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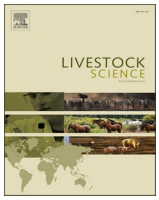

Dietary influence of de-oiled lecithin on broiler growth, Blood parameters, Antioxidant status, Thyroid hormones and immunoglobulins: An analysis across two distinct strains of broilers

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HIGHLIGHTS

- *Improved nutrient absorption*: lecithin contains phospholipids, which can enhance the digestibility and absorption of dietary fats. This can potentially improve the overall energy efficiency of the diet and promote better growth performance in broilers.
- *Enhanced immune function*: lecithin can modulate the immune response, potentially improving the health status of broilers and reducing the incidence of diseases and infections.
- *Alternative to antibiotic growth promoters*: as the poultry industry moves away from the use of antibiotics for growth promotion due to concerns about antibiotic resistance, lecithin could serve as a valuable alternative, offering growth-promoting effects without the same risks.

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ABSTRACT

The objective of this study was to examine the impact of adding an emulsifier, de-oiled soybean lecithin (DOL) in diet, on growth performance, blood parameters, antioxidant potential, thyroid hormones (T_3 & T_4), and serum immunoglobulins in two distinct strains of commercially raised broilers, Ross 308 (S1) and Evian 48 (S2). A total of 800 one-day-old broiler chicks from both strains ($N = 400$ each) were assigned to a control group (T0) and three dietary treatments, each with five replicates of 20 birds. Experimental treatments included a basal diet supplemented with varying levels of de-oiled lecithin (DOL): T1 = 0.5 g/kg, T2 = 1 g/kg, and T3 = 1.5 g/kg. The study used a 2 × 4 factorial design, which allows for the examination of the effects of two factors (in this case, strains and dietary treatments) on the outcome variables. Following 35 days, the broilers of strain S1 and those fed the T3 supplemented diet exhibited superior body weight (BW), body weight gain (BWG), cumulative feed intake (CFI), cumulative feed conversion ratio (CFCR), performance index (PI%), and lower mortality rate (Mr %) ($P < 0.05$) compared to those of strain S2 and those fed other dietary levels. A significant disparity ($P < 0.05$) was observed between the two strains regarding the plasma concentrations of ALB, GLB, A/G ratio, CHO, HDL, AST, and ALT, with strain S1 showing better levels. Similarly, the broiler chicks in group T3, along with the interaction between groups S1 × T3 and S2 × T3, demonstrated a significant ($P < 0.05$) improvement in blood biochemical parameters. Broilers of strain S1 and those on the T3 supplemented diet, along with the interactions between the groups S1 × T3 and S2 × T3, demonstrated substantial improvements ($P < 0.05$) across assessed parameters of antioxidant status and thyroid hormones, when compared to other dietary treatments and the control group. In conclusion, the addition of DOL as a supplement improved growth performance, blood parameters, antioxidant capacity, thyroid hormones (T_3 & T_4), and serum immunoglobulins in both broiler strains.

Abbreviations: S1, ross 308 strain; S2, evian 48 strain; DOL, de-oiled lecithin; T0, control group (basal diet); T1, basal diet + 0.5g/kg DOL; T2, basal diet+1g/kg DOL; T3, basal diet +1.5g/kg DOL; ALB, albumin; GLB, globulin, A/G, albumin/ globulin ratio; CHO, cholesterol; HDL, high density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T_3, triiodo thyronine; T_4, thyroxine.

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1. Introduction

Broilers are commercially produced in short periods and require a lot of energy. In order to meet their nutritional needs, lipids must be added to their diet. It is generally established that adding fats and oils to an animal's diet may enhance productive performance and help meet the demands of the poultry sector (Blanch et al., 1995; Liu et al., 2020). Presently, broiler diets frequently use a variety of fat sources, including vegetable oils and animal tallow (Poorghasemi et al., 2013; Schiavone et al., 2017, 2018). In order for young animals to reach their maximum growth potential, it is crucial for lipid digestion and absorption to occur (Sun et al., 2019). Generally, young animals produce and secrete less bile salt and lipase, which results in inadequate digestion and absorption of fat (Sun et al., 2019; Tancharoenrat et al., 2014). The metabolism of a meal is influenced by the chemical and physical characteristics of lipids, particularly the saturation level and length of fatty acids, which determine the amount of metabolizable energy that can be utilized (Smink et al., 2010). Several factors such as the age of the animal, genetics, the activity of lipase, the status of microbiome, and the composition of meals were found to have an impact on the digestion of lipids (Tancharoenrat et al., 2013; 2014; Zampiga et al., 2016). Thus, including an emulsifying agent in the diet of fast-growing poultry can potentially address the challenges in effectively using and absorbing fat.

Lecithin is a type of fatty substance that can be found naturally in several food sources, including soybean, whole grains, egg yolk, milk and marine (Sun et al., 2019; Liu et al., 2020). In 1845, a French scientist discovered lecithin, which is widely regarded as a superb emulsifying agent (Gobley, 1846). Soy-lecithin, which is produced during the manufacturing of soybean oil, is primarily composed of phosphatides. These include 8–20 % phosphatidyl ethanolamine, 19–21 % phosphatidylcholine, and 20 to 21 % inositol phosphatides. Additionally, it contains other types of phosphatides, which make up 5–11 % of its composition (Scholfield, 1981). Lecithin has numerous advantages, one of which is enhancing the consumption of fat in animals by combining phospholipid molecules, emulsifying dietary fat, and supplying them with energy (Kim et al., 2008; Rovers and Excentials, 2014; Xing et al., 2004). Earlier trials that incorporated lecithin as an emulsifying agent demonstrated beneficial outcomes in terms of animals' nutritional absorption and digestion (Smulders, 2008; Xing et al., 2004). Meanwhile, Lecithin added to broiler diets improves the development of those birds by enhancing the utilization of energy and nutrients (Chen et al., 2019; Zhao and Kim, 2017). In their study, Attia et al. (2018) found that adding either 1 % or 1.5 % soya lecithin to rabbit feed led to enhanced growth performance and improved fat digestibility during both summer and winter seasons. To obtain high-purity lecithin products, it is necessary to de-oiled raw lecithin (Wu and Wang, 2003). De-oiled lecithin (DOL), is a combination of phospholipids that are both hydrophilic and hydrophobic. It possesses effective emulsification capabilities. Crude soybean lecithin undergoes industrial processes like filtration, hydrogen peroxide bleaching, and acetone solvent extraction to produce a more concentrated form of lecithin (DOL) Its similar in composition to crude soybean lecithin but is more efficient in emulsification (Liu et al., 2020). Numerous studies have been conducted to explore the impact of various emulsifier agents on the growth performance and physiological response of broiler chickens through their feed. However, there is limited information available on the use of DOL supplement in broilers. The study of Jansen et al. (2015) revealed that including lysolecithin in the basic diet along with saturated fatty acid (SFA) source such as pig lard improved the nitrogen retention and apparent metabolizable energy (ME) of the feed for broiler chickens in their growing phase. Emulsifiers are believed to enhance the absorption of nutrients to promote optimal growth and productivity by encouraging the growth of epithelial cells in the small intestine and safeguarding the surface of villi from harm (Boontiam et al., 2017). A study conducted on broilers Zhao and Kim (2017) revealed that the levels of triglycerides and total cholesterol were reduced in birds that were fed diets containing an emulsifier (lipidol sourced from soybean lecithin) on day 14 of their age. However, the difference in these levels was comparatively lower on day 39 of their age. The protective impact of emulsifiers against oxidative stress was validated by improvements in the broilers' blood antioxidant status (Zangeneh et al., 2020). According to hypotheses, DOL could enhance broilers' performance by acting as an exogenous emulsifier. The primary aim of this research was to assess the effect of DOL on the productive performance, blood parameters, antioxidant potential, thyroid hormones (T_3 & T_4), and serum immunoglobins, in two distinct commercially raised broiler chicken strains.

2. Materials and methods

2.1. Ethics pertaining to animals

The Institutional Animal Care and Use Committee (IACUC) of the Faculty of Agriculture at Benha University, Egypt approved the animal husbandry and experimental procedures. The experimental birds were treated humanely and all possible measures were taken to reduce any discomfort to them.

2.2. De-oiled lecithin source

This research used BergaPur™ from Berg+Schmidt in Singapore. De-oiled lecithin (DOL) has purity levels of 95 % and is made from soy beans. The production process involves extracting and refining phospholipids from natural liquid lecithin, resulting in a dry powder. The composition of BergaPur™ consists of 84 % phospholipid complex, 11 % lysophospholipid, 3.5 % triglycerides, and 1.5 % water.

2.3. Animal management and experimental design

In a feeding experiment that lasted for 35 days, a total of 800 (1-day-old) unsexed broiler chicks obtained from two commercial strains (Ross 308 and Evian 48 strains, $N = 400$ chicks per each) were used. At the start of the experiment, the chicks had an average weight of (43.07 and 41.21 ± 0.1 g), respectively. The chicks from different strains were randomly divided into four groups. These groups included a control group (T0) fed a basal diet ($n = 100$ chicks) and three experimental groups with varying levels of DOL supplements in their basal diet (T1 = 0.5, T2 = 1 and T3 = 1.5 g/kg). Each experimental group had five replicates (20 birds in each). The birds were kept in experimental pens located in the same geographical area, where the environmental and sanitary conditions were carefully regulated. In the study, all pens were situated in a same location. Initially, electric lighting was provided continuously for the first five days. Then, for the subsequent 30 days, the lighting schedule consisted of 23 h of light and one hour of darkness. The air temperature was maintained at 33 °C, with a relative humidity of 63% three days prior to placing the chicks in the pens. During the first week of the experiment, the temperature remained at 33 °C. Subsequently, the temperature was gradually reduced by 2–3 °C each week until reaching a final range of 20–22 °C. The chicks in the study were immunized against diseases such as Newcastle, Gumboro disease, Pullorum, and Avian influenza. The vaccination program was implemented following guidelines and protocols under the supervision of a licensed veterinarian. Table 1 displays the nutrient composition of the experimental diets used during various phases, which has been calculated and analyzed. The birds were fed a diet in the form of mash and was carefully formulated to fulfill all their nutrient needs and even surpass them slightly (NRC, 1994). Diets and water were available *ad libitum* throughout the experimental period.

2.4. Performing measurements and sampling

Every week, the chicks in each group of the experiment were weighed individually to determine their body weight (BW) and body

Table 1
The composition of ingredients in the experimental diets.

Ingredients,%	Starter (days 0–21)	Gower (days 22–35)
Yellow Corn	54.6	60.4
Soybean meal	35.2	30.2
Corn gluten meal	3.18	2.0
Soybean oil	2.65	3.52
Calcium hydrogen phosphate	2.0	1.65
Limestone	1.25	1.25
NaCl	0.35	0.35
Mineral premix [1]	0.2	0.2
Vitamin premix [2]	0.03	0.025
Aureomycin	0.03	0.03
Choline chloride (50%)	0.26	0.2
L-lysine (78%)	0.08	0.075
Methionine	0.17	0.1
Total	100	100
Energy and nutrient composition		
Calculated values*		
ME (kcal/kg)	2950	3050
Crude protein (%)	22.28	19.78
Crude fat (%)	5.40	6.30
Ca (%)	1.17	1.05
Available phosphorus (%)	0.59	0.51
Lys (%)	1.18	1.04
Met (%)	0.50	0.40
Analyzed values		
Crude protein (%)	21.51	19.23
Crude fat (%)	5.23	6.16
Ca (%)	1	0.91
Available phosphorus (%)	0.46	0.4
Lys (%)	1.15	1.01
Met (%)	0.50	0.40

[1] The mineral premix included the following amounts (mg/kg): Fe, 80; Mn, 100; Cu, 8; Zn, 75; Se, 0.15; I, 0.35.
[2] This diet contains a vitamin premix that offers the following per kilogram: 12,500 IU of vitamin A, 2500 IU of vitamin D3, 30 IU of vitamin E, 2.65 mg of vitamin K3, 2 mg of vitamin B1, 6 mg of vitamin B2, 50 mg of vitamin B3, 12 mg of vitamin B5, 0.0325 mg of vitamin B7, 1.25 mg of vitamin B9, and 0.025 mg of vitamin B12. *The calculations are based on the feedstuff values outlined in NRC (1994).

weight gain (BWG) were assessed using the method outlined by (Broody, 1949). Additionally, feed intake (FI) was recorded twice a week to calculate the feed conversion efficiency ratio (FCR) in grams of feed per gram of gain. The mortality rate was also observed and recorded throughout the experiment, and the number of live birds was subtracted from the original number at the end of the experiment to calculate the mortality rate. The performance index (PI) was determined following the below listed equation proposed by (North and Bell, 1984).

$$\mathbf{PI} = \frac{\textbf{Live body weight (kg)}}{\textbf{Feed conversion ratio}} \times 100$$

2.5. Blood analysis

Once the experiment was completed (35 days), blood samples were collected from the wing vein of chicks (10 samples per replica). Samples were placed into hygienic, heparinized tubes and tubes with a serum separator for the purpose of analyzing serum hormones and immunoglobulins. The blood samples underwent centrifugation at 3500 rpm for 10 min at 4 °C, and the resulting plasma was preserved in a deep freezer at approximately -20 °C until it was ready for chemical analysis. Commercial kits based on a method developed by Gornall et al. (1949) were utilized to measure plasma protein fractions including total protein (TP), albumin (AL), globulin (GL), and albumin/globulin(A/G) ratio. Additionally, the total lipid profile including total cholesterol (Ch), triglycerides (TRI), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) were measured. Liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as kidney function tests including creatinine (Cr) and uric acid (UA), were also measured using a colorimetric approach (Sirois, 2014).

2.6. Assessment of antioxidant activity

Using a commercial kit (Randox, UK) based on a method developed by Miller et al. (1993) to examined the total anti-oxidant (T-AOC) concentration of plasma. Using easily available GPx kits (Randox, Crumlin, UK) and adhering to the manufacturer's instructions, blood glutathione peroxidase activity (GPx, EC 1.11.1.9) was assessed using the method outlined by Paglia and Valentine (1967). Using kits from Randox Laboratories Ltd. (Crumlin, UK), we evaluated the superoxide dismutase (EC 1.1.5.1; SOD) activity in erythrocyte lysates by following the guidelines in (Woolliams et al., 1983). Malondialdehyde (MDA) levels in plasma were determined using a modified fluorometric approach, according to Jo and Ahn (1998).

2.7. Assessment of triiodothyronine (T_3) and thyroxine (T_4) hormones

The quantities of T_3 and T_4 in serum samples were determined by using radioimmunoassay (RIA) kits adhering to the manufacturer's instructions, as outlined in the methods described by Renden et al. (1994).

2.8. Assessment of serum immunoglobulins

The levels of immunoglobulin M (IgM), immunoglobulin E (IgE), immunoglobulin A (IgA) and immunoglobulin G (IgG) in the serum were determined by employing the analysis method described by El Basuini et al. (2016). The kits supplied by (Alpha Diagnostic international) were utilized and the readings were obtained using an ELISA reader as per the manufacturer's guidelines.

2.9. Statistics

The General Linear Models (GLM) approach of two-way ANOVA (SAS, 2004) was used to analyze the data. A factorial design (2 × 3) was employed to evaluate the interaction between the components of the main factors, which included strains and dietary de-oiled lecithin levels. In instances where significant differences between treatments were observed, Duncan's multiple range test was employed to analyze them (Duncan, 1955). The provided linear model is as follows:

$$\mathbf{Y_{ijk} = \mu + ST_i + L_j + (STL)_{ij} + e_{ijk}}$$

"Y_{ijk}"= the k^{th} observation; μ= overall mean; "ST_i" effect of the i^{th} strains (ROSS 308 and Evian 48); "L_j" effect of the j^{th} de-oiled lecithin treatment levels (0. 0.5. 1. 1.5 g/kg diet); "$(STL)_{ij}$" the interaction between i^{th} strains and j^{th} lecithin treatment. "e_{ijk}"= the experimental error, accordingly zero mean and variance = $\sigma^2 e$. Unless otherwise noted, statistical significance was determined using a P value of 0.05.

3. Results

3.1. Growth performance

The mean results (BW, BWG, CFI, FCR, and PI) and mortality rate (Mr) for broiler chicks reared to 35 days of age are shown in Table 2. A statistically significant difference was found between all performance parameters or mortality observed due to strain over the whole period ($P < 0.05$). All growth performance traits and mortality over the study were strongly impacted ($P < 0.001$) by dietary DOL compared to those in T0, despite its lack of significant variations on initial body weight. A significant interaction ($P < 0.05$) was observed between strain (ST) and dietary de-oiled lecithin (DOL) levels on growth performance and mortality. The interactions between S1 × T3 and S2 × T3 improved ($P < 0.05$) growth performance more than those between the various

Table 2
Effect of strain, DOL, and their interactions on the growth performance of broiler chickens.

Items		BW 0	BW 0–35d	BWG 0–35d	CFI 0–35d	CFCR 0–35d	PI 0–35d	Mr 0–35d
Strain (S)	S1	43.07^{a}	2408.36^{a}	2365.29^{a}	3582.84^{a}	1.52^{b}	157.96^{a}	3.416a
	S2	41.21^{b}	2143.82^{b}	2102.60^{b}	3461.84^{b}	1.64^{a}	131.04^{b}	1.250^{b}
	SEM	0.159	10.499	15.628	12.350	0.0087	1.477	0.062
De-oiled lecithin (DOL) g/kg diet	T0	42.34	2175.94^{c}	2133.59^{c}	3340.89^{c}	1.57^{b}	136.31^{b}	4.33^{a}
	T1	41.93	2243.09^{b}	2201.15^{b}	3620.14^{a}	1.62^{a}	137.70^{b}	3.00^{b}
	T2	42.06	2217.25^{b}	2175.18^{bc}	3579.45^{ab}	1.64^{a}	134.88^{b}	1.50^{c}
	T3	42.21	2468.08^{a}	2425.86^{a}	3548.89^{b}	1.46^{c}	169.09^{a}	0.50^{d}
	SEM	0.222	14.623	19.769	15.622	0.0110	1.868	0.078
S × DOL	S1 × T0	42.24^{c}	2251.48^{c}	2209.24^{c}	3245.78^{d}	1.48^{f}	146.83^{b}	6.66^{a}
	S1 × T1	43.49^{a}	2345.00^{b}	2301.50^{b}	3712.20^{a}	1.61^{cd}	145.38^{b}	6.00^{b}
	S1 × T2	43.19^{ab}	2353.75^{b}	2310.55^{b}	3684.25^{a}	1.59^{d}	147.61^{b}	1.00^{d}
	S1 × T3	43.34^{ab}	2683.21^{a}	2639.86^{a}	3689.13^{a}	1.39 g	192.00^{a}	0.00^{e}
	S2 × T0	42.45^{c}	2100.40^{c}	2057.94^{d}	3435.99^{c}	1.66^{ab}	125.80^{b}	2.00^{c}
	S2 × T1	40.37^{d}	2141.18^{d}	2100.81^{d}	3528.08^{b}	1.64^{bc}	130.01^{b}	0.00^{e}
	S2 × T2	40.93^{d}	2080.75^{d}	2039.81^{d}	3474.66^{bc}	1.70^{a}	122.15^{b}	2.00^{c}
	S2 × T3	41.90^{b}	2252.94^{d}	2211.85^{c}	3408.65^{c}	1.54^{e}	146.19^{b}	1.00^{d}
	SEM	0.314	20.678	27.957	22.093	0.0220	2.462	0.111
Two-way ANOVA P- Value	S	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	DOL	0.6185	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	S × DOL	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001	0.0002

The data is the mean value derived from 5 replications.
Means within the same column of each factor and interactions that have differing letters represent a significant difference ($P < 0.05$).
Abbreviations: Abbreviations: S1: Ross 308 strain, S2: Evian 48 strain, DOL: de-oiled lecithin, T0: control group (basal diet), T1: basal diet + 0.5 g/kg DOL T2: basal diet+1 g/kg DOL, T3: basal diet +1.5 g/kg DOL, SEM = standard error mean. BW: body weight (g), BWG: body weight gain (BW at 35d – BW at 0 day), CFI: cumulative feed intake (g feed/ bird), CFCR: cumulative feed conversion ratio (g feed/g gain), PI (%): performance index, Mr (%): mortality rate.

interactions applied. The mortality rate in the interaction of S1 × T3 and S2 × T1 decreased compared with other groups ($P < 0.05$).

3.2. Blood analysis

The findings from Table 3 present the results of plasma biochemical parameters. At 35 days old, there were no significant differences ($P > 0.05$) in the plasma levels of TP, TRIG, LDL Cr, and UA between the birds from S1 and S2. However, notable diversity ($P < 0.05$) was observed between the two strains in terms of the plasma concentrations of ALB, GLO, A/G ratio, CHO, HDL, AST, and ALT. Interestingly, the addition of DOL to the diet did not lead to any changes ($P > 0.05$) in the levels of ALB in the blood plasma. The broilers in group T3, which received a diet supplemented with DOL at a concentration of 1.5 g/kg, experienced

Table 3
Effect of strain, DOL, and their interactions on the blood biochemical analysis of broilers chickens.

Items		TP g/dl	ALB g/dl	GLO g/dl	A/G g/dl	CHO mg/dl	TRIG mg/dl	LDL mg/dl	HDL mg/dl	AST g/dl	ALT g/dl	Cr g/dl	UA g/dl
Strain (S)	S1	8.68	5.94^{a}	3.74^{b}	1.58^{a}	157.82^{b}	115.06	70.02	102.60^{b}	31.99^{b}	14.06^{b}	19.44	2.77
	S2	8.42	5.48^{b}	3.94^{a}	1.39^{b}	188.49^{a}	116.23	71.19	103.77^{a}	32.68^{a}	14.74^{a}	19.76	2.83
	SEM	0.117	0.116	0.018	0.023	1.554	1.192	0.656	0.301	0.168	0.140	0.115	0.071
De-oiled lecithin (DOL) g/kg diet	T0	7.78^{d}	5.40	3.38^{b}	1.59^{c}	200.63^{a}	131.93^{a}	75.93^{a}	91.23^{c}	37.07^{a}	17.00^{a}	21.28^{a}	2.98^{a}
	T1	8.46^{c}	5.67	3.79^{a}	1.49^{d}	170.35^{b}	113.72^{b}	68.86^{b}	98.45^{b}	31.34^{b}	14.11^{b}	19.37^{b}	2.79^{b}
	T2	8.86^{b}	5.84	3.02^{d}	1.93^{a}	168.71^{b}	113.44^{b}	68.90^{b}	97.91^{b}	31.30^{b}	14.09^{b}	19.34^{b}	2.76^{b}
	T3	9.12^{a}	5.96	3.16^{c}	1.88^{b}	152.93^{c}	103.47^{c}	68.73^{b}	125.13^{a}	29.61^{c}	12.42^{c}	18.42^{c}	2.66^{c}
	SEM	0.166	0.166	0.258	0.034	2.198	1.685	0.928	0.425	0.237	0.198	0.162	0.100
S × DOL	S1 × T0	7.91^{f}	5.63^{f}	3.28^{h}	1.71^{c}	185.05^{bc}	113.35^{a}	75.35^{a}	90.65^{d}	36.73^{a}	16.66^{a}	21.12^{a}	2.95
	S1 × T1	8.59^{d}	5.90^{c}	3.69^{c}	1.59^{e}	159.35^{d}	116.75^{bc}	73.15^{a}	91.15^{cd}	31.58^{bc}	14.27^{bc}	19.54^{bc}	2.79
	S1 × T2	8.99^{b}	6.07^{b}	3.92^{a}	1.54^{f}	147.05^{e}	109.25^{de}	68.15^{bc}	104.05^{b}	30.38^{d}e	13.26^{de}	18.86^{de}	2.71
	S1 × T3	9.25^{a}	6.19^{a}	3.06 g	1.69^{d}	139.85^{e}	102.25^{e}	63.45^{b}	124.05^{a}	29.27^{ef}	12.08^{f}	18.26^{e}	2.63
	S2 × T0	7.65 g	5.17^{h}	3.48^{d}	1.48 g	216.22^{a}	132.52^{a}	76.52^{a}	91.82^{cd}	37.42^{a}	17.34^{a}	21.44^{a}	3.01
	S2 × T1	8.33^{e}	5.44 g	3.89^{b}	1.39^{h}	193.65^{b}	118.19^{b}	74.28^{a}	92.86^{c}	32.30^{b}	14.97^{b}	19.88^{b}	2.87
	S2 × T2	8.73^{c}	5.61^{e}	3.12^{f}	1.79^{a}	178.08^{c}	110.14^{cd}	69.32^{b}	104.67^{b}	31.03^{cd}	13.91^{cd}	19.15^{cd}	2.74
	S2 × T3	8.99^{b}	5.73^{d}	3.26^{e}	1.75^{b}	166.02^{d}	104.06^{de}	64.65^{cd}	125.72^{a}	29.96^{f}	12.76^{ef}	18.58^{de}	2.69
	SEM	0.240	0.229	0.037	0.047	3.109	2.384	1.312	0.602	0.336	0.280	0.230	0.142
Two-way ANOVA P- Value	S	0.1167	0.0058	0.0001	0.0001	0.0001	0.4884	0.2087	0.0065	0.004	0.007	0.060	0.551
	DOL	0.0001	0.0892	0.0001	0.0024	0.0001	0.0001	0.0001	0.0001	0.001	0.001	0.001	0.057
	S × DOL	0.0156	0.0001	0.0001	0.0001	0.0001	0.0153	0.0001	0.0001	0.004	0.003	0.025	0.290

The data is the mean value derived from 5 replications (10 samples per each).
Means within the same column of each factor and interactions that have differing letters represent a significant difference ($P < 0.05$).
Abbreviations: Abbreviations: S1: Ross 308 strain, S2: Evian 48 strain, DOL: de-oiled lecithin, T0: control group (basal diet), T1: basal diet + 0.5 g/kg DOL T2: basal diet+1 g/kg DOL, T3: basal diet +1.5 g/kg DOL, SEM = standard error mean. TP: total protein, ALBU: albumin, GLO: globulin, A/G: albumin/globulin ratio, CHO: cholesterol, TRIG: triglycerides, LDL: low density lipoprotein, HDL: high density lipoprotein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, Cr: Creatinine, UA: uric acid.

notable increases in plasma levels of TP, ALB, and HDL. Conversely, they exhibited decreased levels of CHO, TRIG, LDL, AST, ALT, Cr, and UA compared to the broilers in groups T2, T1, and T0. Simultaneously, broiler chicks in the T1 and T2 groups showed raised plasma levels of ALB and the A/G ratio ($P < 0.05$), respectively. There were no significant ($P>0.05$) changes observed in plasma levels of UA when comparing different interactions. However, broiler chicks in the S1 × T3 and S2 × T3 interactions exhibited higher plasma levels of TP, ALB, GLO, A/G ratio, and HDL. Additionally, they showed decreased plasma levels of CHO, TRIG, LDL, AST, ALT, Cr, and UA compared to the other interactions studied ($P < 0.05$).

3.3. Antioxidants and thyroid hormones

As indicated in Table 4 there were notable differences ($P < 0.05$) observed among broiler strains concerning antioxidants status, and thyroid hormones. The serum levels of TAO, GPX, SOD, MDA, T_3, and T_4 of broiler chicks derived from S1 were significantly higher than those derived from S2. Based on the findings, broiler chicks in the T3 group showed significant improvements ($P < 0.05$) in all studied parameters of antioxidant status and thyroid hormones compared to the other treatments and control group. The combination of broiler strain and dietary DOL treatment resulted in the best ($P < 0.05$) values of antioxidant status and thyroid hormones, as indicated by the statistical interaction analysis. Broiler chicks from both strains that were fed the T3 diet exhibited the highest levels of TAO, GPX, SOD, T3, and T4. Additionally, they had the lowest levels of MDA compared to the other interactions that were examined.

3.3. Serum immunoglobulins

According to the data depicted in Fig. 1, the serum immunoglobulin levels are notably influenced by the strain, levels of DOL supplementation, and their interactions. The levels of immunoglobulins (IgM, IgE, IgA, and IgG) were noticeably higher ($P < 0.05$) in the broiler chicks of S1 compared to those in S2. The T1 group exhibited a significantly elevated level of IgM compared to the control group ($P < 0.05$). Conversely, the serum levels of IgE, IgA, and IgG were significantly higher in the T3 group compared to the control group ($P < 0.05$). As a result of the interactions, there was a significant increase in serum IgM levels observed in the S1 × T3 and S2 × T1 groups, respectively. Additionally, the serum levels of IgE, IgA ($P > 0.05$), and IgG were found to be increased in both the S1 × T3 and S2 × T3 groups ($P < 0.05$).

4. Discussion

This research focused on the performance response of the broilers, exploring the impact of dietary DOL supplementation on different broiler strains and their interactions. The results implied that the inclusion of DOL in the feed could potentially boost ($P < 0.05$) growth performance. The body weight (BW), body weight gain (BWG), cumulative feed intake (CFI), cumulative feed conversion ratio (CFCR), performance index (PI), and mortality rate (Mr %) showed improvement in S1 chicks that received basal diets supplemented with DOL, with a notable enhancement observed at 1.5 g/kg of DOL. Commercial broilers have substantial energy needs, yet the source of fat is relatively constrained in a conventional diet. According to reports, adding emulsifiers to feed can enhance performance, particularly in the early stages of growth (Khonyoung et al., 2015; Polycarpo et al., 2016; Zhao and Kim, 2017). This is likely because young birds have limited ability to digest and absorb fat due to low levels of colipase-dependent lipase and bile acids in their digestive system (Ghasemi et al., 2016). Due to its emulsifying effect, DOL facilitates the combination of nutrients and digestive enzymes, leading to improved nutrient absorption and growth performance (Gunther, 1994). The external addition of lipids is becoming an essential trend in broiler production to enhance performance. Nonetheless, the impact of lipid or emulsifier additives continues to show variability (Shen et al., 2021). In various reports, the external addition of phospholipids, like lecithin, has been shown to have diverse effects on performance enhancement. Earlier studies have demonstrated that the addition of lecithin to diets resulted in improved performance metrics in broilers (Allahyari-Bake and Jahanian, 2017). The addition of lecithin positively influenced the growth performance of broilers that were given a soybean oil diet (Yang et al., 2005). The average daily gain (ADG) of

Table 4
Effect of strain, DOL, and their interactions on the antioxidants, and thyroid hormones, of broilers.

Items		TAO (Mm/ L)	GPX (U/ ml)	SOD (U/ ml)	MDA (nmol/ mL)	T_3 μg/ml^{-1}_	T_4 μg/ml^{-1}
Strain (S)	S1	1.05^{a}	310.00^{a}	6.63^{a}	4.40^{a}	3.54^{a}	7.71^{a}
	S2	0.90^{b}	291.00^{b}	5.13^{b}	3.17^{b}	1.84^{b}	6.20^{b}
	SEM	0.018	2.371	0.095	0.108	0.039	0.130
De-oiled lecithin (DOL) g/kg diet	T0	0.911^{b}	289.66^{b}	5.60^{b}	4.09^{a}	2.65	6.63^{b}
	T1	0.918^{b}	290.94^{b}	5.63^{b}	4.06^{a}	2.70	6.75^{b}
	T2	0.925^{b}	295.60^{b}	5.82^{b}	3.92^{a}	2.70	6.87^{b}
	T3	1.155^{a}	325.80^{a}	6.46^{a}	3.08^{b}	2.71	7.56^{a}
	SEM	0.025	3.354	0.134	0.152	0.055	0.184
S × DOL	S1 × T0	0.89^{d}	280.60^{e}	5.89^{c}	5.24^{a}	3.47	6.82^{b}
	S1 × T1	1.09^{b}	319.00^{b}	6.85^{ab}	4.15^{bc}	3.57	7.82^{a}
	S1 × T2	1.00^{bc}	305.10^{bc}	6.57^{b}	4.54^{b}	3.56	7.75^{a}
	S1 × T3	1.23^{a}	335.30^{a}	7.21^{a}	3.70^{cd}	3.58	8.45^{a}
	S2 × T0	0.93^{cd}	298.72^{cd}	5.31^{de}	2.95^{ef}	1.84	6.44^{cd}
	S2 × T1	0.74^{e}	262.88^{f}	4.42^{e}	3.97^{bc}	1.84	5.69^{e}
	S2 × T2	0.85^{d}	268.10^{de}	5.07^{f}	3.31^{de}	1.4	6.00^{d}
	S2 × T3	1.08^{b}	316.30^{b}	5.71^{cd}	2.47^{f}	1.85	6.67^{bc}
	SEM	0.036	4.743	0.190	0.216	0.078	0.261
P- Value	S	0.001	0.001	0.001	0.001	0.001	0.001
	DOL	0.001	0.001	0.001	0.001	0.093	0.002
	S × DOL	0.001	0.001	0.001	0.001	0.087	0.005

The data is the mean value derived from 5 replications.
Means within the same column of each factor and interactions that have differing letters represent a significant difference ($P < 0.05$).
Abbreviations: S1: Ross 308 strain, S2: Evian 48 strain, DOL: de-oiled lecithin, T0: control group (basal diet), T1: basal diet + 0.5 g/kg DOL T2: basal diet+1 g/kg DOL, T3: basal diet +1.5 g/kg DOL, SEM = standard error mean. TAO: total anti-oxidants, GPx: glutathione peroxidase, SOD: superoxide dismutase, MDA: malondialdehyde, T_3: triiodo thyronine, and T_4: thyroxin hormone.

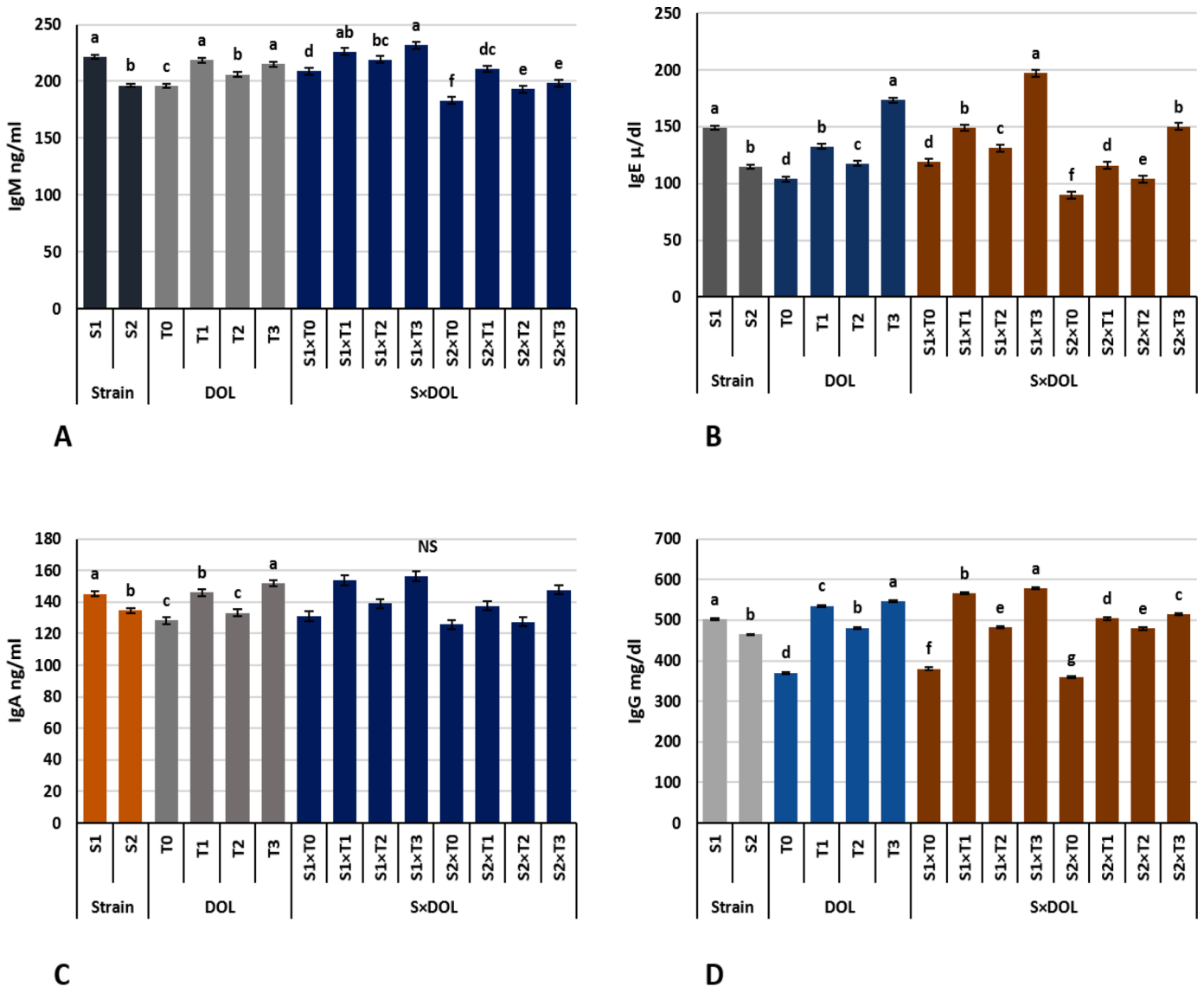

Fig. 1. Serum levels of IgM (A), IgE (B), IgA (C), and IgG (D) in broiler chickens as affected by strain, DOL, and their interactions.
The data is the mean value derived from 5 replications. The distinctive letters at the top of the columns represent significant deviations, at $P < 0.05$ evaluated using the Duncan test. Abbreviations: S1: Ross 308 strain, S2: Evian 48 strain, DOL: de-oiled lecithin, T0: control group (basal diet), T1: basal diet + 0.5 g/kg DOL T2: basal diet+1 g/kg DOL, T3: basal diet +1.5 g/kg DOL, SEM = standard error mean.

broilers fed a palm oil diet with soy lecithin saw an increase during the periods of days 0–21, days 21–42, and days 0–42. Simultaneously, the feed conversion ratio (FCR) decreased during the days 0–42 period (F. A. Siyal et al., 2017). Hassanabadi et al. (2015) demonstrated that the feed conversion ratio (FCR) improved when soy lecithin was added to the broilers' diet. Other studies have found that the introduction of crude soybean lecithin as an alternative energy source had no adverse effects on the productive performance of laying chickens and broilers (Mandalawi et al., 2015; Viñado et al., 2019). Soybean lecithin can be incorporated either in conjunction with or as a substitute for soybean oil. This ingredient is capable of sustaining the growth performance of broiler chickens during their grower and finisher phases (Viñado et al., 2020). The present research found the most favorable results with the DOL diet, endorsing the hypothesis that the inclusion of a high dose of DOL (1.5 g/kg) in the feed can have a positive impact on broiler growth performance. Greater emulsification can enhance fat absorption, which could have played a role in boosting the growth performance in the DOL groups in comparison to the control group. The findings of this study align with those of Nemati et al. (2021) and Drażbo et al. (2019), who conducted experiments on turkeys. They observed a numerical increase in protein digestibility with the addition of DOL. This might have contributed to an enhanced performance in the DOL-fed groups, complementing the other factors. External emulsifiers could be beneficial in balancing the state of adsorption-desorption, influenced by the presence of amphiphilic molecules such as bile salts, phospholipids, and proteins at the interphase (Majdolhosseini et al., 2019; Singh et al., 2009). Our physiological findings indicate that including DOL in the diet of broiler chickens has a beneficial impact on their health. This, in turn, may enhance nutrient absorption and ultimately result in better growth performance. Thus, it can be concluded that the modifications brought about by adding DOL to the broiler diet increased the absorption of fat and nutrients across the enterocyte membrane. This resulted in a higher availability of energy, which is crucial for improving productive performance.

4.1. Blood parameters

Assessing blood biochemical parameters in poultry is utilized to

identify metabolic and nutritional shifts, playing a crucial role in determining the health status (Ghasemi et al., 2013; Zhan et al., 2007). The results of the present study revealed significant variations ($P < 0.05$) in the blood biochemical parameters attributed to the effects of strain, dietary DOL supplementation, and their interactions, as indicated in Table 3. Broiler chicks from both strains that received 1.5 g/kg DOL exhibited increased levels of plasma total protein (TP), albumin (ALB), albumin/globulin (A/G) ratio, and high-density lipoprotein (HDL). At the same time, they showed reduced levels of cholesterol (CHO), triglycerides (TRIG), low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cr), and uric acid (UA). The findings of the present study contrast with those of an experiment conducted by Nemati et al. (2021) on turkeys. In their study, the addition of DOL, did not have any effect on total protein (TP), albumin (ALB), and globulin (GLO). Concurrently, an increase in the dietary supplementation of DOL resulted in a linear decrease in the levels of cholesterol (CHO), triglycerides (TRIG), and uric acid (UA). This might be connected to alterations in the catabolism of lipids and proteins within the body. Uric acid is recognized as the primary end-product of nitrogen metabolism in poultry. A lower uric acid level is often used to evaluate the utilization of amino acids (Ding et al., 2016). This could be tied to enhancements in protein digestibility. In their study, Dierick and Decuypere (2004), Xing et al. (2004) evidenced that the addition of lysolecithin progressively improved the digestibility of crude protein and energy in weaned pigs. These results are in line with previous studies that have shown that the addition of exogenous emulsifiers can reduce levels of cholesterol, triglycerides and low-density lipoprotein in broilers (Huang et al., 2007). It's proposed that dietary emulsifiers could potentially boost lipid metabolism in the liver and reduce blood triglyceride levels by stimulating hepatic lipoprotein lipase activity (Ge et al., 2019a). This phenomenon might be due to the accelerated removal of chylomicrons from the blood and a decelerated rate of their release into the bloodstream. Exogenous emulsifiers have the capacity to hasten the emulsification of lipids within the small intestine and enhance lipase activation (Shen et al., 2021). Furthermore, it has been documented that lecithin aids in the secretion of endogenous bile acid, thereby enhancing the rate of fat utilization. Past research has established a positive link between the activities of the lipase enzyme and the accumulation of lipids in both broiler and layer breeds of chickens (Griffin et al., 1987). In theory, supplementing with an emulsifier could improve the effective use of energy, lower concentrations of cholesterol, LDL, and triglycerides, and raise HDL levels (Upadhaya et al., 2018; Zhao and Kim, 2017). In the present research, the inclusion of DOL (1.5 g/kg) resulted in a decrease in LDL concentrations and an increase in HDL concentrations in both broiler strains. Corresponding with this study's findings, Huang et al. (2008) reported that in broiler chickens, soy-lecithin reduced the ratio of LDL while enhancing the ratio of HDL. In broiler chickens, it was observed that the group given a diet with 0.1 % soybean lecithin had lower LDL to cholesterol ratios compared to the group given a basal diet (Siyal et al., 2017). Potentially, the rise in HDL could be attributed to the enhanced emulsification due to DOL supplementation, which allows for more effective and complete utilization of fat. The levels of HDL and LDL in blood serum corroborate this information about lipid metabolism. HDL is instrumental in the reverse transportation of cholesterol. It assists in extracting surplus cholesterol that has accumulated on the inner walls of blood vessels and carries it back to the liver. Once in the liver, it is eliminated via the gastrointestinal tract (Lund-Katz and Phillips, 2010). Having a lower LDL level is preferable as LDL can be converted into ox-LDL. When LDL levels are high, the cholesterol it carries can accumulate in the artery walls (Toth, 2005). Oxidation of lipids could potentially result in inflammation. Furthermore, decreased LDL and elevated HDL aid in maintaining dilated blood vessels, facilitating superior blood circulation (Ansell et al., 2005; Toth, 2005). This also suggests that DOL could have a beneficial effect on enhancing the health of broilers. The disparity in findings could be attributed to factors such as the age of the broilers, their strain, and the purity and concentration of DOL in their diet. However, further investigation is necessary to substantiate these findings and to understand the impact of DOL supplementation on the profiles of blood metabolites.

4.2. Antioxidants and thyroid hormones

In bird physiology, the status of oxidation is significant in observing shifts in health status. The antioxidant status in birds is typically determined by examining the blood levels of Total Antioxidant Capacity (TAOC) and malondialdehyde, as well as the activities of enzymatic scavengers like superoxide dismutase and glutathione peroxidase (Ghasemi et al., 2020). Soybean lecithin is made up of alpha, gamma, and delta tocopherols (Wang and Wang, 2008). It's thought that the primary antioxidant mechanism of lecithin stems from the synergistic action between amino-alcohol phospholipids and gamma and delta tocopherols (Judde et al., 2003). Based on the findings of the present study, groups supplemented with DOL exhibited elevated serum levels of TAC, GPX, SOD, and decreased MDA concentrations in comparison to the control group. Additionally, the serum antioxidant status of growing broilers showed more positive changes when supplemented with a diet containing 1.5 g/kg DOL, further validating its protective role in combating oxidative stress. In line with the present findings, a recent study showed that the dietary inclusion of lysophospholipid in broilers under cold stress enhanced free radical scavenging activity and reduced lipid peroxidation (Zangeneh et al., 2020). The antioxidant properties of polar headgroups, such as choline and amino groups, present in phospholipids, make them suitable for use as lipophilic antioxidants in food products. These polar headgroups can effectively combat oxidative stress and protect against the harmful effects of free radicals (Sun et al., 2018). Lecithin has the potential to enhance the activities of GSH, GPx, SOD, and GST in the blood plasma of male rabbits (Ahmed et al., 2016). This suggests that lecithin supplementation can positively impact the antioxidant defense system, potentially protecting against oxidative stress (Al-Daraji et al., 2010; Butt et al., 2016). Soy lecithin displays antioxidant and neuroprotective characteristics, which play a role in its capacity to lessen liver damage and boost oxidative resilience (Aabdallah and Eid, 2004; Das and Vasudevan, 2006). Lecithin has the potential to improve the oxidative stability of oils and fats. This beneficial effect can be attributed to the presence of phospholipids, which are the primary components of lecithin (Nemati et al., 2021; Siyal et al., 2017). According to previous studies, soy lecithin has been found to be a valuable source of dietary phospholipids. Therefore, incorporating soy lecithin into poultry or animal feed has been shown to improve feed utilization and enhance growth rates.

Thyrotropin-releasing hormone (TRH), a hormone composed of three amino acids, is produced by the hypothalamus. It has the capacity to stimulate the release of thyrotropic hormone from the thyrotropin-secreting cells in the pituitary gland (Zhang et al., 2008). Thyroid hormones, specifically T_3 and T_4, play a crucial role in promoting gluconeogenesis and hepatic glycogen synthesis in poultry. These hormones facilitate the production of glucose from non-carbohydrate sources and support the synthesis and storage of glycogen in the liver of poultry (Duntas and Brenta, 2018). According to the results of the current study, groups that were supplemented with DOL showed increased serum concentrations of T_3 and T_4 in comparison with the control group. Corroborating the current findings, recent research indicated that the addition of 2 % crude soybean lecithin to broiler diets led to an increase in the circulating levels of thyroid-stimulating hormone (TSH) (Siyal et al., 2017 a). A significant improvement ($P < 0.05$) in the serum level of triiodothyronine (T3) was observed in the soy lecithin group compared to the control (Huang et al., 2007). Ge et al. (2019b) discovered that diets high in energy could decrease T_3 levels in broiler breeders, thereby stimulating lipid metabolism. In the present study, this enhancement in thyroid hormone levels is believed to contribute to the overall improvement in poultry performance.

4.3. Serum immunoglobulins

Immunoglobulins and components of the complement system serve as valuable indicators of an animal's immune status due to their essential functions within the immune system. These biomolecules play critical roles in defending against pathogens, promoting immune responses, and maintaining overall immune health. By assessing the levels and activity of immunoglobulins and complement components, we can gain insights into the immune status and potential immune challenges faced by animals (Rajput and Li, 2012). The findings of the present study revealed that groups given a diet supplemented with DOL exhibited enhanced serum concentrations of immunoglobulins compared to the control group, as illustrated in Fig. 1. While there is no direct research on the effect of lecithin on immunoglobulins in broilers, it is plausible that lecithin could have an indirect effect through its influence on gut health and nutrient absorption. The gut is a major site of immune activity, and good gut health can enhance the immune response. Additionally, better nutrient absorption could improve overall health and thus potentially boost immune function. Shabani et al. (2021) concluded that the supplementation of lecithin and lipase in broilers during their early stages positively bolstered their performance and immune response throughout the finisher rearing phase. levels of IgA (Immunoglobulin A) in both sows and piglets showed an increase, especially when their diets were enriched with 2% and 3% Soy Lecithin (SL) supplementation (Shi et al., 2019). Soy Lecithin (SL) contains significant amounts of linoleic acid, linolenic acid, and various unsaturated fatty acids, along with essential nutrients such as choline, inositol, and Vitamin E. These constituents have a significant impact on promoting and improving immune function by providing essential building blocks for immune cells and supporting antioxidant activity. The presence of these components in Soy Lecithin contributes to its ability to enhance the immune system. Earlier studies have demonstrated that the inclusion of Soy Lecithin (SL) in rat diets can boost the proliferation of T-cells, augment the quantity and conversion of lymphocytes, and promote macrophages' phagocytosis of tumor cells, thereby improving the body's immune capabilities (Shao, 1956). This could provide a reason for the observed increase in serum immunoglobulin concentrations when broiler diets are supplemented with DOL. Nonetheless, there has been limited research exploring the impact of DOL supplementation on immunoglobulin levels in broiler diets, indicating a need for further investigation.

5. Conclusion

Based on the aforementioned findings, it can be concluded that, supplementing diets with de-oiled lecithin (DOL) improved growth performance and increased blood parameters such as plasma total protein (TP), albumin (ALBU), globulin (GLO), A/G ratio, and high-density lipoprotein (HDL) concentrations. It also reduced cholesterol (CHO), triglycerides (TRIG), low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cr), and uric acid (UA) concentrations. Furthermore, dietary supplementation with DOL enhanced antioxidant status, thyroid hormones (T_3&T_4), and serum immunoglobulins in both broiler strains. Therefore, dietary supplementation of DOL with a purity level of 95% or more exhibits promising advantages as a feed supplement in the broiler sector. Still, additional research is needed to uncover the fundamental processes through which DOL impacts blood characteristics and immune responses.

CrediT authorship contribution statement

Hamada Okasha, Gaafar EL-Gendi, Zangabel Saad Mohamed and **Kamal Eid** prepared the original text, participated in the experimental design, conducted out the experimental, and performed statistics. **Hamada Okasha, Waleed Abdelmoez, Osama Abo-Emera** helped with both the statistics and the experimental deigning. **Hamada Okasha, Gaafar EL-Gendi, Zangabel Saad Mohamed, Waleed Abdelmoez, Osama Abo-Emera** and **Kamal Eid** interpret and discuss the results of the findings, and write a final essay.

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Data availability statement

The datasets collected and analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Declaration of Competing Interest

Potential conflicts of interest were not reported by the authors of the study. The authors have stated that they do not have any conflicts of interest related to the research or its findings.

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