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The Effect of Dietary Supplementation of Prebiotic and Probiotic on Performance, Humoral Immunity Responses and Egg Hatchability in Broiler Breeders

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Abstract

In this experiment, the influence of prebiotic and probiotic supplementation in the broiler breeder diets on body weight, mortality, feed intake, egg production, hatchability and humoral immunity response was investigated. A total number of 13140 female and 1260 male breeders (Cobb 500) with 26 wks of age were allocated to three treatments with six replicates (800 birds each replicate). Breeders were fed control basal diet, basal diet supplemented with prebiotic (mannan oligosaccharide) or probiotic (Protexin®) for 17 weeks. Body weight, feed intake and egg production were measured weekly during 26-40 wks of age. The hatchability of eggs was recorded on weeks 38, 39, and 40. Antibody production was recorded after 8 wks of prebiotic and probiotic supplementation. Prebiotic supplementation did not affect feed intake, the percentages of egg production and settable eggs percents. Prebiotic increased egg hatchability and reduced the percentages of infertile eggs, as well as dead embryo-in-shells. Antibody titers against influenza and reovirus were higher in prebiotic fed group, but there were no significant differences among the other blood antibody titers. Probiotic had no significant effect on the considered parameters. In conclusion, findings of present study showed that prebiotic improved egg hatchability and humoral immunity of broiler breeders.

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Introduction

Nowadays, using antibiotics at sub-therapeutic levels has caused concerns about antibiotic residues in the animal productions which lead to the development of drug-resistant bacteria in animals and human. Thus, medical and public concerns focused on the complete removal of the antibiotics from animal feed in the European Union at the beginning of 2006 (Nollet, 2005; Wakeman, 2005; Cervantes, 2006). Therefore, poultry industry has been looking for the substances that could replace antibiotic growth promoters (AGP) in the feed (Bach Knudsen, 2001). Application of feed additives has two objectives: controlling pathogenic microorganisms and enhancing beneficial microorganisms in the digestive content of the gut (Vahdatpour *et al.*, 2011).

Prebiotics are defined as nondigestible food ingredients that can be utilized by intestinal microflora, which beneficially affect the host (Gibson and Roberfroid, 1995). Prebiotics alter the intestinal microbes and immune system to reduce colonization by the pathogens in certain conditions (Hajati and Rezaei, 2010). Some oligosaccharides, carbohydrates composed of short chains of mono-saccharides, enhance the growth of beneficial organisms in the gut and the others are thought to function by competing with the pathogenic bacteria for attachment sites in the lumen. Thus, prebiotic oligosaccharides may improve the animal health. The two most commonly oligosaccharides with prebiotic characteristics are fructo-oligosaccharides (FOSs) and mannan oligosaccharides (MOSs). FOSs are found naturally in some cereal crops and onions (Bailey *et al.*, 1991), and MOSs are obtained from the cell walls of yeast (*Saccharomyces cerevisiae*). FOSs can be fermented by bifidobacteria and lactobacilli (Bouhnik *et al.*, 1994; Gibson and Roberfroid, 1995), which are generally classified as beneficial bacteria (Gibson and Wang, 1994; Flickinger *et al.*, 2003). FOSs may help to control or reduce the growth of harmful bacteria such as *Clostridium perfringens* that is important to the poultry industry because it is a primary cause of necrotic enteritis (Hofacre *et al.*, 2005). Mannose is the main component of MOSs. It is bound by the type I fimbriae being used by many enteric bacteria to attach to host cells. Thus, mannose can result in the movement of undesirable bacteria through the intestine without colonization (Newman, 1994). Supplementation of MOSs increased the production of IgA in laying hens (Kim *et al.*, 2009). Immunoglobulin A inhibits the attachment and penetration of bacteria in the lumen, increases the production of mucus (McKay and Perdue, 1993), and prevents inflammation that could cause epithelial tissue damage (Russell *et al.*, 1989).

Probiotics are microbial cell preparations. They are mono or mixed cultures of live, protective microorganisms that beneficially affect the host animal by competing with the other microorganisms for adhesive sites of the gastrointestinal tract. They can stimulate appetite, improve host's intestinal microbial balance and intestinal environment for the processes of digestion and absorption of nutrients.

They also inhibit the growth of certain pathogens that produce toxic compounds (Patterson and Burkholder, 2003).

Protexin is a multi-strain probiotic containing live microbes to establish, enhance or re-establish essential microflora in the gut. Protexin is a highly concentrated pre-mix containing seven strains of bacteria and two yeasts (*Lactobacillus plantarum* 1.89 × 10^{10} CFU / Kg, *Lactobacillus delbrueckii* subsp. *bulgaricus* 3.09 × 10^{10} CFU / Kg, *Lactobacillus acidophilus* 3.09 × 10^{10} CFU / Kg, *Lactobacillus rhamnosus* 3.09 × 10^{10} CFU / Kg, *Bifidobacterium bifidum* 3.00× 10^{10} CFU / Kg, *Streptococcus salivarius* subsp. *thermophilus* 6.15 × 10^{10} CFU / Kg, *Enterococcus faecium* 8.85 × 10^{10} CFU / Kg, *Aspergillus oryza* 7.98 × 10^{9} CFU / Kg, *Candida pintolopesii* 7.98 × 10^{9} CFU / Kg). All the microorganisms in Protexin are naturally occurring and have been isolated from a wide range of feed, plant, animal, bird and human sources. Protexin is reported to be safe, non-toxic and residual free. There are no risks of overdosing and it is compatible with all feeds, feed ingredients like vitamins and minerals, and some antibiotics (International Animal Health, 1999). Therefore, the purpose of this study was to compare the effects of supplementing the prebiotic of Bio-MOS (Alltech, Inc. USA) and probiotic of Protexin to broiler breeders' diet on performance, mortality, hatchability and humoral immunity.

Materials and Methods

Animals

In this study, 14400 Cobb 500 broiler breeders (13140 female and 1260 male) with 26 wks of age were used. The birds were reared on a wood shaving litter and randomly allocated into 18 pens, each of 800 birds (730 females and 70 males).

Experimental diets

Two basal diets (Table 1) were formulated according to the Cobb 500 broiler breeder recommendations (Cobb-Vantress, 2013) and offered to female and male birds as the control treatment. Bio-MOS (Alltech, Inc. USA) and protexin® (Probiotic International Limited, UK) were added to the basal diet as 0.1 and 0.01 percent, respectively, and offered to the female birds as prebiotic and probiotic treatments. Each of the three treatments was allocated to six replicates. Feed additives were first added to wheat bran, blended with premixes and then to other ingredients. The experimental data was collected from 26 to 40 wks of age in summer days in a private farm (Mazandaran Province, North of Iran). The birds were under feed and water restriction to control their body weight.

Measuring performance

The live body weight of breeders, feed intake and egg weight were recorded weekly. Feed conversion ratio was calculated by dividing the amount of feed intake by the average egg weight production. Mortality was recorded daily and

egg production, settable eggs, and double-yolked eggs were measured weekly. The hatchability of eggs was recorded on weeks 38, 39, and 40 as a percentage of total settable eggs.

Table 1. Ingredients and nutrient composition of the basal diets

Ingredients (%)	Female breeders	Male breeders
Corn	57.40	57.80
Soybean meal	17.00	11.00
Barley	7.00	12.00
Canola meal	5.00	1.00
Wheat bran	0.50	11.40
Soybean oil	1.30	-
Dicalcium phosphate	1.90	1.60
Oyster shell	6.10	1.40
Anzimit	2.00	2.00
Sodium chloride	0.33	0.30
Mineral premix[1]	0.30	0.30
Vitamin premix[2]	0.30	0.30
Vitamin A	0.10	0.10
Vitamin B	0.20	0.20
Vitamin E	0.20	0.20
Vitamin D3	0.10	0.10
Choline Chloride	0.10	-
DL-Methionine	0.10	0.30
L-Lysine	0.05	-
L-Threonine	0.02	-
Calculated composition		
ME (Kcal / Kg)	2750	2700
CP (%)	14.61	13.00

[1]Mineral premix supplied the following per Kg of diet: Cu, 2400 mg; Fe, 32000 mg; Mn, 39700 mg; Se, 80 mg; Zn, 24000 mg.

[2]Vitamin premix supplied the following per Kg of diet: vitamin A, 3,800,000 mg; vitamin D3, 800,000 mg; vitamin E, 8000 mg; vitamin K3, 1000 mg; vitamin B1, 788 mg; vitamin B2, 2,400 mg; vitamin B3, 4,712 mg; vitamin B5,9,900 mg; vitamin B6, 1,182 mg; vitamin B9, 320 mg; vitamin B12, 5.6 mg; vitamin H2, 40 mg.

Humoral immunity

To evaluate the antibody titer, blood samples were collected from the wing vein of 10 birds in each pen at the end of 34 wks of age. Serum samples were stored at -20°C in separate sterile vials. To measuring the anti-body titer against Newcastle disease virus, the hemaglutination inhibition test was done to determine the antibody titer as $\log_2$ of the reciprocal of the last dilution (Marquardt *et al.*, 1984). For measuring the anti-body titer against Infectious Bursal Disease Virus (IBDV) and Infectious Bronchitis Virus (IBV), serum samples were thawed at 22°C,

diluted 500-fold with diluents. 100 µL of the diluted sera was added to 96-well microplates coated with either IBV or IBD (IDEXX Inc., Westbrook, USA). Then, they were covered and incubated at 22°C for 30 min. The microplates were aspirated and washed with 350 µL of sterile distilled water. Then, 100 µL of substrate was dispensed into the wells to develop a color reaction after incubation for 15 min at 22°C. The enzymatic reaction was ended by adding the stop solution. The plates were read on a microplate reader at 650 nm (Molecular Devices, Sunnyvale, CA 94089) to evaluate the antibody titer against IBV and IBD (Kidd *et al.*, 2001). The antibody titer against influenza, reovirus, and *Ornitobacterium rhinotracheal* (ORT) was measured by the enzyme-linked immunosorbent assay (ELISA) with a commercial kit (IDEXX Inc., Westbrook, USA). The test procedure and analysis of the results were performed as recommended by the manufacturer.

Statistical Analysis

The data were analyzed using a completely randomized design (SAS, 2000). The Means were compared using Duncan's new multiple range test (Duncan, 1955).

Results and Discussion

Breeder Performance

The Effects of prebiotic and probiotic on body weight and mortality in female breeders are shown in Table 2. The results showed that dietary treatments had no significant effect on body weight and mortality (*P>0.05*).

Table 2. Effects of prebiotic and probiotic on body weight and mortality of female broiler breeders (26 to 40 wk)

Age	Body Weight (g)			SEM	Mortality (%)			SEM
(wk)	Control[1]	Prebiotic[2]	Probiotic[3]		Control[1]	Prebiotic[2]	Probiotic[3]	
26	3200	3210	3213	18.61	0.0009	0.0008	0.0008	0.00005
27	3247	3270	3282	22.42	0.001	0.001	0.001	0.00008
28	3406	3445	3458	22.80	0.001	0.001	0.001	0.00008
29	3453	3461	3477	22.51	0.002	0.001	0.001	0.00007
30	3508	3479	3501	23.04	0.001	0.001	0.003	0.00008
31	3520	3526	3532	23.56	0.003	0.002	0.001	0.00007
32	3538	3542	3551	25.12	0.002	0.001	0.001	0.00006
33	3581	3589	3603	26.35	0.001	0.001	0.001	0.00008
34	3639	3653	3663	29.07	0.001	0.001	0.001	0.00007
35	3672	3690	3698	29.45	0.004	0.001	0.002	0.00008
36	3698	3701	3712	29.33	0.001	0.001	0.001	0.00008
37	3730	3740	3745	31.47	0.003	0.001	0.001	0.00009
38	3751	3760	3768	29.18	0.001	0.002	0.001	0.00008
39	3760	3771	3779	28.05	0.002	0.001	0.001	0.00008
40	3770	3784	3790	34.18	0.001	0.001	0.001	0.00008

[1]Control treatment was the basal diet and free of antibiotics; [2]Prebiotic treatment was prepared by adding 0.01% mannan oligosaccharide to the basal diet; [3]Probiotic treatment was prepared by adding 0.1% Protexin® to the basal diet.

The effects of prebiotic and probiotic on feed intake and feed conversion ratio during the 26-40 wks are shown in Table 3. The results showed that prebiotic and probiotic supplementations improved feed conversion ratio of broiler breeders numerically, however, the differences were not significant statistically (*P>0.05*). It has been claimed that the benefits of MOS is based on its specific properties such as modification of the intestinal flora, reduction in turnover rate of the intestinal mucosa and modulation of the immune system (Hajati *et al*., 2012).

Table 3. Effects of prebiotic and probiotic on feed intake and feed conversion ratio of female broiler breeders (26 to 40 wk)

Age (wk)	Feed intake (g/bird/week)			SEM	Feed conversion ratio (g feed intake/g egg production)			SEM
	Control[1]	Prebiotic[2]	Probiotic[3]		Control[1]	Prebiotic[2]	Probiotic[3]	
26	862	862	862	7.53	17.58	15.45	16.14	0.069
27	902	902	902	7.68	5.32	5.14	5.05	0.035
28	945	945	945	7.85	3.63	3.51	3.57	0.034
29	990	990	990	7.31	3.16	3.12	3.12	0.034
30	1080	1080	1080	8.49	3.21	3.06	3.10	0.035
31	1171	1171	1171	8.12	3.30	3.25	3.23	0.034
32	1155	1154	1154	8.04	3.28	3.18	3.16	0.036
33	1155	1154	1154	8.68	3.24	3.14	3.15	0.035
34	1155	1154	1154	8.65	3.22	3.10	3.11	0.034
35	1155	1154	1154	8.67	3.18	3.04	3.07	0.033
36	1148	1146	1147	8.85	3.19	3.11	3.11	0.034
37	1141	1139	1140	8.83	3.17	3.06	3.09	0.035
38	1141	1139	1140	8.79	3.20	3.12	3.17	0.035
39	1141	1139	1140	9.02	3.33	3.12	3.23	0.034
40	1141	1139	1140	9.11	3.31	3.16	3.21	0.034

[1]Control treatment was basal diet and free of antibiotics; [2]Prebiotic treatment was prepared by adding 0.01% mannan oligosaccharide to the basal diet; [3]Probiotic treatment was prepared by adding 0.1% Protexin® to the basal diet.

It is well known that the gastrointestinal tract plays a vital role in the digestion and absorption of nutrients required for the maintenance and growth of animals. The proliferation of pathogens in the intestines often results to inflammatory responses that cause productivity losses, increased mortality, as well as contamination of poultry products. Sub-therapeutic antibiotics have long been used in broiler diets for growth improvement and control of intestinal pathogens (Baurhoo *et al*., 2009). Gram-negative pathogens that express type I fimbriae, such as *Salmonella* and *Escherichia coli*, recognize D-mannose receptor sites on the intestinal epithelium. The adhesive properties of type I fimbriae are determined by the fimbrial tip containing a mannose-specific lectin, FimH (Thomas *et al*., 2004). Mannose, either in the pure form (Oyofo *et al*., 1989) or yeast cell wall (Spring *et al*., 2000), competitively binds to the FimH lectin of gram-negative pathogens. This concept was demonstrated by reduced intestinal colonization of *Salmonella* and *Escherichia coli* after the addition of MOS to chicken diets (Baurhoo *et al*., 2007a).

The addition of a commercially available MOS product (BioMos, Alltech Inc., Nicholasville, KY) to broiler diets significantly increased goblet cell number in the intestinal villi (Baurhoo *et al.*, 2007b). Goblet cells are specialized cells that secrete mucins glycoprotein compounds which bind pathogenic microorganisms and reduce their adherence to the intestinal mucosa (Blomberg *et al.*, 1993).

Results concerning the influences of Bio-MOS and Protexin supplementation on the egg production, settable eggs, and double-yolked eggs in broiler breeders during 26-40 wks of age are shown in Table 4. There were not any significant differences in the mention parameters among treatments. This may be related to the breeder rearing condition (hot summer days which caused heat stress), or the status of the birds gut microflora. It seems that the amount of prebiotic and probiotic supplemented in this study was not sufficient to improve egg production, settable eggs and double-yolked eggs. However, prebiotic and probiotic supplementation could improve numerically egg production from 30 wks of age, and settable eggs were higher from 28 wk of age. Sultan and Abdul-Rahman (2011) reported that probiotic supplementation improved egg weight, yolk weight and egg production of broiler breeders. They concluded that this improvement may be due to the improvement of hormonal status, especially FSH which enhances follicle growth and LH which enhances ovulation rate. They also reported that the improvement of gut ecosystem and metabolic activities (such as digestion, absorption and assimilation of nutrient) helps the birds to perform better. Berry and Lui (2000), and also Stanley *et al.* (2000) have reported considerable improvements in egg production of the MOS-fed birds. MOS reduces the pathogenic bacteria load in the intestine and prevents the acute immune response against such bacteria (Spring *et al.*, 2000). It was hypothesized that nutrients are efficiently diverted toward production in MOS-fed birds, which might improve egg production in layers and breeders. Shashidhara and Devegowda (2003) stated that the inconsistency in results of using feed additives such as prebiotics may be due to the variation in breed and age of birds. In this experiment, prebiotic supplementation could numerically reduce double-yolked eggs in the whole experiment. Hajati and Rezaei (2010) reported that beneficial effects from addition of prebiotics is reflected in the presence of antagonism towards pathogens, competition with pathogens, promotion of enzyme reaction, reduction of ammonia and phenol products, increasing resistance to colonization, improvement in the gut health (improved intestinal microbial balance) and performance, enhanced nutrient utilization (*e.g.* amino acids and proteins), as well as decreasing the environmental pollution and production costs.

Table 4. Effect of prebiotic and probiotic on egg production, settable eggs and double-yolked eggs of female broiler breeders (26 to 40 wk)

Age (wk)	Egg Production (%)			SEM	Settalbe Eggs (%)			SEM	Double-yolked Eggs (%)			SEM
	Control[1]	Prebiotic[2]	Probiotic[3]		Control[1]	Prebiotic[2]	Probiotic[3]		Control[1]	Prebiotic[2]	Probiotic[3]	
26	13.21	15.04	14.4	0.48	53.87	54.13	55.14	0.98	1.34	1.26	1.36	0.10
27	44.06	45.65	46.5	0.41	77.2	79.28	76.60	0.94	1.31	1.26	1.34	0.10
28	66.4	65.56	67.8	0.42	79.9	82.14	80.99	1.01	1.31	1.24	1.33	0.09
29	76.54	77. 4	77.4	0.42	84.2	85.06	84.27	1.1	1.29	1.20	1.33	0.09
30	80.2	84.26	83.3	0.45	86.8	87.06	86.87	1.1	1.28	1.18	1.30	0.09
31	83.4	85.02	85.4	0.45	88.4	89.18	88.04	1.12	1.27	1.18	1.29	0.08
32	81.7	84.4	84.8	0.45	89.3	90.40	89.8	1.12	1.25	1.16	1.28	0.09
33	81.5	84.4	84.2	0.46	89.8	91.3	91.0	1.14	1.21	1.00	1.24	0.07
34	81.1	84.4	84.2	0.47	89.7	91.0	90.7	1.13	1.21	0.89	1.18	0.07
35	81.0	84.8	83.9	0.47	89.7	91.0	90.3	1.13	1.17	0.85	1.09	0.08
36	79.5	81.6	81.5	0.48	89.1	90.5	90.2	1.08	1.16	0.82	1.05	0.08
37	79.2	81.8	81.2	0.48	89.0	89.7	89.5	1.07	1.01	0.85	0.89	0.09
38	78.4	79.82	77.8	0.53	88.9	89.7	89.4	1.05	0.93	0.84	0.86	0.07
39	74.5	79.43	76.9	0.54	88.5	89.7	89.0	1.05	0.93	0.80	0.85	0.08
40	74.4	77.88	76.8	0.54	88.0	89.4	88.8	1.04	0.9	0.79	0.82	0.08

[1]Control treatment was the basal diet and free of antibiotics; [2]Prebiotic treatment was prepared by adding 0.01% mannan oligosaccharide to the basal diet; [3]Probiotic treatment was prepared by adding 0.1% Protexin® to the basal diet.

Table 5. Effect of prebiotic and probiotic on hatchability, infertile eggs and dead-in-shells of broiler breeders (26 to 40 wk)

Age (wk)	Hatchability (%)			SEM	Infertile Eggs (%)			SEM	Dead-in-shells (%)			SEM
	Control[1]	Prebiotic[2]	Probiotic[3]		Control[1]	Prebiotic[2]	Probiotic[3]		Control[1]	Prebiotic[2]	Probiotic[3]	
38	89.4	90.91	89.8	0.36	4.6	4.2	4.5	0.16	6.0[a]	4.89[b]	5.7[a]	0.14
39	89.0	90.62	89.5	0.34	5.0	4.4	4.8	0.15	6.0[a]	4.98[b]	5.7[a]	0.13
40	88.5b	90.75[a]	88.7[b]	0.35	5.9[a]	4.7[b]	5.2[a]	0.15	5.6[ab]	4.55[b]	6.1[a]	0.14

[1]Control treatment was the basal diet and free of antibiotics; [2]Prebiotic treatment was prepared by adding 0.01% mannan oligosaccharide to the basal diet; [3]Probiotic treatment was prepared by adding 0.1% Protexin® to the basal diet.

[a,b]For each characteristics, means within a row with different superscripts differ significantly ($P<0.05$).

Hatchability

The effects of prebiotic and probiotic on hatchability, infertile eggs and dead-in-shells in broiler breeders are shown in Table 5. Prebiotic supplementation increased hatchability of eggs and decreased infertile eggs in 40 wk of age. Dead-in shells was reduced during 38-40 wks by prebiotic supplementation. Probiotic supplementation also had beneficial effects and numerically increased hatchability and decreased infertile eggs, as well as dead-in shells. There are several factors that affect hatchability. Breeder factors that affect hatchability include health, nutrition and egg size, weight and quality. Egg weight, shell thickness, and shape index are the most important factors among egg parameters that influence hatchability (King'ori, 2011). Shashidhara and Devegowda (2003) reported that mannan oligosaccharides increase fertility and hatchability in broiler breeders. Abd El-Samee *et al.,* (2013) reported that supplementing diets with Bioplex Zinc up to 40 mg/Kg alone or in combinations with 1.0 g mannan oligosaccharides/Kg significantly improved egg hatchability in quails. At the present study, it is presumed that the effects of environmental factors on the difference of hatchability among treatments are negligible because hatching eggs from all groups were subjected to the same environmental variation during the storage and incubation. The differences in hatchability may be due to a higher sperm density in MOS-females. Although reports pertaining to MOS supplementation and its effect on hatchability in breeders are limited, similar improvements in hatchability have been observed in birds fed a yeast culture supplement (McDaniel and Sefton, 1991).

Humoral Immunity

The effects of prebiotic and probiotic on humoral immunity are shown in Table 6. Antibody titer against influenza and reovirus was significantly higher in prebiotic fed group ($P<0.05$), but there was no significant difference among other antibody titers. Hajati *et al.* (2012) reported that MOS has the capacity to bind pathogenic organisms such as *Salmonella* and *Escherichia coli*, and can stimulate the immunity system. Antibody responses have been used as measures of the humoral immune status of birds (Sklan *et al.*, 1994). One hypothesis is that defensive cells in the gut associated with the lymphoid tissue (GALT) detect the presence of microbes by recognizing molecules unique to microorganisms that are not associated with host cells. These unique molecules are called pathogen associated molecular patterns. They include yeast cell wall components such as mannan and glucan along with other microbial molecules such as peptidoglycan, lipopolysaccharide and glycolipids (Salyers and Whitt, 2001). Mannan and glucan of the yeast cell wall may bind to pattern recognition receptors on a variety of defensive cells of the GALT and activate immune defenses such as phagocytosis, the alternative complement pathways, and the lectin pathway.

Raid *et al.*, (2010) reported that adding prebiotic to broiler diets resulted in a significant increase of antibody titer against SRBC. Abd El-Samee *et al.,* (2012) reported that supplementing diets of growing Japanese quails reared during summer in Egypt with 20 or 40 mg Bioplex Zinc/Kg alone or in combination with 1.0 g/Kg prebiotic (mannan oligosaccharides) had no significant effect on the productive performance, but improved their immune response. Similar to our results, Sadeghi *et al.*, (2013) reported that prebiotic supplementation improved the immune responses and health of the chicks infected with pathogens.

Table 6. Effect of prebiotic and probiotic on humoral immunity of broiler breeders on 32 wk (log $_2$)

	Control[1]	Prebiotic[2]	Probiotic[3]	SEM
Influenza antibody titer	4.7[b]	6.6[a]	4.8[b]	0.22
Newcastle antibody titer	6.6	7.1	6.8	0.25
IBV[4] antibody titer	13.2	13.4	13.2	0.31
Reovirus antibody titer	5352[b]	6912[a]	5015[b]	40.9
ORT[5] antibody titer	4912	5032	4920	35.8
IBDV[6] antibody titer	8507	8543	8530	46.3

[1]Control treatment was the basal diet and free of antibiotics; [2]Prebiotic treatment was prepared by adding 0.01% mannan oligosaccharide to the basal diet; [3]Probiotic treatment was prepared by adding 0.1% Protexin® to the basal diet; [4]Infectious Bronchitis Virus; [5]*Ornitobacterium rhinotracheal*; [6]Infectious Bursal Disease Virus.

[a,b]Means within a row with different superscripts differ significantly (*P<0.05*).

Conclusion

In the condition of present experiment, prebiotic supplementation had better effect on performance, hatchability and humoral immunity compared to probiotic in broiler breeders. However, further studies are needed to evaluate how different factors such as age, genetic, nutrition, health status, rearing conditions and also gut microbial population of the broiler breeders can affect probiotic and prebiotic responsiveness. Furthermore, optimized levels of these additives in breeders should be examined.

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