Effects of probiotic feed additives (biosol and Zemos) on growth and related genes in broiler chickens

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ABSTRACT
The goal of this study was to look into the effect of two probiotics as feed additives: Biosol (lipotropic factor containing probiotics) and/or Zemos, and to determine the best regimen for using both probiotics to improve growth performance, carcass traits, blood parameters, and transcription of some growth and immunity-related genes in broiler chickens. A commercial hatchery provided 400 one-day-old Cobb broiler chicks. The chicks were divided into four equal groups at random, each with 100 birds. Group 1 served as a negative control. Group 2 was given Probiotic 1 (Biosol) in drinking water at a dose of 120 g/10,000 chick each day. Group 3 received probiotic 2 (Zemos) at a dose of 0.25 mL/L of drinking water for 3 days/week. Group 4 was given combined probiotics (Biosol + Zemos), with the same weekly dose of each probiotic alternately. During the experiment, all chickens were fed and hydrated ad libitum and exposed to 24 h of light. Our findings revealed that chicks fed on diets supplemented with Biosol and Biosol + Zemos showed an increase in body weight gain and lower feed conversion rates. Total protein levels were elevated while cholesterol and triglyceride levels dropped, whereas, no effect was detected in the uric acid levels among those groups. Furthermore, the carcass traits of Biosol and Biosol + Zemos increased as a result of the dietary supplementations, however, the weight of the drumstick showed no difference among the four groups. Both probiotics influenced the cecal count of lactobacilli, total aerobes, E. coli, and Enterococci in, as well as the transcript levels of the MTOR, SMYD, TLR-4, and NBN genes. It is possible to conclude that supplementing broilers with probiotics and Biosol, in particular, can increase their growth performance, and improve the biochemical characteristics of the blood and transcript levels of the genes under investigation.

HIGHLIGHTS
- Supplementation of the broiler diet with probiotics is essential
- Biosol and Zemos increased the growth performance
- Both probiotics improved the biochemical blood parameters
- Biosol up-regulated growth and immunity related genes

Introduction
The poultry industry in Egypt is a developing investment, and the government has always put strategic plans in place to expand this sector. Our research focussed on two probiotics (Biosol and Zemos) that have lately become widely used in Egyptian farms. The two probiotics are different in composition, as one contains a lipotropic factor and only one type of bacteria, and the other contains a different group of beneficial bacteria. The study includes the effect of probiotic supplementation on some growth and immunity-related genes expression of broiler chickens that did not take much attention from researchers.

The genes of the study were selected based on newly discovered genes that occupy a great interest of researchers.

Probiotics are live, non-pathogenic microbial supplements that enhance the intestine’s microbial balance. Their administration has become increasingly popular around the world, as seen by the growing number of foods and supplements on the market that contains billions of living microbial cells (Abd Al-Fatah 2020; Taverniti et al. 2021). The use of Probiotics
reduces intestinal inflammation, influences growth promotion in chicken (Broiler) meat production, boosts broiler efficiency and increases production sustainability without negative side effects on immune responses (Giannenas et al. 2014; Asgari et al. 2018; Elgeddawy et al. 2020). The effectiveness of these components is often due to complex microbial ecological variables that influence the competitive pressures that the gut microbial population faces (Gulmez et al. 2019).

Poultry farming is a significant part of agriculture around the world. Native animals in general, and the poultry sector in particular, are attracting more attention these days due to their high meat quality and long-term viability (Habimana et al. 2020). The key factor contributing to economically efficient broiler production is rapid growth, which results in a shorter rearing period to marketing and a better feed conversion ratio (FCR). Geneticists have made rapid genetic changes in broiler chickens for selecting unique biological traits, resulting in continuous improvements in body weight, growth rate, meat yield in meat-type birds, and marketing age (the period when broilers hit 2000 g) (Havenstein et al. 1994). As a result, in the chicken breeding program, the candidate gene strategy has proved to be an effective technique for genetic improvement. Using a candidate gene may increase the efficiency of detecting the traits that are required to improve production output.

Precise collaborative consequences of probiotic combination on the broiler chicken growth and immunity have not established much concern. The divergence of the bioactive components of probiotics necessitates the need to study the interactions between different probiotics. Therefore, the current study aimed to evaluate the effects of two probiotics: Biosol and/or Zemos as feed additives on the growth performance, carcass traits, blood parameters, and the transcription of some growth and immunity-related genes of broiler chickens and to recommend the best regime for using both probiotics to achieve better performance in broiler chickens.

Materials and methods

Experimental design

400 one-day-old cobb broiler chickens purchased from a commercial hatchery. The chicks were split randomly into four equal groups, each with 100 birds. Group 1 (Negative/Control) has received basal water and diet with no treatment. Group 2 has supplemented probiotic 1 (Biosol) in dose 120 g/10,000 chick per day in drinking water, which consists of 3.0% (1.5 \times 10^{12}\text{cfu}) Enterococcus faecium, 93.8% Betaine (lipotropic factor), 2.2% Dextrose and 1.0% Lactose in each kilogram. Group 3 has administered probiotic 2 (Zemos) in dose 0.25 mL/L of drinking water for three days per week, that contains in each litre 10 billion cfu/g of active yeast, 100 billion cfu/g of Bacillus subtilis, 100 billion cfu/g of Bacillus amyloliquafaciens, 100 billion cfu/g of Lactobacillus acidophilus, Biotin 0.5 gm, 50 g of Humic acid (HumateDeposite), 50 g of Citric acid, and deinised water up to 1 L. Group 4 was given mixed probiotics (Biosol + Zemos) with alternative doses per week for each probiotic.

They were held in an environmentally controlled room (32 – 28 °C according to age). Using management guide recommendations, chickens were raised and fed until they were 35 days old. The experimental diets were designed to meet the nutritional needs of broiler chicken. Following the Animal Experimental Ethical Committee’s instructions, all chickens were fed and watered ad libitum and exposed to 24 h of light.

Chickens were weighed and feed intake was registered on the 0th, 7th, 14th, 28th, and 35th days of their lives. To assess the growth performance of each group, the feed conversion rate (FCR) was determined by dividing feed consumption by weight gain (Yi et al. 2018).

Carcass characteristics

Thirty birds from each group were randomly selected and slaughtered on the 35th day. Chickens were eviscerated before the carcass weighs. The breast, drumsticks, thigh, and giblet were all weighed. Muscle samples from the breast, leg, and heart were taken and immediately frozen at – 40 °C (Pourakbari et al. 2016).

Microbial count

The contents of the chickens’ intestines were collected in sterilised sampling tubes after they were dissected. The contents of the intestine were used to make a 10-fold serial dilution. (1 g of sample was rendered in a phosphate buffer solution in a series of 10^{-1} – 10^{-6}) Following that, 100 μL of each of the 10^{-4}, 10^{-5}, and 10^{-6} dilutions were removed and poured into Petri dishes containing the culture media. Total aerobes, E. coli, and Enterococci were cultured in nutrient agar, eosin methylene blue agar, and Slanetz-Bartley agar, respectively, and incubated at 37°C under aerobic conditions for 48 h. Lactobacilli were cultured in De Man, Rogosa, and Sharpe agar and incubated for 72 h.
at 37°C in anaerobic conditions. A colony counter was used to count the number of bacterial colony-forming units (CFU) in the Petri dishes. The counts were given in log_{10} CFU per gram of sample (Pourakbari et al. 2016).

**Blood sampling**

On the 35th day, 30 chickens were chosen at random from each group and 5 mL of blood was extracted from the wing vein in plain tubes to measure blood serum metabolites. Serum was extracted from blood samples after centrifugation (3000 × g, 20 min at room temperature) and preserved in Eppendorf tubes at −20°C before analysis at Centre of Laboratory Analysis and Applied Veterinary Study – Faculty of Veterinary Medicine – Cairo University. Biochemical analysis was performed using an Automated Fuji DRI-CHEM (FDC NX500V V3.2). Total protein, triglyceride, cholesterol, and uric acid were assessed parameters.

**Determination of the transcript level of mTOR, Smyd1, TLR-4 and NBN genes**

**Total RNA extraction**

The ‘Gene JET RNA purification kit’ by Thermo Scientific was used to extract total RNA. In lysis buffer, samples were homogenised with guanidine thiocyanate, a chaotropic salt that protects RNA from endogenous RNases. After that, the lysate was combined with ethanol before being placed into a purification column. While the lysate was spinning through the column, the chaotropic salt and ethanol caused RNA to bind to the silica membrane. By washing the column with wash buffer, impurities are effectively removed from the membrane. Pure RNA is then eluted with nuclease-free water under low ionic strength conditions (Boom et al. 1990).

**Reverse transcriptase polymerase chain reaction (RT-PCR)**

RT-PCR was done using Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, Cat. No. #K1622).

**Quantitative real-time PCR (qPCR):**

Real-time PCR, Smyd1 and TLR using Luminaris Colour HiGreen Low ROX qPCR Master kit (Thermo Scientific, Cat. No. #K0371). Primer Premier 5 software was used to design the primer sets based on the chicken mRNA sequences of the studied genes (Table 1). Each assay was conducted twice (Abdelhady et al. 2021) and a no-template negative control sample was added. Data were normalised using the B-actin gene as a housekeeping gene. The fold change compared to control samples was calculated using CT, ΔCT, ΔΔCT methods.

**Intestinal histopathology and histomorphometry**

The intestinal morphology of thirty birds from each group was analysed at the end of the experimental study. Briefly, a 5 cm section of the ceci was dissected out and preserved in 10% neutral buffered formalin. The tissue samples were dried, embedded in paraffin, and stained with Haematoxylin and Eosin (H and E) after being fixed (Bancroft and Gamble 2008). Using ImageJ software (NIH, USA), the villus height (m) was measured from the villus tip to the villus crypt junction, and the crypt depth (m) was measured from the basement membrane to the mouth of the crypt (Shokryazdan et al. 2017). The villi to crypt ratio (V: C) was estimated. There were twenty measurements taken. Each sample yielded a total of twenty measurements.

**Statistical analysis**

The data were analysed using the SPSS 25 program’s one- and two-way ANOVA procedures and the Post Hock test (Tukey test) was used for multi-comparison between raw means. p Values ≤ .05, are considered significant. Microsoft Office Excel was used to draw graphs.

**Results**

**Blood parameters**

**Triglyceride and cholesterol**

All probiotic supplemented groups showed a significant decline in serum cholesterol and triglyceride levels, however, there was an insignificant difference among the treated groups (Figure 1).

**Uric acid**

The Biosol group was the only group that showed a significant increase in the uric acid level (Figure 2).
Total protein
In all probiotic supplemented groups, serum total protein has risen sharply, with the highest level in the Biosol group (Figure 3).

Growth performance
Body weights, weight gain, feed intake and feed conversion rate (FCR)/chick for all broiler groups fed on diets containing no probiotics (control) or probiotics (Biosol, Zemos, and Biosol + Zemos) were reported weekly during the experiment.

There was an increase in the growth performance in all probiotic supplemented groups starting on the first week. The Zemos group showed the highest body weight gain and the lowest FCR. Whereas, at the fifth week the highest weight and weight gain in both Biosol and Zemos groups, as well as the lowest FCR in Biosol were recorded (Figures 4 and 5).

Cecal bacterial counts
Cecal microbial count was affected by the probiotic administration. The Lactobacilli count increased significantly in all probiotic supplemented classes, with the highest level in the Biosol. The number of Enterococci was higher in the Biosol and Biosol + Zemos groups, but lower in the Zemos group. In all probiotic supplemented groups, E.coli was down-regulated, with the lowest count in the Biosol. Total aerobes are reduced in Biosol + Zemos and Zemos compared to control and Biosol groups (Figure 6).

Intestinal measurements
The villus height and crypt depth were measured in the intestines of different classes.
Figure 3. The total protein content. Means ± SE (error bars) were used to express the data. Statistically significant variations $p \leq 0.05$ are shown by different superscript letters on the bars, whereas similar superscript letters indicate non-significant differences among the compared groups. $n = 30$.

Figure 4. From day 0 to the 21st day, the growth output of various groups was measured. Means ± SE (error bars) were used to express the data. Statistically significant variations $p \leq 0.05$ are shown by different superscript letters on the bars, whereas similar superscript letters indicate non-significant differences among the compared groups. $n = 30$. FCR: feed conversion rate.

Figure 5. From the 22nd to the 35th day, the growth output of various groups was measured. Means ± SE (error bars) were used to express the data. Statistically significant variations $p \leq 0.05$ are shown by different superscript letters on the bars, whereas similar superscript letters indicate non-significant differences among the compared groups. $n = 30$. FCR: feed conversion rate.
Results showed a significant increase in the villus height in groups Biosol and Biosol + Zemos classes compared with group Zemos and control. Crypt depth showed no significant difference was recorded among different experimental groups (Figures 7–9).

The transcript levels of growth and immunity-related genes

The transcript level of mTOR showed a significant upregulation in the Biosol and Biosol + Zemos groups compared to the control and Zemos groups. The highest transcript was recorded in the heart, breast and thigh respectively. Whereas, the smyd1 transcript was significantly up-regulated in the Zemos group compared to the other groups mainly in the heart. As for the TLR-4, the Biosol showed a significant up-regulation compared to other groups mainly in the heart muscle. Moreover, the NBN showed a significant down-regulation among all probiotic groups compared to the control, however, the Biosol + Zemos group recorded the least transcription (Figure 10).

**Discussion**

Probiotics exert their effects in diverse directions to achieve beneficial effects on their hosts if utilised appropriately. Several studies showed that ex vivo stimulation supports a beneficial role for probiotic supplementation, (Hutchinson et al. 2021). The consequences of the probiotics supplementation are plenty, where they modify the intestinal function, have immune responses on gut lymphoid tissue. These impacts involve the intestinal barrier pathogens competitive exclusion, T cell polarisation and intestinal inflammation suppression.

They work via pro-inflammatory cytokine release down-regulated from immune cells and through up-regulation and motivation of endogenous suppressors, cross-regulation and suppression of signalling pathways, including (PI3K) phosphatidylinositol 3-kinase
and (MAPK) mitogen-activated protein kinases (Llewellyn and Foey 2017). Our results proved that the probiotic-supplemented groups exhibited considerably greater serum total protein than the negative control group. Comparing the two probiotics understudy, the broilers supplemented with the Biosol had considerably greater serum total protein than those given Zemos or the combination of the two probiotics. The pathogenic bacteria are competitively excluded by the lactic acid bacteria, which...
diminish protein breakdown to nitrogen and dietary protein efficiency (Yazhini et al. 2018) which results in improvement of amino acid and protein consumption. Furthermore, we reported higher villi height in the probiotic-supplemented groups that might have resulted in enhanced protein absorption.

The current findings are consistent with those of (Yazhini et al. 2018), who found that probiotic administration raised plasma protein and improved broiler chick development performance.

All probiotic-treated groups showed a significant decrease in the blood triglycerides and cholesterol levels compared to the control group. The decline in both triglycerides and cholesterol may be attributed to the role played by the lactic acid bacteria which lower cholesterol levels by decreasing its reabsorption through the intestinal wall and preventing cholesterol synthesis in the digestive system (Alaqil et al. 2020).

Interestingly, the lactic acid bacteria downregulate the expression of Niemann-Pick C1-like 1 protein which is found on the surface of enterocytes and restricts cholesterol absorption (Huang and Zheng 2010). Lactic acid bacteria create bile salt hydrolase, an enzyme that aids in the excretion of additional bile acids in the faeces by deconjugating bile salts (Yazhini et al. 2018; Alaqil et al. 2020).

In terms of kidney function, our findings demonstrated that serum levels of uric acid were coherent between groups subjected to Zemos or Biosol + Zemos combination. On the other hand, the Biosol-supplemented group revealed a nonsignificant increase in blood uric acid levels, as well as no gross abnormalities in the kidneys. Therefore, the relatively stable serum levels of uric acid may be associated with the renal protective effects of the probiotic. Our data partially agree with a previous study conducted

Figure 10. The m-RNA level of the studied genes. (a) mTOR; (b) Symd1; (c) TLR; (d) NBN in different groups. Statistically significant variations $p \leq 0.05$ are shown by different superscript letters on the bars, whereas similar superscript letters indicate non-significant differences among the compared groups. $n = 30$. 
by Strompfova and co-workers, (Strompfová et al. 2006) who reported that there was no effect on serum uric acid levels by the addition of probiotics (*Saccharomyces cerevisiae*) as compared with the control.

In this investigation, the comparative efficiency of different probiotics did not progress at the same rate, which could be owing to differences in composition and delivery routine for each probiotic. Biosol supplemented group had a large rise in *Lactobacilli* and *Enterococci* count, with no effect on total aerobes and a substantial drop in *E.coli* count. In contrast to the Zemos class, there was a substantial increase in *Lactobacilli* and a little decrease in *Enterococci* count, as well as a significant decrease in total aerobes and *E.coli* count. *Lactobacilli* and *Enterococci* increased significantly in the Biosol + Zemos group, while *E.coli* decreased slightly and total aerobes decreased significantly. Decades of studies have reported the positive impacts of probiotics supplementation on gut immunity and gastrointestinal health furthermore, inhibition pathogenic bacteria colonisation.

Early exposure to microbial preparations has been discovered as a method of modulating the microbiota to promote beneficial bacterial growth and reduce pathogen colonisation (Rodrigues et al. 2020). Our findings are partially consistent with those of Pourakbari and co-workers, who found that probiotic supplementation did not influence cecal *Lactobacilli* counts, but that total aerobe counts increased, *Enterococci* counts increased, and *Escherichia coli* counts reduced (Pourakbari et al. 2016). These findings might affect the intestinal measures which revealed in the histopathological examination a considerable increase in the villi height in groups supplemented with Biosol and Biosol + Zemos, however, there was no effect on the villi height in the Zemos treated group. On the other hand, there was no difference in the crypt depth between the control and probiotic-treated groups. This may be attributed to the increase in nutrient absorption by increasing the available surface area for nutrient absorption.

This data agrees with numerous previous studies, where it is documented that, probiotic supplementation has been successfully linked to GIT development by stimulating the growth of villus surface area (Teague et al. 2017; Rodrigues et al. 2020).

The probiotic administration influenced the carcass characteristics considerably such that a substantial increase was recorded in living, carcass, breast, and giblet weight in the probiotic (Biosol, Zemos, Biosol + Zemos) supplemented groups compared to the control group. Whereas, the thigh weight increased significantly in the (Biosol and Biosol + Zemos) groups but the Zemos group showed no difference compared to the control group. The weight of the drum sticks is the same for all groups. The addition of probiotics to the broiler diet enhanced carcass features, which could be connected to the suppression of intestinal pathogen colonisation and enhanced nutritional use.

Our findings are consistent with the studies conducted by Pourakbari and co-workers, who found a positive linear response (to increasing amounts of probiotics) in final BW, carcass characteristics, and organ weights (Pourakbari et al. 2016). However, Rehman and co-workers observed that utilising probiotics did not affect the weights of the thigh, liver, heart, carcass, abdominal fat, and gizzard (Rehman et al. 2020). Bodyweight progressed significantly in the (Biosol, Zemos, and Biosol + Zemos) classes throughout the trial, then became consistent with the control on the fourth week, before increasing again final week. Weight gain increased significantly at the beginning of the experiment, then levelled off with the control group at the end, and it was obvious that weight gain in the (Biosol + Zemos) group reduced on the third week before levelling off with the control group. Feed intake in the beginning phase declined dramatically, then climbed somewhat in the third week in the (Biosol, Zemos) classes, before returning to the normal level in the completing phase. FCR declined dramatically in the first two weeks, then increased somewhat in the (Biosol + Zemos) group on the third week, became comparable to the control in the fourth week, and then decreased again in the final week. The addition of probiotics to the broiler diet resulted in significant improvements in growth performance and FCR throughout the experiment, as well as improved digestion, absorption, and availability of nutrients, as well as a positive effect on intestinal activity and an increase in the activity of some enzymes such as amylase, protease, and lipase. Improvements in carbohydrate, protein and fat digestion are attributed to an increase in enzymatic activity, which is reflected in increased growth and feed conversion ratios (Elbaz and El-Sheikh 2020). Nonetheless, our feed intake findings were in contrast to those of (Elbaz and El-Sheikh 2020; Rehman et al. 2020), who found that supplementing probiotics and prebiotics enhanced feed intake. On the other hand, several reports indicated that dietary addition of probiotics did not affect broiler feed intake (Salehimanes et al. 2016; Sarangi et al. 2016). Differences in outcomes within the trial in
our data could be due to a variety of factors including birds, sex, external stress factors, probiotic strain, probiotic mode of action, and dosing rate.

The promising candidate genes mTOR gene (mammalian Target of Rapamycin), SMYD1 (histone-lysine N-methyltransferase), TLR (Toll receptor) and NBN (nibrin) are evaluated in the present study for chicken growth, carcass quality traits and immune-regulation.

The transcript level of mTOR showed a significant upregulation in the Biosol and Biosol and Zemos groups compared to the control and Zemos groups. The highest transcript was recorded in the heart, breast and thigh respectively. The protein encoded by this mTOR gene is one of the phosphatidylinositol kinase-related kinase family of protein kinases. It is a key component of two protein complexes, 1 and 2, that function as intracellular ATP sensors through the highly conserved Serine-Threonine kinase. It regulates cell development, proliferation, protein synthesis, and insulin and IGF-1 receptor activation (Lee and Aggrey 2015). The mTOR mainly controls protein synthesis by phosphorylation of translational regulators such as eukaryotic translation initiation factor 4E–binding protein (4E-BP) 1 and 4E-BP2 and S6 kinase (S6K), which improves mRNA biogenesis, translational initiation, and elongation, and thus protein synthesis (Ma and Blenis 2009). MTOR has the potential to direct the balance between autophagy and cell multiplication. Nonetheless, autophagy would be down-regulated in the presence of growth factors or nutrients due to the up-regulation of the mTOR downstream enzymes. Autophagy will be up-regulated in the lack of nutrition and growth factors, as well as in the presence of other stresses because Akt/mTOR activation is inhibited (Mohseni et al. 2021).

Probiotics modified energy and protein and lipid metabolism due to the up-regulation of the mTOR (Emami et al. 2020). mTOR abundance might play a crucial role in cellular metabolism and promotes growth (Sampson et al. 2016). Moreover, it regulates the crypt-villus axis responsible for the renewal of the intestinal epithelial cells by controlling the protein synthesis and antioxidant capability of the intestinal epithelium (Yang et al. 2016).

Enterococcus faecalis has been shown to promote macrophage autophagy in vitro by inhibiting the phosphorylation of mTOR and Akt through inducing autophagy in pathogen-loaded macrophages, probiotics can help maintain the immune response (Mohseni et al. 2021). An increased skeletal muscle mass has been demonstrated in rats administered a probiotic, possibly mediated by increased phosphorylation of Akt/mTOR signalling intermediates along with AMPK (Toda et al. 2020).

SMYD1 transcript was significantly up-regulated in the zemos group compared to the other groups mainly in the heart. SMYD is a group of newly discovered proteins that have been linked to the assembly of the myofibrils of both the skeletal and cardiac muscles (Hu et al. 2020). It belongs to the HMT (histone methyltransferases) family, which catalyses the transfer of 1,2 or 3 methyl groups from s-adenosyl methionine to histone protein lysine that primarily functions as a protein-protein interaction module in their protein sequences (Leinhart and Brown 2011). Smyd is involved in the formation of myofibrils such that it serves as a transcriptional repressor in the presence of histone deacetylase (HDAC) (Sirinupong et al. 2010). Moreover, Smyd1 may play a role in myoblast differentiation into myotubes and the organisation of myofibrils in skeletal and cardiac muscles through transcriptional regulation (Tracy et al. 2018).

TLR-4, the biosol showed a significant up-regulation compared to other groups mainly in the heart muscle. Toll-like receptors (TLRs) are a group of receptors that are involved in at least three physiologic processes, including epithelial cell proliferation, antimicrobial factor secretion, and immune response control, all of which contribute to epithelial barrier integrity (Asgari et al. 2018). It acts as the body’s first line of defense against microbes. TLRs are type I transmembrane proteins with 20–27 extracellular leucine-rich repeats (LRR) for the recognition of PAMP(pathogen-associated molecular patterns)/DAMP (damage-associated molecular patterns) and play a key role in connecting innate and adaptive immunity TLRs are type I transmembrane proteins with 20–27 extracellular leucine-rich repeats (LRR) for the recognition of invading pathogens and endogenous danger molecules released from dying cells and damaged tissues. the NBN showed a significant down-regulation among all probiotic groups compared to the control, however, the biosol + zemos group recorded the least transcription. Probiotics inhibit inflammatory responses by inducing gut homeostasis via controlling nuclear factor-B (NF-B) activity, in contrast to pathogenic microorganisms, which launch a pro-inflammatory cascade after TLR triggering (Asgari et al. 2018).

NBN (Nibrin) is a protein-coding gene that is also known as NBS1 (Cell Cycle Regulatory Protein P95) and is found on chromosome 8 (8q21 in Homo sapiens) on chromosome 8. The MRN/NMR complex, also known as the Double strand DNA break complex,
contains 754 amino acids that govern the cellular response to DNA breakdown and the maintenance of chromosomal stability. It’s critical for detecting DNA strands that have been damaged. As well as assisting in their repair. The Nibrin protein regulates the activity of this complex by transporting the MRE11A and RAD50 proteins through the cell nucleus to the site of DNA damage (Nithya and ChandraSekar 2019).

NBN is the complex’s principal regulator, supporting the enzymatic activity of other proteins in the complex and recruiting them to the DNA damage site early on (Wang et al. 2010). NBN also serves as a nuclear focus point and triggers the cell cycle checkpoint, ensuring genomic stability (Lu et al. 2006). probiotic lactic acid bacteria enhanced the production of a large number of pro- and anti-inflammatory mediators and prevented oxidative DNA damage and cellular oxidation (de Oliveira Coelho et al. 2019).

**Conclusion**

Based on our findings, we recommend supplementation of the broiler diet by the probiotic-containing lipotrophic factor Biosol because of its enhancement effect on the growth performance, hematobiochemical parameters, and transcript levels of the examined genes. Interestingly, it is economically worthy to use one probiotic instead of a mixture as the regimen to attain the best results.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Author contributions**

H.A, M.A.I and M.A.E. designed and supervised the study. N.S. and I.M. performed the experiments. N.S., M.A.I and I.M. analysed the data. M.A.I. and N.H. wrote the draft with approval from all authors

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