EFFECTS OF DIETARY LYSOZYME SUPPLEMENTATION ON PERFORMANCE, CARCASS TRAITS, IMMUNITY AND GUT MICROBIAL OF BROILER CHICKENS

Eman S. Ashmawy

Animal and Poultry Nutrition Department, Desert Research Center, Mataria, Cairo, Egypt.

Corresponding author: Eman S. Ashmawy; Emanashmawy@yahoo.com

(*Received 7/3/2024, accepted* 25/3/*2024*)

SUMMARY

Lysozyme is a potential alternative for antibiotic growth promoters in poultry production. The current study investigated the effects of different levels of lysozyme on broilers' performance, immunity, and gut health. A 35-day feeding trial was conducted with a total of 375 one-day-old Ross 308 chicks, which were randomly divided into five dietary treatments: CON) the basal diet; VIR) a basal diet with 200 mg/kg virginiamycin; and LYS) a basal diet with three levels from lysozyme (50, 100, 150 mg/kg diet, respectively). The results showed significant improvement in body weight gain, feed conversion ratio, dressing (%), and digestibility of crude protein in chickens fed 100 and 150 mg lysozyme than other groups. In comparison with the control group, the 150 mg lysozyme group had higher IgGand IgM levelsin serum compared to other groups. Dietary 150 mg lysozymes significantly increased the Lactobacillus and decreased the Clostridium perfringens and total coliforms in the cecum. Enhanced growth performance could be attributed to the positive effects of lysozymes on microflora modification, as well as immune response capacity and improving crude protein digestibility. Therefore, it can be concluded that supplementary up to 150 mg of lysozymes could be used as an effective alternative to antibiotics to promote productivity index and gut health in a broiler diet.

Keywords: *Broiler, lysozymes, performance, immunity, microbial count.*

INTRODUCTION

For many years, antibiotics have been used in the poultry industry as growth promoters to increase meat production leading to the practice has the emergence of antibiotic-resistant pathogens, which pose a potential threat to human health, (resistance to bacterial susceptibility to antibiotics, the creation of antibiotic-resistant strains), that bans their use in animal feed (David *et al.*, 2012; Franz *et al.*, 2010).The European Union and most of the world banned the use of antibiotics due to their risk to the chickens and the consumer (Elbaz *et al.*, 2022). Despite this, many broiler breeders are still forced to use antibiotics, as they develop digestive disorders and intestinal problems, which cause deterioration in performance and health, consequently, the poultry industry needs antibiotic-effective alternatives to enhance animal health and feed efficiency. Therefore, nutrition experts began searching for safe and effective alternatives such as probiotics, herbs, exogenous enzymes, etc. (Elbaz *et al.*, 2021; Abdel-Moneim *et al.*, 2020).

Lysozyme is a natural antibacterial enzyme, lysozyme exerts bacteriolytic activity directly by hydrolyzing the β-1,4-glycosidic linkage between N-acetylmuramic acid and N-acetyl glucosamine of bacterial peptidoglycans in the cell wall (Ma *et al.*, 2017), in addition to impact indirectly by enhancing immune function including in stimulating macrophage phagocytic (Xia *et al.*, 2019). Dietary lysozyme has been reported to improve the immune response, help maintain gut barrier function, and improve growth performance in broilers (Gong *et al.*, 2017). It has also been shown to reduce pathogen counts in the ceca of broiler chickens (Lee *et al.*, 2009), enhance their antioxidant status and nonspecific immunity, and

improve their growth performance (May *et al.*, 2012). Limited studies have been carried out on the effect of dietary lysozyme on growth performance, immune-antioxidant status, and gut microbiota in broiler chickens. This study hypothesizes that adding dietary lysozyme may improve growth performance and intestinal integrity, in addition to enhancing the immune status of chickens. Consequently, in this study, we used different levels of lysozyme in a broiler diet to study the effect on growth performance, immune-antioxidant status, and gut microbial count.

MATERIALS AND METHODS

Experimental design, chicks, and diets:

A total of 375 one-d-old (Ross 308) chicks were obtained from a local commercial hatchery and randomly divided into five dietary groups with 75 chicks (5 replicate/ group) placed in each group based on similar body weights (42±3 g). Birds in each group were arranged in five identical stainless-steel cages (15 birds in each cage) with plastic mesh floors (1.5 m2 floor area/pen). The experimental groups were as follows: CON) the basal diet (corn and soybean), without feed additive, VIR) CON+ 200 mg/kg virginiamycin, LYS1) CON+ 50 mg/kg lysozyme, LYS2) CON+ 100 mg/kg lysozyme, and LYS3) CON+ 150 mg/kg lysozyme. The broiler chicks were exposed to continuous light. Room temperature was maintained at 32˚C for the first 3 d and then gradually reduced to 22˚C until the 5th week. Corn-soybean-based basal diets were formulated as follows; the starter phase (1–21 d, 23% CP and 3000 kcal/kg ME) and the grower phase (22–35 d, 21% CP and 3100 kcal/kg ME diet), nutrients in the experimental diets met the requirements for chicks as recommended by the National Research Council (NRC, 1994), as shown in Table 1. Chicks offered feed and freshwater *ad libitum.*

Table (1): Composition and calculated analysis of the experimental basal diet.

Feed ingredient %	Starter (1-21d)	Grower (22-35d)
Yellow corn	58.18	62.19
Soybean meal (%)	32.62	27.61
Corn gluten meal (%)	4.00	4.00
Dicalcium phosphate	1.80	2.00
Calcium bicarbonate	1.40	1.40
L-Lysine HCl	0.05	0.05
DL-Methionine	0.15	0.15
Soya oil	1.20	2.00
Premix*	0.30	0.30
Salt (NaCl)	0.30	0.30
Chemical composition		
ME (kcal/kg)	3000	3100
Crude protein, %	23.00	21.00
Calcium, %	1.029	1.012
Available phosphorus, %	0.461	0.475
Lysine, %	1.114	1.105
Methionine, %	0.578	0.491

** Provided per kilogram of diet: vitamin E, 44.1 IU; vitamin D3, 6636 IU; vitamin K, 4.5 mg; vitamin A, 13,233 IU; niacin, 88.2 mg; thiamine, 2.21 mg; riboflavin, 6.6 mg; pantothenic acid, 24.3 mg; pyridoxine, 3.31 mg; folic acid, 1.10 mg; biotin, 0.33 mg; vitamin B12, 24.8 µg; choline, 669.8 mg; iron from ferrous sulfate, 50.1 mg; manganese from manganese oxide, 125.1 mg; iodine from ethylene diaminedihydroiodide, 2.10 mg; zinc from zinc oxide, 125.1 mg; copper from copper sulfate, 7.7 mg; selenium from sodium selenite, 0.30.*

Performance index:

Birds and feed were weighed weekly (each bird individually) and the mortality was recorded daily. Average feed intake (AFI), body weight gain (BWG), and the feed conversion ratio (FCR) were calculated. At the age of 35 days, ten chickens were slaughtered from each treatment to estimate the specifications of the carcass, including dressing, breast, thigh, liver, and abdominal fat. Lymphoid organs such as the thymus, spleen, and bursa of Fabricius were weighed as an immune index.

Digestibility trial:

At the age of 35 days, the digestion experiment started. Three broilers from each treatment group were weighed, housed individually in metabolic cages then starved for 12 hours. During the age of 35 to 39 days, unpolluted excreta were collected, three times a day from the bottom of each cage, weighed after being dried to analyze and measure the digestibility of crude protein (CP**)**, crude fiber (CF), and dry matter (DM) according to Association of Official Analytical Chemists, (AOAC, 2000).

Immune responses:

On day 35, before slaughter (10 birds/ group), blood samples were drawn from the jugular vein and then centrifuged (3000 rpm for 15 min) to obtain the serum. Blood immunoglobulin (IgA, IgM, and IgG) concentrations were determined using chicken-specific IgA, IgM, and IgG ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX, USA).

Microbial count:

At the end of the experiment (35 d), 5 birds per group were selected and slaughtered, and 3 g of cecum content was collected in sterilized sampling tubes and frozen at −20◦C, respectively, for subsequent analysis. Then, 10-fold serial dilutions of one g of sample were serially made in phosphate buffer solution. Subsequently, 100 µl were removed from 10^{-4}, 10^{-5}, and 10^{-6} dilutions, and poured onto Petri dishes containing the agar (culture media). *Clostridium perfringens*, *Escherichia coli*, and *total coliforms* were cultured in tryptose sulfite cycloserine agar, MacConkey agar, and MacConkey agar, respectively, and incubated at 37°C for 24- 48 hours under aerobic conditions. *Lactobacilli* were cultured in De Man, Rogosa, and Sharpe's agar and incubated at 37 °C for 24 hours under anaerobic conditions. Cecal contents were counted for microbial populations using a conventional method (spread plate method) by Casagrande Proietti *et al.,* (2009).

Statistical Analysis:

All data were analyzed as a one-way ANOVA using the GLM procedure of the SAS system (SAS, 2004) by performing the Duncan Multiple Range Test have been done to determine the differences among treatment means (Duncan, 1955). The chosen level of significance for all comparisons was $p < 0.05$.

RESULTS AND DISCUSSION

Growth performance:

The impact of different levels of lysozyme on the growth performance of broiler chickens is shown in Table 2. There was a significant improvement in BWG ($P<0.05$) with increasing levels of lysozyme in all stages of the experiment ($P<0.05$), with an enhancement in FCR in broiler fed 150 mg/kg lysozyme in the second stage, also, an enhancement ($P<0.05$) in FCR was observed in broiler fed on 100 and 150 mg/kg lysozyme in the overall stage. Despite that, different levels of lysozyme did not affect ($P<0.05$) the AFI of broilers during all stages of the trial. The LYS3 group had the best BWG and FCR in comparison to the other groups. This result was supported by numerous studies, Oliver *et al.* (2014) found that lysozyme positively impacted feed efficiency and weight gain in pigs. Deng *et al.* (2021) stated that lysozyme supplementation significantly increased BW, ADG, and AFI and enhanced FCR in weaned piglets. The improvement in the final BWG and FCR of broiler chickens is due to the dietary inclusion of lysozyme to promote nutrient utilization by modulated gut microbial. Many reports confirmed the enhancement in nutrient digestibility and intestinal microbes in nursery pigs or rabbits fed lysozyme (El-Deep*et al.,* 2020; Oliver and Wells, 2015). In addition, it significantly reduced diarrhea incidence in piglets. Lysozyme has natural antimicrobial properties, which play an important role in improving animal performance (Abdel-Latif *et al.,* 2017). Furthermore, many studies suggested that the lysozyme effect would regulate metabolism and the modulation of the immune response (Petruschke*et al.,* 2021). All the above findings confirm the beneficial effect of supplementing lysozyme to broiler feed as a growth promoter and an effective and safe alternative to antibiotics.

Carcass traits:

Supplementation of lysozyme significantly increased dressing percentage ($P<0.05$), in addition to the insignificantly increase in breast ($P=0.063$), however, the other carcass characteristics (thigh, liver, and abdominal fat) were not affected by the experimental treatments, as indicated in Table 3. Furthermore,

supplementation of lysozyme enhanced immune organs, including increased (P<0.05) relative weight of bursa of Fabricius. However, the relative weight of the thymus and spleen were not affected (P<0.05) by the experimental diet. Our result was supported by Abu Hafsa *et al.* (2022) who found that lysozyme positively impacted dressing percentage in growing rabbits. Also, our results were consistent with the report, by Hanieh *et al.* (2010) and Elbaz *et al.* (2023) who illustrated that probiotics, such as lysozyme which has an antimicrobial property, supplementation in broilers increased the relative weights of the immune organs, resulted in enhanced immunity. Such a positive effect on carcass traits can be attributed to enhanced nutrient digestibility via the improvement of intestine microbial balance and morphology which leads to an improve in feed digestion and absorption (Abu Hafsa *et al.,* 2022; Fang *et al.,* 2020). Thus, the productive performance of the broiler improves, including the carcass characteristics.

Table (2): Growth performance of broilers fed different levels of lysozyme at 35 days' experimental period.

Item	CON	VIR	LYS1	LYS2	LYS3	SEM	P-value
			0–21d				
BWG (g)	751^{b}	768^{ab}	748b	780^{ab}	812^{a}	7.011	0.001
AFI (g)	938	945	937	941	928	5.340	0.127
FCR	1.249^{a}	1.123^{b}	1.253^{a}	1.206^{ab}	1.143^{b}	0.008	0.001
			22–35d				
BWG (g)	1062	1106	1087	1099	1115	3.975	0.058
AFI (g)	2405	2.439	2441	2443	2436	9.244	0.094
FCR	2.264^{a}	2.205^{b}	2.246^{a}	2.242^{a}	2.184^{b}	0.011	< 0.001
			0–35d				
BWG (g)	1813^{c}	1874^{b}	1835^{c}	1879^{b}	1927^{a}	8.025	< 0.001
AFI (g)	3343	3385	3378	3382	3364	10.34	0.163
FCR	1.844^{a}	1.806^{b}	1.841^{a}	1.811^{b}	1.746^{c}	0.032	< 0.001

$^{a–b}$ Means within a row with different superscripts are significantly different at P < 0.05. CON) Chickens fed a basal diet supplemented without supplementation, VIR) Chickens fed a basal diet supplemented with virginiamycin, LYS1) Chickens fed a basal diet supplemented with 50 mg lysozyme, LYS2) Chickens fed a basal diet supplemented with 100 mg lysozyme, LYS3) Chickens fed a basal diet supplemented with 150 mg lysozyme, AFI) average feed intake, BWG) body weight gain, FCR) feed conversion ratio.

Table (3): Carcass traits (%) of broilers fed different levels of lysozyme at 35 days.

Item	CON	VIR	LYS1	LYS2	LYS3	SEM	P-value
Dressing	71.4^{c}	72.1b^{c}	71.2^{c}	72.6^{b}	73.6^{a}	2.216	< 0.001
Thigh %	15.8	16.1	15.6	15.9	15.7	0.947	0.950
Breast %	24.7	24.3	24.8	24.6	25.1	1.025	0.063
Liver %	4.26	4.31	4.22	4.27	4.25	0.023	0.115
Abdominal fat %	3.74	3.68	3.71	3.73	3.65	0.051	0.097
Thymus %	0.311	0.324	0.315	0.319	0.328	0.003	0.081
Spleen %	0.157	0.162	0.155	0.156	0.161	0.007	0.153
Bursa of Fabricius %	0.204^{b}	0.201^{b}	0.208^{b}	0.219^{ab}	0.235^{a}	0.011	0.001

$^{a–b}$ Means within a row with different superscripts are significantly different at P < 0.05. CON) Chickens fed a basal diet supplemented without supplementation, VIR) Chickens fed a basal diet supplemented with virginiamycin, LYS1) Chickens fed a basal diet supplemented with 50 mg lysozyme, LYS2) Chickens fed a basal diet supplemented with 100 mg lysozyme, LYS3) Chickens fed a basal diet supplemented with 150 mg lysozyme.

Nutrients digestibility:

In this study, results indicate that the digestibility of dry matter (DM) and crude protein (CP) in broilers were significantly increased (P<0.05) by the addition of different levels of lysozyme, as shown in Table (4). Nevertheless, there was no effect (P<0.05) on digestibility of crude fiber among experimental groups. These results are similar to those obtained by Abu Hafsa *et al.* (2022) who found that significant

improvements were observed in nutrient digestibility when including lysozyme in feeding growing rabbits. The improvement in nutrient digestion may also be due to the effects of lysozyme in modifying the microbial content of the animal gut by increasing beneficial microbes and decreasing harmful microbes, which boosts gut health, and thus enhances nutrient metabolism (Deng *et al.,* 2021). Consequently, the addition of lysozyme has a potent effect on increasing the useful microbials in the gut, which enhances nutrient digestion and absorption and improves growth performance.

Table (4): Nutrient digestibility (%) of broilers fed different levels of lysozyme at 35 days of age.

Item	CON	VIR	LYS1	LYS2	LYS3	SEM	P-value
Dry matter	66.2^{b}	66.5^{b}	66.3^{b}	67.1^{a}	67.4^{a}	0.030	0.020
Crude protein	69.4^{b}	70.8^{ab}	70.3^{b}	71.7^{a}	72.1^{a}	0.054	0.001
Crude fiber	57.7	57.4	57.9	57.6	58.3	0.019	0.054

$^{a–b}$ Means within a row with different superscripts are significantly different at P < 0.05. CON) Chickens fed a basal diet supplemented without supplementation, VIR) Chickens fed a basal diet supplemented with virginiamycin, LYS1) Chickens fed a basal diet supplemented with 50 mg lysozyme, LYS2) Chickens fed a basal diet supplemented with 100 mg lysozyme, LYS3) Chickens fed a basal diet supplemented with 150 mg lysozyme.

Immunoglobulin:

Serum immunoglobulins (IgG, IgA, and IgM) are important effectors of humoral immunity. IgM is the earliest-produced immunoglobulin in humoral immunity, as well as IgG plays the important role in the secondary immune response. In this study, the effect of supplementation lysozyme on immunoglobulin is shown in Table 5. The serum IgG and IgM content were significantly higher (P<0.05) in the LYS2 and LYS3 groups than in the other group on day 35 ($p < 0.05$). The lysozyme diets had no significant effect (P<0.05) on the IgA content of chickens compared to that of the control and VIR group (P<0.05). Our results supported those obtained by Xu et al. (2022) who found that dietary supplementation with 500 mg/kg coated lysozyme increased the IgG concentration in weaned piglets. Similarly, May *et al.* (2012) confirmed the positive role in stimulating the immune response through the addition of lysozyme. This finding shows that supplemental lysozyme can promote the synthesis of proteins (immunoglobulins), which can promote the immune index of broilers.

Table (5): Immunoglobulin of broilers fed different levels of lysozyme at 35 days of age.

Item	CON	VIR	LYS1	LYS2	LYS3	SEM	P-value
IgA	167	162	168	165	171	13.41	0.078
IgG	297^{b}	301^{b}	287^{b}	325^{ab}	364^{a}	7.024	0.003
IgM	107^{c}	114^{c}	109^{c}	134^{b}	158^{a}	5.253	< 0.001

$^{a–b}$ Means within a row with different superscripts are significantly different at P < 0.05. CON) Chickens fed a basal diet supplemented without supplementation, VIR) Chickens fed a basal diet supplemented with virginiamycin, LYS1) Chickens fed a basal diet supplemented with 50 mg lysozyme, LYS2) Chickens fed a basal diet supplemented with 100 mg lysozyme, LYS3) Chickens fed a basal diet supplemented with 150 mg lysozyme.

Cecum microbial:

Intestinal bacteria form a complex microbial system with important various functions, like digestion, absorption, metabolism, and immunity (Kelly *et al.,* 2005). According to our results, dietary supplementation of lysozyme significantly increased (P<0.05) the count of *Lactobacilli* and significantly decreased (P<0.05) the count of *Clostridium perfringens* and *Total coliforms* in the cecum of broilers. This result is consistent with the result that the supplementation of lysozyme significantly reduces the harmful bacterial content (Xia *et al.,* 2019). These results are also in line with those stated that the addition of lysozyme was efficient in modulating the bacterial content in the duodenum and ileum of both goats and piglets Maga *et al.* (2006). Also, Liu et al. (2010) found that lysozyme inclusion significantly increased the *Lactobacillus* counts and reduced the *E. coli* counts in the ileum in pigs. Therefore, lysozyme could suppress the growth of *Clostridium perfringens* and *Total coliforms*, in addition to supporting the growth of Lactobacilli, resulting in healthy intestinal development in broiler chickens.

Table (6): Cecum microbial count and antioxidant capacity of broilers fed different levels of lysozyme at 35 days of age.

Item	CON	VIR	LYS1	LYS2	LYS3	SEM	P-value
Lactobacilli	5.250^{c}	4.361^{b}	5.108^{c}	5.757^{b}	6.611^{a}	0.047	0.020
C. perfringens	1.842^{a}	1.241^{c}	1.527^{b}	1.034^{cd}	0.872^{d}	1.052	0.001
E. coli	1.342	1.374	1.325	1.366	1.321	0.039	0.227
Total coliforms	3.521^{a}	3.465^{b}	3.518^{a}	3.349^{b}	3.102^{c}	0.974	0.001

$^{a–b}$ Means within a row with different superscripts are significantly different at P < 0.05. CON) Chickens fed a basal diet supplemented without supplementation, VIR) Chickens fed a basal diet supplemented with virginiamycin, LYS1) Chickens fed a basal diet supplemented with 50 mg lysozyme, LYS2) Chickens fed a basal diet supplemented with 100 mg lysozyme, LYS3) Chickens fed a basal diet supplemented with 150 mg lysozyme, C. perfringens: Clostridium perfringens, E. coli: Escherichia coli.

CONCLUSION

In this study, lysozyme dietary supplementation resulted in improved growth performance by increased BWG, dressing percentage, and digestibility of DM and CP. In addition, lysozyme supplementation leads to enhanced immune response via increased serum IgG and IgM levels and relative weight of bursa of Fabricius, furthermore, modulating the cecum microbial content through increased *Lactobacilli* count and decreased *Clostridium perfringens* and *Total coliforms* count. It can be suggested that lysozyme supplementation could be a safe and effective alternative to antibiotics.

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آثار مكملات الليزوزيم الغذائية على الأداء، وصفات الذبيحة، والمناعة، والميكروبات المعوية لدجاج التسمين

إيمان شعبان عشماوي

مركز بحوث الصحراء - وزارة الزراعة - المطرية - القاهرة – مصر

يعد الليزوزيم بديلاً محتملاً للمضادات الحيوية المحفزة للنمو في إنتاج الدواجن. بحثت الدراسة الحالية في آثار مستويات مختلفة من الليزوزيم على أداء دجاج التسمين ومناعته وصحة الأمعاء. تم إجراء تجربة تغذية لمدة 35 يومًا بإستخدام 375 كتكوتًا من سلالة Ross 308 عمرها يوم واحد، والتي تم تقسيمها عشوائيًا إلى خمس معاملات غذائية: CON) العليقة القاعدية؛ VIR) العليقة القاعدية مع 200 ملجم/كجم من فيرجينيا مايسين؛ وLYS) العليقة القاعدية مع ثلاثة مستويات من الليزوزيم (50، 100، 150 ملجم/كجم من العليقة، على التوالي). أظهرت النتائج تحسناً معنوياً في وزن الجسم ومعامل التحويل الغذائي وتصافي الذبيحة (%) وقابلية هضم البروتين الخام في الدجاج المغذى على 100 و150ملجم من الليزوزيم مقارنة بالمجموعات الأخرى. بالمقارنة مع مجموعة الكنترول، كانت المجموعة المغذاة بالليزوزيم بمستوى 150 ملجم لديها مستويات أعلى من IgG وIgM في المصل مقارنة بالمجموعات الأخرى. أدت التغذية على 150 ملجم من الليزوزيم إلى زيادة البكتيريا اللاكتوباسيلاس بشكل ملحوظ وتقليل البكتيريا الضارة في الأعور. يمكن أن يعزى أداء النمو المعزز الذي لوحظ في التجربة إلى التأثيرات الإيجابية لليزوزيم على تعديل البكتيريا، فضلاً عن القدرة على تعزيز الاستجابة المناعية. لذلك، يمكن أن نستنتج أنه يمكن استخدام ما يصل إلى 150 ملجم من الليزوزيم التكميلية كبدائل فعالة للمضادات الحيوية لتعزيز مؤشر الإنتاجية وصحة الأمعاء في علائق دجاج التسمين.