coccidiostat added in the basal diets. For the treatments, synbiotic (Biomin IMBO; Biomin, Herzogenburg, Austria) was included in the starter and grower basal diets at a concentration of 0.1 or 0.2% of the diet. The symbiotic was a combination of probiotic *Enterococcus faecium* (DSM 3530 strain), prebiotic fructo-oligosaccharides, cell wall fragments, and phycophytic substances derived from sea algae.

Table 1
Composition of basal diets.

Item	Starter (1–10 d)	Grower (11–25 d)	Finisher (25–42 d)
Ingredient (%)			
Corn	52.73	53.82	59.67
Soybean meal (44% CP)	37.89	36.04	30.57
Soybean oil	4.05	5.62	5.42
Limestone	1.24	1.01	0.99
Dicalcium phosphate	2.08	1.82	1.7
Vitamin mixture[a]	0.50	0.50	0.5
Mineral mixture[b]	0.50	0.50	0.5
Salt	0.22	0.22	0.22
DL-M et Bicarbonate Na	0.31	0.23	0.20
ThrL-Lys ≅ HCl	0.17	0.17	0.17
	0.07	0.01	0.01
	0.24	0.06	0.05
Calculated chemical composition			
ME (kcal/kg)	3025	3150	3200
CP (%)	22	21	19

[a] Supplied per kilogram of diet: vitamin A, 9000 IU; cholecalciferal, 1500 IU; vitamin E, 10 IU; vitamin K, 0.5 mg; cobalamin, 0.007 mg; thiamin, 0.4 mg; riboflavin, 6 mg; folic acid, 1 mg; biotin, 0.15 mg; pantothenic acid, 12 mg; niacin, 35 mg; pyridoxine, 4 mg; and cholin chloride, 1000 mg.

[b] Supplied per kilogram of diet: Mn, 60 mg; Cu, 5 mg; Zn, 50 mg; I, 0.35 mg; Se, 0.1 mg; and Fe, 40 mg.

Newcastle disease vaccine was administered in drinking water at 9 (V_4 strain), 18, and 27 d (La Sota strain) of age for all groups. The feed offered and refused were weighed and recorded daily in the morning to estimate the intake. Feed consumption and body weight were recorded in each group; feed conversion rate was calculated at the end of experiment. The study was approved by the Ethics Committee of Shahrekord University.

2.2. Blood sampling and antibody response analyses

On d 8, 22, 32 and 42, 12 chickens from each group were randomly selected (4 chickens from each pen on each day) and blood samples were collected into 5-mL vacuum tubes, and serum samples were stored at -20 °C until analysis. Antibody response was measured by the hemagglutination inhibition technique. Briefly, 25 μL of serum containing antibody was serially diluted into a 96-well plate with PBS (pH 7.4, 4 °C). The same volume of Newcastle disease virus (NDV) antigen was added to react and bind with the antibody. Addition of 2% red blood cell solution in each well should show the ability of NDV left to agglutinate with red blood cells. If enough antibodies were to be bound to virus during the incubation period,