The Effect of a Postbiotic Produced by Stabilized Non-Viable *Lactobacilli* on the Health, Growth Performance, Immunity, and Gut Status of Colisepticaemic Broiler Chickens

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Abstract

This work was designed to evaluate the efficacy of a postbiotic compound produced by stabilized non-viable *Lactobacilli* on the health, growth performance, immunity, and gut status against *Escherichia coli* (*E. coli*) challenge of broiler chickens. A total of 400, day-old broiler chicks were allocated into 4 equal groups (1-4) consisting of 100; each assigned into 2 equal replicates (50 each). Chickens in the 1st group were received the dry form of the compound at doses of 1 kg and 0.5 kg /ton feed for starter and grower, and the finisher diets; respectively. Chickens in the 2nd group were given the aqueous form of the compound in a dose of 4 ml/liter of the drinking water during the first 3 days of life and at a day before and after each vaccination. Feed and water treatments regimens were administered to chickens in the 3rd group. Group (4) was kept without treatment. Each bird in the 1st, 2nd, 3rd, and 4th group was challenged with *E. coli* (O78) at one-week-old. All groups were kept under observation till 5-week-old. The results indicated that both treatments with the postbiotic compound induced more significant ($P \leq 0.05$) amelioration of a disease picture, enhancement of growth performance, boosting of immune response, improvement of bursa/body weight ratio, and reduction of intestinal coliform count in challenged chickens when compared with challenged non-treated chickens. In conclusion, the posbiotic compound either in a dry and/or an aqueous form is recommended for improving the health, performance, and immunity of colisepticaemic broiler chickens.

Introduction

Poultry industry is considered one of the most important source of income all over the world. Avian colibacillosis is an infectious disease that caused by *Escherichia coli* (*E. coli*) (Lutful Kabir 2010). The disease is associated with heavy economic losses including mortality, decrease in productivity, and food-borne illnesses (Koutsianos et al. 2021). The most common avian pathogenic serogroups of *E. coli* are O78, O1, O2, and to some extent O15 and O55 (Ali et al. 2019). Antibiotic treatment is commonly used for the controlling such infection. However, excessive usage of these antimicrobials is associated with the production of antibiotic-resistant strains of *E. coli* (Radwan et al. 2021). These resistant strains infect humans either directly or via consumption of contaminated carcass (Valiakos and Kapna 2021). Thus, Food and Drug Administration Veterinary Feed Directive in 2017 prohibited the usage of antibiotics in farm animals as growth promoters (Veterinary Feed Directive 2019). Nowadays, the urgent challenge of poultry industry all over the world is how to promote the production parallel with keeping safe product to the consumer. Some antibiotics alternatives have recently used to improve the health and encourage the production of safe and foodborne disease-free products (Abd El-Ghany 2020).

Competitive exclusion compounds such as probiotics, prebiotics, and synbiotics are extensively used to prevent or reduce the intestinal colonization with *E. coli* especially in young age (Mohamed and Younis 2018). Although probiotics have been used to promote the health of gut environment, growth performance, and immune response of poultry, they may acquire and transfer antibiotic resistance genes among microorganisms (Shazali et al. 2014). For instance, some probiotics of *Lactobacilli* species carry antibiotic's resistant genes for tetracycline and chloramphenicol that transfer among bacteria (Sharma et al. 2014). Besides, probiotics may have a negative influence on the host by increasing the severity of tissues inflammation (Tsilingiri et al. 2012). Subsequently, probiotics as living bacteria might not be used in the future. Recently, soluble non-viable probiotic metabolites or postbiotics, have been used as a promising and potential substitute for both antibiotics and probiotics in poultry industry (Loh et al. 2010). Postbiotics may be part of cell wall or cytoplasmic extracts of *Lactobacilli* species (Johnson et al. 2019). They are not affected by acids, pH, or environmental conditions, so they can be kept safely with longevity. Moreover, postbiotics of *Lactobacilli* origin have valuable components such as organic acids and bacteriocin which improve lactic acid bacteria growth (Loh et al. 2014).

Therefore, the present study was planned to assess the efficacy of a postbiotic compound produced by stabilized non-viable *Lactobacilli* on the health, growth performance, immunity, and gut status of challenged broiler chickens with *E.*

Materials And Methods

The experiment was carried out in strict compliance with the recommendations of the National Regulations on Animal Welfare and following the guidelines approved by the Institutional Animal Care and Use Committee.

Postbiotic Compound

Lactobacillus species fermentation product (Culbac®, TransAgra's International Inc., Chicago, Illinois, USA) is produced either in a dry or an aqueous form. The recommended manufacture dose of the dry form (Culbac® Animal Dry) is 1 kg/ton of the starter and grower diets and 0.5 kg/ton of the finisher diet. However, the dose of the aqueous form (Culbac® Animal Healthy Start) is 4 ml/liter of the drinking water during the first 3 days of age and at one day before and after routine vaccination program.

Chicks And Experimental Design

A total of 410, day-old broiler chicks (Hubbard breed) of mixed sex was obtained from a local hatchery in Giza governorate, Egypt. On arrival, ten birds were sacrificed and subjected for microbiological examination to prove their freedom of *E. coli*. The remaining 400 chicks were allocated into 4 equal groups (1-4) consisting of 100; each assigned into 2 equal replicates (50 each). All the birds were kept in deep litter system under restricted hygienic measures for 5 weeks. Vaccination was done against Newcastle disease virus (NDv) using live HB1 and La Sota strains at 6 and 18-day-old, respectively, inactivated avian influenza (AI) H5N1 strain at 7-day-old, and infectious bursal disease virus using Intermediate live strain at 13-day-old. All the vaccines were given via eye drop method, except AI vaccine was given subcutaneously (S/C) at the back of the neck. Chickens were fed on commercial balanced diets containing local feed ingredients and formulated to meet the National Research Council requirements (NRC 1994) (Table 1). Birds were fed on starter, grower, and finisher diets at ages 1-15, 16-28, and 29-35 days, respectively. Feed and drinking water were provided *ad libitum*. The dry form of Culbac® was applied as feed treatment in groups (1) and (3), while the aqueous form was given in the drinking water for groups (2) and (3). Each chick in groups (1, 2, 3, and 4) was challenged S/C with 0.3 ml nutrient broth of *E. coli* serotype (O78). Identified field strain of *E. coli* serotype (O78) was isolated from chickens with systemic colisepticaemia. The bacterial culture concentration was adjusted to 10^8 colony forming unit (CFU) of *E. coli*/ ml (Fernandez et al. 2002).

Measured Parameters

Clinical picture

All groups were kept under complete observation for 5 weeks recording clinical signs, mortalities, and post-mortem lesions after *E. coli* challenge.

Growth performance

On arrival, random chicks were collected and the initial body weight was measured. The body weights, feed intake, and feed conversion rate (FCR) were weekly assessed in all groups along 5 weeks experimental period (Sainsbury 1984).

Immune response

Ten blood samples were collected from each group at day-old and weekly till 5 weeks of age. The blood was kept in refrigerator for several hrs and then centrifuged for serum separation. The antibody titers against NDv vaccine were estimated using Haemagglutination Inhibition (HI) test with 4 haemagglutinating units (HAU) (OIE 2002). Briefly, in each well of HI plates, 4 HAU of virus/ antigen were added, kept for 30 min, and then 0.050 ml of 0.5% chicken red blood cells was added for 30 min. Serum samples including positive and negative controls were distributed in wells. Inhibition at a dilution of 1/16 or more against 4 HAU was considered as positive HI.

Bursa/ body weight ratio

On weekly basis, 5 randomly selected chickens from each group were sacrificed, weight, and the bursae of Fabricius were incised and weight to determine bursa/body weight ratio.

Total coliform count

Five birds from each group were sacrificed at 5-week-old. One hundred gram of the intestinal contents was homogenized with 0.5 ml of sterile buffered peptone and 10-fold serial dilutions for each homogenate from the initial dilution (10^{-1}) were made. Approximately 0.1 ml of each dilution was incubated on a MacConkey agar plate at 37°C for 24 hr. The results of the count were expressed as the number of the organism $\log_{10}$ CFU/g of the intestinal content (Huang et al. 2019).

Histopathological examination

At 5-week-old, 5 birds from each group were sacrificed. Specimens from liver and intestine were collected, fixed in 10% neutral buffered formalin, dehydrated in different grades of ethyl alcohol, and embedded in paraffin wax. Microtomy of the tissue to 5 μ m thickness was done and stained with hematoxylin and eosin stain for histopathological examination by the light microscope (Bancroft and Gamble 2007).

Statistical analysis

The results were statistically analyzed according to Snedecor and Cochran (1980). One-way ANOVA analysis of variance was adopted using SAS® software general linear models procedure. Significant differences between treatment means were considered significant at level $P \leq 0.05$.

Results

Clinical picture

Signs of depression, off food, ruffling, fever, and greenish diarrhea were observed 2 days post *E. coli* challenge. The severity of signs was markedly decreased in the treated groups as compared to non-treated one. The effect of different postbiotic treatments on the mortality and protection rates of *E. coli* challenged chickens are presented in Table (2). Chicken's mortality began 3 days post-challenge and continued for 4 days in non-treated birds, while it subsided in the treated chickens 2 days post-challenge. Combined feed and water treatments with the postbiotic compound showed the highest protection rate of 93%, whereas non-treated challenged group revealed protection rate of 73%. The protection rates of postbiotic treatment in feed and water were 90% and 88%, respectively. The post-mortem lesions of *E. coli* challenged chickens were severe septicemia, hemorrhages on the internal organs, liver congestion and necrosis, and enteritis. Variable lesions' severities were observed among groups. The severest lesions were recorded in non-treated challenged chickens compared with the lowest lesions in postbiotic treated chickens.

Growth performance

The results of the growth performance parameters of different groups are shown in Table (3). Application of postbiotic compound in feed and water significantly ($P \leq 0.05$) improved the performance parameters of broilers. The highest significant ($P \leq 0.05$) average body weights were recorded in chickens treated with combined postbiotic treatments in feed and water (1360.7), followed by feed (1245.9) and water (1196.5) treatments when compared with non-treated chickens (987.8). The best FCR (1.6) was in postbiotic treated groups, while the worst one (2.2) was in non-treated group.

Immune response

The immune response against NDv vaccine using HI test revealed that the geometric mean maternal antibody titer of day-old-chicks was 6.8. The HI titers against NDv vaccine were significantly ($P \leq 0.05$) higher in chickens treated with combined feed and water postbiotic treatments than non-treated chickens along the whole experimental period (Table 4).

Bursa/body weight ratio

The measured means bursae/body weight ratio in Table (5) indicate that the highest significant ($P \leq 0.05$) ratios were in postbiotic-treated groups when compared with non-treated group. At the end of the study, the means bursae/body weight ratio were 0.291, 0.288, and 0.272 in combined feed and water, feed, and water postbiotic treated groups, respectively, however, this ratio was 0.203 in non-treated group.

Total bacterial count

In comparison with non-treated challenged chickens, postbiotic treated chickens showed significant ($P \leq 0.05$) reduction in the total intestinal coliform count at the end of observation period (Table 6).

Histopathological examination

Figure (1) shows the results of the histopathological examination of liver and intestine in different groups. In group treated with the postbiotic in feed, the liver showed apparently normal hepatic parenchyma (A), while the intestine showed mild inflammation (B). Postbiotic treatment in water revealed focal portal hepatitis (C) and moderate enteritis (D). However, in group received combined feed and water treatments, apparently normal hepatic parenchyma (E) and mild enteritis (F) have been observed. Challenged group with *E. coli* without treatment exhibited disorganization of the hepatic plates admixed with numerous scattered sporadic cells necrosis (G) and severe necrosis of the intestinal villi with accumulation of inflammatory cells and tissue debris in the submucosa (H).

Discussion

The devastating effects of avian colibacillosis are particularly evident in poultry field due to poor biosecurity and husbandry practices especially in developing countries (Ebrahimi-Nik et al. 2018). The disease induces significant economic annual losses in the global poultry industry (Lau et al. 2010). Prevention and control of colibacillosis have commonly been accomplished by the use of antibiotics. The emergence and rapid dissemination of antibiotic-resistant *E. coli* resulted in reduction in the drug effectiveness and might pose substantial risks for humans' health (Belanger et al. 2011). Accordingly, it was necessary to find viable alternative sources to maintain the health of the birds (Seal et al. 2013). One of these substitutes is "biotic feed additives" such as probiotics, prebiotics, and postbiotics (Klemashevich et al. 2014). Postbiotics are defined as a secondary metabolites from probiotics without viable or living cells (Thanh et al. 2009). Components of cell wall and cytoplasmic extracts of numerous *Lactobacillus* (*L.*) species such as *L. acidophilus*, *L. plantarum*, *L. fermentum*, *L. casei*, *L. rhamnosus*, *L. paracasei*, *L. Rhamnosus*, *L. delbrueckii* subsp.

Bulgaricus, *L. gasseri*, *L. helveticus*, *L. reuteri*, and *L. johnsonni* were found to be highly effective postbiotics (Cicenia et al. 2016; Tiptiri-Kourpeti et al. 2016). Bacteria used for postbiotics production should be nonpathogenic, technologically suitable for industrial processes, acid and bile resistant, and good producers for antimicrobial substances that modulate immune responses and influence the metabolic activities of the gut (Dunne et al. 1999). The compound that used in this study (Culbac®) has been produced by fermentation process of proprietary strain of *Lactobacilli* and then stabilization for rendering the bacteria non-viable or dead. The non-viable bacterial fermentation product (postbiotic) is exposed to extensive processing to modify the cell contents and allow exposure of the cell wall to the digestive bacteria.

Here, decreasing in the severity of *E. coli* clinical picture after feed and water postbiotic treatments. Similarly, postbiotic metabolites of *Pediococcus acidilactici*, *Enterococcus faecium*, *L. reuteri*, and *L. acidophilus* reduced mortality and lesion scores of *C. perfringens* challenged broiler chickens as compared to challenged non-treated chickens (Johnson et al. 2019). Moreover, the postbiotic from different *L. plantarum* strains showed a trend of lower mortality in broiler chickens as compared with other treatments such as antibiotic growth promotor and ascorbic acid (Humam et al. 2019).

In this study, postbiotic treatment of broiler chickens improved the weight gains and the FCR. It has been documented that postbiotics can be used as feed additives to promote the health and growth performance in broilers (Kareem et al. 2017) and layers (Choe et al. 2012; Loh et al. 2014). Thanh et al. (2009) reported that chickens fed metabolites combination produced by *L. plantarum* had higher final body weight and weight gain compared with those fed the negative control diet. Heat stressed broiler chickens fed on diet containing postbiotics of *L. plantarum* showed promoting weight gain and feed conversion efficiency as a result of increasing hepatic insulin-like growth factor 1 mRNA expression level (Humam et al. 2019). In addition, a combined mixture of postbiotics and prebiotic in the diet of broiler chickens enhanced the total body weight and the feed efficiency in terms of increasing in liver insulin like growth factor 1 and growth hormone receptor mRNA expressions (Kareem et al. 2016). Improvement in weight gain was observed after treatment of *C. perfringens* challenged broiler chickens with a postbiotic when compared with non-treated control (Johnson et al. 2019). However, Rosyidah et al. (2011) found no significant difference in body weight or weight gain of chickens fed on metabolites and combination of metabolite and acidifier and those fed positive and negative control diets.

These findings may be owing to the ability of postbiotics to reduce the number of pathogenic intestinal microorganisms, leading to better gut health and growth performance. Postbiotics may be also similar to probiotics regarding the enhancement of nutrient transporter gene expression (Na⁺-dependent glucose, galactose transporter, and long-chain acyl CoA dehydrogenase genes) which promote broiler growth performance (Jahromi et al. 2016). Besides, *Lactobacilli* species may increase the utilization of nutrients due to upregulation of nutrient gene expression resulting in improvement of the bodyweight of broilers (Kalavathy et al. 2003). Organic acids and bacteriocins are antimicrobial metabolites of postbiotics that may decrease the pH and prevent the proliferation of pathogens in the gut of animals (Aguilar-Toalá et al. 2018). Moreover, supplementation with postbiotics could enhance the growth performance and health via improving the immune status, gut health, and intestinal villus, increasing lactic acid bacteria, and decreasing *Enterobacteriaceae* and intestinal pH (Loh et al. 2010; Kareem et al. 2016).

The immune response to NDv vaccines was modulated after postbiotic treatments in the present study. Moreover, improved bursa of Fabricius/body weight ratios were observed in postbiotic treated chickens as compared with non-treated chickens. The current results are in agreement with those of Johnson et al. (2019) who found that postbiotics metabolites mixture of *Pediococcus acidilactici*, *L. reuteri*, *Enterococcus faecium*, and *L. acidophilus* was able to stimulate the immune response in *C. perfringens* infected broiler chickens. Moreover, the levels of immunoglobulin M (IgM) and IgG were significantly higher in broilers received postbiotics in feed than those received ascorbic acid. Stimulation of immune response of birds after postbiotic treatment might be related to presence of peptidoglycan (β -

glucan) in 90% of the dry weight of *Lactobacilli* with lipopolysaccharides and teichoic and lipoteichoic acids in the bacterial cell wall (Adams 2010). In addition, after *Lactobacilli* lysis or degradation by host gastric acid, the bacterial DNA (CpG motifs) released and recognized by the host as a foreign antigen, and consequently both the cell mediated and humoral immune responses could be stimulated (Kant et al. 2014).

The current result showed that the total intestinal coliform count was reduced in postbiotic treated groups while it was high in non-treated group. The bacteriostatic and bactericidal activity of postbiotics have been reported. During fermentation of *Lactobacilli* bacteria, metabolites are produced, released, and stimulated the growth of beneficial microorganisms (Caldwell 2015). Some of beneficial bacteria are fastidious and require a lot of nutrients such as amino acids, energy (sugars), and metabolites (vitamin B complex) provided by the host. Accordingly, postbiotics can provide these bacteria by nutrients such as inulin or cellulose that present in their cell walls. In addition, postbiotics can reduce the harmful bacterial multiplication in the gut. Parallel results were found in pigs which showed reduction in *E. coli* count after treatment with cell free extracts of *Lactobacilli* fermentation process (Pollman et al. 1982; Blomberg et al. 1993; Thu et al. 2011). A postbiotic metabolite of *L. plantarum* either alone or in combination with a prebiotic showed inhibitory effects of some pathogens such as *E. coli*, *Salmonella typhimurium*, vancomycin-resistant *enterococci*, and *Listeria monocytogens* (Thanh et al. 2009; Van Thu et al. 2011; Choe et al. 2013; Kareem et al. 2014). A significant increase in lactic acid bacteria and decrease in *Enterobacteriaceae* count were observed in broiler chickens fed on *L. plantarum* (Rosyidah et al. 2011) or a mixture of postbiotic and inulin (Kareem et al. 2016). Furthermore, the postbiotic produced from a mixture of *L. plantarum* strains significantly increased the total bacteria and *Lactobacilli* counts but decreased *Salmonella*, *E. coli*, and *Enterobacteriaceae* counts in broilers when compared to control groups (Humam et al. 2019). In human, lipoteichoic acids of *L. acidophilus* and *L. johnsonni* maintained human's gut homeostasis and used treatment of diseases caused by Gram-negative bacteria (Vidal et al. 2002). Also, *L. paracasei* cells supernatant protected tissues from invasive *Salmonella* (Tsilingiri et al. 2012).

The histopathologic examinations revealed that *E. coli* challenged group exhibited disorganization and necrosis of the hepatic cells plus severe necrosis of the intestinal villi with inflammatory cells. The current observation is in tandem with that of Abalaka et al. (2017). In contrast, chickens treated with combined postbiotic treatment in feed and water or in feed alone showed apparently normal hepatic parenchyma and mild intestinal inflammation. Improvement of intestinal villi height was observed in broiler chickens treated with metabolite combinations in feed (Thanh et al. 2009; Loh et al. 2010). Moreover, addition of postbiotics and inulin combinations to the diet of broiler chickens beneficially altered the intestinal mucosal architecture in terms of longer villi (Kareem et al. 2016). Recently, Humam et al. (2019) demonstrated that a mixture of *L. plantarum* metabolites significantly increased the intestinal villus height to crypt depth ratio.

In conclusion, this study showed that the used postbiotic (Culbac®) either in a dry or an aqueous form was effective in amelioration of colisepticaemia in broiler chickens. Moreover, the synergistic beneficial action of both treatment regimens when supplemented together was proposed. Combined feed and water treatment with the postbiotic was even better than a separate treatment. Under present experiment conditions, reduction of clinical disease picture, improvement of performance parameters, modulation of immune response, and decreasing in intestinal coliform count were proven after postbiotic treatment of *E. coli* challenged chickens. This may indicate the growth promoting, immunomodulatory, and the antibacterial activities of the tested natural compound (Culbac®) which may be used in the near future for opposing this serious devastating infection in poultry.

Declarations

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Author contribution

The idea for the paper was conceived by WAA and FHE. The experiments were designed by WAA and FHE. The experiments were performed by WAA. The data were analyzed by WAA. The paper was written by WAA. The paper was revised by FHE. All authors read and approved the manuscript.

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Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was conducted according to the ethical standards applied to animal research and approved by Animal Welfare and Institutional Animal Ethical Committee.

Conflict of interest

The authors declare no competing interests.

Code availability

Not applicable

Consent for publication

All authors hereby declare that they participated in the development of the manuscript titled (Assessment of the capacity of stabilized non-viable lactobacillus to counteract colisepticaemia in broiler chickens). They have read the final version and give the consent for the article to be published in Tropical Animal Health and Production.

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Tables

Table (1). Composition of diets ingredients given for Hubbard broiler chickens during 5 weeks observation period

Composition	Starter	Grower	Finisher
Metabolized energy (kcal/kg)	3000	3150	3200
Crude protein %	23.0	22.0	19.0
Soybean meal (45%)	330.5	302.7	250.9
Yellow maize (9%)	57.94	57.94	57.94
Maize gluten meal (60%)	70.2	70.1	65.7
Fat	32	45	41
Lysine	2.9	2.7	3.5
Methionine	2.2	2.0	2.2
Dicalcium phosphate	18	18	18
Sodium chloride	4	4	4

Each g of the mineral mixture of the diet contained: IU: vit. A 9000, vit. D3 2500, vit. E 17; mg, vit. K3 2.5, vit. B1 1.7, vit. B2 6.6, vit. B6 2.4, vit. B12 0.015; mg choline chloride 400, Mn 80, Fe 40, Zn 70, Cu 8, Se 0.3.

Table (2). Effect of postbiotic treatments on clinical status in different groups

Group	Treatment	<i>E. coli</i>	Mortality %	Protection %
1	Feed	+	10	90
2	Water	+	12	88
3	Feed and water	+	7	93
4	-	+	27	73

Number of birds/group (n=100)

Mortality %= Number of dead birds/total number of birds (n=100)

Protection %= Number of survived birds/total number of birds (n=100)

Table (3). Effect of postbiotic treatments on performance parameters in different groups

Group	Treatment	<i>E. coli</i>	Average body weight/gm (Mean±SD) /age/week					FCR
			1 st	2 nd	3 rd	4 th	5 th	
1	Feed	+	140.1±7.6 ^a	290.3±6.9 ^b	578.9±22.6 ^b	890.6±65.3 ^a	1245.9±65.0 ^a	1.61 ^b
2	Water	+	138.9±3.2 ^a	270.9±8.1 ^{ab}	556.1±25.4 ^{ab}	863.9±54.7 ^b	1196.5±46.8 ^b	1.63 ^b
3	Feed and water	+	142.5±5.4 ^a	320.6±5.7 ^a	607.5±27.1 ^a	910.9±49.6 ^a	1360.7±40.2 ^a	1.61 ^b
4	-	+	135.1±3.1 ^a	299.9±7.9 ^b	530.6±19.8 ^{ab}	815.7±54.8 ^b	987.8±70.1 ^c	2.2 ^a

Means with different letters (a, b, c) within the same column are significantly different at P≤0.05

FCR= Feed conversion rate

Table (4). Effect of postbiotic treatments on haemagglutination inhibition titers to Newcastle disease virus vaccine in different groups

Group	Treatment	<i>E. coli</i>	HI titers (Mean±SD) (log ₂)/age/week				
			1 st	2 nd	3 rd	4 th	5 th
1	Feed	+	7.71±0.2 ^a	7.56±0.2 ^a	7.30±0.1 ^{ab}	7.06±0.1 ^{ab}	6.75±0.1 ^{ab}
2	Water	+	7.37±0.1 ^b	7.20±0.1 ^{ab}	7.07±0.2 ^{ab}	6.79±0.3 ^b	6.28±0.1 ^b
3	Feed and water	+	7.97±0.2 ^a	7.80±0.1 ^a	7.76±0.1 ^a	7.21±0.1 ^a	6.96±0.2 ^a
4	-	+	6.88±0.1 ^b	6.98±0.3 ^b	6.79±0.1 ^b	6.22±0.2 ^b	5.89±0.1 ^b

Means with different letters (a, b, c) within the same column are significantly different at P≤0.05

Haemagglutination inhibition: (HI)

Table (5). Effect of postbiotic treatments on bursa/body weight ratio in different groups

Group	Treatment	<i>E. coli</i>	Bursa/ body weight ratio (gm) (Mean±SD)/age/week				
			1 st	2 nd	3 rd	4 th	5 th
1	Feed	+	0.086±0.02 ^{ab}	0.192±0.05 ^a	0.199±0.01 ^{ab}	0.226±0.07 ^{ab}	0.288±0.03 ^a
2	Water	+	0.080±0.06 ^{ab}	0.156±0.01 ^{ab}	0.194±0.07 ^{ab}	0.220±0.08 ^{ab}	0.272±0.07 ^{ab}
3	Feed and water	+	0.099±0.05 ^a	0.194±0.08 ^a	0.228±0.02 ^a	0.253±0.06 ^a	0.291±0.09 ^a
4	-	+	0.069±0.09 ^b	0.135±0.07 ^b	0.151±0.03 ^b	0.193±0.01 ^b	0.203±0.05 ^b

Means with different letters (a, b, c) within the same column are significantly different at $P \leq 0.05$

Bursa/body weight ratio = [Bursa weight (g)/body weight (g)] × 100.

Table (6). Effect of postbiotic treatments on intestinal coliform count in different groups

Group	Treatment	<i>E. coli</i>	Intestinal coliform count $\log_{10}$ (CFU/g)
1	Feed	+	$4.10 \times 10^5 \pm 0.01^a$
2	Water	+	$4.72 \times 10^5 \pm 0.03^a$
3	Feed and water	+	$3.98 \times 10^5 \pm 0.02^a$
4	-	+	$8.06 \times 10^6 \pm 0.03^b$

Means with different letters (a, b, c) within the same column are significantly different at $P \leq 0.05$

Figures

Figure 1

Caption not included with this version.