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Original Research Article

Dietary synbiotic supplementation improves the growth performance, body antioxidant pool, serum biochemistry, meat quality, and lipid oxidative stability in broiler chickens

Kapil Dev [a, b], Nasir Akbar Mir [a], Avishek Biswas [a, *], Jyoti Kannoujia [c], Jubeda Begum [d], Rajiv Kant [b], Asitbaran Mandal [a]

[a] ICAR-Central Avian Research Institute, Izatnagar, Bareilly 243122, India
[b] Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad 211007, India
[c] CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, India
[d] G.B. Pant University of Agriculture & Technology, College of Veterinary & Animal Sciences, Pantnagar 263145, India

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ABSTRACT

The present study investigated the effects of *Lactobacillus acidophilus* (LBA) and mannan-oligosaccharides (MOS) supplementation on the production performance, serum biochemistry, antioxidant profile, health indices, meat quality, and lipid oxidative stability of broiler chicken. A total of 252 commercial broiler chickens at 1 d old of uniform body weight were randomly allocated to 6 maize-soybean-based dietary treatments: T_1 (control diet), T_2 (antibiotic bacitracin methylene di-salicylate [BMD] at 20 mg/kg diet), T_3 (MOS at 0.1% + LBA at 10^6 CFU/g feed), T_4 (MOS at 0.1% + LBA at 10^7 CFU/g feed), T_5 (MOS at 0.2% + LBA at 10^6 CFU/g feed), and T_6 (MOS at 0.2% + LBA at 10^7 CFU/g feed). Each treatment was assigned to 6 replicates of 7 birds. The samples for meat quality and serum biochemistry analysis were taken from 12 birds per treatment (2 birds/replicate). The results revealed better ($P < 0.01$) growth performance and production efficiency of birds fed either T_5 or T_6 diet compared to control or BMD supplemented diet and BMD-supplemented birds superseded the control birds. Higher ($P < 0.01$) serum and liver antioxidant enzyme activities, meat antioxidant capacity (2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid [ABTS] and 1, 1-diphenyl-2-picrylhydrazyl [DPPH] assays], serum total protein, high-density lipoproteins (HDL) cholesterol ($P < 0.05$), and globulin levels ($P < 0.01$) were observed in birds fed either T_5 or T_6 diet compared to control or BMD supplemented birds, whereas, lower lipid oxidation ($P < 0.01$), cardiac risk ratio, atherogenic coefficient, atherogenic index of plasma, serum glucose, triglyceride, total cholesterol levels ($P < 0.01$), and serum albumin-to-globulin ratio ($P < 0.05$) were observed in the chickens. The pH of meat from birds fed T_4, T_5 or T_6 diet was lower ($P < 0.01$) compared to control and other treatments. The extract release volume (ERV), water holding capacity (WHC), and protein content of meat were higher ($P < 0.05$) in birds fed either T_5 or T_6 diet compared to control or BMD supplemented birds. Thus, it was concluded that the supplementation of 0.2% MOS along with LBA at 10^6 CFU/g is optimum for better growth performance, serum biochemistry, antioxidant profile, health indices, meat quality, and lipid oxidative stability of broiler chickens.

1. Introduction

The selection pressure imposed by the use of antibiotic growth promoters in agricultural settings for better health and productivity of animals has hastened the evolution and spread of the resistance genes in pathogens (Begum et al., 2018). This has led to a strong resentment against the use of antibiotic growth promoters which compelled the animal scientists to arrive at the alternative

* Corresponding author.
E-mail address: drbiswas007@rediffmail.com (A. Biswas).
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strategies to maintain gut health and productivity of broiler chickens (Amerah et al., 2013). Thus, the mixtures of probiotic and prebiotic, called synbiotics, are gaining popularity and scientific credibility as functional feed supplements in poultry nutrition. The prebiotics are non-digestible carbohydrates which selectively affect the intestinal bacteria and immunity of broiler chicken (Bozkurt et al., 2014). The most commonly used prebiotic is manann-oligosaccharide (MOS) which inhibits the colonization of enteric pathogens, enhances immunity, modifies microflora fermentation to favor nutrient availability for the host, enhances the brush border mucin barrier, reduces enterocyte turnover rate, and enhances the integrity of the gut lining (Ferket, 2003). Probiotics, also called direct-fed microbials, improve the health and growth performance of broiler chicken (Lee et al., 2010) by immunomodulation, competitive exclusion of gut pathogens, and by improving the diversity and stability of intestinal microflora (Lee et al., 2010; Patterson and Burkholder, 2003). The different strains of *Lactobacillus* have been reported to improve the growth performance and immunity; and limit the growth of gut pathogens of broiler chicken (Mookiah et al., 2014; Ramasamy et al., 2009).

The physicochemical properties of meat are important since they determine to a great extent the possibilities for its storage or further processing (Popova, 2017). The pH of meat is a significant index of meat quality which is closely related to other important characteristics such as water holding capacity (WHC) or extract release volume (ERV) and the effect of probiotics on them depends on the type of microorganisms used (Popova, 2017; Welglarz, 2010). Further, lipid oxidation is one of the major causes for food quality deterioration which is generally accompanied by development of off-odours and flavours, and also formation of substances considered cancerogenic (Popova, 2017). The use of different probiotics and prebiotics has shown reduced lipid oxidation in chicken meat by displaying lower thiobarbituric acid reactive substances (TBARS) (Bobko et al., 2015; Zhang et al., 2005; Capcarova et al., 2010). The meat of broiler chicken fed probiotics like *Lactobacillus acidophilus* (LBA) and *Lactobacillus casei* had higher content of moisture, protein, and ash compared to the control (Khaksefidi and Rahimi, 2005). Also, the synbiotic supplementation has been reported to exert hypocholesterolemic effect by altering the pathways of cholesteryl esters and lipoprotein transporters (Liong et al., 2007).

The use of the combination of prebiotics and probiotics produce synergistic effects in broiler chicken because the prebiotics enhance the survival and multiplication of probiotics by increasing their tolerance to high temperature, oxygen, and low pH (Sekhon and Jairath, 2010; Alloui et al., 2013). However, the synergetic effects of synbiotics in broiler chicken have not been reported consistently in previous studies, which may be due to the variations in the compatibilities of probiotics with prebiotic oligosaccharides in in vitro studies, followed by their evaluation in broiler chicken directly (Mookiah et al., 2014). Thus, the present study investigated the production performance, serum biochemistry, antioxidant profile, health indices, meat quality, and lipid oxidative stability of broiler chickens fed diet supplemented with LBA probiotic along with prebiotic MOS.

2. Materials and methods

2.1. Ethics statement

The experimental procedures carried out in the study were approved by the Institutional Animal Ethics Committee (IAEC) following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) 2012 established under the Prevention of Cruelty to Animals Act 1960 of Indian Penal Code.

2.2. Supplements

The antibiotic bacitracin methylene di-salicylate (BMD) with 44% bacitracin activity was purchased from ALPHARMA Animal Health Division New Jersey-USA. The MOS was purchased from Kothari Fermentation & Biochem Ltd. India. The LBA (UBLA-34 MTCC 5401) was purchased from Unique Biotech Ltd. India. The LBA UBLA-34 was of healthy human fecal origin, characterised by Whole Genome Sequencing and deposited at DDBJ/ENA:GenBank under the accession number RBHY00000000. The LBA UBLA-34 was certified genetically safe as it did not contain any putative virulence factors, antibiotic resistant genes and plasmid. The LBA UBLA-34 used in the study were Gram positive rods in the form cream to brown coloured powder with water activity of less than one. Pathogens like *Escherichia coli*, *Salmonella*, *Staphylococcus*, and *Pseudomonas* were absent in 10-g powder, and yeast mould count was not more than 100 CFU/g. As far as the knowledge of authors, this was the first study of its kind to use this LBA strain as a potential probiotic in poultry nutrition.

2.3. Birds, experimental design and management

The experiment was conducted as per a completely randomized design. A total of 252 straight run (sex ratio $\approx$ 1) CARIBRO Vishal commercial broiler chickens at 1 d old of uniform body weight were randomly divided in to 36 replicate groups with 7 birds in each. The BMD, MOS, and LBA were used in broiler chicken diets to formulate 6 maize-soybean meal based dietary treatments viz., T_1 (negative control diet), T_2 (positive control diet containing antibiotic BMD at 20 mg/kg diet), T_3 (MOS at 0.1% + LAB at 10^6 CFU /g feed), T_4 (MOS at 0.1% + LAB at 10^7 CFU/g feed), T_5 (MOS at 0.2% + LAB at 10^6 CFU/g feed), and T_6 (MOS at 0.2% + LAB at 10^7 CFU/g feed). Each treatment was assigned to 6 replicates of 7 birds. Birds were housed in specially designed battery brooder cages providing 0.093 ft^2 per bird. The ingredients and nutrient composition of basal diet of broiler chicken in mash form is given in Table 1. The birds were vaccinated according to the routine vaccination programme followed at the concerned research institute and provided ad libitum respective feed and fresh water throughout the feeding trial of 42 d. The birds were provided 24 h of light on d 1 followed by a decrease of 1 h per day till it reached 18 h of light period which was continued till the end of trial. The initial cage temperature was 35 °C which was reduced by 2.78 °C every week to provide thermo comfort environment to the birds.

2.4. Growth monitoring and measurements

The weighed amount of feed was offered ad libitum daily and body weight of birds was taken on weekly basis to arrive at overall body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) under respective dietary treatments. Furthermore, the growth efficiency parameters such as production efficiency factor (PEF), protein efficiency ratio (PER), and energy efficiency ratio (EER) were calculated as follows (Mir et al., 2019).

$$PEF = [\text{Final body weight (kg)} \times \text{Livability (\%)} \times 100]/\text{Age in days} \times \text{FCR}$$

$$PER = \text{Weight gain (g)}/\text{Protein intake (g)}$$

$$EER = [\text{Weight gain (g)}/\text{Total energy intake (ME kcal)}] \times 100$$

Table 1
Ingredients and nutrient composition of broiler pre-starter, starter, and finisher diets (DM basis, g/kg).

Item	Pre-starter (0–7 d)	Starter (8–21 d)	Finisher (22–42 d)
Ingredients			
Maize	443	460	505
Soyabean	410	380	342
Rape seed meal	30	30	30
Fish meal	50	50	30
Oil	42	55	65
Limestone	6.0	6.0	7.0
Di-calcium phosphate	13.5	13.6	15.5
Salt	3.0	3.0	3.0
DL-Methionine	0.2	0.2	0.2
TM premix[1]	1.0	1.0	1.0
Vitamin premix[2]	1.5	1.5	1.5
Vitamin B complex[3]	0.15	0.15	0.15
Choline chloride	0.50	0.50	0.50
Nutrient composition of diets (analysed)			
Crude protein	231	220	200
Metabolizable energy, kcal/kg	3,001	3,101	3,200
Calcium	10.0	10.0	10.0
Available P	4.9	4.8	4.6
Lysine	13.3	12.1	10.6
Methionine	5.0	5.0	4.6

[1] Trace mineral (TM) mixture (100 g): $FeSO_4 \bullet 7H_2O$ 8 g, $ZnSO_4 \bullet 7H_2O$ 10 g, $MnSO_4 \bullet H_2O$ 10 g, $CuSO_4 \bullet 5H_2O$ 1 g, KI 30 g.
[2] Vitamin premix (1 g): vitamin A 82.5 IU, vitamin E (50%) 160 mg, vitamin D_3 12,000 U, vitamin K 10 mg.
[3] Vitamin B complex (1 g): vitamin B_1 8 mg, vitamin B_2 50 mg, vitamin B_6 16 mg, vitamin B_{12} 80 μg, niacin 120 mg, calcium panthotheonate 80 mg, L-lysine 10 mg, and DL-methionine 10 mg.

At the end of 42-d experimental period, after 12 h of fasting with ad libitum drinking water, 12 birds from each treatment (2 birds per replicate pen) were selected randomly and slaughtered for assessment of carcass characteristics and organ weight. Equal proportion of male and female birds was selected for slaughter to avoid sex as a possible confounding factor.

2.5. Sample collection

At the time of slaughter of birds, blood samples were collected in non-heparinised tubes from individual birds followed by serum harvesting and storage at −20 °C until biochemical analysis. The breast and thigh meat samples were collected individually from each bird for the study of antioxidant status, lipid oxidation, and physicochemical parameters. Liver samples of the respective birds were also collected to study its antioxidant enzyme activities.

2.6. Antioxidant and lipid oxidation status of meat

The assessment of antioxidant status of broiler chicken meat was done by 2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assays. The spectrophotometric (PerkinElmer, Model: Lambda EZ 201) analysis of ABTS and DPPH radical scavenging activity of meat was done by the methods of Shirwaekar et al. (2006) and Kato et al. (1988). The lipid oxidation status of meat samples were assessed by estimation of TBARS value (Witte et al., 1970), free fatty acid value, and peroxide value (Koniecko, 1979). The TBARS value was calculated as mg malondialdehyde (MDA) per kilogram of sample by multiplying O.D value with K-factor of 5.2.

2.7. Physicochemical properties of meat

The estimation of pH of breast and thigh meat sample was done by homogenizing 5-g meat sample in 25-mL distilled water (Trout et al., 1992). The WHC of breast and thigh meat samples was done by homogenizing 10 g of meat samples in 0.6 mol/L NaCl solution (Wardlaw et al., 1973). For the determination of ERV of breast and thigh meat, 15 g of each meat sample was homogenized in 60 mL of 0.05 mol/L phosphate buffer solution (Jay, 1964). The percentage of protein in breast and thigh meat was calculated by the method described by Association of Analytical Chemists (AOAC, 1995).

2.8. Serum biochemistry, health indices, and antioxidant status

The serum triglyceride (Fossati and Prencipe, 1982), total cholesterol (Flegg, 1973), and high density lipoproteins (HDL) cholesterol (Lopes Virella et al., 1977) were estimated. The atherogenic indices of serum like cardiac risk ratio (CRR), atherogenic coefficient (AC), and atherogenic index of plasma (AIP) were calculated as described by Frolich and Dobiasova (Frohlick and Dobiasova, 2003).

CRR = Total cholesterol/HDL cholesterol

AC = (Total cholesterol − HDL cholesterol)/HDL cholesterol

AIP = log (Triglycerides/HDL cholesterol)

The serum glucose (Barham and Trinder, 1972), alkaline phosphatase (ALP) (McComb and Bowers, 1972), acid phosphatase (ACP) (Hillmann, 1971), total protein (TP) (Doumas, 1975), albumin (ALB) (Doumas et al., 1971), globulin, albumin-to-globulin (A:G) ratio were estimated. The liver function was assayed by measuring serum glutamic oxaloacetate (SGOT) and serum glutamic pyruvic transaminase (SGPT) (Reitman and Frankel, 1957). The serum and liver TBARS were estimated by the method of Yagi (1998) using Cayman diagnostic kits and expressed in terms of malondialdehyde (MDA) concentration. The body antioxidant defence system comprises of mainly superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GSH-Px), and glutathione reductase (GR). The activities of these enzymes in serum and liver samples were determined by the method described by Wheeler et al. (1990) using the Cayman diagnostic kits. All the samples and standards were measured in triplicate.

2.9. Statistical analysis

For the data analysis of feed intake and FCR each replicate was taken as an experimental unit, whereas, for the analysis of body weight gain, lipid oxidation, antioxidant activity, physicochemical properties of meat, serum biochemistry, and serum health indices, each bird was taken as an experimental unit. The data were analysed by one-way ANOVA for a completely randomized design, using the General Linear Model procedure (IBM SPSS softeware-20). The Tukey post-hoc analysis was done to test the significant mean differences between the groups with significance level defined at $P < 0.05$.

3. Results

3.1. Growth performance and carcass traits

The birds in dietary treatments T_6 and T_5 resulted in better ($P < 0.01$) BWG, FCR, PEF, PER, and EER followed by treatment T_3 and T_4 compared to T_1 (Table 2). The growth performance of birds in treatment T_5 was similar to that that of T_6, except the significantly higher PER and EER in T_5 compared to T_6. Furthermore, treatment T_2 resulted in better overall growth performance of birds compared to T_1. Among the carcass traits only live weight was significantly ($P < 0.05$) lower in birds fed control diet (T_1) and higher in birds fed diet T_5 or T_6 which did not differ from each other, whereas, other treatments resulted in intermediate values (Table 3). The other carcass traits did not show any significant dietary treatment effects.

3.2. Lipid oxidation and antioxidant parameters

The TBARS value, peroxide value, and free fatty acid value of chicken meat have shown a decreasing ($P < 0.01$) trend from treatment T_1 to T_6 (Table 4). The treatment T_1 showing higher lipid oxidation was statistically similar to T_2 and the treatment T_6 showing lower lipid oxidation status was similar to T_5. The treatment T_3 and T_4 resulted in intermediate values. On the other hand, the antioxidant status of chicken meat depicted an increasing trend from treatment T_1 to T_6, however, the trend was not significant in case of ABTS values of chicken breast meat. The ABTS value of chicken thigh ($P < 0.01$) and DPPH values of breast ($P < 0.05$) and thigh ($P < 0.01$) meat were lower in treatment T_1 and higher in treatment T_6 which was statistically similar to T_5. No significant difference was observed between T_1 and T_2.

Table 2
Effect of different dietary combination of LBA and MOS on productivity index of broiler chickens.[1]

Item	Treatments[2]						SEM	*P*-value
	T_1	T_2	T_3	T_4	T_5	T_6		
BWG, g	1,671^{a}	1,726^{b}	1,828^{c}	1,844^{c}	1,910^{d}	1,908^{d}	15.8	< 0.01
FI, g	3,120	3,002	3,158	3,148	3,072	3,141	20.9	> 0.05
FCR	1.86^{c}	1.74^{b}	1.69^{b}	1.71^{b}	1.61^{a}	1.62^{a}	0.016	< 0.01
PEF	210^{a}	233^{b}	251^{c}	252^{c}	278^{d}	274^{d}	4.1	< 0.01
PER	2.73^{a}	2.97^{bc}	2.94^{b}	3.06^{c}	3.22^{d}	3.09^{c}	0.040	< 0.01
EER	18.5^{a}	20.3^{b}	20.1^{b}	20.9^{c}	22.1^{d}	21.1^{c}	0.211	< 0.01

LBA = *Lactobacillus acidophilus*; MOS = mannan-oligosaccharides; BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio; PEF = production efficiency factor; PER = protein efficiency ratio; EER = energy efficiency ratio.
[a to d] Within a row, mean values bearing different superscripts differ significantly ($P < 0.05$).
[1] Data is the mean of 6 replicates per treatment.
[2] T_1 (control), T_2 (bacitracin methylene disalicylate at 20 mg/kg), T_3 (MOS at 0.1% + LBA at 10^6 CFU/g), T_4 (MOS at 0.1% + LBA at 10^7 CFU/g), T_5 (MOS at 0.2% + LBA at 10^6 CFU/g), T_6 (MOS at 0.2% MOS + LBA at 10^7 CFU/g).

3.3. Physicochemical parameters

The pH of breast and thigh meat has shown a decreasing ($P < 0.01$) trend from treatment T_1 to T_6, however, statistical similarity was observed among T_1, T_2, and T_3 and among T_4, T_5, and T_6 (Table 5). The ERV and WHC of breast and thigh meat showed an increasing ($P < 0.05$) trend from treatment T_1 to T_6. Higher values were observed in T_5 and T_6 which were statistically similar to each other, whereas, lower values were observed in T_1 and T_2 which did not differ significantly from each other. The protein contents of breast and thigh meat were lower ($P < 0.05$) in treatments T_1 and T_2, which did not differ from each other, and were higher in T_5 and T_6, which were statistically similar to each other. In general, an increasing trend in protein content was depicted from T_1 to T_6.

3.4. Serum biochemistry

Among the serum biochemistry parameters, only glucose, total protein, globulin, and A:G ratio have shown significant treatment effects (Table 6). A declining ($P < 0.01$) trend was observed in serum glucose from treatment T_1 to T_6, with a higher value in T_1 and a lower value in T_6, whereas, T_3 and T_4 were similar to both T_2 and T_5. The serum total protein ($P < 0.05$) and globulin ($P < 0.01$) were lower in statistically similar T_1, T_2, and T_3 followed by T_4 compared to T_5 and T_6 which did not differ from each other. The serum A:G ratio was higher ($P < 0.05$) in treatment T_1 followed by T_2 and T_3 which did not differ significantly from each other and a lower ratio was observed in T_5 and T_6 which were statistically similar to each other. However, SGPT, SGOT, ALP, ACP, and albumin in serum were not influenced by dietary treatments.

3.5. Serum health indices

The serum health indices (Table 7) have shown that TG, TC, CRR, AC, and AIP depicted a decreasing trend ($P < 0.01$) from treatment T_1 to T_6. The higher values were observed in T_1 which was statistically similar to T_2 and lower values were observed in T_5 which did not differ significantly from T_6. The serum HDL cholesterol concentration was lower in T_1 followed by statistically similar T_2 and higher concentration was observed in T_5 which did not differ significantly from T_6. The treatment T_3 and T_4 resulted in intermediate values of serum health indices.

3.6. Antioxidant enzyme activities of serum and liver

The serum antioxidant enzymes have shown an increasing ($P < 0.01$) trend from T_1, and there were no significant differences between T_1 and T_2 in SOD and GSH-Px activities (except GR activities) and between T_5 and T_6 in SOD, GSH-Px and GR activities (Table 8). However, serum CAT activity was significantly lower in T_1 compared to T_2 and higher in T_5 compared to T_6. Similarly, the liver antioxidant enzymes depicted an increasing trend from T_1 to T_6, with no significant difference between T_5 and T_6, except serum GSH-Px, which was significantly higher in T_5 compared to T_6. The serum and liver TBARS value (MDA concentration) showed a decreasing trend ($P < 0.01$) from T_1 to T_6. However, the TBARS levels in T_5 and T_6 did not differ significantly from each other.

4. Discussion

The use of synbiotic supplementation is reported to be superior to the individual use of probiotics or prebiotics because prebiotic acts a necessary food source for probiotic and also increase their resistance to temperature, oxygen, and low pH (Sekhon and Jairath, 2010) which results in better growth performance in broiler

Table 3
Effect of different dietary combination of LBA and MOS on carcass traits of broiler chickens (%).[1]

Item	Treatments[2]						SEM	*P*-value
	T_1	T_2	T_3	T_4	T_5	T_6		
Live weight, g	1,614^{a}	1,669^{b}	1,771^{c}	1,787^{c}	1,853^{c}	1,851^{c}	20.2	< 0.01
Eviscerated weight	68.3	64.6	67.4	66.0	66.2	66.8	4.93	> 0.05
Dressed weight	74.2	71.1	73.1	72.2	72.4	72.7	3.81	> 0.05
Liver	2.73	3.08	2.88	3.01	3.04	2.54	0.605	> 0.05
Heart	0.68	0.69	0.67	0.66	0.71	0.59	0.087	> 0.05
Gizzard	2.47	2.72	2.20	2.48	2.42	2.72	0.363	> 0.05
Abdominal fat	0.87	0.63	0.94	1.10	0.79	1.32	0.267	> 0.05
Breast	17.6	16.7	18.0	16.9	17.0	17.2	0.939	> 0.05
Drum stick	10.5	10.4	10.0	10.3	10.4	9.9	0.388	> 0.05
Thigh	9.86	8.93	9.68	9.22	9.50	9.67	0.525	> 0.05

LBA = *Lactobacillus acidophilus*; MOS = mannan-oligosaccharides.
[a to c] Within a row, mean values bearing different superscripts differ significantly ($P < 0.05$).
[1] Data is the mean of 12 birds per treatment.
[2] T_1 (control), T_2 (bacitracin methylene disalicylate at 20 mg/kg), T_3 (MOS at 0.1% + LBA at 10^6 CFU/g), T_4 (MOS at 0.1% + LBA at 10^7 CFU/g), T_5 (MOS at 0.2% + LBA at 10^6 CFU/g), T_6 (MOS at 0.2% MOS + LBA at 10^7 CFU/g).

chicken (Awad et al., 2009; Hassanpour et al., 2013). On the similar lines the present study revealed better growth performance of birds fed 0.2% MOS along with LBA at either 10^6 or 10^7 CFU/g compared to control or BMD supplemented birds and BMD supplemented birds superseded the control birds. A number of mechanisms have been put forward to explain the positive effects of synbiotic supplements such as antimicrobial effects, regulation of host immune system, competitive exclusion of pathogens, conducive environment for growth of favourable microbiota, nutrient sparing effect, improved energy utilization, and enhancement of gut integrity (Lee et al., 2010; Vamanu and Vamanu, 2010; Ferreira et al., 2011) but none of them has been well established until now. However, in the present study improved BWG and FCR without changes in FI of birds can be justified based on the fact that synbiotic supplementation improves the nutrient utilization of the birds. The significant changes in live weight of birds in the present study reflected the trend of BWG of birds. The non-significant effects on other carcass traits signify that the

Table 4
Effect of different dietary combination of LBA and MOS on lipid peroxidation and antioxidant activity of broiler chicken meat.[1]

Item	Treatments[2]						SEM	*P*-value
	T_1	T_2	T_3	T_4	T_5	T_6		
TBARS (MDA), mg/kg								
Breast	0.49^{c}	0.47^{bc}	0.46^{ab}	0.45^{a}	0.45^{a}	0.45^{a}	0.003	< 0.01
Thigh	0.44^{d}	0.43^{cd}	0.42^{c}	0.41^{bc}	0.40^{a}	0.40^{a}	0.003	< 0.01
PV, mEq/kg								
Breast	3.95^{d}	3.66^{d}	2.80^{c}	2.70^{bc}	2.40^{ab}	2.30^{a}	0.114	< 0.01
Thigh	3.60^{c}	3.50^{bc}	3.30^{bc}	3.20^{c}	2.80^{a}	2.70^{a}	0.068	< 0.01
FFA, %								
Breast	0.31^{c}	0.30^{c}	0.24^{b}	0.23^{b}	0.19^{a}	0.18^{a}	0.008	< 0.01
Thigh	0.70^{c}	0.68^{cb}	0.65^{b}	0.64^{b}	0.56^{a}	0.57^{a}	0.009	< 0.01
ABTS, % inhibition								
Breast	92.0	92.5	93.4	93.2	95.7	95.9	1.26	> 0.05
Thigh	89.4^{a}	88.9^{a}	88.7^{a}	88.0^{a}	91.0^{b}	91.1^{b}	0.32	< 0.01
DPPH, % inhibition								
Breast	22.8^{a}	22.9^{a}	23.2^{a}	23.3^{a}	26.1^{b}	25.5^{b}	0.22	< 0.05
Thigh	18.3^{a}	19.4^{b}	20.1^{c}	20.6^{d}	22.1^{e}	22.0^{e}	0.23	< 0.01

LBA = *Lactobacillus acidophilus*; MOS = mannan-oligosaccharides; TBARS = thiobarbituric acid reactive substances; MDA = molondialdehyde; PV = peroxide value; FFA = free fatty acid; ABTS = 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); DPPH = 2,2-diphenyl-1-picrylhydrazyl.
[a to d] Within a row, mean values bearing different superscripts differ significantly ($P < 0.05$).
[1] Data is the mean of 12 birds per treatment.
[2] T_1 (control), T_2 (bacitracin methylene disalicylate at 20 mg/kg), T_3 (MOS at 0.1% + LBA at 10^6 CFU/g), T_4 (MOS at 0.1% + LBA at 10^7 CFU/g), T_5 (MOS at 0.2% + LBA at 10^6 CFU/g), T_6 (MOS at 0.2% MOS + LBA at 10^7 CFU/g).

Table 5
Effect of different dietary combination of LBA and MOS on physicochemical parameters of broiler chicken meat. [1]

Item	Treatments[2]						SEM	*P*-value
	T_1	T_2	T_3	T_4	T_5	T_6		
pH								
Breast	5.89^{b}	5.91^{b}	5.89^{b}	5.81^{a}	5.83^{a}	5.82^{a}	0.009	< 0.01
Thigh	6.11^{b}	6.13^{b}	6.09^{ab}	6.1^{ab}	6.07^{a}	6.07^{a}	0.006	< 0.01
ERV, mL								
Breast	32.8^{a}	33.0^{a}	33.6^{b}	33.9^{b}	34.9^{c}	34.8^{c}	0.162	< 0.05
Thigh	30.5^{a}	30.8^{ab}	31.0^{b}	31.1^{b}	31.6^{c}	31.8^{c}	0.126	< 0.05
WHC, %								
Breast	81.6^{a}	82.8^{ab}	84.4^{bc}	84.8^{c}	87.5^{d}	87.3^{d}	0.408	< 0.05
Thigh	74.8^{a}	74.6^{a}	75.2^{b}	75.3^{b}	76.9^{c}	76.7^{c}	0.197	< 0.05
Protein, %								
Breast	22.4^{a}	22.5^{a}	22.8^{ab}	22.9^{b}	25.0^{c}	25.0^{c}	0.192	< 0.05
Thigh	13.9^{a}	13.9^{a}	14.5^{b}	14.8^{c}	15.9^{d}	15.9^{d}	0.143	< 0.05

LAB = *Lactobacillus acidophilus*; MOS = mannan-oligosaccharides; ERV = extract release volume; WHC = water holding capacity.
[a to d] Within a row, mean values bearing different superscripts differ significantly ($P < 0.05$).
[1] Data is the mean of 12 birds per treatment.
[2] T_1 (control), T_2 (bacitracin methylene disalicylate at 20 mg/kg), T_3 (MOS at 0.1% + LBA at 10^6 CFU/g), T_4 (MOS at 0.1% + LBA at 10^7 CFU/g), T_5 (MOS at 0.2% + LBA at 10^6 CFU/g), T_6 (MOS at 0.2% MOS + LBA at 10^7 CFU/g).

Table 6
Effect of different dietary combination of LBA and MOS on serum biochemistry of broiler chickens.[1]

Item	Treatments[2]						SEM	*P*-value
	T_1	T_2	T_3	T_4	T_5	T_6		
Glucose, mg/dL	187^{d}	174^{c}	172^{bc}	170^{bc}	167^{b}	157^{a}	2.2	< 0.01
SGPT, U/L	148	153	159	160	168	188	17.7	< 0.05
SGOT, U/L	189	184	197	196	215	219	13.2	> 0.05
ALP, U/L	26.1	28.9	31.1	31.6	32.3	32.6	2.74	> 0.05
ACP, U/L	27.5	30.2	31.2	29.9	25.5	28.0	1.95	> 0.05
TP, g/dL	3.80^{a}	4.00^{a}	3.90^{a}	4.60^{b}	5.10^{c}	4.90^{c}	0.080	< 0.05
ALB, g/dL	2.10	2.10	2.00	2.10	2.00	2.00	0.051	> 0.05
GLB, g/dL	1.70^{a}	1.90^{a}	1.90^{a}	2.50^{b}	3.10^{c}	2.90^{c}	0.150	< 0.01
A:G ratio	1.24^{d}	1.11^{c}	1.05^{c}	0.84^{b}	0.65^{a}	0.69^{a}	0.051	< 0.05

LBA = *Lactobacillus acidophilus*; MOS = mannan-oligosaccharides; SGPT = serum glutamic pyruvic transaminase; SGOT = serum glutamic oxaloacetate; ALP = alkaline phosphatase; ACP = acid phosphatase; TP = total protein; ALB = albumin; GLB = globulin; A:G ratio = albumin-to-globulin ratio.
[a to d] Within a row, mean values bearing different superscripts differ significantly ($P < 0.05$).
[1] Data is the mean of 12 birds per treatment.
[2] T_1 (control), T_2 (bacitracin methylene disalicylate at 20 mg/kg), T_3 (MOS at 0.1% + LBA at 10^6 CFU/g), T_4 (MOS at 0.1% + LBA at 10^7 CFU/g), T_5 (MOS at 0.2% + LBA at 10^6 CFU/g), T_6 (MOS at 0.2% MOS + LBA at 10^7 CFU/g).

Table 7
Effect of different dietary combination of LBA and MOS on serum health indices of broiler chickens.[1]

Item	Treatments[2]						SEM	*P*-value
	T_1	T_2	T_3	T_4	T_5	T_6		
TG, mg/dL	127^{d}	126^{d}	118^{c}	115^{bc}	108^{a}	111^{ab}	1.8	< 0.01
TC, mg/dL	97.3^{c}	98.6^{c}	87.3^{b}	88.5^{b}	80.8^{a}	82.6^{a}	1.6	< 0.01
HDLCho, mg/dL	50.9^{a}	52.0^{a}	53.9^{b}	53.5^{b}	55.3^{c}	54.1^{bc}	0.61	< 0.05
CRR	1.91^{d}	1.89^{d}	1.63^{c}	1.66^{c}	1.46^{a}	1.56^{b}	0.030	< 0.01
AC	0.91^{d}	0.89^{d}	0.62^{bc}	0.66^{c}	0.46^{a}	0.56^{b}	0.037	< 0.01
AIP	0.40^{d}	0.39^{d}	0.34^{c}	0.33^{bc}	0.29^{a}	0.31^{ab}	0.009	< 0.01

LBA = *Lactobacillus acidophilus*; MOS = mannan-oligosaccharides; TG = triglyceride; TC = total cholesterol; HDL Cho = high density lipoprotein cholesterol; CRR = cardiac risk ratio; AC = atherogenic coefficient; AIP = atherogenic index of plasma.
[a to d] Within a row, mean values bearing different superscripts differ significantly ($P < 0.05$).
[1] Data is the mean of 12 birds per treatment.
[2] T_1 (control), T_2 (bacitracin methylene disalicylate at 20 mg/kg), T_3 (MOS at 0.1% + LBA at 10^6 CFU/g), T_4 (MOS at 0.1% + LBA at 10^7 CFU/g), T_5 (MOS at 0.2% + LBA at 10^6 CFU/g), T_6 (MOS at 0.2% MOS + LBA at 10^7 CFU/g).

synbiotic supplementation does not have differential effects on the different parts of the body in broiler chicken. The results of present are corroborated by a number of previous studies which have shown improved growth performance of broiler chicken (Amerah et al., 2013; Awad et al., 2009; Hassanpour et al., 2013; Ghasemi et al., 2016). On the other hand, there are various other studies showing no positive effects of probiotic or prebiotic supplementation on growth performance and carcass characteristics of broiler chicken (Erdoğan et al., 2010; Baurhoo et al., 2009; Manafi, 2015). The variations in the results pertaining to growth performance of broiler chicken in response to synbiotic supplementation can partly be explained by the difference in genetics of the birds used for the experiment, the strain and amount of probiotic, the source and inclusion level of prebiotic, etc.

The present study revealed significantly improved serum and liver antioxidant pool of birds fed 0.2% MOS along with LBA at either 10^6 or 10^7 CFU/g compared to control or BMD supplemented birds and the similar trend was reflected in the antioxidant capacity (ABTS and DPPH values) of broiler chicken meat. However, the reverse trend was observed in peroxide value (PV) and FFA of broiler chicken meat and in the TBARS value of serum, liver, and meat of broiler chicken. The mechanism of antioxidant role of probiotics is not well established yet, but it has been linked to the inhibition of ascorbate autoxidation, scavenging of free radicals, and metal ion chelation (Ejtahed et al., 2012; Lin and Chang, 2000). The oxidative defense mechanisms of probiotics were stimulated and enhanced in turkeys due to dietary MOS, resulting in improved growth performance of the birds (Ognik and Krauze, 2012). The direct neutralization of oxidants in the intestinal tract by the possible expression of antioxidant enzymes such as SOD, CAT, and GPx explains the antioxidant mechanism of synbiotics (Kleniewska et al., 2016). The enhanced absorption of nutrients, including antioxidants, which reduce the postprandial lipids connected with oxidative damage (Martarelli et al., 2011; Mikelsaar and Zilmer, 2009) is another proposed antioxidant mechanism of probiotic *Lactobacillus*. In line with the results of present study, an increased activity of SOD has been reported due to synbiotic administration (Shen et al., 2011). The administration of *Lactobacillus plantarum* increased CAT activity of chicken tissues (Shen et al., 2014). The increased GPx activity was confirmed by Shen et al. (2011, 2014) in chicken and Ejtahed et al. (2012) in humans. Similarly, an increase in CAT activity was observed in the blood of turkeys fed feed additives rich in MOS with bio-stimulating properties (Konyalioglu and Karamenderes, 2005; Gutowicz et al., 2008).

The enrichment of diets with probiotics and prebiotics favourably improve the oxidative stability of broiler chicken meat (Capcarova et al., 2010) which supports the higher ABTS and DDPH values observed in the present study. The TBARS estimation is a most widely used comprehensive assay of MDA levels in the body and a sequela of diminished antioxidant protection against free radicals in the body (Aluwong et al., 2013). The *Bifidobacterium longum* and LBA exerted the antioxidative activity by inhibiting the linoleic acid peroxidation (Lin and Chang, 2000) and a reduction in oxidative damage has been reported in other studies as well (Koller et al., 2008). It has been reported that probiotics (LBA and *L. casei*) reduce the streptozotocin induced oxidative damage in pancreatic tissues by inhibiting lipid peroxidation and preserving the antioxidant enzyme pool in rats (Yadav et al., 2008). However, there is no literature available pertaining to the effects of symbiotic supplementation on the PV and FFA values of animal tissues.

The physicochemical properties of meat like pH, WHC, ERV, and protein content are the determinants of the broiler chicken meat quality which affect further processing suitability of chicken meat (Mir et al., 2017; Popova, 2017). The present study revealed that pH of meat from birds fed 0.2% MOS along with LBA at 10^6 or 10^7 CFU/g or 0.1% MOS along LBA at 10^7 CFU/g was lower compared to control and other treatments. The ERV, WHC, and protein content of meat was higher in birds fed 0.2% MOS along with LBA at 10^6 or 10^7 CFU/g compared to control or BMD supplemented birds. The pH of meat is strongly correlated with the WHC or ERV of meat (Popova, 2017) because it has a direct bearing on the protein stability. The pH lower than 5.5 cause's protein denaturation and the meat suffers from water loss (Mir et al., 2018). The increase in protein content of chicken meat reflects the trend of WHC and ERV of meat in the present study. However, the reports on pH of meat are highly conflicting, where some researchers report decreasing trend (Mazaheri et al., 2014), some report increasing (Zheng et al., 2015), and some report nonsignificant effects (Pelicano et al., 2003) depending on the strain of probiotic used. The lower drip loss in broiler chicken meat was reported due to dietary probiotic supplementation (Zheng et al., 2015) indicating better WHC, whereas, Pelicano et al. (2003) reported nonsignificant effect of probiotics on the WHC of broiler chicken meat. Similar to the results of present study higher protein content of broiler chicken meat was reported due to probiotic supplementation (Liu et al., 2012).

The present study depicted that birds supplemented with 0.2% MOS along with LBA at 10^7 CFU/g had lower blood glucose followed by the birds supplemented with 0.2% MOS along with LBA at 10^6 CFU/g compared to control and BMD supplemented birds. This decline of blood glucose can be attributed to the antioxidant effect of the synbiotics as shown above in this study which reduce the stress level in the birds. In humans the significant decline of blood glucose due to the improvement of insulin sensitivity has been documented by prebiotic and probiotic supplementation which has been shown to reduce the risk of obesity and diabetes (Barboza et al., 2013; Dixit et al., 2016). Furthermore, the supplementation of probiotics mixture elevated the concentrations of immunoglobulin G and immunoglobulin M in turkeys which have been linked to better growth performance and disease resistance in animals (Cetin et al., 2005). Similarly, in the present study, the birds supplemented with 0.2% MOS along with 10^6 or 10^7 CFU LBA/g resulted in higher serum globulin concentration compared to control or BMD supplemented birds which resulted in corresponding positive effect on the serum total protein and globulin-to-albumin ratio. The higher globulin/albumin ratio indicates better immune status of the birds resulting in their better growth performance.

The present study revealed significantly higher serum HDL cholesterol and lower triglyceride and total cholesterol in birds

Table 8
Effect of different dietary combination of LBA and MOS on antioxidant enzyme activity in broiler chickens.[1]

Item	Treatments[2]						SEM	*P*-value
	T_1	T_2	T_3	T_4	T_5	T_6		
Serum								
SOD, U/mL	12.0[a]	12.1[a]	13.2[b]	13.8[c]	15.2[d]	15.1[d]	0.22	< 0.01
CAT, U/mL	4.35[a]	4.46[b]	4.72[c]	4.89[d]	5.47[f]	5.18[e]	0.067	< 0.01
GSH-Px, U/mL	198[a]	202[a]	212[b]	215[b]	232[c]	228[c]	2.3	< 0.01
GR, U/L	16.7[a]	18.2[b]	21.1[cd]	22.2[d]	28.8[e]	28.2[e]	0.68	< 0.01
TBARS value, nmol MDA/mL	8.03[d]	7.88[c]	7.74[b]	7.68[b]	7.34[a]	7.38[a]	0.044	< 0.01
Liver								
SOD, U/mL	19.5[a]	22.8[b]	26.5[c]	27.0[c]	29.8[d]	29.5[d]	0.621	< 0.01
CAT, U/mL	21.3[a]	21.9[ab]	22.1[b]	22.2[b]	25.7[c]	25.7[c]	0.311	< 0.01
GSH-Px, U/mL	24.6[a]	25.7[b]	26.6[c]	26.8[c]	30.8[e]	30.2[d]	0.388	< 0.01
GR, U/L	7.23[a]	7.64[b]	8.05[cd]	8.18[d]	9.87[e]	9.74[e]	0.17	< 0.01
TBARS value, nmol MDA/mL	2.11[e]	1.87[d]	1.52[c]	1.46[bc]	1.23[a]	1.30[a]	0.053	< 0.01

LBA = *Lactobacillus acidophilus*; MOS = mannan-oligosaccharides; SOD = superoxide dismutase, CAT = catalase; GSH-Px = glutathione peroxidase; GR = glutathione reductase; TBARS = thiobarbituric acid reactive substances; MDA = molondialdehyde.
[a to f] Within a row, mean values bearing different superscripts differ significantly ($P < 0.05$).
[1] Data is the mean of 12 birds per treatment.
[2] T_1 (control), T_2 (bacitracin methylene disalicylate at 20 mg/kg), T_3 (MOS at 0.1% + LBA at 10^6 CFU/g), T_4 (MOS at 0.1% + LBA at 10^7 CFU/g), T_5 (MOS at 0.2% + LBA at 10^6 CFU/g), T_6 (MOS at 0.2% MOS + LBA at 10^7 CFU/g).

supplemented with 0.2% MOS along with LBA at 10^6 CFU/g followed by 0.2% MOS along with LBA at 10^7 CFU/g compared to other treatments. This hypocholesterolemic and hypolipidemic effects resulted in consequent lower CRR, AC, and AIP in broiler chicken. It has been reported that the enzymatic deconjugation of bile acids (Begley et al., 2006) or conversion of cholesterol to coprostanol in the intestines (Chiang et al., 2008) by the probiotics causes their elimination via faeces. This elimination directs more cholesterol to synthesis of new bile acids in a homeostatic response, resulting in lowering of serum cholesterol. On the other hand, prebiotics increase the viscosity of the digestive tract and increase the thickness of mucus layer in the small intestine which inhibits cholesterol uptake and in turn leads to a higher cholesterol catabolism in the liver that contributed to a hypocholesterolemic effect (Dikeman et al., 2006). The prebiotics have also been reported to increase the short chain fatty acid concentration which inhibits or limits the cholesterol or triglyceride synthesis in the liver (Trautwein et al., 1998). However, these mechanisms have been proved via in vitro studies and have not been well established in in vitro studies. In the existing literature, the hypocholesterolemic and hypolipidemic effects of prebiotics and probiotics have been reported in various studies (Liong et al., 2007; Ooi et al., 2010). The cardioprotective indices like CRR, AC, and AIP observed in the present study may also be attributed to the enhanced absorption of micro and macronutrients, including antioxidants, which reduce postprandial lipids associated with oxidative damage and various cardiovascular pathologies (Martarelli et al., 2011; Mikelsaar and Zilmer, 2009).

5. Conclusion

This study concludes that supplementation of broiler chicken diets with 0.2% MOS along with LBA at 10^6 CFU/g feed is optimum for better growth performance, meat yield, and improvement of body antioxidant defense system with significant inhibition of lipid peroxidation in the body of broiler chicken. The better physicochemical properties of meat are observed in birds fed ration supplemented with 0.2% MOS along with LBA at 10^6 CFU/g feed. The supplementation of dietary 0.2% MOS along with LBA at 10^6 CFU/g feed results in hypocholesterolaemia and hyperlipidaemia with better health indices in broiler chickens.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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