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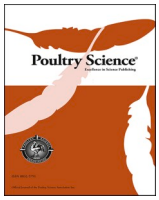

Dietary supplementation of lysozyme can improve growth rate, laying performance, blood biochemistry, and mRNA levels of some related genes in different plumage-colored quails

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ABSTRACT

The impact of dietary lysozyme (LZ) supplementation on the growth and laying performance was investigated over 4 weeks of growing and 6 weeks of laying periods in two different plumage color (white and brown-feathered) Japanese quail varieties. For each variety, 240 birds were randomly assigned into four groups with four replicates for each group. The first group (control) was fed a basal non-supplemented diet (BD). Whereas the 2nd, 3rd, and 4th received the BD supplemented with commercial LZ (CLZ) at 100 mg/kg diet, and natural LZ (NLZ) at 100 and 200 mg/kg diet, respectively. The main findings included significant increases in body weights and gains in the white-feathered quails supplemented with NLZ1 compared to the control and NLZ2. However, there were no significant differences in the case of brown-feathered quails in all LZ supplementations. Moreover, the different dietary LZ lowered FI in both quails with the lowest intake observed in the brown-feathered quails. Accordingly, enhanced FCR was reported in the CLZ groups for both quail varieties and in NLZ1 and NLZ2 for the white-feathered and brown-feathered quails, respectively. In both quail varieties, the NLZ2 significantly lowered serum creatinine and urea and increased albumen and globulin levels compared with other groups. Histologically, the best hepatic histological features were found in both quail' varieties fed the NLZ1-supplemented diet. Accompanying LZ-induced modulations in the expression levels of *GHR, IGF-1, leptin, CCK, FAS,* and *ACC* genes in both quail varieties were reported. Besides, both quail varieties in NLZ1& NLZ2 supplementation exhibited significant increases in hen day egg production, egg weight, egg mass, and hatchability percentages along with differences in external and internal egg qualities compared with LZ-free diet or CLZ. Therefore, NLZ could be used as an effective feed supplement to enhance the growth and egg performance of Japanese quail with caution being drawn to the supplementation dose about quail variety.

Introduction

The exponential enlargement of the human population greatly increases the human demand for animal-source protein, such as meat and eggs which intensifies the need to produce more varieties of food forms (Henchion, et al., 2017). Quail production is one of the fastest-growing sectors in the poultry industry which gained popularity as an alternative agricultural enterprise. Quails are characterized by their small bodies, rapid growth rate, stress, disease resistance, short generation times, and high egg production rate (Elsaidy, et al., 2021; Kirrella, et al., 2023). Therefore, quails could be reared as an inexpensive animal protein source to fill the shortage of the other sources especially in the developing countries (Elkhaiat, et al., 2023; Kirrella, et al., 2021). Moreover, as a result of the reported plumage color mutations, numerous varieties

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of Japanese quails, including those of grey, white, and brown feather-colored quails have been recognized (Bagh et al. 2016), which exhibit distinguished productive and reproductive traits.

For a long time, antibiotics have been extensively used in livestock production to treat diseases and as growth promoters enhancing animals' performance (Abd El-Hack, et al., 2022b; Dibner and Richards, 2005). However, their inappropriate use in animal production has resulted in severe public health concerns because of their residues in animal byproducts and the development of antibiotic resistance (Zhu, et al., 2021). Thus, producing antibiotic-free and safe food products has become a crucial demand (Mohammadi, et al., 2023). Therefore, efforts are underway worldwide to find alternatives that are safe for public health, cost-effective, and environmentally friendly growth promoters (El-Kasrawy, et al., 2023).

Currently, several alternatives have been recognized and received attention for their ability to boost animal production raised in commercial settings, including probiotics, organic acids, prebiotics, symbiotics, enzymes, and phytogenic compounds (Abd El-Hack, et al., 2022b). Lysozyme (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 peptidoglycans in the bacterial cell wall (Ibrahim, et al., 1996) and indirectly by promoting the phagocytic activity of macrophage (Biggar and Sturgess, 1977). As a result of its antimicrobial activity, lysozyme is considered an endogenous antibiotic that gained considerable interest for use in several applications including; medicine, (the treatment of infectious diseases, wound healing, and anti-biofilm), veterinary, food preservation, and crop protection (Ferraboschi, et al., 2021).

Several beneficial effects of lysozyme dietary inclusion have been documented in different animals. In broiler chickens, LZ lowered *C. perfringens* colonization and enhanced the function of the intestinal barrier and growth performance (Liu, et al., 2010). Besides, it enhanced the broiler's gut antioxidant status, and nonspecific immunity and improved their growth performance (Abdel-Latif, et al., 2017). In rabbits, LZ increased growth rate, blood health, total edible parts, digestive enzyme activities, and nutrient digestibility (Abdelazeem, et al., 2023; EL-Deep, et al., 2020). In addition, LZ also improves the growth and immune response, maintains gut barrier function, and regulates the gut microflora in weaned pigs (Ma, et al., 2017; May, et al., 2012). Moreover, LZ has antibacterial, antiviral, antimetastatic, and anti-inflammatory effects, indicating its function as a natural immunostimulant that could replace antibiotics (Saurabh and Sahoo, 2008).

LZ is widespread in all living organisms' tissues and secretions with the avian egg white being the richest source. A variety of methods, including, direct crystallization, ion exchange, ultrafiltration, two-phase system separation, reverse micelle extraction, and affinity membrane chromatography have been developed for the extraction of lysozyme (Yao, et al., 2022). Because of their comparatively prohibitive cost and excessive experimental operation, some of these technologies are challenging to attain industrial mass production. In this study, we hypnotized that LZ would modulate quail's growth, productive and reproductive performance, blood biochemistry and public health. Therefore, the current study aimed to explore the effects of dietary LZ supplementation on growth performance, blood biochemistry, liver histology, and the expression levels of certain growth-, feed intake (FI)-, and lipid metabolism-linked genes during the growing period, as well as assessing the laying performance and egg quality parameters during the egg production period in two Japanese quail varieties (Brown-feathered and White-feathered).

Materials and methods

Ethical statement

All experimental procedures, management, and bird husbandry followed the regulations and instructions of the Animal Care and Ethics Committee, Kafrelsheikh University, Egypt and all methods were performed in accordance with the relevant guidelines and regulations (Approval number: KFS-IACUC/164/2023).

Quail care and experimental design

This study depended on mixed-sex, one-day-old Japanese quail chicks with two plumage colors (brown- and white-feathered quails). Birds were obtained from the poultry facility, Faculty of Agriculture, Kafrelsheikh University, Egypt. To control the chicks' survival rate, they were housed collectively during a two-week adaption period. Then at 14 days old, each bird was weighed separately (average initial body weight 76.01 g ± 5.81), and randomly clustered into 4 groups with four replicates each. For each quail variety, 240 birds were allocated into four treatments (15 bird/ replicate), the first group (control) fed the basal diet (BD) with no lysozyme added; the second group (CLZ) received the BD supplemented with commercial lysozyme (100 mg/kg diet); third and fourth group (NLZ1 & NLZ2) fed the BD supplemented with egg-extracted natural lysozyme added at 100 and 200 mg/kg diet, respectively. The commercial lysozyme (lysozyme ten%®, Nan Chang Lifeng Industry and Trading Co., Ltd., Jiangxi, China) was added at the dose rate of 100 mg per kg BD. Each 1 kg of the commercial lysozyme 10 % contained 100 g of lysozyme, 50 g of glycine, 10 g of a sporadic acid, 8 g of water, and 832 g of glucose). Whereas the natural form of LZ was purified from non-fertile hen egg white using the extraction method described by (Guérin-Dubiard, et al., 2005; Luding, et al., 2011). Briefly, the egg white was gathered from fresh hen eggs and diluted with equal volume of ultrapure water (Milli-Q water). Then, the pH of the mixture was adjusted to 6 using 1 $molL^{-1}$ of HCl. After that the mixture was gently stirred overnight at 2 °C and centrifuged for eight min at 8000 rpm at 4 °C to remove the precipitate. The supernatant was used as a source for lysozyme that was purified using the chromatography at 280 nm with pH of the supernatant was adjusted to 7.8 with 1 $molL^{-1}$ of NaOH.

The housing temperature at the beginning of the experiment (brooding period) was 33-34 °C, which steadily dropped to 22-25 °C on the 21st day of the bird's age and continued throughout the experiment. Throughout the whole experiment, all birds were housed under identical housing conditions with free access to food and water. The ingredient composition of the basal diet used was formulated according to (NRC 1994) to meet the dietary needs of quail during both periods (illustrated in Table 1). Birds were maintained in traditional cages (90 × 40 × 40 cm) for four weeks of growth (2-6 wk of age) then a further six weeks laying (6-12 wk of age).

Growth performance

Bird's body weights (BW) (initial, weekly, and final) and FI of each quail variety were monitored once a week to evaluate its growth performance. Based on the BW and FI, the body weight gain (WG) (final weight – initial weight) and feed conversion ratio (FCR, g feed/g gain) were calculated.

Sample collection and measurements

Twelve males from each group (three from each replicate) were randomly selected at 6 weeks old (the end of the growing period) and used for blood and tissue sample collections. Blood samples were collected from birds' jugular veins and used for the separation of blood sera. Following that, birds were killed under mild anesthetic by using intramuscular injection with 1 ml/kg of ketamine xylazine mixture (2:1). Then, liver tissue samples were collected, quickly frozen in liquid nitrogen, and stored at -80 °C for gene expression. Another hepatic sample was collected for histological examination. Sampled birds were dissected and eviscerated and the weight of carcass and internal organs (gizzard, liver, and heart) were recorded. Carcass weight was described

Table 1
Ingredient composition of the used basal diets during both periods of the experiment.

Ingredients (%)	Growing diet	Laying diet
Yellow corn	51.17	57.40
Corn Gluten Meal	6.80	4.00
Soybean meal (47 %)	36.19	28.10
Soybean Oil	2.00	1.60
DCP[1]	1.47	1.70
Limestone [2]	1.59	6.40
Premix [3]	0.30	0.00
Premix [4]	0.00	0.20
Common Salt	0.25	0.25
DL-methionine [5]	0.10	0.20
Lysine HCl [6]	0.03	0.05
Choline chloride	0.05	0.05
Mycotoxin adsorbent	0.05	0.05
Calculated Composition (%)		
Crude protein	23.79	19.04
Calcium	0.98	2.69
Available Phosphorus	0.32	0.31
Digestible lysine	1.16	0.95
Digestible Methionine	0.47	0.46
ME (Kcal/kg)[7]	2975	2872

[1] DCP=Dicalcium phosphate (17 % Phosphorus and 21 % Calcium).
[2] Limestone (contain 35 % calcium).
[3] Growing Premix: each 3 kg vitamin and mineral mixture contains: 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] Laying Premix added (0.1 % vitamin premix + 0.1 % mineral premix produced by Devenish Nutrition Company, UK; each containing 1kg of vitamin premix composed of Vit A (24000000 IU), Vit D (8000000IU), Vit E(80000 mg), Vit K_3 (6000 mg), Vit B_1 (4000 mg), Vit B_2 (12000 mg), vitB_6 (10000 mg), Vit B_{12} (10gm), nicotinic acid (90000 mg), pantothenic acid (24000 mg), folic acid (3000 mg), biotin (200 mg), BHA/BHT% (10000 mg), and carrier (calcium carbonate) up to 1kg; each 1kg contains mineral premix contains: iron (60000 mg), copper (10000 mg), zinc (120000 mg), manganese (20000 mg), iodine (1000 mg), selenium (400 mg), cobalt (200 mg) and carrier up (calcium carbonate) to 1kg.
[5] DL-Methionine (Produced by Evonik Co. and containing 99 % methionine).
[6] Lysine = lysine hydrochloride (containing 98 % Lysine).
[7] calculated composition according to NRC (NRC, 1994).

relative to bird live weight.

Serum biochemical variables

Using a non-heparinized syringe, blood samples were collected and placed in sterile vials for centrifugation at 3000 rpm for ten minutes to separate the serum, which was stored at -20 °C until further analysis of biochemical constituents. The measured serum biochemical indices included: total protein, albumin, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), urea and creatinine, and lipid indicators such as cholesterol, triglycerides, plus the low and high-density lipoprotein (LDL and HDL, respectively). Following the manufacturer's guidelines, these parameters were measured by spectrophotometer using commercial kits (Bio-diagnostic Co, Egypt). The difference between the albumin and total protein readings of the same sample was used to compute the serum globulin levels.

Liver morphology

Liver tissue samples (n = 12/ group) were also collected for morphological analysis, washed with physiological saline, and then fixed in 10 % formalin for 24 h. Slides were prepared and stained with hematoxylin and eosin (H&E) following the method of Bancroft, et al. (2013) and examined under the light microscope.

Real-time PCR

For RNA extraction, liver samples (n = 12/ group) were homogenized in PBS. The total RNA was extracted using Trizol (iNtRON Biotechnology, Inc.). The RNA quality and quantity were evaluated using 2 % ethidium bromide-stained agarose gel electrophoresis and Nanodrop (UV-Vis spectrophotometer Q5000, Quawell, USA), respectively. Then, a fixed amount of RNA (~2μg) was used for reverse transcription to synthesize the complementary DNA (cDNA) from each mRNA sample using (Thermo Scientific first-strand cDNA synthesis kits). As illustrated in Table 2, specific primers were used to amplify some genes related to growth (*GHR* and *IGF1*), fat metabolism (*FAS* and *ACC*), and feed intake regulation (*CCK*, and *Leptin*). Gene amplification was done using Stratagene MX300 P real-time PCR system (Agilent Technologies) and SensiFast™ SYBR green (Bioline, United Kingdom). The total reaction volume was twenty μl including ten μl of SensiFast™ SYBR master mix, two μl of cDNA, and 0.5 μM of each primer. The amplification 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 amplification of each gene was run in duplicate. The results were normalized against the *B-actin* (as a housekeeping gene) and the control birds fed the BD to calculate the expression fold changes according to (Livak and Schmittgen, 2001).

Egg production and egg quality parameters

At six weeks old, thirty females and fifteen males (2 female: 1 male) from each group were randomly distributed in three replicates (10 females+5 males /replicate) to evaluate the laying performance and hatchability percentage for a six-week laying period. Hen-day egg production and internal and external egg quality attributes were evaluated. In this context, daily egg collection was conducted, and the hen day egg production percentage (HDEP) was computed using the formula (number of eggs produced / number of laying females on that day × 100). At the end of the laying period, forty-five eggs per group (15 eggs per replicate) were collected and weighed using a digital scale. Egg quality parameters including internal (length, width, and height of yolk and albumen, and yolk color) and external (shell thickness) were measured following (Kirrella, et al., 2023). Eggs were collected daily from the distinct groups and were taken to incubation on the same day. Then, a total of 400 eggs (100 eggs/treatment) were held for 17 days at 75 % relative humidity and 18- 21 °C temperature. Eggs were then delivered to the hatchery facility in separate egg trays for each group as they were kept at a temperature of 37.5 °C and 55-60 % relative humidity in the hatcher. After hatching, chicks were counted and non-hatched eggs were broken to determine the fertility and hatchability percentages. Hatchability (%) = (number of hatched chicks/ numbers of fertile eggs) × 100.

Statistical analysis

The results of this study were statistically analyzed by Two-way ANOVA using SPSS-22 ((©IBM Corp. Released 2013, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM). The analysis assessed the effects of quail varieties (white- and brown-feathered quails), dietary supplementation of various sources of lysozyme (CLZ and NLZ), and their interactions. Multiple comparisons using Tukey's test were done. Graph pad prism9 (©GraphPrism Software, La Jolla, CA, USA) was used to create the figures of qPCR results. The results were expressed as means ± SE. Statistical significances were considered at $P < 0.05$.

Results

Growth performance

Fig. 1 illustrates the effects of LZ dietary supplementation either CLZ or the natural one on the growth performance of the two studied quail

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)
GHR	F: AACACAGATACCCAACAGCC R: AGAAGTCAGTGTTTGTCAGGG	NM_001001293.1	60	(El-Naggar, et al., 2019)
GF1	F: CACCTAAATCTGCACGCT R: CTTGTGGATGGCATGATCT	NM_001004384.2	60	
CCK	F: CAGCAGAGCCTGACAGAACC R: AGAGAACCTCCCAGTGGAACC	NM_001001741.1	58	Kirrella, et al. (2021)
Leptin	F: CGTCGGTATCCGCCAAGCAGAGGG R:CCAGGACGCCATCCAGGCTCTCTGGC	AF082500	60	
FAS	F: GGAAGATCTGGAGGCTCGTG R: AAAGGAAGCAGCAGCAAAGC	NM_205155.2	63	El-Naggar, et al. (2019)
ACC	F: AATGGCAGCTTTGGAGGTGT R: TCTGTTTGGGTGGGAGGTG	NM_205505	63	

GHR= growth hormone receptor; *IGF*= Insulin like growth factor; *CCK*= cholecystokinin; *FAS*= fatty acid synthetase; *ACC*=acetyl Co A carboxylase

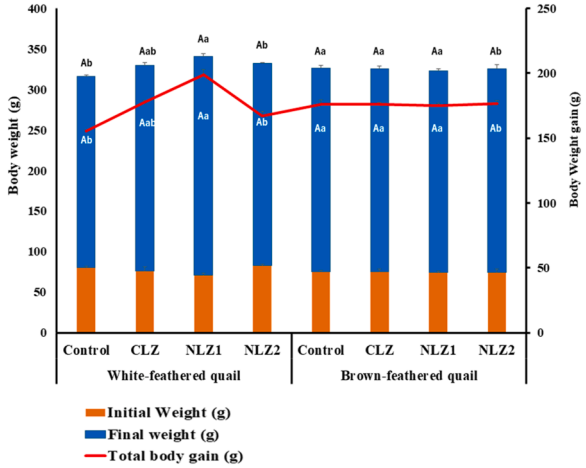

Fig. 1. Body weight and body weight gain in the two different varieties of Japanese quail (Brown-feathered & White-feathered) supplemented with lysozyme.

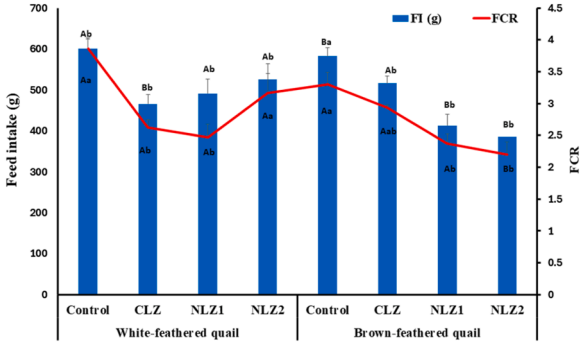

Fig. 2. Feed intake and feed conversion ratio in the two different varieties of Japanese quail (Brown-feathered & White-feathered) supplemented with lysozyme.

varieties. LZ supplementation similarly improved quail's growth performance between white- and brown-feathered quails ($P < 0.05$) with significant statistical interaction between quail varieties and supplemental lysozyme ($P < 0.05$). Among the white-feathered quails, the heaviest body weights were recorded for quails supplemented with a 100 mg/kg diet of natural LZ (NLZ1) compared with the control (LZ-free diet), and 200 mg/kg diet of natural LZ (NLZ2) ($P < 0.05$). However, no significant differences were found in the final weights of brown-feathered quails with LZ supplementation. The total body gain followed the same trend as the final body weight. Feed intake and FCR were also altered by the dietary addition of commercial and natural LZ in both white- and brown-feathered quails (Fig. 2). Both commercial and natural LZ at both levels lowered the FI in the two studied quails with the lowest FI recorded in the case of brown-feathered quails ($P< 0.05$). As a result, improvement of the FCR was recorded. Enhanced FCR was reported for commercial (CLZ) and NLZ1 in the case of white-feathered quails. Supplementing the natural LZ in a brown quail diet resulted in improved FCR.

Carcass traits

Without variation across the two quail varieties under study, LZ supplementation also affected the carcass characteristics (Table 3). In this regard, supplementing LZ to quail diet significantly altered male carcass characteristics depending on the LZ type ($P < 0.05$). Using CLZ and natural LZ (NLZ1) induced the highest carcass weights and the relative carcass weight compared to the control and natural LZ (NLZ2). Whereas the liver, gizzard, and heart weights were not affected by the LZ addition.

Biochemical profile

Total protein showed no variation in response to LZ supplementation in both quail varieties (Table 4). However, significant increases in albumen and globulin levels were measured in quails supplemented with LZ. These changes in albumen and globulin levels were combined with distinct alterations of kidney function indices (creatinine and urea levels). Both quail varieties exhibited less creatinine and urea levels in response to feeding on LZ supplemented diet. The liver function did not change among the two studied quails nor with LZ supplementation ($P > 0.05$). This response was ascertained by the non-significant changes in liver function indicators AST and ALT. Moreover, the supplemental LZ did not change the lipid profile confirmed by the non-significant changes in cholesterol, TG, LDL, and HDL levels.

Hepatic histological characteristics

The histological features of the liver in both white- and brown-feathered quails were investigated in response to LZ supplementation (Fig. 3). The liver of the two studied quail varieties demonstrated almost normal spongy architecture of the hepatic parenchyma (Fig. 3) except slight vacuolation and degeneration within the hepatocytes of quail with white feather which received 200 mg of natural LZ (NLZ2) (Fig. 3D). In general, the best histological features were reported in both varieties

Table 3
Carcass characteristics of white- and brown-feathered quails in response to dietary lysozyme supplementation.

Item	White-feathered quail				Brown-feathered quail				P values		
	Control	CLZ	NLZ1	NLZ2	Control	CLZ	NLZ1	NLZ2	Q	LZ	Q*LZ
CW (g)	148.00 ±1.53^{c}	182.5 ± 3.18^{a}	177.00 ± 1.15^{ab}	165.5 ± 6.06^{bc}	157 ± 1.15^{b}	161 ± 7.51^{ab}	177.5 ± 10.68^{ab}	199.5 ± 4.33^{a}	0.176	<0.001	0.001
RC (%)	72.11 ± 0.86^{c}	79.05 ± 0.60^{ab}	79.61 ± 1.03^{a}	73.99 ± 0.71^{bc}	75.85 ± 0.08^{b}	77.94 ± 0.94^{a}	74.40 ± 1.42^{b}	78.42 ± 0.34^{a}	0.450	0.001	<0.001
LW(g)	3.50 ± 0.224^{a}	3.67 ± 0.211^{a}	3.83 ± 0.167^{a}	3.67 ± 0.197^{a}	3.33 ± 0.211^{a}	3.67 ± 0.333^{a}	3.50 ± 0.224^{a}	3.67 ± 0.211^{a}	0.443	0.619	0.865
GW (g)	3.50 ± 0.224	4.33 ± 0.211	3.5 ± 0.224	3.83 ± 0.307	4.00 ± 0.365	3.83 ± 0.307	3.83 ± 0.167	3.5 ± 0.224	0.921	0.338	0.169
HW (g)	2.17 ± 0.167	2.67 ± 0.211	2.17 ± 0.167	2.17 ± 0.167	2.17 ± 0.167	2.33 ± 0.211	2.17 ± 0.167	2.67 ± 0.211	0.751	0.175	0.175

CW= carcass weight; RC= relative carcass; LW= liver weight; GW= gizzard weight; HW= heart weight. CLZ= commercial lysozyme (100mg/kg diet); NLZ1= egg-extracted lysozyme (100mg/kg diet); NLZ2= egg-extracted lysozyme (200mg/kg diet); Q represents quail's varieties effect; LZ represents the source of lysozyme. Q*LZ= represents the interaction between quail's varieties source of lysozyme. 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 $P < 0.05$.

Table 4
Biochemical profile of white- and brown-feathered quails in response to dietary lysozyme supplementation.

Item	White-feathered quail				Brown-feathered quail				*P* values		
	Control	CLZ	NLZ1	NLZ2	Control	CLZ	NLZ1	NLZ2	Q	LZ	Ql*LZ
TP (g/dL)	4.12 ± 0.08	4.28 ± 0.22	5.24 ± 0.41	4.82 ± 0.09	4.66 ± 0.21	4.16 ± 0.15	3.87 ± 0.48	4.94 ± 0.11	0.280	0.111	0.013
Glob. (g/dL)	2.47 ± 0.12^{b}	2.53 ± 0.19^{b}	3.34 ± 0.40^{a}	2.95 ± 0.08^{ab}	2.92 ± 0.12^{ab}	2.18 ± 0.24^{b}	2.40 ± 0.20^{b}	3.10 ± 0.12^{a}	0.257	0.031	0.021
Alb. (g/dL)	1.65 ± 0.20^{b}	1.75 ± 0.04^{a}	1.90 ± 0.06^{a}	1.87 ± 0.04^{a}	1.57 ± 0.08^{b}	1.98 ± 0.09^{a}	1.81 ± 0.08^{a}	1.84 ± 0.03^{a}	0.890	0.039	0.295
Urea (mg/dL)	5.02 ± 0.17^{a}	4.53 ± 0.22^{ab}	4.32 ± 0.16^{ab}	2.99 ± 0.51^{b}	4.19 ± 0.11^{a}	3.82 ± 0.08^{b}	3.94 ± 0.10^{b}	4.09 ± 0.09^{a}	0.214	0.002	0.002
Creat.(mg/dL)	3.02 ± 0.08^{Aa}	2.89 ± 0.05^{Aab}	2.70 ± 0.18^{Ab}	2.77 ± 0.07^{Aab}	2.87 ± 0.13^{Ba}	2.91 ± 0.12^{Aa}	2.01 ± 0.02^{Ab}	2.52 ± 0.08^{Aab}	0.002	<0.001	0.021
AST (U/L)	34.75 ± 3.38	35.81 ± 2.42	30.81 ± 1.62	27.59 ± 4.64	29.99 ± 1.39	29.89 ± 1.87	29.79 ± 4.95	22.83 ± 1.13	0.064	0.070	0.848
ALT (U/L)	23.42 ± 1.81	20.45 ± 1.15	20.75 ± 2.52	20.37 ± 0.93	23.46 ± 3.37	20.77 ± 1.09	23.45 ± 3.35	23.54 ± 1.13	0.589	0.451	0.903
CHO (mg/dL)	93.48 ± 2.55	92.63 ± 4.01	107.76 ± 8.30	97.56 ± 1.64	99.32 ± 1.39	97.40 ± 1.63	97.84 ± 3.76	96.89 ± 1.37	0.999	0.222	0.186
TG (mg/dL)	84.90 ± 3.61	96.23 ± 2.81	95.28 ± 4.47	91.66 ± 3.43	91.39 ± 2.07	89.31 ± 4.36	100.23 ± 2.70	94.63 ± 2.27	0.450	0.086	0.240
LDL (mg/dL)	35.57 ± 3.72	35.37 ± 6.00	43.52 ± 2.98	37.11 ± 3.09	40.89 ± 1.74	35.41 ± 4.80	34.67 ± 2.78	35.93 ± 1.94	0.654	0.741	0.311
HDL (mg/dL)	40.93 ± 2.29	38.01 ± 2.11	45.18 ± 5.15	42.12 ± 2.12	40.16 ± 0.69	44.14 ± 2.99	43.13 ± 1.99	42.04 ± 2.11	0.680	0.568	0.460

TP= total protein; Glob= globulin= alb= albumin; creat= creatinine; AST= aspartate aminotransferase; Alt= Alanine aminotransferase; CHO= cholesterol; TG= triglycerides; LDL=low density lipoprotein; HDL= high density lipoprotein. CLZ= commercial lysozyme (100mg/kg diet); NLZ1= egg-extracted lysozyme (100mg/kg diet); NLZ2= egg-extracted lysozyme (200mg/kg diet); Q represents quail's varieties effect; LZ represents the source of lysozyme. Q*LZ= represents the interaction between quail's varieties source of lysozyme. 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) fed on BD supplemented with 100 mg/kg diet of natural LZ (NLZ1, Fig. 3; C, c) that revealed perivascular leukocytic infiltration. Furthermore, there was no significant improvement in the liver of brown-feathered quails between NLZ1 and NLZ2 (Fig. 3c and d).

qPCR responses of some growth-, feed intake-, and fat metabolism-regulating genes

Fig. 4 represents the relative mRNA levels of *GHR* and *IGF1* genes in response to LZ supplementation (commercial or natural LZ) in white- and brown-feathered quails. Using natural LZ at a concentration of 100 mg/kg diet (NLZ1) significantly up regulated the mRNA copies of both *GHR* and *IGF1* genes ($P < 0.05$). This effect was noticeable in quail with white feathers compared with the brown-feathered ones which did not display any alterations in the mRNA levels of *GHR* and *IGF1* in response to supplemental LZ. Moreover, the up-regulatory effect of NLZ1 on *IGF1* gene transcriptomic level was significantly higher in quail with white-feather compared to the brown-feathered ones.

The response of the FI regulatory genes, *leptin,* and *CCK* to LZ in both white- and brown-feathered quails was illustrated in Fig. 5. In this regard, in white-feathered quails, using commercial (CLZ) or natural LZ at 100mg/kg diet (NLZ1) significantly increased the mRNA copies of *Leptin* gene compared to BD only or the higher supplementary dose of natural LZ (NLZ2) ($P < 0.05$). In brown-feathered quails, the commercial LZ, and the higher dose of natural LZ (NLZ2) induced significant up-regulation of the expression level of the *leptin* gene in comparison with BD and NLZ1. For the CCK gene, supplementing egg-extracted LZ at 200 mg/kg diet (NLZ2) induced the highest expression level of *CCK* gene in both quail varieties compared to the other supplemented dose of natural LZ or synthetic LZ as well as the control ($P < 0.05$).

Fig. 6 illustrates the relative expression levels of some fat metabolism-regulating genes such as *FAS* and *ACC*. For FAS, supplementing the white-feathered quails with natural LZ, especially at 200 mg dose, significantly unregulated its hepatic mRNA levels ($P < 0.05$). Whereas in brown-feathered quails, commercial LZ markedly increased its mRNA copies compared with the control and the two supplemented

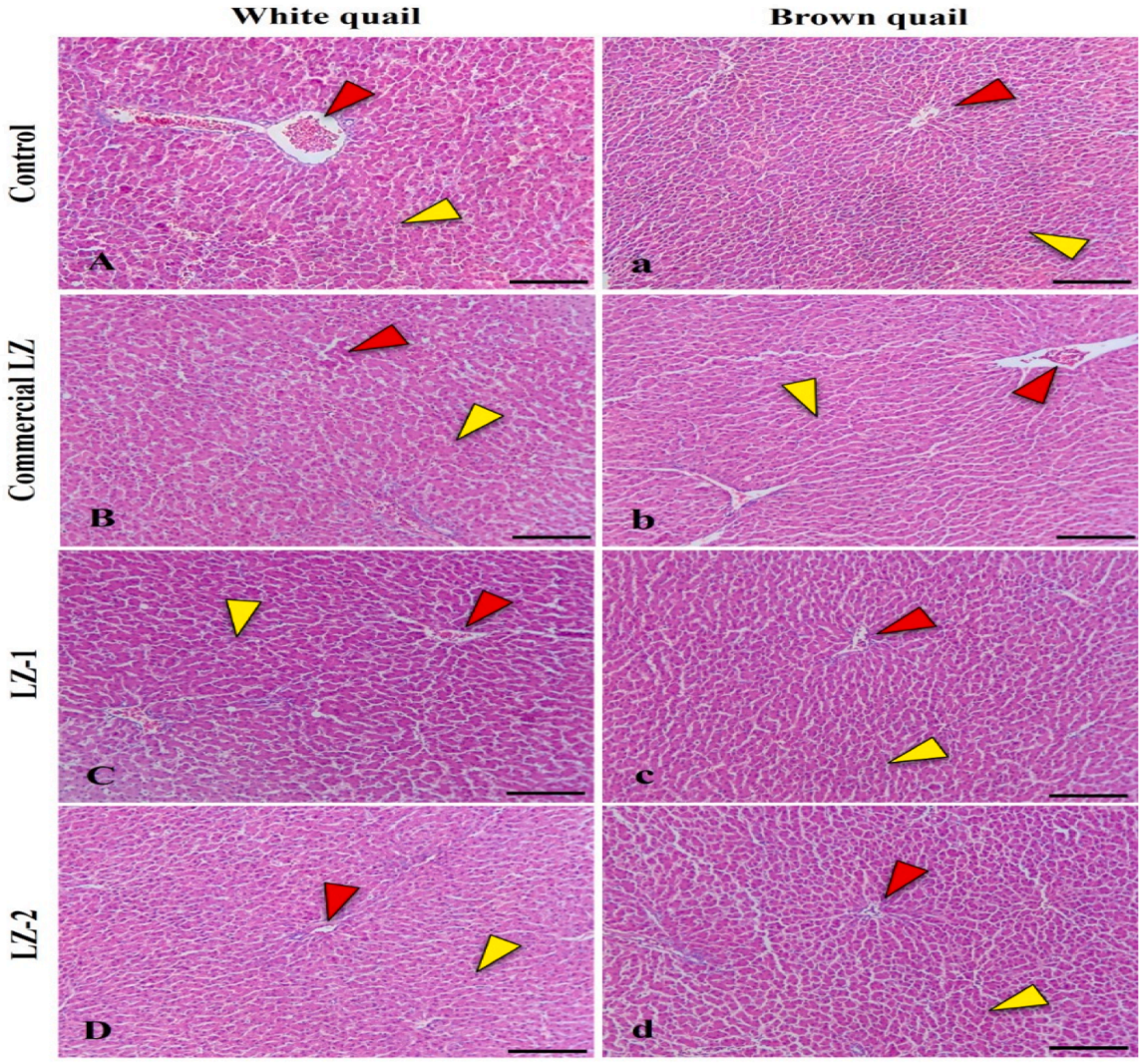

Fig. 3. The histological features of liver in white- and brown-feathered quails in response to dietary supplementation with lysozyme

doses of natural LZ ($P < 0.05$). The expression levels of the *ACC* gene differentially changed in response to supplemented LZ in a quail diet. In this regard, in white-feathered quails, the dietary supplementation of either the commercial LZ (at 100 mg/kg diet) or the natural LZ at 200 mg/kg diet distinctly increased the relative *ACC* mRNA levels ($P < 0.05$). While, in brown-feathered quails, there were slight increases in the mRNA levels *ACC* gene in all supplemented groups compared to the non-supplemented one (control) ($P > 0.05$). Besides, the upregulating effects of CLZ and NLZ2 were significantly higher in white-feathered quails compared to the brown-feathered ones ($P< 0.05$).

Egg production and egg quality

Egg production and quality (Fig. 7 and Table 5) were examined in both varieties of quail (white- and brown-feathered) in response to LZ dietary supplementation. In this line, significant increases in HDEP were recorded. Compared to birds that received the LZ-free diet (control) and those fed on CLZ, both white- and brown-feathered quails that received both levels of natural LZ (NLZ1& NLZ2) displayed a significant increase of HDEP ($P < 0.05$). This effect was associated with marked increases in egg weight and mass ($P < 0.05$). Additionally, natural LZ supplementation resulted in obvious increases in hatchability percentages in both studied quail varieties ($P < 0.05$). The external and internal egg qualities were also, modulated. A noticeable increase in egg length was recorded in both white- and brown-feathered quails that received natural LZ compared to commercial and LZ-free diets ($P < 0.05$). However, egg width and shell thickness were not altered by the LZ addition ($P > 0.05$). Internally, the characteristics of egg whites and yolk were changed because of the commercial and natural LZ supplementation in quail's diet. Egg-white length and width were not altered by LZ supplementation but significantly differed across the two studied varieties of quails. The brown-feathered quails that received the natural LZ in their diet had higher values of EWL and EWW compared to the white-feathered quails. The height of the egg white was changed in response to the LZ supplementation. Using both commercial and natural LZ at 100 mg/kg diet induced higher heights of egg whites ($P < 0.05$). For yolk features, only yolk weight was significantly altered by the dietary supplementation of commercial and natural LZ with the higher weights measured for those who received the natural LZ compared with the commercial and BD. Besides, the brown-feathered quails displayed higher weights compared with the white-feathered quails.

Discussion

As far as we know, no research has been done on how dietary LZ supplementation with diverse levels would affect the growing performance of Japanese quail with varied colored feathers.

The current investigation revealed an improved growth performance

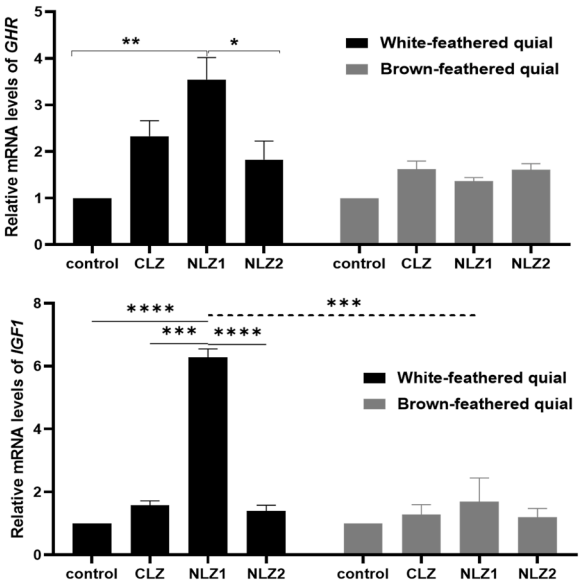

Fig. 4. Relative mRNA expressions of *GHR, IGF* in the liver of white- and brown-feathered quails in response to dietary lysozyme supplementation (n = 12 /group). Values are mean ± SEM, statistical significance at $P < 0.05$.

in both quail varieties in response to LZ supplementation with a clear response in quail with white feathers than the brown feathered ones. Higher body weights were recorded for the white feather quails supplemented with 100 mg/kg diet of the natural LZ compared to the CLZ and the higher dose of NLZ (200 mg/kg diet), while all types of LZ showed no significant effect on the growth of brown feathered quail. A similar improved growth in broiler chickens was reported by (Abdel-Latif, et al., 2017; Liu, et al., 2010) and in growing rabbits by (EL-Deep, et al., 2021; EL-Deep, et al., 2020). While our findings disagree with Xia, et al. (2019) who documented that LZ dietary addition from 0 to 200 ppm did not contribute to broiler growth, and only a numerical increase was observed in birds' body gain. The enhanced performance with LZ addition could be associated with their up-regulatory effect on the expression of some assessed growth-regulating genes (*GHR and IGF-I*).

Growth hormone (GH) is an essential controller of growth and body composition, as it regulates the differentiation of muscle cells, adipocytes, and the other cells required for improving development and growth (Kim, 2010). When GH binds to its receptor (GHR), hepatic synthesis and release of IGF-I into the bloodstream are triggered which in turn promote cell development, proliferation, and metabolism in chickens (Kita, et al., 2005; Scanes, 2009). In the same regard, Scanes (2009) found a positive correlation between the blood *IGF-1* concentrations with broiler chicken BW.

According to our findings, supplemental LZ increased the *GHR*

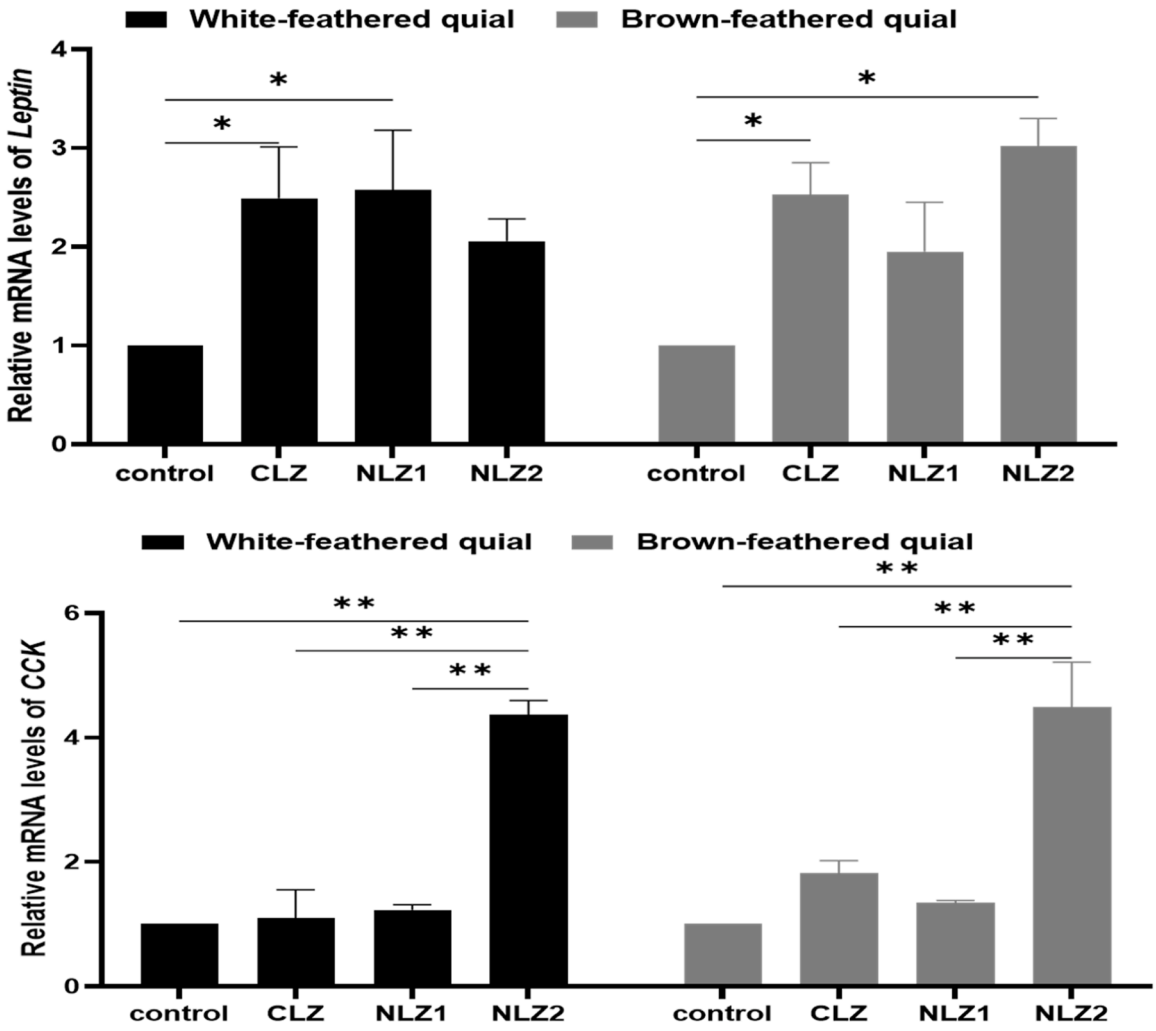

Fig. 5. Relative mRNA expressions of *Leptin* and *CCK* in white- and brown-feathered quails in response to dietary lysozyme supplementation (n = 12 /group). Values are mean ± SEM, statistical significance at $P < 0.05$.

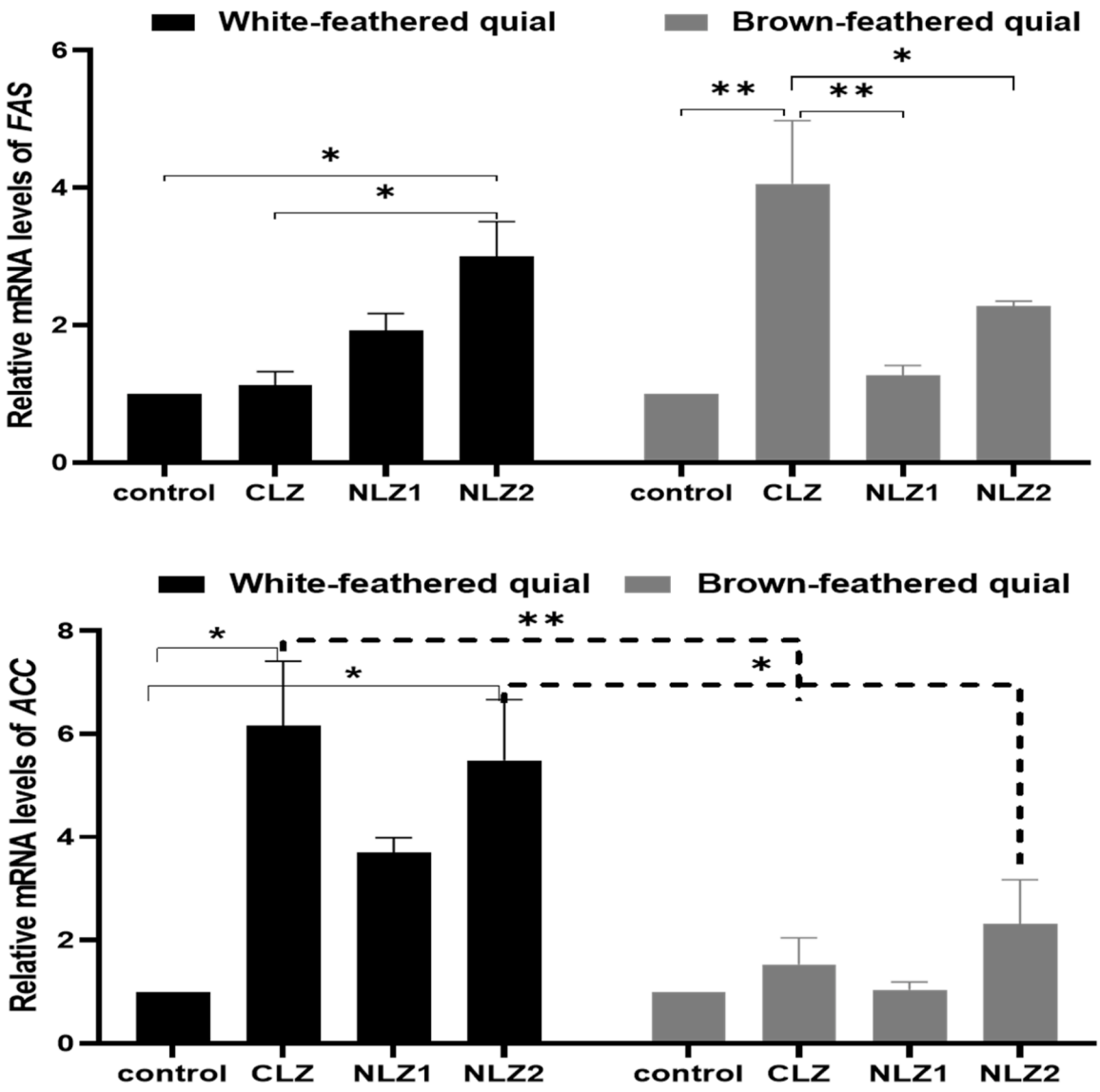

Fig. 6. Relative mRNA expressions of *FAS* and *ACC* in white- and brown-feathered quails in response to dietary lysozyme supplementation ($n = 12$ /group). Values are mean ± SEM, statistical significance at $P < 0.05$.

expression in white feathered quail which was noticeable in those supplemented with the 100 mg/kg diet of natural LZ. This suggests that LZ stimulated the direct binding of GH to its receptor (GHR), which in turn stimulated higher gain by increasing the synthesis and release of *IGF* as evidenced by its higher expression. Besides, the effect of supplemental LZ on the growth-regulatory gene expression (*GHR* and *IGF-I*) appeared clearly in quail with white feathers than the brown feathered ones. The different response between the two studied quail varieties could be connected to the genotype differences which affect their way of expression. In the same regard, Beccavin, et al. (2001) found that genotype has a considerable impact on *IGF-I* expression, which in turn influences the growth rate in broiler chickens. In addition, the decreased growth performance observed with the higher level of NLZ (200 mg/ kg diet) in the case of the white-feathered quail could be linked to the lower *GHR* expression observed in this group. This result could suggest that LZ supplementation at this dose had a negative effect in this variety of quail which confirmed by the slight vacuolation, and degeneration observed within the hepatocytes. Also, it could be associated with an inhibitory effect of this supplementation level on the growth of some beneficial lactic acid bacteria such as *Lactobacillus*. These results are consistent with the findings of (Abdel-Latif, et al., 2017), who recommended 90 g of LZ/ kg diet for the better growth of broilers. Because increasing this level to 120 g LZ/ kg diet induced adverse effects on broiler's growth by reducing the total *Lactobacillus* count and increasing the development and colonization of the harmful organisms, that compete for nutrient with the host (Jin, et al., 1996). Thus, further investigations are recommended to identify how the LZ dietary supplementation differentially alters the bacterial population and gut health among different plumage-color quail varieties.

Dietary LZ supplementation resulted also, in a reduction in FI in both quail varieties. These reductions in FI and the alterations in WG were reflected in FCR which displayed marked enhancement with LZ supplementation. These obtained results are in line with those of (Zaili, et al., 2020) who reported reduction in the average daily FI of egg-producing hens received different levels of LZ (200, 350, and 500 mg/kg) in their diet with the 200 mg LZ/kg decreased the feed to egg ratio by 3.40 % . However, (Abdel-Latif, et al., 2017) found unaffected FI response with LZ supplementation in broilers.

To understand the effect of the experimental factors (quail varieties and LZ dietary addition) on the reported FI response, we assessed the expression level of some peripheral FI-regulatory genes including *Leptin and CCK*. The FI regulatory effect of *leptin* hormone is mediated by

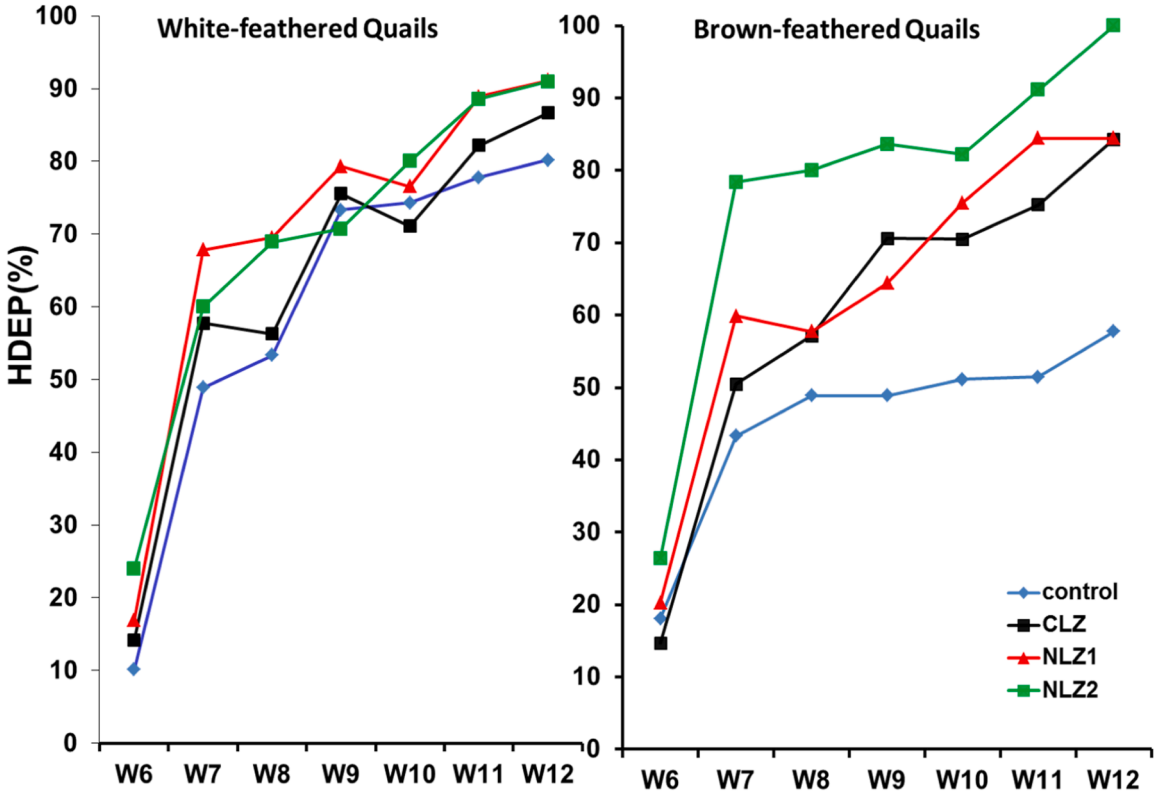

Fig. 7. Egg production curve in the two different varieties of Japanese quail (Brown-feathered & White-feathered) supplemented with lysozyme.

Table 5
Egg production and quality of white- and brown-feathered quails in response to lysozyme supplementation.

Item	White-feathered quail				Brown-feathered quail				P values		
	Control	CLZ	NLZ1	NLZ2	Control	CLZ	NLZ1	NLZ2	Q	LZ	Q*LZ
HDEP	56.37 ± 8.06^{b}	63.40 ± 6.98^{ab}	70.02 ± 6.68^{a}	69.02 ± 6.98^{a}	45.47 ± 3.01^{b}	60.42 ± 2.79^{ab}	63.84 ± 3.34^{a}	77.71 ± 8.22^{a}	0.452	0.004	0.314
EW	11.85 ± 0.63^{ab}	11.77 ± 0.48^{ab}	11.43 ± 0.34^{b}	12.23 ± 0.70^{a}	10.96 ± 0.16^{ab}	10.34 ± 0.50^{b}	11.13 ± 0.22^{ab}	12.73 ± 0.48^{a}	0.133	0.038	0.248
EM	6.68 ± 2.04^{b}	7.42 ± 0.99^{ab}	8.00 ± 0.81^{a}	8.44 ± 0.59^{a}	4.98 ± 0.33^{b}	6.25 ± 0.65^{ab}	7.11 ± 0.50^{a}	9.89 ± 1.37^{a}	0.389	0.006	0.310
H%	61.67 ± 1.67^{b}	75.00 ± 1.20^{ab}	73.33 ± 0.83^{a}	80.00 ± 1.44^{a}	61.67 ± 3.00^{b}	73.33 ± 3.01^{ab}	73.33 ± 1.67^{a}	79.17 ± 5.46^{a}	0.743	<0.001	0.987
EL	3.32 ± 0.06^{a}	3.33 ± 0.04^{a}	3.34 ± 0.05^{a}	3.37 ± 0.05^{a}	3.29 ± 0.02^{b}	3.26 ± 0.04^{b}	3.50 ± 0.06^{a}	3.46 ± 0.04^{a}	0.225	0.005	0.048
EW	2.64 ± 0.03	2.63 ± 0.04	2.67 ± 0.03	2.69 ± 0.02	2.58 ± 0.02	2.65 ± 0.03	2.73 ± 0.02	2.72 ± 0.02	0.542	0.053	0.14
Sh. Th.	221.67 ± 4.41	222.50 ± 3.05	221.67 ± 3.66	217.50 ± 4.79	221.67 ± 4.58	220.83 ± 4.68	224.12 ± 3.79	227.50 ± 3.29	0.350	0.986	0.498
EWL	3.81 ± 0.19^{A}	3.83 ± 0.22^{A}	3.80 ± 0.17^{B}	3.46 ± 0.13^{B}	3.97 ± 0.16^{A}	3.76 ± 0.13^{A}	4.14 ± 0.08^{A}	4.13 ± 0.14^{A}	0.015	0.636	0.133
EWH	4.78 ± 0.29^{b}	5.24 ± 0.22^{a}	5.70 ± 0.53^{a}	4.91 ± 0.26^{ab}	5.07 ± 0.30^{ab}	4.49 ± 0.57^{b}	6.13 ± 0.20^{a}	6.13 ± 0.28^{a}	0.244	0.012	0.061
EWW	3.15 ± 0.13^{Aa}	3.45 ± 0.12^{Aa}	3.38 ± 0.11^{Aa}	3.06 ± 0.11^{Ba}	3.36 ± 0.10^{Aa}	3.40 ± 0.10^{Aa}	3.56 ± 0.10^{Aa}	3.60 ± 0.07^{Aa}	0.004	0.200	0.057
YW	2.40 ± 0.07	2.31 ± 0.09	2.32 ± 0.04	2.50 ± 0.06	2.23 ± 0.03	2.33 ± 0.05	2.45 ± 0.03	2.39 ± 0.06	0.436	0.086	0.046
YH	12.14 ± 0.30	11.82 ± 0.40	12.18 ± 0.32	11.69 ± 0.50	12.00 ± 0.22	11.21 ± 0.68	12.99 ± 0.21	12.50 ± 0.25	0.427	0.063	0.189
YC	5.75 ± 0.45^{A}	5.67 ± 0.41^{A}	6.17 ± 0.40^{A}	5.83 ± 0.32^{A}	5.67 ± 0.40^{A}	4.83 ± 0.21^{A}	4.33 ± 0.22^{B}	4.83 ± 0.17^{B}	<0.001	0.477	0.088
YW	4.15 ± 0.21^{Ab}	4.00 ± 0.12^{Ab}	4.33 ± 0.14^{Bab}	4.42 ± 0.15^{Ba}	4.17 ± 0.21^{Ab}	4.27 ± 0.11^{Ab}	4.80 ± 0.16^{Aa}	4.83 ± 0.17^{Aa}	0.021	0.001	0.399

HDEP= Hen-day egg production; EW= Egg weight; EM= Egg mass; H%= Hatchability %; EL= Egg Length; EW= Egg Width; Sh.Th.= shell thickness; EWL= Egg-white Length; EWH= Egg-white height; EWW= Egg-white width; YW= yolk width; YH= yolk height; YC= yolk color; YW= Yolk weight. CLZ= commercial lysozyme (100mg/kg diet); NLZ1= egg-extracted lysozyme (100mg/kg diet); NLZ2= egg-extracted lysozyme (200mg/kg diet Q represents quail's varieties effect; LZ represents the source of lysozyme. Q*LZ= represents the interaction between quail's varieties source of lysozyme. Results 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 $P < 0.05$.

stimulating the melanocyte-stimulating hormone (α-MSH) expression and inhibiting *AgRP* production (Dridi, et al., 2005) which in turn reduces chicken FI. On the other hand, the *CCK* hormone (polypeptide secreted from the GIT mucosa) regulates FI by connecting to its receptors, CCKAR and CCKBR (El-Kassas, et al., 2016), to trigger the POMC neurons and cause appetite suppression (Fan, et al., 2004) resulted in reduction in the FI. In the present study, both commercial LZ and 100 mg/kg diet of natural LZ dietary in white-feathered quails and

the commercial LZ and 200 mg/kg diet of natural LZ in the brown-feathered ones induced higher *Leptin* expression. In addition, significant upregulations of the *CCK* gene in both quail varieties received the 200 mg/kg diet of natural LZ was measured. As both *Leptin and CCK* work on reducing feed consumption, so, the reported higher expression levels of these genes would explain the reduction of FI obtained in the LZ-supplemented quails. Additionally, the two quail varieties exhibited varied responses indicating that the different plumage colors of quail (white, dark brown, golden, and wild-type) are significantly related to the variations in live weight, FI, FCR, and carcass yield (Inci, et al., 2015).

Serum biochemical constituents are important diagnostic tools, that contribute to assessing the effect of nutritional additives on animal health status (El-Naggar, et al., 2019). In the current study, LZ addition stimulated significant increases in albumen and globulin, with no differences in total protein concentrations in both quail varieties. A similar result of increased globulin levels was obtained by documented in broilers (Abdel-Latif, et al., 2017), and rabbits (EL-Deep, et al., 2021) fed LZ in their diet revealing its immune stimulatory role.

Liver function-related enzymes including ALT and AST are routinely used as diagnostic indicators for animal hepatic injury. The non-significant alterations in the activity of these enzymes in response to dietary LZ supplementation in both quails suggest that LZ has no negative impacts on the liver function. This finding was confirmed by the normal hepatic parenchyma's architecture. Similar results were obtained with (Abdelazeem, et al., 2023; EL-Deep, et al., 2020; Szymonowicz, 2008), who reported unchanged serum levels of these enzymes in growing rabbits. These reported hepatic protecting effects of LZ could be linked to its antioxidant activity, which can stimulate higher expression levels of antioxidants (*SOD* and *GPX*) genes in the liver. The antioxidants can defend against free radicals, reduce their toxicity, and potentially protect the liver health (EL-Deep, et al., 2021). Regarding the kidney function-related parameters; creatinine and urea, lower levels of these serum indices were obtained in the LZ-treated quails from the two varieties. The obtained results suggested that the used LZ levels exerted a healthful effect on the liver and kidney which help in maintaining their function. A similar response was obtained by (EL-Deep, et al., 2021; EL-Deep, et al., 2020).

Moreover, no significant differences were found in serum lipid profile constituents in LZ-supplemented quails, which is consistent with (Abdel-Latif, et al., 2017), who found no changes in the serum levels of triglyceride and total cholesterol of broiler chickens fed on different levels of LZ (ranged from 0 to 20g / ton of the basal diet. Also, (EL-Deep, et al., 2021) found no alteration of triglyceride and total cholesterol in LZ-fed rabbits. Unlike, (Ibne Khalil, et al., 2020) found higher levels of total cholesterol along with lower triglyceride and LDL levels in the LZ-supplemented broilers. Differences between trials could be associated with the differences in experimental designs including the supplemental levels of LZ and the species under the study.

To deeply illustrate the influence of LZ dietary supplementation on fat metabolism, some of the fat metabolism-related genes including *FAS* and *ACC* were investigated. The FAS and ACC are essential enzymes in lipogenesis (Huang, et al., 2008). ACC enzyme is involved in the conversion of acetyl-CoA into malonyl-CoA and then into palmitate; and this pathway is mediated by FAS and results in TG synthesis (Richards, 2003). The natural LZ supplementation stimulated higher expression of hepatic *FAS* in a dose-dependent manner, with the highest mRNA copies of *FAS* were measured in the case of commercial LZ followed by the higher dose of natural LZ (200 mg/kg diet) in brown-feathered quails. Similar response was reported for the *ACC* gene expression, which was clear with supplemental commercial LZ or at 200 mg/kg diet of natural LZ in white quail to induce a higher *ACC* expression. The upregulating response was more pronounced in the white-feathered quails compared to brown-feathered ones. Although LZ supplementation promoted the expression of these fat metabolism-regulating genes, it did not induce significant differences in the serum lipid profile parameters. The obtained results suggest that LZ particularly the commercial one and the highest dose of the natural LZ (200 mg/kg diet) has an important stimulatory effect on enzymes involved in lipogenesis, and this response was more prominent in the white-feathered quails than those of brown-feathered ones which showed only slight numerical increase in serum triglyceride. The reported increases in the lipid indicators in the white-feathered quails in comparison to brown-feathered quails, may be explained by the higher affinity of this variety to deposit more fat (Inci, et al., 2015; Nasr, et al., 2017). Exploring the expression levels of other lipolysis- and lipogenesis-related genes is advised to comprehend the underlying theory of the regulatory action of LZ supplementation on lipid metabolism in the different quail varieties.

As little information on the influence of LZ supplementation on quail's laying performance is available, so the experimental design was continued during the laying period. Dietary LZ supplementation increased the HDEP, egg weight, egg mass, and hatchability percentages in both quail varieties, especially those who received the natural LZ groups compared to the CLZ or birds fed on the LZ-free diet. Unlike these results, (Sindaye, et al., 2023) found that LZ supplementation did not affect laying rate, egg mass, and egg weight in laying chicken hens. Variation between trials could be related to species difference, LZ method of production, and basal diet used in each study. In both quails, the highest laying performance indices were reported in the two studied quail varieties that received the highest dose of natural LZ (200 mg/kg diet). These findings come in agreement with the previous trials that recommended 200 to 300 mg LZ/kg in the laying hens' diets (Sindaye, et al., 2023; Zaili, et al., 2020). Moreover, higher egg length and yolk weights were recorded in quails received the natural LZ compared with CLZ and control birds. Though, no significant differences in egg width, egg-white length, and width and shell thickness with LZ supplementation were found. Similarly, (Zaili, et al., 2020) reported no significant effect on the egg quality of laying hens fed different levels of LZ (200, 350, and 500 mg/kg) in their diet. Also, (Sindaye, et al., 2023) found that LZ addition (100, 200, 300, and 400 mg/kg) didn't affect the egg quality traits. Besides, the quail variety did not impact all assessed egg quality parameters (egg width, egg-white height, yolk width, and shell thickness) except egg white length and width, and egg yolk weight which were improved in the quail with brown feather compared to the white-feathered quails. In the same aspect, Al-Kafajy, et al. (2018) reported that brown feathered quail presented better albumen quality (in terms of albumen height) than the white and black lines of quails. Also, Kirrella, et al. (2023) found that egg quality criteria were not influenced by quail variety except for the relative weight of eggshell and yolk, and yolk index, which were all different in quail with white feather compared to the brown-feathered ones. On the other hand, Bagh, et al. (2016) reported no difference in external and internal egg quality among different quail varieties (brown, white, and gray). Yılmaz, et al. (2011) concluded that variation in external and internal quality characteristics of eggs from quails of different plumage color lines results from the different genetic background of these quail lines. Again, the dissimilarity in laying performance results between studies could be linked to the differences in the experimental design used in each study and the bird growing performance which in turn influenced their performance during the laying period.

Conclusion

Considering the former results, it could be concluded that lysozyme supplementation in quail diet either commercial or egg extracted boosted their growth performance through upregulating the growth, feed intake, and fat metabolism-related genes, and maintaining the serum biochemistry, and the liver and kidney function. A dose rate of 100 mg lysozyme/kg diet of either the commercial or egg-extracted lysozyme is recommended for quail during the growing period. During the laying period, lysozyme supplementation particularly the egg-extracted LZ enhanced both quail laying performance, hatchability,

and some egg quality parameters, with the 200 mg lysozyme/kg diet recommended level during this period.

Declaration of competing interest

There were no conflict of interests.

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