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# Leverage of lysozyme dietary supplementation on gut health, hematological, antioxidant, and immune parameters in different plumage-colors Japanese quails

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ABSTRACT

The current study was conducted on two different feather-colored Japanese quail varieties (brown and white) to examine the impact of lysozyme (LZ) dietary supplementation on growth performance, hematological profile, serum lysozyme, phagocytic and antioxidant activities, along with the gut status and the relative expression of some antioxidant- and immune-related genes. Two forms of LZ; extracted from egg white (natural LZ (NLZ)), and the commercial LZ (CLZ) were included in this experiment. For each quail variety, 240 birds were randomly allocated into four groups with four replicates per group. The first group (control) ate the basal diet (BD) only. The other groups ate the BD supplemented with commercial lysozyme (CLZ, at 100 mg/kg diet), NLZ at 100 (NLZ1) and 200 (NLZ2) mg/kg diet. Different LZ treatments differentially modulated the quail's growth performance with significant increases in the final body weight of white-feathered quails fed the NLZ1 compared to other treatments. The NLZ2 and CLZ noticeably increased the total antioxidant activity (TA) in the white- and brown-feathered quails, respectively. Also, all LZ groups displayed distinct increases in the serum lysozyme and phagocytic activities. For gut status, both varieties exhibited increases in intestinal villi length and goblet cell count with significant reductions in the total lactobacillus, total coliform, and total bacterial counts. These effects were linked with marked modulations of *SOD, CAT, GPX*, and*IL-1β*gene expression levels in both quail varieties. Therefore, the LZ could differentially impact quail growth, immune and antioxidant status as well as gut health.

## Introduction

The continuous increase in the human population requires an inevitable growth and development of poultry production to ensure a sustainable supply of safe, nutritious, and affordable protein for humans (Jahejo, et al., 2021). A bird's productive performance is influenced by many factors such as the bird's genetic background including its species, breed, and sex, and the gut development and health (Naga Raja Kumari and Veerasamy, 2021). Gut health is very important for poultry to attain its maximum production as it affects nutrient absorption and assimilation (Aruwa et al., 2021; Elnesr et al., 2022). Gut health involves complex network of interactions including the intestinal barrier, its structural integrity on a larger and micro scale, immunity, microbiota balance, susceptibility to enteric infection, and the immune status of the host (Aruwa et al., 2021; Alagawany et al., 2022).

The fast growth and highly efficient feed conversion of the high-performing poultry lines such as the meat-producing ones, is accompanied by increased feed consumption which places tremendous pressure on the gastrointestinal tract (GIT) to maintain their health and performance (Svihus, 2014; Abou-Kassem et al., 2019). Moreover, intensifying

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poultry production is associated with intestinal problems such as leakage of the gut mucosal barrier, inflammation, and gut microbiome dysbios resulting in performance impairment (Zhu et al., 2021). Gastrointestinal leakage may have a partial or complete negative effect on the health of poultry and hinder the absorption and utilization of nutrients, thus delaying the growth of affected birds (Aruwa et al., 2021). These problems forced the producers to rely on antibiotics to control the production-associated infections and to boost birds' growth performance resulting in significantly higher productivity (Chattopadhyay 2014; El-Kasrawy et al., 2023). However, the inappropriate and excessive use of antibiotics resulted in increasing antibiotic residues in animal products and the development of antibiotic resistance, which grave concerns on public health (Zhu et al., 2021).Therefore, great attention has been paid to the search for new alternatives to antibiotics that act as growth promoters, maintain a healthy intestinal environment, safe for public health, cost-effective, and environmentally friendly (Zhu et al., 2021; El-Ratel et al., 2024).

Recently, several functional feed additives including probiotics, organic acids, prebiotics, symbiotic, enzymes, and phytogenic compounds were established as alternatives to antibiotics and showed the ability to maintain gut health and improve animal performance. In this line, lysozyme (LZ) was recently used as a potential alternative to antibiotics and was approved as an effective natural growth-promoting and antibacterial agent in animal production (Ferraboschi et al., 2021). LZ is a naturally occurring antibacterial enzyme that exerts its activity both directly by hydrolyzing the β-1,4-glycosidic linkage between N-acetylmuramic acid and N-acetyl glucosamine of bacterial peptidoglycans in the cell wall and indirectly via promoting the phagocytic activity of macrophages (Roy et al., 2024). Incorporating LZ into the diet was shown to have several positive impacts in a variety of animal studies. In broiler chickens, LZ lowered *C. perfringens* colonization enhanced the function of the intestinal barrier (Liu et al., 2010), and improved the gut antioxidant status, nonspecific immunity, and growth performance (Abdelazeem et al., 2023). Moreover, it effectively reduced necrotic enteritis-associated mortality and improved intestinal integrity (Du and Guo, 2021). An *in vitro* study conducted by (Zhang et al., 2006) suggested that 200 ppm of LZ prevented *Clostridium perfringens* growth and α-toxin production, which produced lesions connected to necrotic enteritis in chickens. In rabbits, LZ increased growth rate, blood health, digestive enzyme activities, and nutrient digestibility (Abdelazeem et al., 2023; EL-Deep et al., 2020). It was shown to improve the growth and immune response, maintain gut barrier function, and regulate the gut microflora in weaned pigs (Ma et al., 2017; May et al., 2012).

Rearing Japanese quails have gained popularity in poultry industry as an alternative protein source for human beings in developing countries because of its fast growth rate, small body size, high immune competence, short life cycle, and high egg laying rate (Elkhaiat et al., 2023; Elsaidy et al., 2021; Kirrella et al., 2021; Kirrella et al., 2023). The reported plumage color related-genetic mutations resulted in various quail varieties such as brown and white feather-colored quails (Bagh et al., 2016). These varieties are characterized by their different growing and reproductive performance (Elkhaiat et al., 2023; Ibrahim et al., 2021). In this study, we hypnotized that LZ would modulate quail's growth, immune, and antioxidant capacities as well as gut health. Therefore, the current experiment was designed to examine the effect of dietary supplementation of different forms of lysozyme (commercial and egg-extracted) on gut health, innate immune response, antioxidant status, and the overall growth performance in growing Japanese quail (Brown-feathered & White-feathered). This aim was assessed by investigating the intestinal and splenic tissue morphology, cecal microbiology, expression levels of some antioxidant-, and immune-related genes as well and the hematological parameters.

## Materials and methods

### *Ethical approval*

Quail rearing management adhered to the regulations and guidelines of the Animal Care and Ethics Committee, Kafrelsheikh University, Egypt (KFS-IACUC/164/2023). All methods were carried out following relevant guidelines and regulations of KFS- IACUC.

### *Quail care and experimental design*

For this feeding trial, mixed-sexes, one-day-old Japanese quail chicks from two different plumage colors (brown and white) were obtained from the poultry facility at the faculty of agriculture, Kafrelsheikh University, Egypt. These chicks were exposed to two weeks of adaptation phase to manage their survival rate (Kirrella, et al., 2023). At two weeks old, a total of 480 quails (240 for each quail variety) were weighed separately (average initial body weight=76.01 g ± 5.81) and randomly assigned into four groups, with four replicates for each group (15 birds per replicate). The experimental treatment groups included the control group in which birds were fed on a basal diet (BD) without any supplements. In the second group (CLZ), the birds received BD supplemented with a commercial form of lysozyme at a supplementation level =100 mg/kg diet (according to the manufacturer recommendations). While, the third and fourth groups (NLZ1 and NLZ2, respectively), the quails were fed the BD supplemented with an egg-extracted lysozyme form which was added to diet at supplementation levels equal 100, and 200 mg/kg diet, respectively. The source of the commercial lysozyme (CLZ) was lysozyme 10 %® (Nan Chang Lifeng Industry and Trading Co., Ltd., Jiangxi, China). Each 1 kg of lysozyme 10 % contained 100 g of lysozyme, 50 g of glycine, 10 g of sporadic acid, 8 g of water, and 832 g of glucose).The natural-egg extracted lysozyme was obtained from hen egg albumin (non-fertile egg) using the extraction method described by (Guérin-Dubiard, et al., 2005; Luding, et al., 2011) with some modifications. Briefly, egg white was carefully separated from egg yolk and placed in a clean container, and thoroughly mixed with equal volume of ultrapure water (Milli-Q water). After that the lysozyme was precipitated using 0.5 M NaCl at pH 6 using HCL (1 $mmol^{-1}$). Then, the mixture was centrifuged at 8000 rpm for 8 min to separate the protein including lysozyme from the supernatant. Lysozymes was then purified by slowly adding ammonium sulfate, stir gently and kept on ice for 1h then centrifuged again to collect the precipitated lysozyme that then diluted in phosphate buffer (7.8 pH).

The birds from the two quail varieties were housed at temperature of 33–34 °C at the start of the experiment (the brooding phase); then it slowly declined to 22–25 °C, by day twenty-one of the bird's age which remained constant throughout the feeding trial. All birds were kept in standard cages (90 × 40 × 40 cm) and kept in the same rearing conditions with unrestricted access to food and water for a 4-week experimental period. The basal diet (BD) ingredient composition (Table 1) was formulated following the guide of NRC (NRC, 1994) to satisfy the quail's nutritional requirements.

### *Growth performance*

For each quail variety, growth parameters including weekly body weight (BW), body weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were assessed for all birds in each group. The WG was calculated by subtracting the initial boy weight from the final body weight. Whereas the FCR were computed using the WG and FI based on this equation$(FCR) = \frac{Feed\ consumed\ (g)}{weight\ gain(g)}$.

### *Hematological parameters and non-specific immune response*

At the end of the growing period (at 6-week-old), twelve males/

**Table 1**
Ingredient composition of the used basal diet.

| Ingredients (%) | |
|---|---|
| Yellow corn | 51.17 |
| Corn Gluten Meal | 6.80 |
| Soybean meal (47 %) | 36.19 |
| Soybean Oil | 2.00 |
| DCP[1] | 1.47 |
| Limestone [2] | 1.59 |
| Premix [3] | 0.30 |
| Common Salt | 0.25 |
| DL-methionine [4] | 0.10 |
| Lysine HCl [5] | 0.03 |
| Choline chloride | 0.05 |
| Mycotoxin adsorbent | 0.05 |
| Calculated Composition (%) | |
| Crude protein | 23.79 |
| Calcium | 0.98 |
| Available Phosphorus | 0.32 |
| Lysine | 1.26 |
| Methionine | 0.51 |
| ME (Kcal/kg)[6] | 2975.13 |

[1] DCP=Dicalcium phosphate (17 % Phosphorus and 21 % Calcium).
[2] Limestone (contain 35 % calcium).
[3] Premix: each 3 kg vitamin and mineral mixture contain: vitamin A 12,000,000 IU; vitamin D3 2,500,000 IU; vitamin E 10,000 mg; vitamin K3 2000 mg; vitamin B11000 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).
[4] DL-Methionine (Produced by Evonik Co. and containing 99 % methionine).
[5] Lysine = lysine hydrochloride (containing 98 % Lysine).
[6] calculated composition according to NRC (NRC, 1994).

group (three/replicate) were randomly selected for blood and tissue samples collection. Blood samples were collected from birds' jugular vein in anticoagulant-containing tubes (sodium citrate 3.8 %) for determination of red blood cells (RBCs), hemoglobin (Hb), total and differential leukocytic count (WBCs), packed cell volume (PCV), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The RBCs and WBCs counts were done using hemocytometer. Heterophil/lymphocyte ratio (H/L) was also calculated. Using the same whole blood samples, phagocytic activity (PA) and phagocytic index (PI) were done according to (El-Kassas et al., 2018; Kawahara et al., 1991). Another blood samples were collected and placed in clean vials without anticoagulant for serum separation by centrifugation at 3000 rpm for ten minutes. Serum samples were stored at -20°C until further analysis of the total antioxidant (TA) and serum lysozyme activity. Total antioxidants were determined by spectrophotometer using commercial kits (Bio-diagnostic Co, Egypt). The serum lysozyme activity was measured following the method described by (Engstad et al., 1992).

### *Intestinal and splenic morphology and cecal microbiology*

After blood sampling, birds were killed by cervical dislocation under mild anesthesia, dissected, and splenic tissue and jejunum (2 cm) (n =12/group) were collected for morphological analysis. Tissue samples were cleaned with physiological saline and preserved in 10 % formalin for 24 h. Slides were prepared and stained with hematoxylin and eosin (H&E) in accordance with Bancroft et al. (2013) and examined using the light microscope. Using an image computer analysis system (Image J software, Bethesda, MD, USA), the villus length, and width and crypt depth were examined, and measurements were taken.

For the microbial examination, caecal contents were collected (n=12 birds/group) in sterile Eppendorf tubes under aseptic conditions and kept at -40 °C till analysis. Ten-fold serial dilation from each sample was prepared according to (Erener, et al., 2011). Nutrient agar (Oxoid MRS (Man, Rogosa, Sharpe), and Violet Bile Agar plates were used for bacterial enumeration for the total bacterial count, *lactobacillus* count and *Coliform,* respectively according to (Erener, et al., 2011; Sorescu, et al., 2019). The plates were inoculated with specific volume of the serial dilutions from each sample and incubated at 37 °C for 18 to 24 h. The colony forming unite in one gram of collected sample (CFU/g) was calculated according to (Barnes and Impey, 1970; Garrity, et al., 2010).

### *Real time PCR*

Samples of intestine (jejunum) were taken from each bird (n=12 birds/group), quickly frozen in liquid nitrogen, and stored at -80°C for gene expression. For RNA extraction, intestinal samples were ground in sterile mortars using liquid nitrogen then the total RNA was extracted using Trizol (iNtRON Biotechnology, Inc.). The RNA quality and quantity were assessed using 2 % ethidium bromide-stained agarose gel electrophoresis and Nanodrop (UV-Vis spectrophotometer Q5000, Quawell, USA). Reverse transcriptase to was utilized to synthesize the complementary DNA (cDNA) for each RNA sample using cDNA synthesis kits (SensiFAST™ cDNA Synthesis Kit (Bioline, United Kingdom)).Specific primers (illustrated in Table 2) were used to amplify the intestinal antioxidants(*SOD, CAT* and *GPX*) and immune response-related genes (*IL1-β*). Gene amplificationwas done using Stratagene MX300 P real-time PCR system (Agilent Technologies) and SensiFast™ SYBR green (Bioline, United Kingdom). A total reaction volume was twentyμl included, tenμl of SensiFast™ SYBR master mix, 2 μl of cDNA, and 0.5 μM of each primer. Theamplification program initiated with a pre-denaturation step at 95 °C for 30 s, then forty cycles of 95 °C for 10 s and annealing temperatures listed in Table 2. The results were normalized against the *B-actin* (as a house keeping gene) and the control samples fed the BD to calculate the expression fold changes according to (Livak and Schmittgen, 2001).

### *Statistical analysis*

In this study, Two-way ANOVA was used for the statistical analysis of the obtained results using SPSS ((©IBM Corp. Released 2013, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM). The Two-way ANOVA was used to assess the effect of quail variety (white- and brown-feathered quails), the LZ dietary supplementation (commercial and egg-extracted) and their interactions. Tukey'smultiple comparisons test was then used. The figures of qPCR results were created by Graph pad prism9 (©Graph Prism Software, La Jolla, CA, USA). The results were shown as means ± SE with the $P < 0.05$ for the statistical significances consideration.

## Results

### *Growth performance*

Table 3 illustrates the effects of LZ dietary supplementation on growth performance of the two studied quail varieties. LZ supplementation similarly improved quail's growth performance between white- and brown-feathered quails ($P < 0.05$) with significant statistical interaction between the quail variety and the supplemented lysozyme ($P < 0.05$). Among the white-feathered quails, the heaviest body weights were recorded for quails supplemented with 100 mg/kg diet of egg-extracted lysozyme (NLZ1) compared with the control (BD only), and 200 mg/kg diet of egg-extracted lysozyme (NLZ2) ($P < 0.05$). However, no alterations in the final weights of brown-feathered quails following the dietary supplementation of lysozyme. Consequently, higher total body gains were calculated in the case of white-feathered quails fed on BD supplemented with 100 mg/kg diet of egg-extracted lysozyme

**Table 2**
Primer sequences (5′-3′) used in real-time PCR.

| Gene | Primer sequence Gene | Accession NO | Ann Tm | /Ref. |
|---|---|---|---|---|
| *B- actin* | F: ACCTGAGCGCAAGTACTCTGTCT R: CATCGTACTCCTGCTTGCTGAT | NM_205518.1 | 60 | (El-Kassas, et al., 2018) |
| *SOD* | F:CGGGCCAGTAAAGGTTACTGGAA R:TGTTGTCTCCAAATTCATGCACATG | NM_205064.1 | 60 | (El-Kassas, et al., 2019) |
| *CAT* | F: ACTGGTGCTGGCAACCC R: ACGTGGCCCAACTGTCAT | NM_001031215 | 60 | |
| *GPX* | F: GCGACTTCCTGCAGCTCAACGA R: CGTTCTCCTGGTGCCCGAAT | | 60 | (Li and Sunde, 2016) |
| *IL1-β* | F: TGCCTGCAGAAGAAGCCTCG R:GACGGGCTCAAAAACCTCCT | NM_204524.1 | 62 | (Adu-Asiamah, et al., 2021) |

SOD= sodium oxide dismutase, CAT= catalase, GPX= glutathione peroxidase. IL1-β= interleukin 1 beta

**Table 3**
Growth performance of white- and brown-feathered quails in response to dietary lysozyme supplementation.

| | White-feathered quail | | | | Brown-feathered quail | | | | *P* values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | CLZ | NLZ1 | NLZ2 | Control | CLZ | NLZ1 | NLZ2 | Q | LZ | Q*LZ |
| IW (g) | 80.32 ± 1.63$^{a}$ | 76.06 ± 4.70$^{a}$ | 70.74 ± 3.06$^{a}$ | 82.57 ± 1.98$^{a}$ | 74.94 ± 1.84$^{a}$ | 74.76 ± 3.77$^{a}$ | 74.18 ± 1.65$^{a}$ | 74.52 ± 3.87$^{a}$ | 0.226 | 0.262 | 0.325 |
| FW (g) | 235.78 ± 1.62$^{b}$ | 253.79 ± 3.30$^{ab}$ | 269.86 ± 3.28$^{a}$ | 249.41 ± 1.48$^{b}$ | 251.14 ± 3.81$^{a}$ | 250.81 ± 3.54$^{a}$ | 249.05 ± 2.27$^{a}$ | 251.29 ± 4.54$^{a}$ | 0.467 | 0.001 | <0.001 |
| TBG (g) | 155.46 ± 1.00 $^{b}$ | 177.73 ± 1.25$^{ab}$ | 199.12 ± 3.77$^{a}$ | 166.84 ± 3.76$^{b}$ | 176.19 ± 3.54$^{a}$ | 176.05 ± 1.21$^{a}$ | 174.88 ± 1.93$^{a}$ | 176.77 ± 2.17$^{a}$ | 0.729 | 0.003 | 0.002 |
| FI (g) | 600.35 ± 27.28 $^{Aa}$ | 465.74 ± 23.72 $^{Bb}$ | 491.56 ± 35.28 $^{Ab}$ | 525.94 ± 37.87 $^{Ab}$ | 582.67 ± 20.47 $^{Ba}$ | 516.37 ± 17.14 $^{Ab}$ | 413.46 ± 26.61 $^{Bb}$ | 384.99 ± 18.33 $^{Bb}$ | 0.044 | 0.001 | 0.033 |
| FCR | 3.86 ± 0.15 $^{Aa}$ | 2.62 ± 0.13 $^{Ab}$ | 2.47 ± 0.21 $^{Ab}$ | 3.17 ± 0.30 $^{Aa}$ | 3.30 ± 0.187 $^{Aa}$ | 2.93 ± 0.05 $^{Aab}$ | 2.37 ± 0.15 $^{Ab}$ | 2.20 ± 0.20 $^{Bb}$ | 0.023 | <0.001 | 0.019 |

IW= Initial weight; FW=Final weight at 6weeks old; TBG= total body gain; FI= feed intake; FCR= Feed conversion ratio. CLZ= commercial lysozyme (100mg/kg diet); NLZ1= natural lysozyme (100mg/kg diet); NLZ2= natural lysozyme (200mg/kg diet); Q represents quail's varieties effect; LZ represents the form of lysozyme. Q*LZ= represents the interaction between quail's varieties and lysozyme supplementation. Results are expressed as means ± SE. Different uppercase letters donate statistical significances at $P < 0.05$ between the white- and brown-feathered quail varieties. While the lowercase letters donate statistical significance between the various sources of lysozyme at $P < 0.05$.

(NLZ1) compared with their contemporaries received the BD only or BD supplemented with 200 mg/kg diet of egg-extracted lysozyme ($P < 0.05$). In the case of brown-feathered quails, neither the commercial lysozyme (CLZ) nor the egg-extracted lysozyme (100 & 200 mg/kg diet) resulted in alterations in body gains ($P > 0.05$). Both commercial and egg-extracted lysozymes (at 100 and 200mg/kg diet supplementation levels) lowered the feed intake in the two studied quail varieties with the lowest FI was recorded in the case of brown-feathered quails (Table 3, $P < 005$). The lowest FCR was reported for commercial (CLZ) and NLZ1 in the case of white-feathered quails. While, supplementing the egg-extracted lysozyme at 100 and 200mg/kg diet of brown-feathered quails resulted in the lowest FCR.

### *Hematological and immune responses*

The CLZ and NLZ2 induced higher HB concentrations in white-feathered quails compared to the control and NLZ1 (Table 4). Whereas, in the brown-feathered quails, the two supplemented egg-extracted LZ concentrations (NLZ1 & NLZ2) resulted in significant higher HB concentration compared to the control and CLZ. The other hematological parameters including RBCs, MCHC, MCH, MCV, and PCV ( %) (Table 4) did not change in response to the LZ dietary supplementations in both quail varieties ($P > 0.05$). Among differential leucocytic counts, WBCs, lymphocytes, basophils, or eosinophil counts did not display any significant changes in response to LZ ($P > 0.05$). Only monocytes showed prominent increased in response to the LZ supplementations. Both commercial (CLZ) and egg extracted (NLZ1 & NLZ2) induced significant reduction of H/L ratio in the two studied quail varieties ($P < 0.05$). Besides, both CLZ and NLZ (the two doses) increased the total antioxidant activity (TA) in the two quail varieties with the NLZ2 and CLZ had the highest activity in the white-feathered and brown-feathered, respectively. Interesting increases in the serum LZ levels were also, reported in response to the LZ dietary supplementation. The CLZ and NLZ2 in white-feathered quails and CLZ and NLZ1 in brown-feathered quails induced distinct higher serum LZ levels compared to the other treatments ($P < 0.05$). The response of the white-feathered quails was higher than that of the brown-feathered ones in the case of NLZ2 supplementing group (P <0.05). For the phagocytic activities, the CLZ, NLZ1, NLZ2 in white-feathered quails and NLZ1 in the case of brown-feathered quails induced the highest PA ($P < 0.05$). No changes were reported in the PI in all supplemented groups in the two quail varieties ($P > 0.05$).

### *Intestinal histological, morphometric, and microbial characteristics*

The histological features of jejunum in both white- and brown-feathered quails were investigated in response to dietary supplementation of lysozyme (Fig. 1). Both white- and brown-feathered quails fed the BD displayed healthy appearance of intestinal villi, submucosal glands, and tunica muscularis (Fig. 1A& a, respectively). However, there was slight degeneration of intestinal mucosa with a little epithelial vacuolation in the case of white-feathered quails fed on BD supplemented with 200mg/kg diet of egg-extracted lysozyme (NLZ2) (Fig. 1D) and brown-feathered quail fed on BD supplemented with commercial lysozyme (CLZ) (Fig. 1b). In contrast, other groups showed a normal histological appearance of intestinal villi with intact epithelial lining and plentiful goblet cells (Fig. 1). Moreover, the morphometric analysis (Table 5) revealed a significant improvement in villi length, width, crept depth as well as the goblet cell count in the intestine of both studied quail varieties. In this context, the white-feathered quails exhibited significant reduction of jejunum villi length in response to commercial LZ dietary supplementation. However, the two supplementation doses of egg-extracted LZ (NLZ1 & NLZ2) showed better length compared to CLZ and control group ($P < 0.05$). The brown-feathered quails displayed significant increases in villi length in all supplemented groups with CLZ and NLZ (NLZ1 & NLZ2). Villus width also, showed noticeable variations between the two studied quail varieties and the different LZ treatments (P <0.05). Normally, the white-feathered quails, fed only on BD, had significant smaller villi width than brown-feathered one. Both CLZ and NLZ1 significantly increased the villus width compared to the control

**Table 4**
Hematological picture and non-specific immunity of male white- and brown-feathered quails in response to different sources of lysozyme.

| Quail's strain | White-feathered quail | | | | Brown-feathered quail | | | | *P* values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Control | CLZ | NLZ1 | NLZ2 | Control | CLZ | NLZ1 | NLZ2 | Q | LZ | Q*LZ |
| HB (g/100mL) | 10.54 ± 0.27$^{b}$ | 11.75 ± 0.21$^{a}$ | 10.56 ± 1.07$^{b}$ | 12.69 ± 0.41$^{a}$ | 11.60 ± 0.38$^{b}$ | 12.07 ± 0.14$^{b}$ | 14.31 ± 0.24$^{a}$ | 13.95 ± 0.76$^{a}$ | 0.058 | <0.001 | 0.003 |
| RBCs(× $10^{6}$ /$mm^{3}$) | 3.49 ± 0.32$^{a}$ | 3.74 ± 0.10$^{a}$ | 3.32 ± 0.47$^{a}$ | 3.99 ± 0.51$^{a}$ | 3.78 ± 0.78$^{a}$ | 3.79 ± 0.14$^{a}$ | 4.59 ± 0.22$^{a}$ | 4.64 ± 0.24$^{a}$ | 0.067 | 0.400 | 0.494 |
| MCHC | 33.39 ± 0.92 | 34.25 ± 0.94 | 33.86 ± 0.86 | 33.27 ± 0.87 | 32.69 ± 0.70 | 33.68 ± 1.89 | 33.40 ± 1.43 | 31.44 ± 0.58 | 0.269 | 0.497 | 0.918 |
| MCH | 30.23 ± 0.17 | 31.49 ± 0.79 | 32.14 ± 1.32 | 31.77 ± 0.24 | 30.81 ± 0.42 | 31.93 ± 1.59 | 31.30 ± 1.22 | 30.06 ± 0.14 | 0.562 | 0.479 | 0.556 |
| MCV | 90.67 ± 2.58 | 92.04 ± 2.88 | 94.85 ± 1.66 | 95.59 ± 1.87 | 94.33 ± 2.46 | 94.91 ± 1.94 | 93.77 ± 1.24 | 95.66 ± 1.30 | 0.360 | 0.502 | 0.630 |
| PCV (%) | 31.67 ± 3.18$^{B}$ | 34.33 ± 2.64$^{A}$ | 31.33 ± 23.84$^{B}$ | 38.00 ± 4.51$^{B}$ | 35.33 ± 2.32$^{A}$ | 36.00 ± 1.53$^{A}$ | 43.00 ± 2.08$^{A}$ | 44.33 ± 1.79$^{A}$ | 0.030 | 0.183 | 0.519 |
| WBCs (× $10^{3}$/$mm^{3}$) | 34.17 ± 2.22 | 41.16 ± 2.38 | 42.31 ± 2.76 | 49.66 ± 2.93 | 43.13 ± 2.44 | 46.07 ± 2.75 | 36.51 ± 3.36 | 45.00 ± 1.22 | 0.863 | 0.572 | 0.653 |
| Monocyte (× $10^{3}$/$mm^{3}$) | 2.27 ± 0.20$^{b}$ | 3.36 ± 0.62$^{ab}$ | 3.19 ± 0.27$^{ab}$ | 4.59 ± 0.47$^{a}$ | 2.54 ± 0.52$^{b}$ | 3.33 ± 0.22$^{ab}$ | 2.91 ± 0.60$^{b}$ | 4.23 ± 0.67$^{a}$ | 0.774 | 0.006 | 0.911 |
| Lymphocytes (× $10^{3}$/$mm^{3}$) | 22.23 ± 1.82 | 26.79 ± 1.53 | 26.08 ± 2.07 | 31.25 ± 1.70 | 27.54 ± 5.00 | 30.26 ± 3.63 | 24.97 ± 6.40 | 29.81 ± 4.51 | 0.633 | 0.577 | 0.842 |
| Heterophils (× $10^{3}$/$mm^{3}$) | 7.40 ± 1.23 | 9.41 ± 1.52 | 10.39 ± 2.77 | 11.59 ± 2.90 | 10.02 ± 2.02 | 9.91 ± 1.39 | 6.56 ± 1.50 | 9.71 ± 1.85 | 0.652 | 0.689 | 0.420 |
| Basophils (× $10^{3}$/$mm^{3}$) | 0.86 ± 0.08 | 0.79 ± 0.36 | 1.60 ± 0.08 | 1.00 ± 0.10 | 1.43 ± 0.26 | 1.50 ± 0.60 | 1.03 ± 0.34 | 0.82 ± 0.14 | 0.525 | 0.608 | 0.142 |
| Eosinophil (× $10^{3}$/$mm^{3}$) | 1.28 ± 0.62 | 0.99 ± 0.31 | 1.06 ± 0.62 | 1.24 ± 0.59 | 0.93 ± 0.27 | 1.07 ± 0.15 | 1.03 ± 0.34 | 0.76 ± 0.25 | 0.541 | 0.996 | 0.904 |
| H/L ratio | 0.45 ± 0.04$^{a}$ | 0.28 ± 0.02$^{b}$ | 0.27 ± 0.02$^{b}$ | 0.28 ± 0.01$^{b}$ | 0.34 ± 0.04$^{a}$ | 0.28 ± 0.03$^{b}$ | 0.27 ± 0.02$^{b}$ | 0.30 ± 0.01$^{ab}$ | 0.344 | 0.001 | 0.040 |
| TA (mM/L) | 1.26 ± 0.37$^{Ab}$ | 1.98 ± 0.09$^{Aa}$ | 1.93 ± 0.03$^{Aa}$ | 2.04 ± 0.08$^{Aa}$ | 1.27 ± 0.21$^{Ab}$ | 1.87 ± 0.11$^{Aa}$ | 1.19 ± 0.09 $^{Bb}$ | 1.76 ± 0.25 $^{Ba}$ | 0.049 | 0.007 | 0.243 |
| LZ (μg/ml) | 7.58 ± 0.76$^{Abc}$ | 10.71 ± 0.81$^{Aa}$ | 8.91 ± 0.48$^{Ab}$ | 11.55 ± 0.50$^{Aa}$ | 6.77 ± 0.59 $^{Ac}$ | 10.77 ± 0.41$^{Aa}$ | 9.43 ± 0.32$^{Aa}$ | 8.15 ± 0.69 $^{Bb}$ | 0.045 | <0.001 | 0.020 |
| Phagocytic index (PI) | 1.45 ± 0.11$^{A}$ | 1.76 ± 0.16 $^{B}$ | 1.81 ± 0.06 $^{B}$ | 1.79 ± 0.06 $^{B}$ | 1.32 ± 0.01$^{A}$ | 2.03 ± 0.09$^{A}$ | 2.06 ± 0.06$^{A}$ | 2.01 ± 0.04$^{A}$ | 0.029 | 0.229 | 0.125 |
| Phagocytic activity (PA) | 8.77 ± 0.57$^{b}$ | 9.08 ± 0.66$^{ab}$ | 10.63 ± 0.97$^{a}$ | 10.03 ± 0.17$^{a}$ | 9.37 ± 0.54$^{b}$ | 10.15 ± 0.22$^{a}$ | 9.23 ± 0.52$^{b}$ | 10.44 ± 0.31$^{a}$ | 0.654 | <0.001 | 0.166 |

CLZ= commercial lysozyme (100mg/kg diet); NLZ1= egg-extracted lysozyme (100mg/kg diet); NLZ2=egg-extracted lysozyme (200mg/kg diet); Q represents quail's strain effect; LZ represents the source of lysozyme. Q*LZ= represents the interaction between quail's strain source of lysozyme. Results expressed as means ± SEM. Different uppercase letters donate statistical significances at $P < 0.05$ between the white- and brown-feathered quail strains. While the lowercase letters donate statistical significances between the different sources of lysozyme at $P < 0.05$.

and NLZ2. The crypt depth showed similar responses in both white- and brown-feathered quails ($P > 0.05$ for quail varieties) with significant effects for the LZ supplementation ($P < 0.05$). In both quail varieties, the commercial and egg-extracted LZ dietary supplementations induced significant deeper crypts compared with the BD only. Moreover, the dietary supplementation of commercial of egg-extracted LZ induced discernible increases in the number of goblet cells in both quail varieties. The improved histological features of jejunum in both white- and brown-feathered quails in response to the commercial and NLZ dietary supplementations were associated with improved microbial population of cecum (Table 5). In this regard, both commercial and egg-extracted LZ significantly lowered the TLC, TCC and TBC in cecal contents in both white- and brown-feathered quails. The splenic structure showed normal appearance of red and white pulps in all groups (Fig. 2) in addition to lymphoid nodules in the brown quails in case of NLZ1 and NLZ2. The number of sheathed arteries within the splenic tissue was increased in white quail fed on NLZ2.

*qPCR levels of some antioxidant and innate immunity genes*

Fig. 3 represents the relative mRNA levels of *SOD* and *CAT* genes in the jejunum of white- and brown-feathered quails supplemented with commercial and egg-extracted LZ in their diets. In white-feathered quails, the dietary supplementation of CLZ induced significant up-regulation of the *SOD* mRNA transcription levels compared to control, NLZ1 and NLZ2 ($P < 0.05$). In the brown-feathered quails, the highest *SOD* transcription levels were reported for those fed diet supplemented with 200mg /kg diet of egg-extracted LZ (NLZ2). Moreover, the response of the white-feathered quails to CLZ dietary supplementation was significantly higher than that of their contemporaries of brown-feathered quails ($P < 0.05$). For *CAT* gene relative expression level, the CLZ, NLZ1 and NLZ2 increased its relative mRNA copiesin white-feathered quails with the prominent effect was in the case of NLZ2. In the case of the brown-feathered quails, the highest relative *CAT* mRNA copies were reported for those fed BD supplemented with CLZ and NLZ2. In addition, the mRNA transcriptomic level of *GPX* (Fig. 4) was significantly altered by the LZ dietary supplementation and quail varieties ($P < 0.05$). In the white-feathered quails, slight increases were reported. Whereas, the two supplemented doses of egg-extracted LZ (NLZ1 and NLZ2) significantly up-regulated the *GPX* mRNA level ($P < 0.05$). The response of the brown-feathered quails to NLZ2 dietary supplementation was significantly higher than that of white-feathered ones ($P < 0.05$). For *IL1-β* gene (Fig. 4), the CLZ, NLZ1, and NLZ2 significantly increased its mRNA levels in both white- and brown-feathered quails with the highest levels were reported for CLZ and NLZ2, respectively ($P < 0.05$).

## DIscussion

Feed efficiency is one of the most determining factors affecting poultry growth (Li et al., 2020). It depends on the quality of the diet and the efficiency of nutrient metabolization which mirror the feed digestion and absorption, and the gut health (Barzegar et al., 2020; Al-Gheffari et al., 2024). In our study, LZ supplementation differentially improved the quail's growth parameters. The white-feathered quails, supplemented with egg-extracted lysozyme at 100 mg/kg diet (NLZ1) showed the heaviest final body weights and higher total body gains. However, in

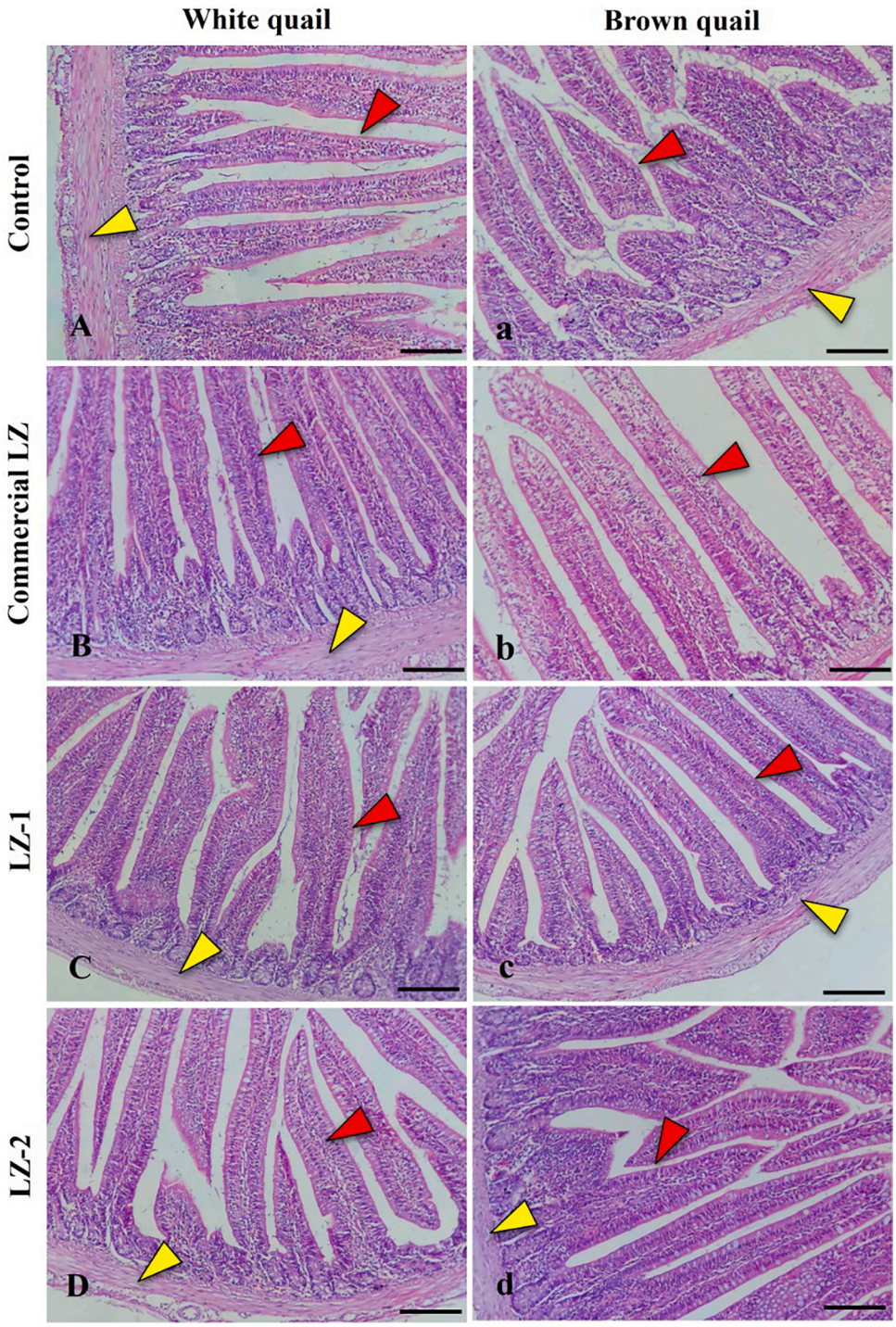


**Fig. 1.** The histological features of jejunum in both white- and brown-feathered quails in response to supplementation of lysozyme.

the brown-feathered quails, neither the commercial lysozyme (CLZ) nor the egg-extracted lysozyme (NLZ1&NLZ2) altered the body weight and gain compared to the control group. In addition, both commercial (CLZ) and egg-extracted lysozyme (NLZ1&NLZ2) lowered the feed intake in the two studied quails with marked improvement in the FCR. First, the variation in the growth rate between the two studied quail varieties in response to the LZ feed additive could be related to the genetic and physiological variations between these two varieties (El-Kassas et al., 2019; Naga Raja Kumari and Veerasamy, 2021). On the other side, the reported LZ effects on quails' growth performance especially the white-feathered ones might be attributed to many factors including enhancing gut antioxidant capacities, This explanation was confirmed, in the current study, by the reported upregulation of mRNA levels of the antioxidant-related genes, *SOD, CAT,* and *GP*x in response to LZ dietary supplementation with the higher expressions found in the case of the 200mg /kg diet of egg-extracted LZ (NLZ2). Moreover, it may be linked with the enhanced serum total antioxidant capacity especially at the 100mg\kg concentration. This antioxidant characteristics of LZ may be correlated with the bioactive peptides of chicken-egg white lysozyme which have potent antioxidant effects (Benedé and Molina, 2020). These peptides could inhibit the lipid peroxidation, suppress the generation of reactive oxygen species (ROS), and have a strong ion chelating activity

**Table 5**
Intestinal morphometric and microbial characteristics of male white- and brown-feathered quails in response to different sources of lysozyme.

| | White-feathered quail | | | | Brown-feathered quail | | | | *P* values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | CLZ | NLZ1 | NLZ2 | Control | CLZ | NLZ1 | NLZ2 | Q | LZ | Q*LZ |
| Villus height | 447.09 ± 11.24 $^{Aa}$ | 397.48 ± 6.63 $^{Ba}$ | 420.69 ± 8.25$^{Aa}$ | 426.07 ± 7.50 $^{Ba}$ | 350.80 ± 11.23$^{Bc}$ | 568.13 ± 8.05$^{Aa}$ | 434.06 ± 10.85$^{Ab}$ | 545.15 ± 11.82$^{Aa}$ | 0.015 | 0.012 | 0.001 |
| Villus Width | 59.68 ± 1.51 $^{Bb}$ | 93.77 ± 5.53$^{Aa}$ | 96.44 ± 8.32$^{Aa}$ | 80.68 ± 5.29$^{Aab}$ | 87.28 ± 1.75$^{Ab}$ | 96.21 ± 5.92$^{Aa}$ | 94.51 ± 5.16$^{Aa}$ | 91.23 ± 3.22$^{Aa}$ | 0.016 | 0.001 | 0.047 |
| Crypt depth | 126.31 ± 6.20$^{a}$ | 96.24 ± 8.53$^{b}$ | 66.84 ± 5.64$^{b}$ | 68.28 ± 5.38$^{b}$ | 104.12 ± 2.93$^{a}$ | 69.15 ± 3.74$^{b}$ | 44.30 ± 1.37$^{bc}$ | 101.42 ± 6.02$^{a}$ | 0.119 | <0.001 | 0.006 |
| Goblet cell count | 9.67 ± 1.20$^{b}$ | 35.67 ± 1.86$^{a}$ | 42.33 ± 2.73$^{a}$ | 45.00 ± 4.16$^{a}$ | 17.67 ± 2.96$^{c}$ | 29.67 ± 4.63$^{b}$ | 49.67 ± 2.03$^{a}$ | 43.33 ± 3.67$^{a}$ | 0.397 | <0.001 | 0.102 |
| TLC | 4.65 ± 0.37$^{Aa}$ | 3.6 ± 0.27$^{Ab}$ | 3.34 ± 0.57$^{Ab}$ | 2.95 ± 0.73 $^{Ac}$ | 2.75 ± 0.41 $^{Ba}$ | 2.98 ± 0.33$^{Aa}$ | 1.93 ± 0.13 $^{Bb}$ | 1.86 ± 0.20 $^{Bb}$ | < 0.001 | 0.018 | 0.491 |
| TCC | 3.58 ± 0.59$^{a}$ | 2.23 ± 0.50$^{b}$ | 2.35 ± 0.39$^{b}$ | 1.48 ± 0.14$^{c}$ | 3.75 ± 0.44$^{a}$ | 3.05 ± 0.41$^{a}$ | 2.45 ± 0.46$^{b}$ | 2.30 ± 0.54$^{b}$ | 0.146 | 0.005 | 0.764 |
| TBC | 5.05 ± 0.55$^{a}$ | 2.35 ± 0.43$^{b}$ | 2.85 ± 0.62$^{b}$ | 2.08 ± 0.28$^{b}$ | 4.68 ± 0.28$^{a}$ | 2.98 ± 0.36$^{b}$ | 1.85 ± 0.47$^{b}$ | 2.06 ± 0.45$^{b}$ | 0.552 | <0.001 | 0.343 |

TLC= Total lactobacilli count; TCC= total coliform count; TBC= total bacterial count. CLZ= commercial lysozyme (100mg/kg diet); NLZ1= egg-extracted lysozyme (100mg/kg diet); NLZ2= egg-extracted lysozyme (200mg/kg diet); Q represents quail's strain effect; LZ represents the source of lysozyme. Q*LZ= represents the interaction between quail's strain source of lysozyme. Results expressed as means ± SEM. Different uppercase letters donate statistical significances at $P < 0.05$ between the white- and brown-feathered quail strains. While the lowercase letters donate statistical significances between the different sources of lysozyme at $P < 0.05$.

(Chen, et al., 2022). Moreover, the LZ-improved growth performance might be correlated with improving the gut histology and intestinal health (improved intestinal morphology) as proven by increasing the jejunum villi length and crept depth especially in the groups supplemented with egg extracted lysozyme (NLZ1&NLZ2). All these factors would augment the intestinal surface area for more nutrient absorption which boosts the bird's performance (Humphrey et al., 2002; Sindaye et al., 2023).The later response may be associated with increasing nutrient absorption due to increasing enterocytes division, lowering the oxidative stress, and increasing its antioxidant capacity (De Grande, et al., 2019). In the same line, Abdel-Latif et al. (2017), Abdelazeem et al. (2023) reported increases in broiler growth performance in response to exogenous lysozyme dietary supplementation by upregulating the intestinal *SOD1* and *GSH-Px* gene expression levels. Plus, they reported that the higher dose of LZ (90 mg/kg diet) had better impacts than the low dose (70 mg/kg diet). In rabbit, the LZ dietary supplementation also, improved its growth performance (EL-Deep, et al., 2020). Brundige et al. (2010), Zou et al. (2019) documented an enhanced growth of pig supplemented with LZ in their diet due to increasing protein synthesis and skeletal growth confirmed by increasing serum metabolites such as methionine, threonine, and hydroxyproline. On the other hand, lowering growth of the brown-feathered quails might be attributed to the exhaustion of gastrointestinal tract and reducing the absorptive capacities of enterocytes in this variety (Ducatelle et al., 2023).

Improving the gastrointestinal tract morphology was another interesting response to LZ dietary supplementation in both quail varieties. The NLZ distinctly increased the jejunum villi length and crept depth. This response may be linked with the LZ-associated improved growth performance. Similarly, Abdel-Latif et al. (2017), Liu et al. (2010) reported improvement in chicken growth in response to higher doses of lysozyme due to improving the intestinal villi length and crypts depth and the consequence increases of the nutrient absorption. Additionally, lysozyme supplementation increased the jejunum goblet cells in both types of quails. The goblet cells secrets mucin which is the main component of luminal mucus and lubricates the intestinal mucosa, facilities food absorption, and protects the cells from injuries that could impair the intestinal integrity and feed utilization (Ducatelle et al., 2023). Additionally, goblet cells are one of the first intestinal defense lines as prevent the invasion of the forging pathogen to the enterocytes. Moreover, they have an essential role in the intestinal immune response as they could identify and present the antigens to the underlying antigen-presenting cells (APCs) (Yang and Yu, 2021; Zhang and Wu, 2020). Similar increases of goblet cell count, and mucous secretion were reported in chicken and fish in response to LZ presence in their diets (Abdel-Latif et al., 2017).

The gut health includes, not only, the cells integrity and histology, but also gut immunity, and microbial balance (Gieryńska et al., 2022). The intestine of chicken contains a proper balance of beneficial and pathogenic bacteria, such as *Clostridium, Coliform*, and *Escherichia coli*, which are vital for the gut health and function (Shang et al., 2018). These intestinal microflora could enhance the bird's performance through assisting the nutrient mobilization and digestion, enhancing the gut immunity, and protection against pathogens (Aruwa et al., 2021). Accordingly, microbiota regulate food digestion via regulating the degradation of the non-digestible carbohydrates producing short chain fatty acids (SCFA) that accelerate enterocyte proliferation, help mineral absorption and the synthesis of some vitamins such as Vit K and B (Hamid and Magray, 2012). Moreover, SCFA and other bacterial products as lactates could strongly enhance the host immune response (Yang et al., 2017). However, the balance of these microbiota is highly affected by several factors, such as the dietary constituents which could impair this balance (Zhu et al., 2021). In this regard, dietary presence of antibacterial substances is crucial to maintain the normal balance and to selectively enhance the growth of beneficial bacteria (El-Ratel et al., 2024) however, they have a draw-backs on poultry and human health including the rapid development of bacterial resistant to conventional antibiotics (Chattopadhyay, 2014). Thus, attention has been drawn toward the naturally antibacterial feed additives such as LZ as effective growth promoter to improve the bird immunity and growth (Krysiak et al., 2021). In this study, all LZ supplementations significantly decreased TLC, TCC and TBC in cecal contents in both white- and brown-feathered quails. This response might be linked with the anti-microbial and antibacterial features of LZ because of its peptides which have bacteriostatic and bactericidal effects against several Gram-negative and Gram-positive bacteria (Mine et al., 2004). The antibacterial features of LZ might be attributed to its ability to modulate the intestinal pH that alters microbial growth. Besides, the immunomodulatory character of LZ enhances the bird's immune system that may inhibit the microbial growth including lactobacillus (Ferraboschi et al., 2021). Thus, LZ was recommended as an alternative to antibiotics in poultry feed to manage intestinal bacterial growth (Xia, et al., 2019). In the same line, feeding lysozyme to birds for 35 days markedly reduced the number of *E. coli* in the ileum compared with the virginiamycin antibiotics (Gong et al., 2016). Besides, LZ lowered the count of *C. perfringens* and limited the growth of *E. coli* and *Lactobacillus* (Liu et al., 2010) restored broiler's normal intestinal morphology and digestive function and improved the bird's growth and nutrient

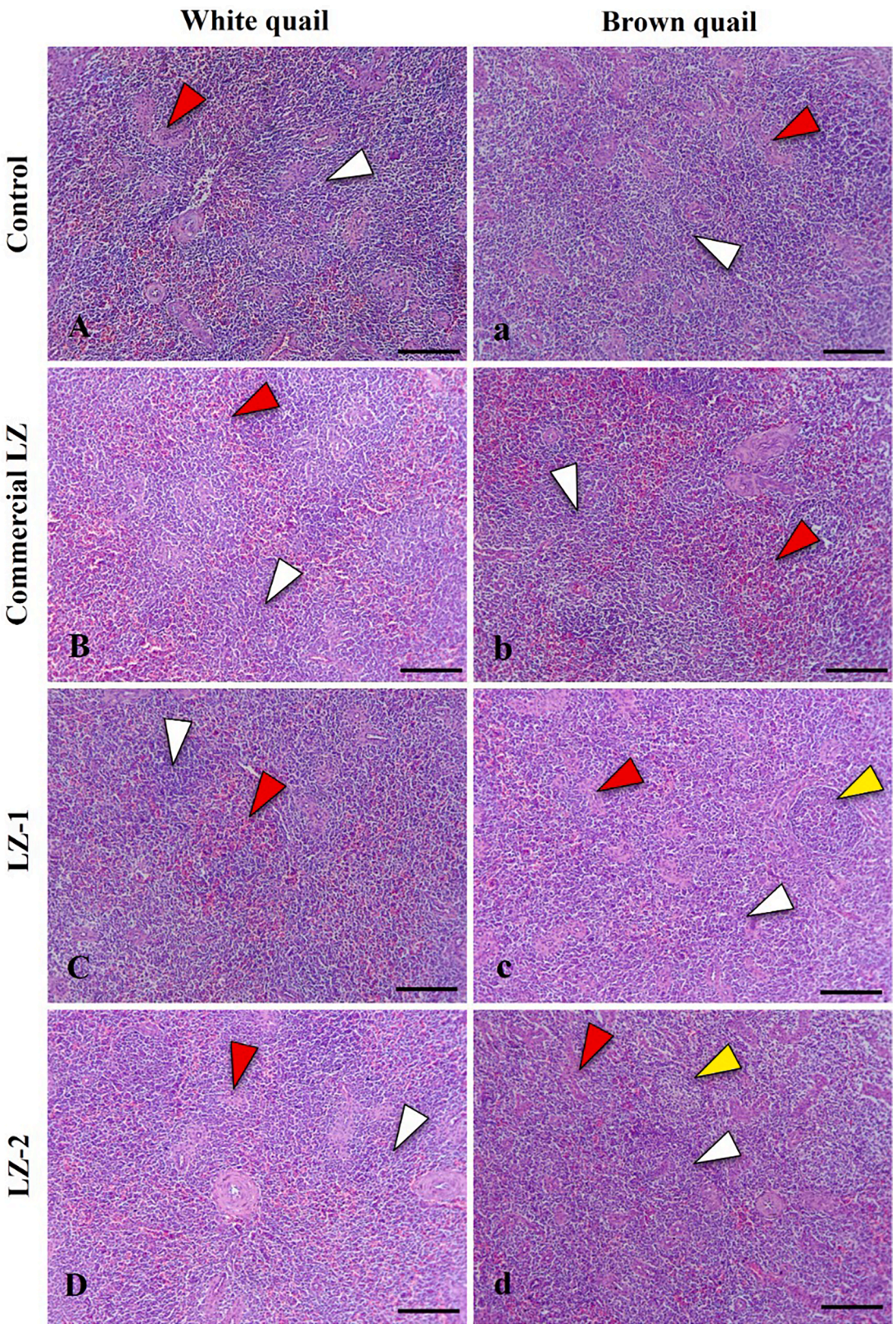


**Fig. 2.** The splenic structure of jejunum in both white- and brown-feathered quails in response to supplementation of lysozyme.

utilization. Additionally, lysozyme alleviated the destructive intestinal lesion associated with *C. peferenges* by inhibiting the production of its toxin (Zhang et al., 2006).

In addition, lysozyme could enhance the gut immune response and regulate the expression of somegut immune-related genes. In this study, lysozyme significantly enhanced the expression level of *ILI-β* gene in all lysozyme supplemented groups. *ILI-β* is a pleiotropic cytokine which plays an important role in the innate and adaptive immunity (Garlanda et al., 2013; Muñoz-Wolf and Lavelle, 2018). The up regulation of IL1 family including *ILI-β*, under normal environmental condition could

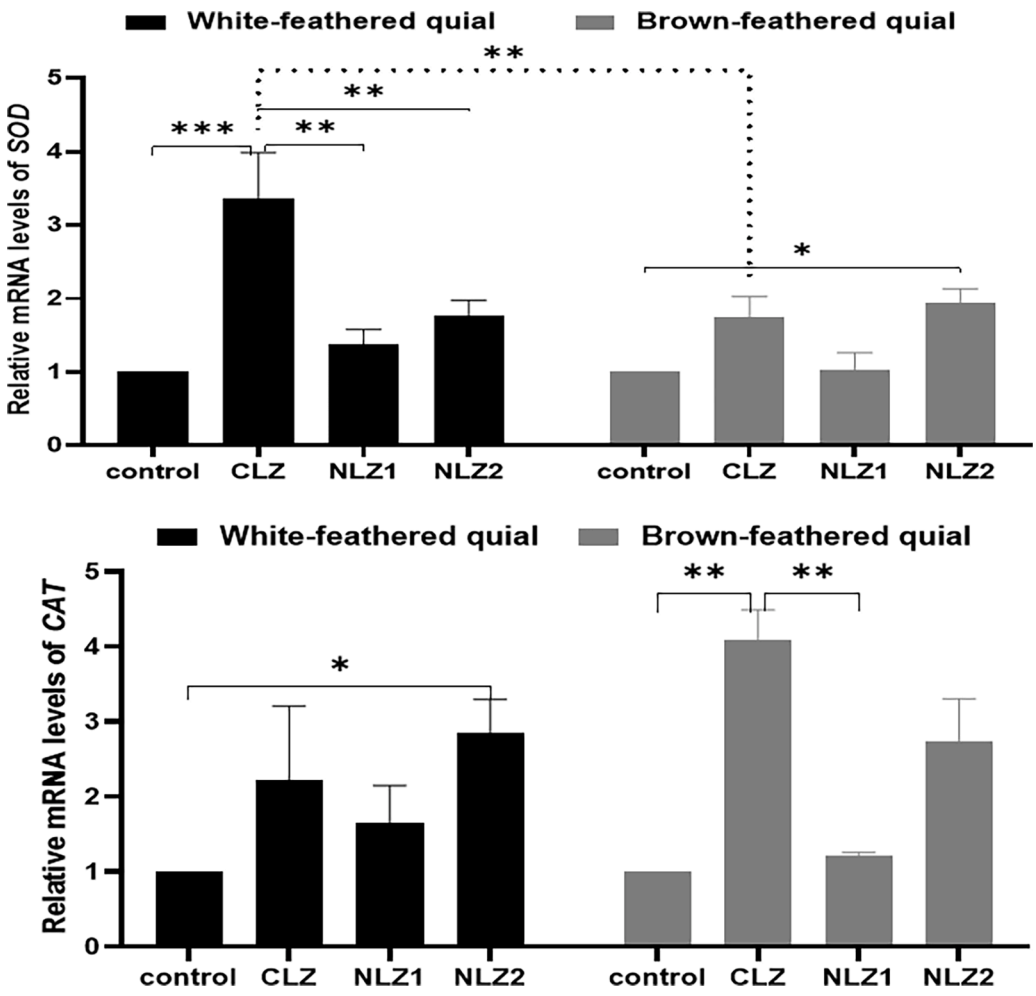


**Fig. 3.** The relative mRNA levels of *SOD* and *CAT* genes in the jejunum of white- and brown-feathered quails supplemented with lysozyme.

refer to active immune system (El-Kassas et al., 2016; Shini et al., 2010). However, the extreme levels of the proinflammatory cytokines expression are mostly observed with infection or stress conditions which could induce inflammatory tissue damages (El-Kassas et al., 2018). In accordance exogenous lysozyme upregulated the intestinal *IL10, IL8* and *INF* expression (Abdel-Latif et al., 2017).

The total and differential WBCs count are widely used to assess birds immune and clinical health status (Grasman, 2002; Abdulazeez et al., 2016). Thus, lysozyme supplementation did not only improve the gut immunity but also showed systematic immune stimulant effects. LZ slightly modified WBCs count besides, the both commercial (CLZ) and egg extracted lysozymes (NLZ1 and NLZ2) induced significant reduction of H/L ratio in the two studied quail strains due to increased lymphocyte count. Likewise, the administration of LZ increased the plasma level of lysozyme which enhanced the WBCs activity and number (Park et al., 2021). In accordance with the current results, LZ improved rabbits immune status supported by elevated counts of WBCs and lymphocytes, increasing the serum total protein and globulin concentrations and lowering the H/L ratio (EL-Deep et al., 2020).These systemic immune stimulant effects of lysozyme included also the enhancement of the immune organ's morphology. The splenic structure showed normal appearance of red and white pulps in all groups in addition to the lymphoid nodules in the brown-feathered quails in the case of NLZ1 and NLZ2. Plus, the number of sheathed arteries within the splenic tissue was increased in white-feathered quails fed on NLZ2.

Moreover, lysozyme not only modulated WBCs number but also enhanced their activities. In our study, lysozyme enhanced the WBCs phagocytic activity and the serum lysozyme activity in all treated groups with the brown-feathered quails showed the highest activities. Increasing the WBCs phagocytic activity and serum lysozyme levels were considered indicators of macrophage and mononuclear phagocytes higher activities(Schulz et al., 2024).

The lysozyme is important for the destruction of the bacterial cells, where the in vitro incubation of macrophages with LPS markedly increased lysozyme c-type gene expression and enzyme activity (Myrnes et al., 2013).The enhanced immunity and the serum lysozyme in several species after exogenous lysozyme are associated with the improved antioxidant status (Abu Hafsa et al., 2022; Deng et al., 2012).

The hematological parameters are strongly recommended as an indicator about the poultry general health status (Etim, 2014). In this regard, the general improvements of the bird gut health, feed utilization, and immunity and antioxidant status were mostly associated with enhancement of the bird hematological and biochemical profiles. The Lysozyme dietary supplementation slightly increased RBCs count, and PCV (%), and markedly elevated HB concentration in both studied quail types. In the same line, lysozyme improved the blood profile in several species. Lysozyme supplementation to broiler chicken at the rate of - 1 g/4 L drinking water, improved birds performance FCR, growth rate and increased the RBCs, HG and PCV (Khalil et al., 2020). Dietary LZ to rabbits at three different doses obviously improved the total RBC number, Hb, and PCV (EL-Deep et al., 2020). In another study, the lysozyme in a rabbit's diet improved the gut physiological function feed digestibility which reflected on a significantly enhanced hematological analysis (Abdelazeem et al., 2023).

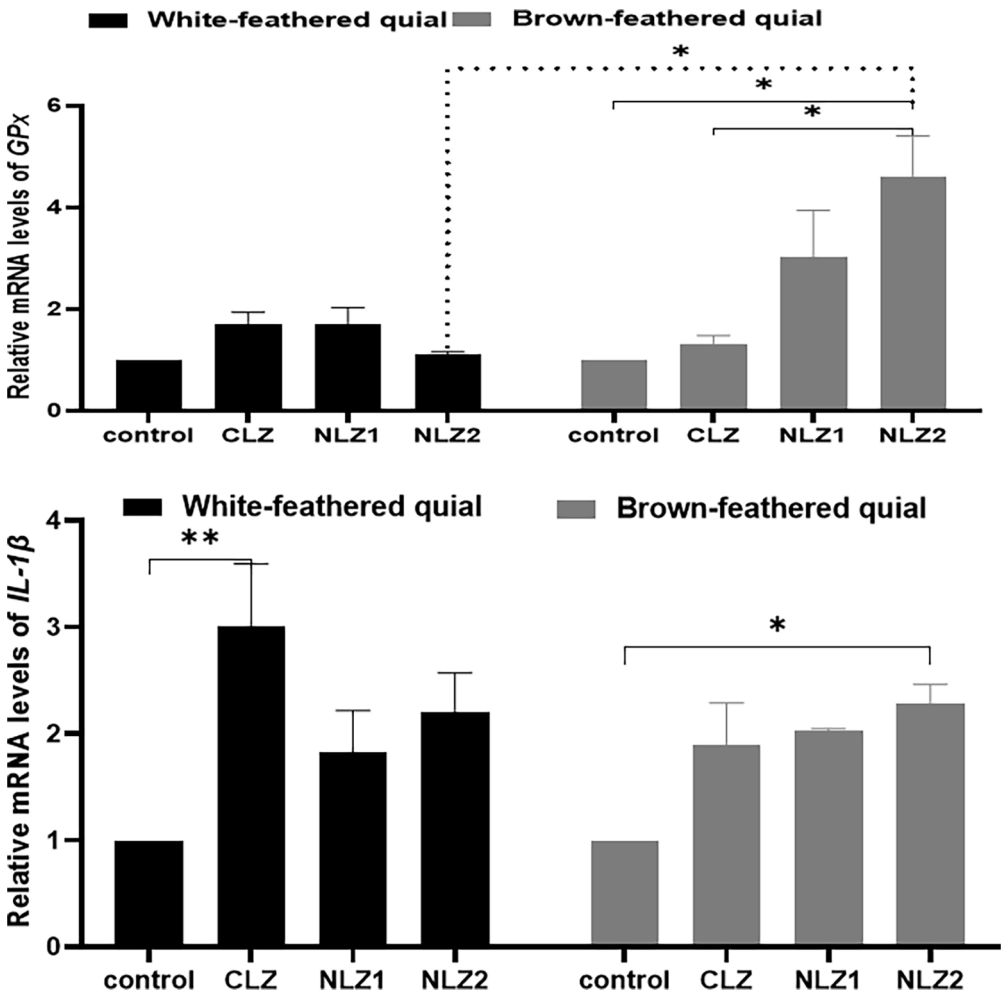


**Fig. 4.** The mRNA transcriptomic level of *GPX* in both white- and brown-feathered quails in response to supplementation of lysozyme.

## Conclusion

In summary, in this feeding experiment the effects of different forms of LZ on quails' performance, immune and antioxidant responses, and gut health were explored in two different quail varieties (brown- and white-feathered Japanese quail). The main results included improved growth performance (especially the white-feathered quails) of quails with significant reduction in feed intake in both quail cultivars in response to the different forms of LZ compared to the control group. There were obvious differences in growth performance between the two studied quail varieties in response to the two forms of LZ. Compared to the control diet, a marked improvement in FCR was reported in the CLZ group for both quail varieties and in NLZ1 and NLZ2 for the white-feathered and brown-feathered quails, respectively. The CLZ and the higher dose of NLZ (NLZ2) resulted in noticeable increases in the total antioxidant activity (TA) in the white-feathered and brown-feathered quails with significant elevations in the serum lysozyme and phagocytic activities in all LZ groups. At the gut level, both CLZ and NLZ increased the intestinal villi length and goblet cell count with significant reductions in the total lactobacillus, total coliform, and total bacterial counts in the two studied quail varieties. These impacts of different LZ forms were associated with prominent changes in the expression levels of *SOD, CAT, GPX,* and *IL-1β* genes in both quail varieties with the NLZ had better responses. Thus, the form of LZ either CLZ or NLZ, could differentially impact quail growth, immune and antioxidant status as well as gut health between different quail varieties.

## Declaration of competing Ineterst

There were no conflict of interests

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