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Exogenous lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology of broiler chickens

R.A. Hassan[1*]; Y.Z. Eid[2] and M. A. M. Mousa[1],
[1]*Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.*
[2]*Department of Poultry Production, Faculty of Agriculture, Kafr El-Sheikh University, Egypt.*
**Corresponding author e-mail address (redaalihasan@yahoo.com)*

Abstract. there have been successful attempts to replace antibiotics in broilers with dietary lysozyme (LYZ), but more study is required to ensure its efficacy. We investigated the effects of LYZ and the feed addition of avelamycin (AVI) on broiler chickens' immunity, gastrointestinal health, and growth performance. Two hundred and seventy-one-day-old broiler chicks (Ross 308) were randomly assigned to three groups, each consisting of six replicates and each consisting of fifteen birds. The standard diet without supplements was applied as control group (I), while chicks in the other groups were provided with 100 mg AVI per kg of diet (AVI, group II), and 90 mg LYZ per kg of diet (LYZ, group III) for five consecutive weeks. In comparison to the CON group, the AVI and LYZ groups showed significant ($p<0.05$) increases in body weight, feed conversion ratio, body weight gain, European production efficiency factor, protein efficiency ratio, and digestibility of crude protein; however, feed intake remained unchanged. IgG and IgM levels in the serum were greater in the lysozyme group than in the AVI and control groups. Comparing the serological hemagglutination inhibition titers of NDV vaccination to the control and AVI groups, the LYZ-treated group showed a substantial increase ($p < 0.05$). Dietary 90 mg lysozymes dramatically reduced the amount of *E. coli* and total coliforms in the cecum while increasing Lactobacillus. Comparing the LYZ group to the AVI and control groups, the VH and VH/CD were significantly greater. In conclusion intestinal integrity, cecum bacterial counts, immunological response, and growth performance were all improved by exogenous dietary lysozyme supplementation at a level of 90 mg/kg broiler diet, and these results were on par with avilamycin. Thus, dietary lysozyme could be a safe substitute for avilamycin in the diet of broiler chickens.
Keywords: Lysozyme, broiler chickens, growth, intestinal morphology

Introduction

Maintaining intestinal health to properly fully utilize dietary nutrients and enable the manifestation of the entire genetic potential for growth is known as intestinal integrity in broilers. Enhancing this integrity is crucial for endogenous material secretion, better nutritional absorption and digestion, effective gut microbial balance, and defense against infectious and non-infectious agents. It was strongly urged by all of the earlier research that a broiler production program should continuously assess intestinal integrity (Fang et al., 2020). Since January 1, 2006, the USA and/or Europe have prohibited the addition of some antibiotics used in human medicine, including avilamycin, monensin, salinomycin, and flavophospholipol, as growth promoters in animal feed (Marshall and Levy, 2011). According to the WHO's 2019 list of the top 10 global public health concerns confronting humanity, the One Health strategy emphasizes the significance of combating antimicrobial resistance (AMR). AMR, often known as the silent tsunami or pandemic, poses a worldwide danger to public and animal health, and bacterial infections are the second leading cause of death globally (Escribano, 2023). Therefore, it is crucial to look for antibiotic substitutes for poultry diets that have comparable One Health advantages (Janardhana et al., 2009). Promising substitutes for antibiotics include probiotics, prebiotics, phytobiotics (Abd El-Ghany et al., 2022), exogenous enzymes (Saleh et al., 2018), and organic acids (Ding et al., 2020). As part of the worldwide effort to combat antibiotic resistance, these substitutes are now required. One of the most promising substitutes for antibiotics is Lysozyme (LYZ). It is an enzyme that derived from avian albumin and is found in many animal tissues and secretions. It is essential in combating a number of bacteria that cause a positive Gram stain test result because it breaks down the β-1,4-glycosidic bond between N-acetyl glucosamine and N-acetyl muramic acid in their cell walls (Ibrahim et al., 1994).Following the beneficial results of previous study of sole LYZ with several levels (Abdel-Latif et al., 2024) and others (Liu et al., 2010), we were motivated to compare the best obtained level of LYZ (90 mg/kg feed) (Abdel-Latif et al., 2024) with the most used growth promoter antibiotic AVI (100 mg/kg) (Wellenreiter et al., 2000). In order to replace growth-promoting antibiotics, the purpose of this innovative study was to examine the effects of dietary LYZ and AVI on intestinal health, growth performance, and viability in broiler production. In order to improve the One Health approach, the study's results can aid in preventing and slowing the spread of antibiotic resistance in people.

Materials and methods

Experimental design

One-day-old, Ross-308 broiler unsexed chicks (obtained from a commercial hatchery), with a total number of 270 were randomly allocated into three groups (90 birds/ group reared in 6 replicates of 15 birds each). The birds were housed in floor penscovered with wood shaving litter (stocking density was 10 birds / m2) and individually weighed and randomly assigned to five groups. The total duration of the experimental trial was 35 days. The temperature of 35±2°C and relative humidity (RH) of 65% was maintained by day 1 of the experiment. The temperature decreased gradually by 3°C per week to reach 26°C at day 21 of the investigation. From day 22 onwards, the temperature of 26°C and RH of 65 ± 5 % and the duration of light exposure was a 23 h of light daily. Chicks offered feed and freshwater ad libitum. The chicks of 3 groups, G1 (control) fed on a commercial basic diet; G2 (AVI) supplemented with commercial basic diet containing 100 mg of avilamycin/kg which is the maximum dose recommended by the producer company, (Hangzhou Well Sunshine Biotech.Co. Ltd., China), and G3 (LYZ) fed the same basal diet supplemented with 90 mg of lysozyme 10%/kg diet (Nanchang Lifeng Industry and Trading Co., Ltd., Jiangxi, China) according to Abdel-Latif et al. (2024).The rearing cycle was 35 days. The used corn–soybean based diet was formulated according to the nutrient requirements for Ross-308 broiler chickens (Ross Nutrition Specifications, 2019). The nutritive analysis of the ingredients was done according to AOAC (2005). The ingredients and chemical-nutritional characteristics of the basal diet is shown in Table 1. Gradual and effective mixing of both feed supplements was confirmed. The chicks, at day 7, were injected subcutaneously in the back of the neck with inactivated Newcastle disease (NDV) plus inactivated avian influenza (H9N1) vaccines while they were vaccinated against infectious bursal disease (IBD) at day 14.

Measurements

Performance index:

Performance metrics such as feed consumption (FC), body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR, the ratio between average feed intake and body weight gain). Additionally, mortality rate was noted during the entireexperiment. Furthermore, European production efficiency factor (EPEF) was calculated at the end of the experiment using the following formula: [(viability % × body weight Kg / age (d) × FCR)] ×100 (Marcu et al., 2013).

Table 1 Ingredients' percentage and calculated composition analysis of the experimental starter and grower diets (%, as-fed basis)

Ingredients %	Starter (0–10 d)	Grower (11–21 d)	Finisher (22 35 d)
Yellow corn	54.87	58.88	63.90
Soybean meal 44%	33.5	29.4	24.0
Corn gluten (60%)	5	5	5
Corn oil	2.0	2.65	3.15
Dicalcium phosphate	1.73	1.60	1.50
$CaCo_3$	1.35	1.00	1.00
Nacl	0.4	0.4	0.4
DL-Methionine*	0.15	0.12	0.10
Hcl-Lysine**	0.35	0.30	0.30
Premix***	0.3	0.3	0.3
Toxin binder	0.2	0.2	0.2
Sodium bicarbonate	0.1	0.1	0.1
Choline chloride	0.05	0.05	0.05
Calculated composition			
ME, Kcal/kg diet	3005	3100	3195
CP %	23.0	21.5	19.5
Ca %	1.00	0.87	0.82
Avail. P %	0.47	0.44	0.41
Methionine %	0.56	0.51	0.47
Lysine %	1.44	1.29	1.14
Meth + Cyst.	0.93	0.86	0.78
Na %	0.2	0.2	0.2

ME = metabolizable energy, CP = crude protein, Av. (P) = available phosphorous. * DL—methionine 99% feed grade China. ** L— lysine 99% feed grade. *** Vitamin and mineral premix (Hero mix) produced by Hero pharm and composed (per 3 kg) of vitamin A 12,000,000 IU, vitamin D3 2,500,000 IU, vitamin E 10,000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin

B12 10 mg, niacin 30,000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10,000 mg, manganese 60,000 mg, zinc 50,000 mg, iron 30,000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.

Carcass traits

The carcass parameters, including dressing, breast, thigh, liver, and abdominal fat, were estimated by slaughtering six chickens from each treatment at 35 days of age. As a measure of immunity, lymphoid organs such the spleen, bursa of Fabricius, and thymus were weighed.

Digestibility trial:

At the end of the experimental period, (35 days of age) the digestion experiment started. Six broilers from each treatment group were weighed, housed individually in metabolic cages then starved for 12 hours. During the period of 35 - 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 dry matter (DM), crude protein (CP), and crude fiber (CF) according to AOAC, (2000).

Biochemical parameters

Blood samples (n = 6) were collected during slaughtered at 35 d of age without anticoagulant for serum separation. Samples were centrifuged at 1435 × g for 5 min at 4°C to obtain clear sera for biochemical analyses. The collected sera were analyzed for total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, triacylglycerol (TAG), creatinine, and uric acid concentrations. Serum globulin levels were estimated by subtraction of the albumin value from the total protein value of the same sample (Coles, 1986). Serum total antioxidant capacity (TAC), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) were also determined. Blood biochemistry and antioxidants were assessed calorimetrically using bio-diagnostic kits (Biodiagnostic, Cairo, Egypt), following the manufacturer's directions.

Blood immunoglobulin (IgA, IgM, and IgG) concentrations were assessed by chicken-specific IgA, IgM, and IgG ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX, USA).

Antibody titers for Newcastle disease vaccine (NDV) were determined in each group (n = 6) at 35 d of age, with the HI test as recommended by Brugh (1978).

Microbial count:

At the end of the experiment (35 d), 6 birds per group were selected and slaughtered, and 3 g of cecal content were collected in sterilized sampling tubes and frozen at −20◦C, respectively, for estimate cecum microbiota. Total bacterial count, *Escherichia coli*, *Clostridium*, and *Lactobacillus* count were performed according to Collins and Lyne (1970).

Statistical Analysis:

All data were analyzed using the one-way ANOVA of GLM procedure of the SAS system (SAS, 2004).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

Broiler growth performance

On the first day of age, there were no discernible differences in the live BW of the chicks across the various experimental groups (Table 2). The final body weight (FBW) and body weight gain(BWG) were significantly increased for AVI and LYZ supplemented groups when compared to the control group. Additionally, the FCR, PER and EPEF were significantly ($p<0.05$) enhanced in AVI and LYZ groups compared to control group

Table 2 Effects of avilamycin (AVI) and lysozyme (LYZ) feed supplements on growth performance and economic viability of broilers

Items	Experimental groups			SEM	p-value
	CON	LYZ	AVI		
IBW,(g)	41.21	41.09	41.12	0.392	0.862
FBW, (g)	2000.7[b]	2160.0[a]	2110.5[a]	37.801	0.005
BWG, (1-35 g per chick)	1959.5[b]	2118.91[a]	2069.38[ab]	35.166	0.017
FC, (1-35 g per chick)	3320	3330	3325	22.672	0.062
FCR, (g feed per g BWG)	1.694[a]	1.571[b]	1.606[b]	0.033	0.004
EPEF	323.08[b]	385.22[a]	363.88[ab]	16.182	0.046
Viability, %	97.77	100.00	98.88	--	--

[a-b]Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error means; CON: Chickens fed a basal diet supplemented without supplementation; LYS: Chickens fed a basal diet supplemented with 90 mg lysozyme; AVI: Chickens fed a basal diet supplemented with avilamycin; IBW: Initial body weight; FBW: Final body weight; BWG: Body weight gain; FC: Feed consumption; FCR: Feed conversion ratio and EPEF: European production efficiency factor.

without marked difference between AVI and LYZ groups in all growth parameters. However, feed intake was numerically improved in both supplemented groups compared to the control group.

Nutrients digestibility:

In this study, results indicate that the digestibility of dry matter (DM), crude protein (CP) and crude fiber (CF) in broilers were significantly increased ($P<0.05$) by the addition of lysozyme, as shown in Table (3).

Table 3. Effects of avilamycin (AVI) and lysozyme (LYZ) feed supplements on nutrient digestibility (%) of broilers

Items	Experimental groups			SEM	p-value
	CON	LYZ	AVI		
Dry matter	66.4^{b}	67.5^{a}	66.8^{b}	0.046	0.036
Crude protein	70.5^{b}	72.6^{a}	70.9^{b}	0.062	0.002
Crude fiber	57.0	58.3	57.4	0.021	0.052

$^{a-b}$Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error means; CON: Chickens fed a basal diet supplemented without supplementation; LYS: Chickens fed a basal diet supplemented with 90 mg lysozyme; AVI: Chickens fed a basal diet supplemented with avilamycin

Carcass traits

Results of dressing % and some carcass trait percentages in the experimental groups are displayed in Table 4. Results showed significant enhancement in dressing rate and Thigh and breast weight percentage by lysozyme in the broiler chicken diet compared to AVI additions and supplement-free diet (Table 4).

Table 4. Effects of avilamycin (AVI) and lysozyme (LYZ) feed supplements on carcass traits of broilers

Items	Experimental groups			SEM	p-value
	CON	LYZ	AVI		
Dressing %	71.0^{b}	72.80^{a}	72.0^{ab}	2.181	0.001
Thigh weight %	31.2	32.1	32.3	6.856	0.063
Breast weight %	42.5	41.6	42.6	1.130	0.861
Liver weight %	4.20	4.25	4.13	0.060	0.176
Abdominal fat %	3.70	3.65	3.68	0.026	0.088

$^{a-b}$Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error means; CON: Chickens fed a basal diet supplemented without supplementation; LYS: Chickens fed a basal diet supplemented with 90 mg lysozyme; AVI: Chickens fed a basal diet supplemented with avilamycin.

Biochemical analysis

The blood total protein and globulin of chicks fed dietary lysozyme and AVI additives were meaningfully increased ($p < 0.05$) compared with the control (Table 5). Dietary lysozyme resulted in higher blood total protein and globulin than chicks treated with AVI ($p<0.05$). Blood creatinine and uric acid were numerically lowered by dietary lysozyme compared with the control and AVI-treated chicks (Table 5). The

Table 5. Effects of avilamycin (AVI) and lysozyme (LYZ) feed supplements on some blood constituents and antioxidants capacityof broilers

Items	Experimental groups			SEM	p-value
	CON	LYZ	AVI		
Total protein (mg/dl)	5.20	5.80	5.30	0.185	0.062
Albumin (mg/dl)	3.60	3.80	3.66	0.074	0.326
Globulin (mg/dl)	1.60[b]	2.00[a]	1.64[b]	0.125	0.001
Total cholesterol (mg/dl)	108.5	113.0	110.0	7.861	0.078
Triglycerides (mg/dl)	135.0	132.0	130.0	3.105	0.196
Liver functions					
AST (IU/L)	210.3	210.0	208.0	3.171	0.449
ALT (IU/L)	18.0	17.0	17.8	1.961	0.812
Kidney functions					
Creatinine (mg/dl)	0.52	0.48	0.53	0.044	0.860
Uric acid (mg/dl)	7.86	6.70	7.00	0.56	0.078
Antioxidants capacity					
TAC (ng / ml)	0.29c	0.36a	0.31b	0.024	0.001
GPx (IU /L)	28.2c	46.2a	40.3b	1.532	0.001
SOD (IU / L)	0.26b	0.33a	0.30ab	0.021	0.004
MDA (nmol /ml)	0.46a	0.28c	0.32b	0.015	0.025

[a-b]Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error means; CON: Chickens fed a basal diet supplemented without supplementation; LYS: Chickens fed a basal diet supplemented with 90 mg lysozyme; AVI: Chickens fed a basal diet supplemented with avilamycin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; TAC: total antioxidants capacity; GPx: glutathione peroxidase; SOD: superoxide dismutase; MDA: malondialdehyde.

levels of blood ALT, and AST were numerically lowered (p<0.05) by dietary lysozyme and AVI (Table 4). No meaningful impacts of lysozyme or AVI were seen on the blood cholesterol and triglycerides (p > 0.05) (Table 5).

Data on antioxidant capacity markers and immunity constituents in blood serum are shown in Table 5. Supplementation of AVI, and lysozyme significantly increased TAC, GSH, GPx and SOD and significantly decreased MDA levels compared to the control diet.

Immunity

Data showed in Table 6 shows supplementation of lysozyme enhanced immune organs, including increased (P<0.05) relative weight of bursa of Fabricius and thymus and spleen (Table 6).

Data illustrated in Table 6 shows increment in serum IgG, IgM and NDV antibody titers in lysozyme and AVI groups when compared to control group at 35 d. The antibody titers were higher in LYZ than AVI group.

Table 6. Effects of avilamycin (AVI) and lysozyme (LYZ) feed supplements on immunity of broilers

Items	Experimental groups			SEM	p-value
	CON	LYZ	AVI		
Immune organs					
Thymus %	0.310[b]	0.328[a]	0.325[ab]	0.004	0.048
Spleen %	0.152[b]	0.162[a]	0.162[a]	0.028	0.002
Bursa of Fabricius %	0.205[b]	0.230[a]	0.207[b]	0.030	0.001
Immunoglobulin					
IgG	296[b]	336[a]	305[b]	12.45	0.001
IgM	105[c]	136[a]	110[b]	5.260	0.004
IgA	164	172	162	10.36	0.082
Immune response against NDV					
NDV titers (Log2)	4.2b	5.8a	4.5b	0.522	0.002

[a-b]Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error means; CON: Chickens fed a basal diet supplemented without supplementation; LYS: Chickens fed a basal diet supplemented with 90 mg lysozyme; AVI: Chickens fed a basal diet supplemented with avilamycin

Total cecum bacterial count

Chicks treated with lysozyme and AVI additives had markedly low populations of *Clostridium spp*. and *Escherichia coli* ($p<0.05$) compared with the control (Table 7). However, the counts of *Lactobacillus* and total bacterial were meaningfully increased in the cecum of chicks treated with lysozyme and AVI ($p<0.05$). The inclusion of lysozyme resulted in higher counts of Lactobacillus and total bacterial count than dietary AVI ($p < 0.05$) (Table 7).

Table 7. Effects of avilamycin (AVI) and lysozyme (LYZ) feed supplements on Cecum microbial count of broilers

Items	Experimental groups			SEM	p-value
	CON	LYZ	AVI		
Total Coliform count (log10/g fecal matter)	9.8[a]	7.5[c]	8.2[b]	0.245	0.004
Total clostridial count (log10/g fecal matter)	9.2[a]	6.5[b]	6.2[b]	0.158	0.032
Total lactobacillus count (log10/g fecal matter)	6.4[b]	6.3[b]	7.9[a]	0.255	0.001

[a-b]Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error means; CON: Chickens fed a basal diet supplemented without supplementation; LYS: Chickens fed a basal diet supplemented with 90 mg lysozyme; AVI: Chickens fed a basal diet supplemented with avilamycin

Intestinal morphology

Jejunal histology in response to AVI and LYZ dietary supplements was determined by measuring villi height (VH), crypt depth (CD), and VH/CD (Tables 8). The height of intestinal villi was markedly higher in LYZ than AVI and control groups. As a result, VH/CD was markedly elevated in LYZ and AVI groups respectively, compared with control group.

Table 8. Effects of avilamycin (AVI) and lysozyme (LYZ) feed supplements on villus high and crypt depth of broilers

Items	Experimental groups			SEM	p-value
	CON	LYZ	AVI		
Villus high (VH) µm	1085.2^{b}	1270.4^{a}	1160.0^{b}	36.02	0.002
Crypt depth (CD) µm	215.5^{a}	199.2^{b}	185.4^{b}	12.18	0.048
VH / CD	5.035^{b}	6.377^{a}	6.256^{a}	0.36	0.001

$^{a-b}$Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error means; CON: Chickens fed a basal diet supplemented without supplementation; LYS: Chickens fed a basal diet supplemented with 90 mg lysozyme; AVI: Chickens fed a basal diet supplemented with avilamycin

Discussion

It is commonly recognized that the best land animals in converting energy into meat are broilers (Choct, 2009). However, the intestinal integrity and the microbial community of the bird's gut are intimately linked to the broiler performance (BW, BWG, FCR, and PER). Numerous studies conducted on hens have demonstrated the importance of intestinal integrity and gut microbiota on a number of pivotal functions, including mucosal immunity, gut development, nutrient absorption, and feed digestion by host (Stanley et al., 2016 and fang et al., 2020). Similarly, adding 90 mg/kg of LYZ to the diet of broiler chickens changed the intestinal health and performance of the birds (Abdel-Latif et al., 2017). While there was no discernible difference in FI when compared to the control group, the current study found that both LYZ and AVI supplementation significantly enhanced performance indicators such as BW, BWG, FCR, and EPEF.

Because lysozyme is added to the diet to encourage nutrient utilization by modified gut microbes, broiler chickens' ultimate BWG and FCR have improved. The improvement in intestinal bacteria and nutritional digestibility in nursery pigs or rabbits administered lysozyme has been documented in numerous studies (El-Deep et al., 2020; Oliver and Wells, 2015). Additionally, it dramatically decreased the incidence of diarrhea in pigs. Lysozyme's natural antibacterial qualities are crucial for enhancing animal performance (Abdel-Latif et al., 2017).Additionally, a number of studies indicated that the lysozyme action would control immune response regulation and metabolism (Petruschke et al., 2021). The aforementioned results all support the advantages of adding lysozyme to broiler feed as a growth booster and a secure and efficient substitute for antibiotics.

Similarly, Liu et al. (2010) found that lysozyme tended to promote weight gain in broiler chickens and greatly improved the FCR. Additionally, piglets fed a diet containing 90 mg of lysozyme/kg outperformed compared to the control, antibiotic-treated, and 120 mg of lysozyme/kg groups in terms of growth performance (Long et al., 2016). In addition to the notable increase in intestinal villi, which simultaneously increase the intestinal surface area for nutrients absorption, the effect of lysozyme on growth performance may be caused by improved gut antioxidant and immunological genes (Humphrey et al., 2002). According to Lee et al. (2009), lysozyme and AVI additions both exhibited antibacterial properties and improved defense against the secretions of pathogenic microbes in animals' digestive tracts. In fact, the high numbers of beneficial bacteria populations led to better feed digestion and easier nutrient absorption into the bloodstream across the intestinal barriers (Fang et al., 2020). Remarkably, chicks fed with dietary lysozyme and AVI had a much lower FCR. The results agree with El-Deep et al. (2020) who reported reduced FCR in rabbits treated with lysozyme. Low FCR value means that rabbits efficiently utilized feed in relation to the growth performance (Dawood, 2021).The ability of dietary lysozyme to improve the FCR is likely the reason for its superiority over AVI in terms of boosting growth performance. Through the binding of threonine, methionine, and hydroxyproline, lysozyme has been shown to be in charge of blood protein synthesis, leading to strong feed utilization and growth performance (Brundige et al., 2008). However, AVI has a number of useful polypeptides that have strong growth-promoting properties (Brundige et al., 2008).

Nutrients digestibility:

According to the study's findings, adding lysozyme to broiler feed considerably ($P<0.05$) improved the digestibility of dry matter (DM), crude protein (CP), and crude fiber (CF). The findings of Abu Hafsa et al. (2022) are comparable to these, since they discovered that adding lysozyme to growing rabbit feed significantly increased nutritional digestibility. Improved nutrient digestion could also result from lysozyme's ability to change the animal gut's microbial composition by reducing dangerous microorganisms and boosting helpful ones. This improves gut health and, in turn, improves nutrient metabolism (Deng et al., 2021). Thus, adding lysozyme has a strong impact on the number of beneficial bacteria in the gut, which promotes growth performance and enhanced nutrient digestion and absorption.

Carcass traits:

The experimental treatments had no effect on the some carcass features (thigh, liver, and abdominal fat), but lysozyme supplementation considerably increased the dressing % ($P<0.05$) in addition to the minor rise in breast. Moreover, lysozyme supplementation improved immune organs (bursa, spleen and thymus). According to Abu Hafsa et al. (2022), lysozyme had a good effect on the dressing % of growing rabbits, which corroborated our findings. Additionally, our findings aligned with the findings of Hanieh et al. (2010) and Elbaz et al. (2023), who demonstrated that supplementing broilers with probiotics, including the antibacterial lysozyme, increased the relative weights of the immune organs and improved immunity. Increased nutrient digestibility through improved intestinal microbial balance and morphology, which improves feed digestion and absorption, is responsible for this beneficial effect on carcass characteristics (Abu Hafsa et al., 2022; Fang et al., 2020). As a result, the broiler's carcass qualities and overall productive performance both improve.

Biochemical analysis

Blood biochemical analyses aids in determining how food additives affect an animal's overall health (Attia et al., 2011). ZnB and dietary lysozyme are well documented for their ability to immunomodulate and promote growth in rabbits (El-Deep et al., 2020). Therefore, neither lysozyme nor AVI negatively affect blood metabolites (lipids and proteins), liver (ALT and AST), or renal function (urea and creatinine). These outcomes, which were seen in the current trial setting, demonstrate the positive effects of lysozyme and AVI on the health of chicks. Additionally, Thema et al. (2019) reported that ZnB-treated rabbits exhibited higher blood total proteins. The elevated blood proteins were viewed by the authors as a direct consequence of improved feed utilization and metabolic activity throughout the rabbits' body. The way that dietary lysozyme and AVI regulate kidney and liver function suggests that both additions may play a part in preserving the health and welfare of broiler chicks. Furthermore, chicks fed with lysozyme and AVI showed no abnormal featuresin their blood lipid metabolites (triglycerides and cholesterol), suggesting that these additions are safe to use. However, as total triglycerides and cholesterol are produced in the liver and are thought to be indicators of hepatic synthesis efficiency, we measured their levels. According to Phillips et al. (2003), the results are consistent with the usual requirements for animal feed additives, which include being nutritious, health-improving, and safe for the animal in order to create safe products for humans. Also, El-Deep et al. (2020) observed similar outcomes, showing that lysozyme-treated rabbits had improved blood protein levels and regulated metabolites linked to the liver and kidney.

When compared to the control diet, all dietary supplements in the current study significantly reduced the level of MDA while increasing antioxidant capacity, TAC level, and GPx, and SOD activity. Similarly, the lysozyme (50 and 100 mgkg−1) groups showed notable improvements in MDA and antioxidant enzymes (Hassan et al., 2023).

According to Kambayashi et al. (2009), a biomarker for raising the scavenging oxidative activity against free radicals is an increase in TAC. It was discovered that lysozyme supplementation raised the SOD activity in rabbits (Abu Hafsa et al., 2022). Additionally, exogenous lysozyme increased intestinal overexpression of SOD and GPx activities, which is a sign of an improvement in intestinal detoxification against different xenobiotics (Abdel-Latif et al., 2017).Lysozyme supplementation showed a rise in blood SOD activity and a fall in MDA levels, as well as an increase in goblet cells and microvilli length in the intestine's median area (Chen and Zhang, 2012). lysozyme may enhance the release and biological activity of bioactive peptides as well as the activity of antioxidants (Huang et al., 2010; Chen and Zhang, 2012).

Immunity

The gut immunity is influenced by the intestinal microbiota and antioxidant enzymes, which in turn affect serum globulin levels, disease resistance as determined by the HI test, and survival rates. Humoral immunity is significantly influenced by serum immunoglobulins (IgG, IgA, and IgM). In humoral immunity, IgM is the earliest-produced immunoglobulin to be produced, while IgG is crucial for the secondary immune response. On day 35, the LYZ group's serum IgG and IgM content was considerably higher ($p<0.05$) than that of the AVI and control groups ($p<0.05$). When compared to the control and AVI groups, the lysozyme diet had no discernible impact ($p<0.05$) on the hens' IgA content. Our findings corroborated those of Xu et al. (2022), who discovered that weaned pigs' IgG concentration rose when they were supplemented with 500 mg/kg coated lysozyme. Similarly, May et al. (2012) verified that adding lysozyme had a beneficial effect on boosting the immunological response. According to this research, adding more lysozyme can increase the production of proteins called immunoglobulins, which can raise the broiler's immunological index.

Furthermore, it is important to note that LYZ supplementation plays a crucial part in promoting humoral and cellular immunity (Siwicki et al., 1998). Additionally, oral lysozyme may hydrolyze in the duodenum and produce antimicrobial peptides that may be essential for innate immunity, according to Jiménez-Saiz et al. (2011) and Mine et al. (2004). Here, LYZ's immunomodulatory activity was revealed by a marked rise in serum antibody titers for NDV in the LYZ-supplemented group at 35 days compared to the control and AVI groups, respectively. Additionally, van de Crommenacker et al. (2010) found that adult pigeons' immunological function was enhanced by LYZ administration.

Intestinal morphology

The enhanced gut morphology in the LYZ group's jejunum, which was explained by a much greater VH and VH/CD ratio than in the other groups, may be the cause of the performance improvement in this study. Furthermore, the control group's crypt depth was noticeably higher than that of the LYZ and AVI groups. It was previously proposed that the rise in VH was a measure of intestinal absorption efficiency (Helmy et al., 2021). The small intestine's rates of nutrient absorption are increased by the increased VH and VH/CD ratio (Long et al., 2016). Diet is known to have a significant effect on intestinal architecture and absorption capacity, which in turn affects the growth performance of broilers (Hamedi et al., 2011). Accordingly, more intestinal absorptive area, intestinal absorptive capacity for nutrients and increased development are all associated with longer intestinal villi (Izadi et al., 2013).

Intestinal crypts are the main source of the intestinal stem cells to renew and regenerate intestinal villi. So, deeper intestinal crypts lead to more production of intestinal villi cells and more absorption (Hamedi et al., 2011). Our study's findings thus supported the idea that LYZ helps to improve gut morphology.

Cecum microbial:

Increased cecal microbial diversity, particularly in beneficial populations, is linked to enhanced feed utilization and growth rate (Gidenne et al., 2004). According to the data, chicks given food lysozyme or AVI had lower populations of *Clostridium spp*. and *Escherichia coli* and higher numbers of *Lactobacillus* and total bacteria. According to El-Deep et al. (2020), chicks given lysozyme also had higher levels of Lactobacillus and total bacteria but lower levels of Clostridium spp. and E. coli. Brundige et al. (2008) and Gong (2014) similarly reported that lysozyme-treated pigs had a decreased E. coli count. According to Agnolietti et al. (2007), rabbits given ZnB had strong antibacterial action against *Clostridium perfringens*. According to Ibrahim et al. (2011), lysozyme and AVI exhibit antibacterial efficacy against dangerous bacteria, most likely as a result of their function in hydrolyzing the peptidoglycan layer in bacterial cell walls.According to Ibrahim et al. (2011), who examined the antibacterial properties of lysozyme, peptides found in the lysozyme sequence can target harmful bacteria in the gastrointestinal system by "dissipating bacterial respiration and loss of membrane integrity." Improved feed utilization and thus improved development performance in chicks treated with lysozyme and AVI can be explained by a higher count of beneficial bacteria (*Lactobacillus*) and a lower count of *Clostridium spp*. and *E. coli* in this study. Here, the improvement in feed utilization may be linked to the absence of harmful bacteria, which leads to a rise in helpful bacteria that are known to create digestive enzymes that help the rabbits' intestines break down nutrients more easily (Zhao et al., 2020). Moreover, increased epithelial cell activity and the passage of digested nutrients into the bloodstream may result from the low concentration of dangerous microorganisms.In general, the absence of harmful bacteria and the presence of more beneficial ones may stimulate intestine digestion and immunity, which in turn may stimulate immunity throughout the body (Dawood, 2021). To clarify how lysozyme works to increase the antibacterial capacity in chicks' intestines, more research is necessary.

Conclusion

Broilers supplemented with exogenous dietary lysozyme at a level of 90 mg/kg showed improved similar effects on growth performance, fecal bacterial counts, intestinal health, and immunological response to avilamycin. In order to achieve the objectives of the one health approach, it is advised to employ lysozyme, a promising substitute for avilamycin. More in vitro and in vivo experimental research is necessary to target more gut microbiome populations and investigate the specifics of lysozyme's positive effects in broilers.

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دور إنزيم الليزوزيم كبديل للمضادات الحيوية في تحسين النمو وصفات الذبيحة ووظائف الأمعاء الدقيقة في دجاج اللحم

رضا على حسن[1]*، يحيى زكريا عيد[2]،محمد عبد العظيم محمد موسى[1]

1معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، الدقي، الجيزة، مصر.
2قسم إنتاج الدواجن، كلية الزراعة، جامعة كفر الشيخ، مصر.
* عنوان البريد الإلكتروني (redaalihasan@yahoo.com)

مستخلص. كانت هناك محاولات ناجحة لاستبدال المضادات الحيوية في دجاج التسمين بالليزوزيم الغذائي (LYZ)، ولكن هناك حاجة إلى مزيد من الدراسة لضمان فعاليته. لقد قمنا بدراسة آثار مكملات LYZ وأفيلاميسين (AVI) في العلف على صفات النمو وصفات الذبيحة ومناعة دجاج التسمين وصحة الجهاز الهضمي. تم توزيع مائتين وسبعين كتكوت دجاج تسمين يبلغ من العمر واحدًا يومًا (Ross 308) بشكل عشوائي على ثلاث مجموعات، تتكون كل منها من ستة مكررات وتتكون كل منها من خمسة عشر طائرًا. تم التغذية علي العلف القياسي بدون مكملات تجريبية كمجموعة كنترول (I)، بينما تم اضافة للكتاكيت في المجموعات الأخرى بـ 100 مجم أفيلاميسين / كجم من النظام الغذائي (AVI، المجموعة الثانية) و 90 مجم لليزوزيم / كجم في العلف (LYZ، المجموعة الثالثة) لمدة خمسة أسابيع متتالية. ودلت النتائج انه بالمقارنة مع مجموعة CON، أظهرت مجموعتا AVI وLYZ زيادات كبيرة ($p < 0.05$) في وزن الجسم وتحسن في تحويل العلف وزيادة وزن الجسم معامل كفاءة الإنتاج الأوروبي ؛ ومع ذلك، لم يتغير استهلاك العلف . كانت مستويات IgG و IgM في سيرم الدم أعلى في مجموعة الليزوزيم مقارنة بمجموعتي AVI والكنترول . عند مقارنة الاجسام المضادة في سيرم الدم للقاح فيروس نيوكاسل مع مجموعتي التحكم وAVI، أظهرت المجموعة المعالجة بـ LYZ زيادة معنوية ($p < 0.05$). قلل الليزوزيم الغذائي بجرعة 90 مجم بشكل كبير من إجمالي القولونيات الأعورية مع زيادة Lactobacillus. عند مقارنة مجموعة LYZ بمجموعتي AVI والكنترول، كانت VH و CD / VH أكبر بشكل معنوي. في الختام، تم تحسين صحة الأمعاء وعدد البكتيريا المعويه والاستجابة المناعية وأداء النمو باضافة الليزوزيم للعلف بجرعة 90 مجم / كجم من علف الدجاج اللاحم، وكانت هذه النتائج على قدم المساواة مع أفلامايسين. وبالتالي، يمكن أن يكون الليزوزيم الغذائي بديلاً آمنًا لأفيلاميسين في النظام الغذائي للدجاج اللاحم.

الكلمات المفتاحية: الليزوزيم، دجاج اللحم، صفات النمو، مورفولوجيا الأمعاء