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

Kapil Dev, Nasir Akbar Mir, Avishek Biswas, Jyoti Kannoujia, Jubeda Begum, Rajiv Kant, Asitbaran Mandal

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

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G.B. Pant University of Agriculture & Technology, College of Veterinary & Animal Sciences, Pantnagar 263145, India

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: T1 (control diet), T2 (antibiotic bacitracin methylene di-salicylate [BMD] at 20 mg/kg diet), T3 (MOS at 0.1% + LBA at 10^6 CFU/g feed), T4 (MOS at 0.1% + LBA at 10^7 CFU/g feed), T5 (MOS at 0.2% + LBA at 10^6 CFU/g feed), and T6 (MOS at 0.2% + LBA at 10^7 CFU/g feed). Each treatment was assigned to 6 replicates of 7 birds. The results revealed better (P < 0.01) growth performance and production efficiency of birds fed either T5 or T6 diet compared to control or BMD supplemented diet and BMD-supplemented birds superseded the control birds.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 T5 or T6 diet compared to control or BMD supplemented diet and BMD-supplemented birds. The pH of meat from birds fed T4, T5 or T6 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 T5 or T6 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...
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 mannan-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; Welglzarz, 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 (LAB) and Lactobacillus casei had higher content of moisture, protein, and ash compared to the control (Khaksafdi 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 synergistic 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 = 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., T1 (negative control diet), T2 (positive control diet containing antibiotic BMD at 20 mg/kg diet), T3 (MOS at 0.1% + LAB at 106 CFU/g feed), T4 (MOS at 0.1% + LAB at 108 CFU/g feed), T5 (MOS at 0.2% + LAB at 106 CFU/g feed), and T6 (MOS at 0.2% + LAB at 108 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 ft2 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).

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

\[
\text{PER} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}
\]

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

\[
\begin{align*}
\text{CRR} & = \frac{\text{Total cholesterol}}{\text{HDL cholesterol}} \\
\text{AC} & = \frac{\text{Total cholesterol} - \text{HDL cholesterol}}{\text{HDL cholesterol}} \\
\text{AIP} & = \log(\text{Triglycerides/} \text{HDL cholesterol})
\end{align*}
\]

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.

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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) |
|-----------------------------|---------------------|------------------|--------------------|
| 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                |
| L-s-Methionine              | 0.2                 | 0.2              | 0.2                |
| TM premix1                  | 1.0                 | 1.0              | 1.0                |
| Vitamin premix2             | 1.5                 | 1.5              | 1.5                |
| Vitamin B complex3          | 0.15                | 0.15             | 0.15               |
| Choline chloride            | 0.50                | 0.50             | 0.50               |

**Nutrient composition of diets (analysed)**

- Crude protein: 321
- Metabolizable energy, kcal/kg: 3,001
- Calcium: 10.0
- Available P: 4.9
- Lysine: 13.3
- Methionine: 5.0

1. Trace mineral (TM) mixture (100 g): FeSO₄·7H₂O 8 g, ZnSO₄·7H₂O 10 g, MnSO₄·H₂O 10 g, CuSO₄·5H₂O 1 g, KI 30 g.
2. Vitamin premix (1 g): vitamin A 82.5 IU, vitamin E (50%) 160 mg, vitamin D₃ 12,000 U, vitamin K 10 mg.
3. Vitamin B complex (1 g): vitamin B1 8 mg, vitamin B2 50 mg, vitamin B6 16 mg, vitamin B12 80 μg, niacin 120 mg, calcium panthothenate 80 mg, l-lysine 10 mg, and L-methionine 10 mg.
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 software-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 T6 and T5 resulted in better \((P < 0.01)\) BWG, FCR, PER, and EER followed by treatment T3 and T4 compared to T1 (Table 2). The growth performance of birds in treatment T5 was similar to that of T6, except the significantly higher PER and EER in T5 compared to T6. Furthermore, treatment T2 resulted in better overall growth performance of birds compared to T1. Among the carcass traits only live weight was significantly \((P < 0.05)\) lower in birds fed control diet (T1) and higher in birds fed diet T3 or T5 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 T1 to T6 (Table 4). The treatment T1 showing higher lipid oxidation was statistically similar to T2 and the treatment T5 showing lower lipid oxidation status was similar to T6. The treatment T3 and T4 resulted in intermediate values. On the other hand, the antioxidant status of chicken meat depicted an increasing trend from treatment T1 to T6, 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 T1 and higher in treatment T6 which was statistically similar to T5. No significant difference was observed between T1 and T2.

3.3. Physicochemical parameters

The pH of breast and thigh meat has shown a decreasing \((P < 0.01)\) trend from treatment T1 to T6, however, statistical similarity was observed among T6, T2, and T1 and among T4, T5, and T6 (Table 5). The ERV and WHC of breast and thigh meat showed an increasing \((P < 0.05)\) trend from treatment T1 to T6. Higher values were observed in T5 and T6 which were statistically similar to each other, whereas, lower values were observed in T1 and T2 which did not differ significantly from each other. The protein contents of breast and thigh meat were lower \((P < 0.05)\) in treatments T1 and T2 which did not differ from each other, and were higher in T5 and T6 which were statistically similar to each other. In general, an increasing trend in protein content was depicted from T1 to T6.

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 T1 to T6, with a higher value in T1 and a lower value in T6, whereas, T3 and T4 were similar to both T2 and T5. The serum total protein \((P < 0.05)\) and globulin \((P < 0.01)\) were lower in statistically similar T1, T2, and T3 followed by T4 compared to T5 and T6 which did not differ from each other. The serum A:G ratio was higher \((P < 0.05)\) in treatment T1 followed by T2 and T3 which did not differ significantly from each other and a lower ratio was observed in T5 and T6 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 T1 to T6. The higher values were observed in T1 which was statistically similar to T2 and lower values were observed in T6 which did not differ significantly from T6. The serum HDL cholesterol concentration was lower in T1 followed by statistically similar T2 and higher concentration was observed in T5 which did not differ significantly from T6. The treatment T3 and T4 resulted in intermediate values of serum health indices.

3.6. Antioxidant enzyme activities of serum and liver

The antioxidant enzymes have shown an increasing \((P < 0.01)\) trend from T6, and there were no significant differences between T1 and T2 in SOD and GSH-Px activities (except GR activities) and between T5 and T6 in SOD, GSH-Px and GR activities (Table 8). However, serum CAT activity was significantly lower in T3 compared to T2 and higher in T5 compared to T6. Similarly, the liver antioxidant enzymes depicted an increasing trend from T1 to T6 with no significant difference between T2 and T6, except serum GSH-Px, which was significantly higher in T3 compared to T6. The serum and liver TBARS value (MDA concentration) showed a decreasing trend \((P < 0.01)\) from T1 to T6. However, the TBARS levels in T5 and T6 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
Effect of different dietary combination of LBA and MOS on carcass traits of broiler chickens (%).1

| Item                  | Treatments2 | SEM   | P-value |
|-----------------------|-------------|-------|---------|
|                      | T1          | T2    | T3      | T4      | T5      | T6      |
| Live weight, g        | 1.614a      | 1.669b| 1.771c | 1.787d | 1.853e | 1.851f |
| Elavercated weight     | 68.3        | 64.6  | 67.4    | 66.0   | 65.8   | 66.8   |
| Dresssed weight       | 74.2        | 71.1  | 71.1    | 72.2   | 72.4   | 72.7   |
| Liver                 | 2.73        | 3.08  | 2.88    | 3.01   | 3.04   | 2.54   |
| Heart                 | 0.68        | 0.69  | 0.67    | 0.66   | 0.71   | 0.59   |
| Gizzard               | 2.47        | 2.72  | 2.20    | 2.48   | 2.42   | 2.72   |
| Abdominal fat         | 0.87        | 0.63  | 0.94    | 1.10   | 0.79   | 1.32   |
| Breast                | 17.6        | 16.7  | 18.0    | 16.9   | 17.0   | 17.2   |
| Drum stick            | 10.5        | 10.4  | 10.4    | 10.3   | 10.4   | 9.9    |
| Thigh                 | 9.86        | 8.93  | 9.68    | 9.22   | 9.50   | 9.67   |

Effect of different dietary combination of LBA and MOS on serum biochemistry of broiler chickens.1

| Item                  | Treatments2 | SEM   | P-value |
|-----------------------|-------------|-------|---------|
|                      | T1          | T2    | T3      | T4      | T5      | T6      |
| Glucose, mg/dL        | 187         | 174   | 172e    | 170c    | 167a    | 157b    | <0.05   |
| ALP, U/L              | 148         | 153   | 159     | 160     | 168     | 188     | 17.7    | <0.05   |
| GLB, g/dL             | 184         | 184   | 197     | 196     | 215     | 219     | 13.2    | <0.05   |
| ACP, U/L              | 28.8        | 31.3  | 27.2    | 29.9    | 25.8    | 28.0    | 19.5    | <0.05   |
| TP, g/dL              | 3.80        | 3.80  | 3.40    | 3.80    | 4.60    | 5.10    | 4.00    | <0.05   |
| ALB, g/dL             | 2.10        | 2.10  | 2.10    | 2.10    | 2.00    | 2.00    | 0.05    | <0.05   |
| GLB/g/dL              | 1.70        | 1.90  | 1.90    | 2.50    | 3.10    | 2.90    | 0.15    | <0.05   |
| A/G ratio             | 1.24        | 1.11  | 1.05    | 0.84    | 0.65    | 0.65    | 0.05    | <0.05   |

Effect of different dietary combination of LBA and MOS on carcass traits of broiler chickens.1

| Item                  | Treatments2 | SEM   | P-value |
|-----------------------|-------------|-------|---------|
|                      | T1          | T2    | T3      | T4      | T5      | T6      |
| TBARS (MDA, mg/kg)    | 0.49        | 0.47  | 0.46    | 0.45    | 0.45    | 0.003   | <0.01   |
| PV, meq/kg            | 3.95        | 3.66  | 2.80    | 2.70    | 2.40    | 2.30    | 0.114   | <0.01   |
| FFA, %                | 0.31        | 0.30  | 0.24    | 0.23    | 0.19    | 0.18    | 0.008   | <0.01   |
| ABTS, % inhibition    | 0.70        | 0.68  | 0.65    | 0.64    | 0.56    | 0.57    | 0.009   | <0.01   |
| DPPH, % inhibition    | 92.0        | 92.5  | 93.4    | 93.2    | 95.7    | 95.9    | 1.26    | >0.05   |
| Glucose, mg/dL        | 187         | 174   | 172e    | 170c    | 167a    | 157b    | <0.05   |
| ALP, U/L              | 148         | 153   | 159     | 160     | 168     | 188     | 17.7    | <0.05   |
| GLB, g/dL             | 184         | 184   | 197     | 196     | 215     | 219     | 13.2    | <0.05   |
| ACP, U/L              | 27.5        | 30.2  | 31.2    | 29.9    | 25.8    | 28.0    | 19.5    | <0.05   |
| TP, g/dL              | 3.80        | 4.00  | 3.90    | 4.60    | 5.10    | 4.00    | 0.05    | <0.05   |
| ALB, g/dL             | 2.10        | 2.10  | 2.10    | 2.10    | 2.00    | 2.00    | 0.05    | <0.05   |
| GLB/g/dL              | 1.70        | 1.90  | 1.90    | 2.50    | 3.10    | 2.90    | 0.15    | <0.05   |
| A/G ratio             | 1.24        | 1.11  | 1.05    | 0.84    | 0.65    | 0.65    | 0.05    | <0.05   |

Effect of different dietary combination of LBA and MOS on carcass traits of broiler chickens.1

| Item                  | Treatments2 | SEM   | P-value |
|-----------------------|-------------|-------|---------|
|                      | T1          | T2    | T3      | T4      | T5      | T6      |
| LBA – Lactobacillus acidophilus; MOS – mannan-oligosaccharides; TBA – thio-barbituric acid reactive substances; MDA – malondialdehyde; PV – peroxide value; FFA – free fatty acid; ABTS – 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid); DPPH – 2,2-diphenyl-1-picrylhydrazyl.3

1 Data is the mean of 2 birds per treatment.
2 T1 (control), T2 (bacitracin methylene disalicylate at 20 mg/kg), T3 (MOS at 0.1% + LBA at 106 CFU/g), T4 (MOS at 0.1% + LBA at 107 CFU/g), T5 (MOS at 0.2% + LBA at 106 CFU/g), T6 (MOS at 0.2% MOS + LBA at 107 CFU/g).
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 DPPH 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 10^6 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 (Pelicanos 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, Pelicanos et al. (2003) reported nonsignificant effect of probiotics on the WHC of broiler chicken meat. Similarly 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 10^6 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 antioxidative effect of the symbiotics 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 probiotic and prebiotic 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 7

| Item                      | Treatments | SEM | P-value |
|---------------------------|------------|-----|---------|
| TG, mg/dl                 | T1 127^d  | T2 126^d  | T3 118^b  | T4 108^b  | T5 111^ab | T6 1.8 < 0.01 |
| TC, mg/dl                 | 97.3^b  | 98.6^b  | 87.3^b  | 85.8^b  | 82.6^a  | 1.6 < 0.01 |
| HDLChol, mg/dl            | 50.9^a  | 52.0^a  | 53.9^a  | 53.5^a  | 54.1^b  | 0.61 < 0.05 |
| HDL                      | 1.91^a  | 1.89^a  | 1.65^b  | 1.65^b  | 1.56^b  | 0.030 < 0.01 |
| AC                       | 0.91^d  | 0.89^d  | 0.62^c  | 0.66^c  | 0.46^c  | 0.56^c  | 0.037 < 0.01 |
| AIP                      | 0.40^b  | 0.39^b  | 0.34^b  | 0.33^b  | 0.29^b  | 0.31^b  | 0.009 < 0.05 |

LBA = Lactobacillus acidophilus; MOS = mannooligosaccharides; TG = triglyceride; TC = total cholesterol; HDL Cho = high density lipoprotein cholesterol; CRI = cardiac risk ratio; AC = atherogenic coefficient; AIP = atherogenic index of plasma.

Within a row, mean values bearing different superscripts differ significantly (P < 0.05).

Data is the mean of 12 birds per treatment.

1 Data is the mean of 12 birds per treatment.

2 T1 (control), T2 (bacitracin methylene disalicylate at 20 mg/kg), T3 (MOS at 0.1% + LBA at 10^6 CFU/g), T4 (MOS at 0.1% + LBA at 10^7 CFU/g), T5 (MOS at 0.2% + LBA at 10^6 CFU/g), T6 (MOS at 0.2% MOS + LBA at 10^7 CFU/g).

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 DPPH 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.

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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 probiotics causes their elimination via faeces. This elimination directs more cholesterol to synthesis of new bile acids (Begley et al., 2006) or conversion of cholesterol to bile acids (Baurhoo B, Ferket PR, Zhao X. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal morphology of broiler chickens. Poultry Sci 2009;88(1):49–56; Barboza M, German J, Lebrilla C, Mills D, Freeman S, et al. Prebiotic oligosaccharides. J Chemists; 1995.) in the body of broiler chicken. The better physico-chemical 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 or 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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**References**

Alloui MN, Szczurek W, Swiatkiewicz S. The usefulness of prebiotics and probiotics in modern poultry nutrition: a review. Ann Anim Sci 2013;13(1):17–32; Aluowong T, Kawu M, Raji M, Denda T, Gouwong F, Sankala V, Ayo J. Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens. Antioxidants 2013;2(4):326–39; Amerah AM, Gules A, Medei F, Sanchez J, Lehtinen MJ, Graça MI. Effect of pelleting temperature and probiotic supplementation on growth performance and immune function of broilers fed maize/soy-based diets. Anim Feed Sci Technol 2013;180(1–4):55–63; AOAC. Official methods of analysis. 16th ed. Arlington, VA: Association of Analytical Chemists; 1995. Awd WA, Ghareeb K, Abdel-Raheem S, Bohn J. Effects of dietary inclusion of probiotic and symbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Sci 2009;88(1):49–56; Barbosa M, German J, Lebrilla C, Mills D, Freeman S, et al. Prebiotic oligosaccharides. 2013. US Patent 8,425,930 B2; Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 1972;97(5):55–63; Bauerho B, Ferlet PR, Zhao X. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. Poultry Sci 2009;88(11):2262–72; Begley M, Hill C, Gahan CGM. Bile salt hydrolase activity in probiotics. Appl Environ Microbiol 2005;72:1729–38; Begum J, Mir NA, Dev K, Khan IA. Dynamics of antibiotic resistance with special reference to Shiga toxin-producing Escherichia coli infections. J Appl Microbiol 2018;125(5):1228–37; Bobko M, Hackl P, Bobkova A, Pavkova A, Tkacova J, Trembecka L. Lipid oxidation in chicken meat after application of bee pollen extract, propolis extract and probiotic in their diets. Potravinarstvo 2015;59:342–6; Bozkurt M, Aysul N, Kucukyilmaz K, Ayapak S, Ege G, Cati AU, et al. Efficacy of in-feed preparations of an anticoccidial, multienzyme, probiotic, probiotic, and herbal essential oil mixture in healthy and Eimeria spp.-infected broilers. Poultry Sci 2014;93:389–99;
Caparova M, Weiss J, Hrnčar J, Kolesarova A, Pal G. Effect of Lactobacillus fermentum and Enterococcus faecium strains on internal mielin, antioxidant status and body weight of broiler chickens. J Anim Physiol Anim Nutr 2010;94(5):e215–24.

Cetin N, Gücüli BK, Cetin E. The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys. J Vet Med A 2005;52(1):125–38.

Chang YR, Ismail W, Heintz D, Schaeffer C, van Dorsselaer A, Fuchs G. Study of anoxic and oxic cholesterol metabolism by sterolbacterium denitrificans. J Bacteriol 2008;190:905–14.

Dikeman CL, Murphy MR, Orpin GC. Dietary fibers affect viscosity of solutions and simulated human gastric and small intestinal digesta. J Nutr 2006;136:913–9, DIXIT Y, Wagle A, Vakil B. Patents in the field of probiotics, prebiotics, synbiotics: a review. J Food: Microbiol Safety & Hygiene 2016:1–2. 

Doumas BT, Dornhorst AG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chim Acta 1971;31(1):87–96.

Doumas BT. Colorimetric determination of total protein in serum or plasma. Clin Chem 1973;21(8):1150–1154.

Ejehed HS, Moltaeri NA, H. Homayouni-Rad A, Niafar M, Agha-Charafabadi M, Moffid V. Probiotik yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 2012;28(5):539–43.

Erdogan Z, Erdogan S, Aslantaş O, Celik S. Effects of dietary supplementation of synbiotics and probiotics on performance, caecal coliform population and some oxidant/antioxidant parameters of broiler chickens. J Anim Physiol Anim Nutr 2010;94(5):e40–8.

Ferlert PR. Controlling gut health without the use of antibiotics. In: Proceedings of the annual Carolina poultry nutrition conference. 2003; p. 57–68.

Ferreira CL, Salminen S, Grzeskowiak L, Brizuela MA, Sanchez L, Carneiro H, Ferket PR. Controlling gut health without the use of antibiotics. In: Proceedings of the annual Carolina poultry nutrition conference. 2003; p. 57–68.

Flegg HM. Anees award lecture 1972. An investigation of the determination of serum cholesterol by an enzymatic method. Ann Clin Biochem 1973;10(1):79–84.

Fossati P, Principe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982;28(10):2077–80.

Frohlich J, Dobiasova M. Fractional esterification rate of cholesterol and ratio of triglycerides to HDL-cholesterol are powerful predictors of positive findings on coronary angiography. Clin Chem 2003;49(11):1873–80.

Ghosemi HA, Shavaz M, Micaureanu Rezaii S, Karim Tooshizadeh MA. Effect of synbiotic supplementation and dietary fat sources on broiler performance, serum lipids, muscle fatty acid profile and meat quality. Br Poult Sci 2016;57(1):71–83.

Gutowicz M, Chojczyk M, Pyrzawonka J, Widy-Tyszkiewicz E. Effect of curcumin on turbulent and laminar heat and mass transfer modification in the layers of aging rats. Med Weter 2006;64(8):955–97.

Hasnappour H, Moghadam AZ, Khosravi M, Mayahi E. Effects of synbiotic on the intestinal morphology and humoral immune response in broiler chickens. Livest Sci 2013;153(1):116–22.

Hillmann G. Z Klin Chem Klin Biochem 1971;9:273.

Jay RR. Direct titration of epoxy compounds and aziridines. Anal Chem 1964;36(4):482–8.

Kato K, Terao S, Hirata M. Studies on scavengers of active oxygen species. 1. Synthesis and biological activity of 2-O-alkylascorbic acids. J Med Chem 1988;31:793.

Kleniewska P, Hoffmann A, Pniewska E, Pawliczak R. The in vivo effect of probiotics on bacterial populations of broilers. J Anim Physiol Anim Nutr 2012;96(4):380–2.

Klep MV. Handbook for meat chemists. Wayne: Avery Pub. Group. Inc.; 1979.

Kato K, Terao S, Hirata M. Studies on scavengers of active oxygen species. 1. Synthesis and biological activity of 2-O-alkylascorbic acids. J Med Chem 1988;31:793.

Koniecko ES. Handbook for meat chemists. Wayne: Avery Pub. Group. Inc.; 1979.

Konyalioglu S, Karamenderes C. The protective effects of Achillea L. species native in Turkey against H2O2-induced oxidative damage in human erythrocytes and some oxidant/antioxidant parameters of broilers. J Anim Physiol Anim Nutr 2012;96(4):380–2.

Kung WL, Lowsky A, Stone P, Ellis S, Colwell JA. Cholesterol determination in high density lipoproteins separated by three different methods. Clin Chem 1973;57:19–24.

Kung WL, Lowsky A, Stone P, Ellis S, Colwell JA. Cholesterol determination in high density lipoproteins separated by three different methods. Clin Chem 1973;57:19–24.

Kung WL, Lowsky A, Stone P, Ellis S, Colwell JA. Cholesterol determination in high density lipoproteins separated by three different methods. Clin Chem 1973;57:19–24.

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