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Effects of dietary supplemental lysophospholipids and vitamin C on performance, antioxidant enzymes, lipid peroxidation, thyroid hormones and serum metabolites of broiler chickens reared under thermoneutral and high ambient temperature

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Abstract

The effects of dietary supplemental lysophospholipids (LPLs) and vitamin C (VC) on performance, activity of antioxidant enzymes, and thyroid hormones of broiler chickens reared under thermoneutral and high ambient temperatures were evaluated. A total of 1,680 broiler chicks (Cobb 500) in finishing rearing period (days 21–38 of age) were allotted to two groups: thermoneutral (TN) and heat stress (HS). In the TN group, 480 chicks were subjected to four treatments with four replicates (n = 30) and maintained in usual ambient temperature (24 ± 1°C). In HS group, the remaining 1,200 chicks were subjected to four treatments with 10 replicates (n = 30) and exposed to high ambient temperature (34 ± 1°C for 8 hr daily). In both groups, four iso-caloric and iso-nitrogenous experimental diets based on a 2 × 2 factorial arrangements including supplemental LPLs (0 or 1,000 mg/kg) and VC (0 or 500 mg/kg) were formulated and used. Supplemental LPLs decreased (p < 0.05) body weight gain and increased FCR in the TN and HS groups. In the TN group, increased (p < 0.05) serum glucose was observed in chickens fed with dietary supplemental VC. In the HS group, decreased (p < 0.05) total protein concentration was detected in birds fed with supplemental LPLs. In both TN and HS groups, decreased (p < 0.05) uric acid concentration was detected in chicks fed with the VC-supplemented diets. A significant (p < 0.05) interaction between LPLs and VC on lactate concentration in the TN group was observed. In the HS group, decreased breast malondialdehyde concentration was detected in birds fed with the VC-supplemented diet. In the TN group, increased serum total antioxidant status was detected in birds fed with the LPLs-supplemented diet. In conclusion, LPLs improved oxidative stability and increased the antioxidant capacity of the serum. In addition, vitamin C modified heat stress and reduced lipid peroxidation.

KEYWORDS

broilers, heat stress, lysophospholipids, thermoneutral, vitamin C

1 | INTRODUCTION

Heat stress results in behavioural and physiological changes in poultry and has negative effects on health, productivity and product quality. The adverse effects of heat stress on poultry can be briefly described as reduced feed intake (Abidin & Khatoon, 2013), which is the major reason of decreased weight gain, increased mortality, reduced fertility and hatchability, alteration of the electrolyte balance and blood pH (Borges, Fischer DaSilva, Majorka, Hooge, & Cummings, 2004), impaired secretion and endogenous enzymes activity (Attia, Hassan, & Qota, 2009), decreased serum concentrations of T_3 and T_4, suppressed immune function (Yahav & Mcmurty, 2001) and decreased intestine absorbing capacity (Garriga et al., 2006). Various methods have been established to prevent and alleviate heat stress in birds.

Oxidative stress is defined as an imbalance between oxidants and antioxidants. High ambient temperature increases lipid peroxidation, produces free radicals, and impairs antioxidant status (Lin, Decuypere, & Buyse, 2006). Various nutritional modifications have been reported to reduce the deleterious effects of oxidative stress resulting from heat stress, such as incorporating antioxidants into poultry diets (Yang et al., 2014). Vitamin C (VC) as an antioxidant reduces vascular oxidative stress (Chen, Touyz, Park, & Schiffrin, 2001), and plays an important role in cellular antioxidant defence not only by reacting to all oxygen species through formation of dehydroascorbyl (inert radical), but also by transferring radical equivalents from lipid phases to aqueous compartments (Ciftci, Nihat Ertas, & Guler, 2005). Chickens endogenously produce VC, which is sufficient for normal physiological functions under normal conditions (Pardue & Thaxton, 1986). Levels of VC in the blood are inversely proportional to the environmental temperature within the range of 21 to 31°C (Thornton, 1961), so at elevated environmental temperature, it would be an essential dietary supplement (Attia et al., 2009).

Lysolecithins are mixtures of phospho- and lysophospholipids (LPLs) which differ in phosphatidyl substituent and fatty acid pattern (Van Nieuwenhuyzen & Tom′as, 2008). Lysophospholipids are mono-acyl formative of phospholipids resulting from the action of phospholipase A1 or A2, which hydrolyze the ester bond at sn-1 and sn-2 positions respectively (Joshi, Paratkar, & Thorat, 2006). Due to the removal of one fatty acid, LPLs are more hydrophilic and have better oil-in-water emulsifying properties (Joshi et al., 2006). Lysophospholipids are mentioned to improve gut permeability to macromolecules like proteins and dextrans (Tagesson, Franzen, Dahl, & Westr€om, 1985), regulate the activity of several enzymes (Shier, Baldwin, Nilsen-Hamilton, Hamilton, & Thanassi, 1976; Tagesson et al., 1985), and influence the formation of protein channels (Lundbaek & Andersen, 1994). This is a key application of LPLs in animal nutrition because it implies the possibility of extracting more nutritional value of feed, even when nutrients will normally show a low absorption level. Dietary supplemental LPLs increase AMEn, body weight gain, and antibody titres against Newcastle and infectious bursal diseases, as well as feed efficiency and carcass quality of broilers (Allahyari-Bake & Jahanian, 2017; Melegy, Khaled, El-Bana, & Abdellatif, 2010; Zaefarian, Romero, & Ravindran, 2015; Zampiga, Meluzzi, & Sirri, 2016).

Prolonged heat stress reduces visceral blood supply to the intestine and causes damage to epithelial cells in the gut, thereby affecting feed digestion and nutrient absorption (Cronje, 2007). Therefore, at this time, the use of materials that mean less heat increment and increase the digestion and absorption of nutrients can alleviate the negative effects of stress. It is postulated that together with bile salts, the LPLs act as an emulsifier in the first stages of fat digestion. By improving their emulsification, fat and oils are thought to be better available for lipases to hydrolyze them. LPLs also alter the protein channel formation in the membrane by increasing ion exchanges (Maingret, Patel, Lesage, Lazdunski, & Honor′e, 2000). Change in deformation energy increased the number and size of the membrane pores and consequently increased the flux rate of macromolecules across the cell membrane (Kelkar & Chattopadhyay, 2007; Lundbak, Collingwood, Ing′olfsson, Kapoor, & Andersen, 2010). Both mechanisms induce the transport of nutrients, from small particles such as calcium ions to large components such as polysaccharides to be broken down for absorption, leading to higher nutrient bioavailability for promising broiler performance (Boontiam, Jung, & Kim, 2017).

The objective of this study was to evaluate the effects of dietary inclusion of LPLs and/or VC on performance, oxidative stability, and blood biochemicals of broiler chickens during finishing phase (21–38 days of age) in thermoneutral and heat stress conditions.

2 | MATERIALS AND METHODS

2.1 | Animals, diets, and experimental design

All the experimental protocols adhered to the guidelines of and were approved by the Animal Ethics Committee of Razi University. The total number of 1,800 unsexed day-old broiler chicks (Cobb 500) were obtained from a commercial hatchery (44.02 ± 0.78) and raised for 21 days in the same condition before the beginning of the study. All chicks were vaccinated against infectious bronchitis on the first day, Newcastle disease and influenza on day 7th and infectious bursal disease on day 14th of age. The initial house temperature was 32°C and then gradually decreased to reach 24°C on the 21st day of age. On day 21, 1,680 chickens with uniform body weights (699.85 ± 39.86) were selected and randomly allotted to thermoneutral (TN) and heat stress (HS) groups. In the TN group, 480 broilers were randomly allotted to 16 floor pens with 30 birds in each (four treatments with four replicates). In the HS group, 1,200 broilers were randomly assigned to four experimental treatments with 10 replicates. Pen size for each replicate was 2.5 m^2 and a lighting cycle of 23-hr light:1-hr dark was applied during the experiment.

Lysophospholipids were supplemented to broiler diets in the form of a commercial lysolecithin product containing 4.06% of a standardized mixture of lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylinositol and lysophosphatidic acid. In both groups, based on Cobb 500 production catalogue (Available at: http://www.cobb-vantress.com/academy/

TABLE 1 Ingredients and composition of the experimental diets fed to birds in thermoneutral and heat-stressed groups from 21 to 38 days of age

Lysophospholipids (mg/kg)	0		1,000	
Vitamin C (mg/kg)	**0**	**500**	**0**	**500**
Ingredients (g/kg)				
Corn	639	639	635	635
Soybean meal (440 g/kg CP)	307	307	308	308
Soybean oil	14	14	4	4
Salt	2	2	2	2
Limestone	12	12	12	12
Filler	0	0	13	12.5
Broiler chicken concentrate (2.5%)[a]	25	25	25	25
Lysophospholipids	0	0	1	1
Vitamin C	0	0.5	0	0.5
Sand	1	0.5	0	0
Calculated composition				
ME (MJ/kg)	12.8	12.8	12.8	12.8
CP (g/kg)	190	190	190	190
Ca (g/kg)	8.20	8.20	8.20	8.20
Available P (g/kg)	4.10	4.10	4.10	4.10
Lys (g/kg)	10.5	10.5	10.5	10.5
Met (g/kg)	4.70	4.70	4.70	4.70
MET + Cys (g/kg)	7.90	7.90	7.90	7.90
Thr (g/kg)	7.30	7.30	7.30	7.30
DCAB (mEq/kg)[b]	222	222	222	222

Notes. [a]Broiler chicken concentrate (2.5%) composition: ME, 13.61 MJ/kg; CP, 21.33%; Ca, 12.70%; Available P, 11.34%; Lys, 2.29%; Met, 6.19%; Thr, 1.17%; Na, 2.42%; K, 0.66%; Cl, 2.43%; retinyl acetate, 10,000,000 IU; cholecalciferol, 3,500,000 IU; DL-a-tocopheryl acetate, 40,000 IU; menadione sodium bisulphite, 2,000 mg; thiamine hydrochloride, 2,000 mg; riboflavin, 5,000 mg, nicotinic acid, 35,000 mg; D-calcium pantothenate, 13,000 mg, pyridoxine hydrochloride, 3,000 mg; folic acid, 1,500 mg; cobalamin, 10 mg; biotin, 100 mg, calcium iodate, 1,250 mg; copper sulphate, 16,000 mg; zinc sulphate, 100,000 mg; sodium selenite, 300 mg; manganese oxide, 120,000 mg; iron sulphate, 40,000 mg.
[b]DCAB: dietary cation-anion balance (Na + K − Cl).

product-guides#cobb500), four iso-caloric and iso-nitrogenous experimental diets (metabolizable energy, ME = 12.8 MJ/kg and crude protein, CP = 190 g/kg diet) in a 2 × 2 factorial arrangements including supplemental LPLs (0 or 1,000 mg/kg) and VC (0 or 500 mg/kg) were formulated to get the nutrient requirements. Ingredients and composition of the experimental diet are shown in Table 1.

According to the product catalogue issued by the LPLs manufacturer, 100,000 Kcal/Kg ME was considered for supplemental LPLs in matrix ingredient during diet formulation. All chicks experienced a 3-day feed adaptation period (21–24 days of age), and from day 24 of age different temperature programs were applied in TN and HS groups for a duration of 15 days. The broilers in the TN group were maintained in usual ambient temperature (24 ± 1°C), and broilers in the HS group were reared in 34 ± 1°C for 8 hr daily while the following temperature was set up during 2 hr (between 8:00 and 10:00 a.m.) and gradually increased to 34°C. This high temperature was then maintained for 8 hr (until 18:00 p.m.). After that, the temperature was gradually decreased to the basal level (20:00 p.m.). The relative humidity was kept between 60%–70% during the experimental period. Mash feed and water were provided ad libitum. Mortality and the weight of dead chickens were recorded daily. Body weight gain (BWG) and feed intake (FI) were measured, and feed conversion ratio (FCR) was calculated during the experiment.

2.2 | Sampling

Two blood samples were taken via the wing vein on days 34 and 35 of age to determine parameters in plasma and serum respectively. On each day, ten male chicks per each treatment within the HS group and eight male chicks per each treatment within the TN group were randomly selected. The first samples were collected and placed into tubes containing anticoagulant and centrifuged at 1,372 *g* for 15 min to obtain plasma. The coagulated blood of the second samples was centrifuged (1,008 *g*, 15 min) to obtain sera. Plasma and serum samples were kept at −80°C until subsequent analysis. At the end of the experiment (day 39), after an overnight feed deprivation, chickens from each treatment were slaughtered and dissected. To measure lipid peroxidation and antioxidant status, the liver and pectoral right breast muscle samples were collected and immediately frozen in liquid nitrogen and then stored at −20°C for further analysis.

2.3 | Blood and tissue parameters

Serum metabolites, enzyme activity, and hormone assessment: Triglyceride (TG), total cholesterol (CHO), high-density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), glucose (GLU), albumin (AL), total protein (TP), lactate (LAC), creatinine (CREA), uric acid (UA), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) activity were measured in the serum samples using Pars Azmun kits (Pars Azmun, Iran) via an automatic analyzer (Abbott alcyon 300, USA). All samples were run in a single assay to avoid interassay variation. To measure enzyme activity of CPK and LDH, serum samples were diluted at a ratio of 1 to 10 with distilled water. Plasma concentrations of 3, 5, 3′triiodothyronine (T_3) and thyroxine (T_4) were determined using commercially available ELISA kits (Pishtazteb, Iran).

Antioxidant Status: Total antioxidant status of blood (TAS) was measured using the diagnostic kits (RANDOX/NX 2332, United Kingdom). To evaluate tissue oxidative status, the liver and breast samples were homogenized in 1.15% KCl solution. Hepatic glutathione peroxidase (GSH-Px) activity was measured according to Paglia and Valentine (1967) using Randox (United Kingdom). Liver tissue superoxide dismutase (SOD) was assayed by a spectrophotometric

method based on the inhibition of a superoxide-induced reduced nicotinamide adenine dinucleotide (NADH) oxidation according to Paoletti, Aldinucci, Mocali, and Caparrini (1986). Lipid peroxidation in sera and homogenized liver and breast tissue were assayed by measuring the level of malondialdehyde (MDA) based on the TBARS (thiobarbituric acid reactive substances) method explained by Kaya, Sezik, Ozkaya, Siebzehnrubl, and Wildt (2004). The TBARS content was assayed using a spectrophotometer at 532 nm and expressed as nanomoles of MDA.

2.4 | Statistical analysis

The experimental data of a 2 × 2 factorial arrangement of treatments based on completely randomized design were subjected to ANOVA using GLM procedure of SAS (SAS Institute, 2009). The following model was considered for analysis: $Y_{ijk} = \mu + LPLs_i + VC_j + (LPLs \times VC)_{ij} + \mu_{ijk}$; where Y_{ijk} is the measured characteristic, μ is the overall mean, $LPLs_i$ is the main effect of dietary LPLs, VC_j is the main effect of dietary VC, $(LPLs \times VC)_{ij}$ is the interaction between LPLs and VC, and μ_{ijk} is the random error term. All statements of significance are based on a probability of < 0.05, where the model indicated significance. The least squares means were separated using the PDIFF option of SAS (SAS Institute, 2009).

3 | RESULTS

3.1 | Growth performance

The effects of dietary supplemental LPLs and VC on BWG, FI, FCR, and mortality rate (MR) of broilers are shown in Table 2. Vitamin C had no significant effect on BWG, FI, and FCR in both TN and HS groups. Decreased ($p < 0.05$) BWG and increased FCR were observed in chickens fed with the LPL-supplemented diet in the TN and HS groups, but LPLs had no significant effect on FI. No significant effect of dietary supplemental LPLs and VC was detected on MR in both TN and HS conditions (Table 2).

3.2 | Serum biochemical parameters

The effects of dietary supplemental LPLs and VC on the blood metabolites in TN and HS conditions are presented in Tables 3, 4, and 5. No significant effect of dietary supplemental LPLs and VC was detected on the serum concentration of HDL, LDL, TG, and CHO in chickens in both TN and HS conditions (Table 3). In both TN and HS groups, AL was not significantly affected by dietary treatments. Increased GLU was detected in chickens fed with the VC-supplemented diet in the TN group (Table 4). Decreased ($p < 0.05$) TP was observed in chickens fed with the LPLs-supplemented diet in the HS group (Table 4). No significant effect of dietary supplemental LPLs and VC on CREA in both TN and HS groups was detected (Table 5). Decreased ($p < 0.05$) serum level of UA was observed in chickens fed with VC-supplemented diet in both TN and HS groups, but LPLs had no significant influence on serum UA (Table 5). The main effects of LPLs and VC on serum level of LAC in the HS group were not significant (Table 5). A significant ($p < 0.05$) interaction between LPLs and VC on serum level of LAC was detected in the TN group, so that serum level of LAC was lower in birds fed with the diet supplemented with LPLs and/or VC compared to those fed with the control diet (Table 5).

3.3 | Thyroid hormones and enzyme activity

There were no significant effects of dietary supplemental LPLs and VC on plasma concentrations of T3 and T4 and the activity of CPK and LDH activity in TN and HS groups (Table 6).

TABLE 2 Effects of dietary supplemental LPLs and VC on BWG (g), FI (g), FCR and MR (%) of broiler chickens reared under thermoneutral condition or subjected to heat stress during finishing period (Days 24–38 of age)

	Thermoneutral group				Heat-stressed group			
Treatment	BWG	FI	FCR	MR	BWG	FI	FCR	MR
LPLs (g/kg of diet)								
0	1,042[a]	2,177	2.10[b]	1.05	859[a]	1,847	2.16[b]	1.6
1,000	973[b]	2,153	2.21[a]	0.63	813[b]	1,823	2.25[a]	0.62
VC (mg/kg of diet)								
0	990	2,171	2.20	1.25	825	1,818	2.21	1.04
500	1,025	2,160	2.11	0.42	847	1,852	2.19	1.17
SEM[†]	17.818	41.765	0.038	0.536	16.124	24.050	0.024	0.401
Source of variation (*p*-value)								
LPLs	0.018	0.683	0.047	0.524	0.050	0.481	0.011	0.203
VC	0.191	0.854	0.146	0.305	0.329	0.328	0.615	0.242
LPLs × VC	0.613	0.849	0.513	0.523	0.759	0.648	0.875	0.799

Notes. BWG: Body weight gain; FCR: Feed conversation rate; FI: feed intake; LPLs: Lysophospholipids; MR: Mortality rate; VC: Vitamin C.

Means with different superscript in the same column are significantly different at $p < 0.05$.

[†]Pooled standard error of the mean.

TABLE 3 Effects of dietary supplemental LPLs and VC on HDL (mg/dl), LDL (mg/dl), TG (mg/dl) and CHO (mg/dl) of broiler chickens reared under thermoneutral condition or subjected to heat stress during finishing period (Days 24–38 of age)

	Thermoneutral group				Heat-stressed group			
Treatment	HDL	LDL	TG	CHO	HDL	LDL	TG	CHO
LPLs (mg/kg of diet)								
0	23.25	83.28	63.88	93.75	24.55	95.12	51.10	116.50
1,000	23.75	92.60	62.38	103.88	25.18	91.82	56.82	105.62
VC (mg/kg of diet)								
0	21.88	82.48	62.38	91.88	26.10	96.56	53.30	112.00
500	25.12	93.40	63.88	105.75	23.62	90.38	54.62	110.12
SEM[a]	2.132	4.853	7.359	5.042	2.009	5.535	3.265	6.950
Source of variation (*p*-value)								
LPLs	0.871	0.199	0.887	0.181	0.829	0.680	0.235	0.286
VC	0.302	0.137	0.887	0.075	0.398	0.444	0.778	0.851
LPLs × VC	0.470	0.883	0.943	0.850	0.417	0.658	0.919	0.210

Notes. CHO: cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; LPLs: Lysophospholipids; TG: Triglyceride; VC: Vitamin C.
[a]Pooled standard error of the mean.

TABLE 4 Effects of dietary supplemental LPLs and VC on GLU (mg/dl), AL (g/dl) and TP (g/dl) of broiler chickens reared under thermoneutral condition or subjected to heat stress during finishing period (Days 24–38 of age)

	Thermoneutral group			Heat-stressed group		
Treatment	GLU	AL	TP	GLU	AL	TP
LPLs (mg/kg of diet)						
0	209.25	1.30	3.11	224.00	1.46	3.58[a]
1,000	217.88	1.30	3.16	216.32	1.30	3.12[b]
VC (mg/kg of diet)						
0	195.88[b]	1.28	3.16	225.30	1.46	3.51
500	231.25[a]	1.32	3.11	215.02	1.30	3.19
SEM[†]	6.173	0.071	0.127	7.834	0.070	0.145
Source of variation (*p*-value)						
LPLs	0.342	1.000	0.785	0.499	0.125	0.039
VC	0.001	0.629	0.785	0.368	0.125	0.136
LPLs × VC	0.236	1.000	0.684	0.488	0.691	0.857

Notes. L: Albumin; GLU: Glucose; LPLs: Lysophospholipids; TP: Total protein; VC: Vitamin C.
Means with different superscript in the same column are significantly different at $p < 0.05$.
[†]Pooled standard error of the mean.

3.4 | MDA concentration in tissue and serum and antioxidant status

No significant effect of dietary supplemental LPLs and VC was detected on liver and serum concentrations of MDA in both TN and HS groups (Table 7). Decreased ($p < 0.05$) concentrations of MDA in breast muscle were observed in chickens fed with VC-supplemented diet in the HS groups, but LPLs had no significant influence on breast muscle concentration of MDA (Table 7). Effects of dietary supplemental LPLs and VC on the activity of SOD and GSH-Px and serum level of TAS are presented in Table 8. Dietary LPLs had no significant effect on the activity of SOD and GSH-Px in birds within TN and HS groups, but numerical increased activity of SOD and GSH-Px was observed in broilers fed with LPLs-supplemented diets. Increased ($p < 0.05$) serum concentration of TAS was detected in birds in the TN group fed with LPLs-supplemented diets.

4 | DISCUSSION

4.1 | Growth performance

Chickens performed better in the TN compared to the HS group. Reduced performance, lower feed intake, and smaller growth rate have been well documented in broilers exposed to continuously high temperature or chronic heat stress (Ahmad et al., 2006; Al-Fataftah & Abu-Dieyeh, 2007; Sahin, Sahi, & Kucuk, 2003; Yahav, Straschnow, Plavnik, & Hurwitz, 1996).

TABLE 5 Effects of dietary supplemental LPLs and VC on LAC (mg/dl), CREA (mg/dl) and UA (mg/dl) of broiler chickens reared under thermoneutral condition or subjected to heat stress during finishing period (Days 24–38 of age)

	Thermoneutral group			Heat-stressed group		
Treatment	LAC	CREA	UA	LAC	CREA	UA
LPLs (mg/kg of diet)						
0	86.44	0.08	4.60	61.89	0.07	3.48
1,000	74.21	0.11	5.52	55.01	0.05	4.15
VC (mg/kg of diet)						
0	89.36	0.06	5.76[a]	61.39	0.06	4.29[a]
500	71.29	0.13	4.36[b]	55.51	0.06	3.34[b]
SEM[†]	4.348	0.026	0.426	2.706	0.020	0.306
Source of variation (*p*-value)						
LPLs	0.070	0.305	0.150	0.092	0.342	0.144
VC	0.012	0.103	0.038	0.145	0.878	0.044
LPLs × VC	0.015	0.103	0.469	0.545	0.905	0.379
LPLs-VC	$LPLs_0VC_0$		$LPLs_0VC_{500}$	$LPLs_{1,000}VC_0$		$LPLs_{1,000}VC_{500}$
LAC (Thermoneutral group)	104.18[a]		68.71[b]	74.55[b]		73.88[b]

Notes. CREA: Creatinine; LAC: Lactate; LPLs: Lysophospholipids; UA: Uric acid; VC: Vitamin C.
Means with different superscript in the same column (in main effects) and in the same row (in significant interactions) are significantly different at $p < 0.05$.
[†]Pooled standard error of the mean.

TABLE 6 Effects of dietary supplemental LPLs and VC on CPK (U/L) and LDH (U/L) activity, T_3 (ng/ml) and T_4 (μgEq/ml) of broiler chickens reared under thermoneutral condition or subjected to heat stress during finishing period (Days 24–38 of age)

	Thermoneutral group				Heat-stressed group			
Treatment	CPK	LDH	T_3	T_4	CPK	LDH	T_3	T_4
LPLs (mg/kg of diet)								
0	644.62	162.00	2.98	2.21	519.00	160.90	2.96	2.98
1,000	658.50	165.38	2.94	2.55	396.60	157.55	2.60	2.96
VC (mg/kg of diet)								
0	762.12	171.75	2.90	2.22	489.60	176.20	2.64	3.02
500	541.00	155.62	3.01	2.54	426.00	142.25	2.92	2.92
SEM[a]	114.74	12.93	0.247	0.161	53.060	15.011	0.181	0.224
Source of variation (*p*-value)								
LPLs	0.933	0.856	0.916	0.165	0.125	0.876	0.183	0.944
VC	0.198	0.395	0.753	0.196	0.410	0.130	0.284	0.751
LPLs × VC	0.099	0.664	0.972	0.558	0.635	0.515	0.668	0.143

Notes. CPK: Certain phosphokinase; LDH: Lactate dehydrogenase; LPLs: Lysophospholipids; T_3: 3, 5, 3′triiodothyronine; T4: Thyroxine; VC: Vitamin C.
[a]Pooled standard error of the mean.

Based on our knowledge, no study in the literature investigates the effects of LPLs in heat-stressed poultry during finisher period. Melegy et al. (2010) reported enhanced feed intake and nutrient absorption in broilers fed with the diet which included 0.25 or 0.5 kg lysolecithin/ton feed. Zhang, Haitao, Zhao, Guo, and Barri (2011), who evaluated the effects of three different fat sources (soybean oil, tallow and poultry fat) with or without supplemental LPLs on broiler performance, observed higher body weight gain in chicks fed with LPLs-supplemented diets compared to control birds during the starting period. In another study, adding 3.5 g/kg lysolecithin to broiler diet increased the AMEn during week 2 of age with no significant effect on performance (Zaefarian et al., 2015). Zampiga et al. (2016) reported no improving effect of dietary supplemental LPLs on final body weight and daily weight gain of broilers during different rearing periods. The mentioned inconsistent results may be partly caused by different basal diets,

TABLE 7 Effects of dietary supplemental LPLs and VC on LMDA (nmol/mg Protein), BRMDA (nmol/mg Protein) and BLMDA (nmol/ml) of broiler chickens reared under thermoneutral condition or subjected to heat stress during finishing period (Days 24–38 of age)

	Thermoneutral group			Heat-stressed group		
Treatment	LMDA	BRMDA	BLMDA	LMDA	BRMDA	BLMDA
LPLs (mg/kg of diet)						
0	0.35	0.06	1.38	0.33	0.13	1.41
1,000	0.36	0.11	1.08	0.38	0.08	1.35
VC (mg/kg of diet)						
0	0.34	0.07	1.21	0.36	0.14[a]	1.49
500	0.37	0.12	1.24	0.34	0.07[b]	1.30
SEM[a]	0.034	0.024	0.107	0.018	0.024	0.153
Source of variation (*p*-value)						
LPLs	0.892	0.123	0.071	0.066	0.123	0.882
VC	0.528	0.153	0.871	0.574	0.049	0.389
LPLs × VC	0.779	0.151	0.871	0.066	0.186	0.452

Notes. BLMDA: Blood malondialdehyde; BRMDA: Breast malondialdehyde; LMDA: Liver malondialdehyde; LPLs: Lysophospholipids; VC: Vitamin C.
Means with different superscript in the same column are significantly different at $p < 0.05$.
[a]Pooled standard error of the mean.

TABLE 8 Effects of dietary supplemental LPLs and VC on SOD (U/mg Protein) and GSH-Px (U/mg Protein) activity of liver and TAS (mmol/l) of broiler chickens reared under thermoneutral condition or subjected to heat stress during finishing period (Days 24–38 of age)

	Thermoneutral group			Heat-stressed group		
Treatment	SOD	GSH-Px	TAS	SOD	GSH-Px	TAS
LPLs (mg/kg of diet)						
0	3.76	0.32	1.95[b]	4.44	0.38	1.85
1,000	4.16	0.34	2.28[a]	5.06	0.43	1.88
VC (mg/kg of diet)						
0	3.77	0.33	2.27	4.90	0.38	1.94
500	4.16	0.33	1.96	4.61	0.43	1.80
SEM[a]	0.263	0.015	0.105	0.416	0.026	0.090
Source of variation (*p*-value)						
LPLs	0.302	0.325	0.050	0.316	0.241	0.834
VC	0.317	0.859	0.062	0.636	0.293	0.291
LPLs × VC	0.332	0.325	0.340	0.776	0.720	0.230

Notes. GSH-Px: Glutathione peroxidase; LPLs: Lysophospholipids; SOD: Superoxide dismutase; TAS: total antioxidant status; VC: Vitamin C.
Means with different superscript in the same column are significantly different at $p < 0.05$.
[a]Pooled standard error of the mean.

dietary fat sources and levels, and type and inclusion rate of emulsifiers (Zhang et al., 2011).

In the present experiment, according to the product catalogue issued by the manufacturer of LPLs (as an emulsifier), 100,000 kcal ME/kg LPLs was considered in formulating the experimental diet, so the oil percentage in basal diet was more than the LPLs-included diets. Lack of performance response to dietary supplemental LPLs may be partly due to the lower equivalent ME of the used LPLs suggested by the manufacturer. In other words, in this experiment, LPLs provided less ME for the birds than what was considered as a matrix for it. This may be due to several reasons: the duration of the experiment during which LPLs was added to the diet, the composition of the diet, the type and level of the dietary fat source, and the age of the birds.

Krogdahl (1985) showed the poor synthesis of bile salts in the newly hatched chicks, so dietary supplemental emulsifier may have some advantage during this early stage of age. However, the dietary supplemental LPLs used in the present investigation was given to chickens from day 21 of age (finishing period). Zhang et al. (2011) reported that LPLs supplementation can improve body weight gain of broiler chickens in the starter period.

Although many experiments have been conducted with VC on broiler performance, the data obtained from this study are controversial. In previous studies, dietary VC supplementation could

partially overcome the depression in growth performance caused by high temperature in broiler chickens (Lin et al., 2006; Sahin et al., 2003; Seven, Seven, Yilmaz, & Ugsims, 2008). In contrast, Pardue, Thaxton, and Brake (1985) reported no improving effect of supplemental ascorbic acid (1,000 ppm) in birds maintained at 22°C or exposed to 43°C, which is in parallel with the results of the present experiment. Several reasons might explain the difference between our findings and those in the literature: different strains of chickens used, the model of heat stress, background diet, birds' age when using VC supplement and dosage of VC. It seems that VC supplementation at proper dosage may have positive effects on birds' performance. Sabah, Mohammed, and Abdel (2008) reported that diet supplementation with 250 mg/kg VC improved broiler performance, but adding higher doses of VC (500 mg and 750 mg/kg) decreased performance of broilers compared to the control group. Mirfendereski and Jahanian (2015) reported no difference in egg production and feed intake of laying hens fed with 500 mg/kg VC. On the other hand, lack of response to 500 mg VC/kg in this study might be related to the innate ability of the chicks to synthesize ascorbic acid with age. Horing and Frigg (1979) reported an increase synthesis rate of ascorbic acid with age. The increased concentration of ascorbic acid with age in heart muscle and spleen was also previously detected by Dorr and Nockels (1971).

4.2 | Blood parameters

In the present experiment, serum lipid profile of chickens was not significantly affected by diet supplementation with LPLs, which is consistent with Melegy et al. (2010), who reported no significant effect of dietary supplemental lysolecithin on cholesterol, triglyserid, HDL and LDL in broilers. Zhao, Li, Hossain, and Kim (2015) reported lower serum triglyceride in pigs fed with the LPLs-included diet compared to those fed with the control diet. In this study, the profile of blood lipids was not affected by dietary supplemental VC in both TN and HS groups. Torki, Zangeneh, and Habibian (2014) also reported no significant effect of dietary supplemental VC on serum levels of LDL, cholesterol and triglyceride in laying hens subjected to heat stress. Based on the study by Attia, Hassan, Tag El-Din, and Abou-Shehema (2011), chronically elevated temperature significantly increased the blood level of triglycerides, while dietary supplemental ascorbic acid, as an antioxidant, decreased blood level of triglycerides. Reduced blood concentration of glucose was reported by Sujatha et al. (2010) in heat-stressed broilers fed with the VC-supplemented diet.

In this study, in the TN group, the birds given dietary supplemental VC exhibited significantly higher serum concentration of glucose. Based on the glucose-ascorbate antagonism theory, which was first proposed by Hamel, Santisteban, Ely, and Read (1986), blood glucose and VC compete for the same transport system to enter cells due to the structural similarity between the two molecules (Wilson, 2005). In addition, both glucose and VC depend upon insulin and its signalling effects to get into cells. Therefore, it seems that an increased blood level of VC competes with glucose to enter cells, and as a result, it increases the concentration of glucose in the blood. In this experiment, in the TN group, the level of 500 mg of VC supplement was likely to be higher than the birds' requirement, which increased blood levels of vitamin C and, consequently, increased blood glucose levels. In addition, high-dose VC supplementation results in an increase in blood glucose (Branch, 1999). Braun et al. (1996) reported that glucose can be produced by VC through gluconeogenesis pathway in ascorbate cycle that may increase blood glucose.

Dietary supplemental LPLs had no significant effect on serum glucose concentration. Boontiam et al. (2017) found the increasing blood glucose concentration accompanied by increased supplementation level of LPLs. They believed that dietary supplemental LPLs stimulated uptake of glucose, which is commonly used as an energy source and a metabolic intermediate. The increased availability of glucose was also detected in birds fed with glycerol polyethylene glycol-ricinoleate as a synthetic emulsifier (Roy, Haldar, Mondal, & Ghosh, 2010). Based on the reports by Melegy et al. (2010) and Roy et al. (2010), there was no significant effect of dietary supplemental emulsifier on serum concentration of total protein. In the HS group, chickens given dietary supplemental LPLs exhibited significantly lower serum concentration of total protein. In HS group, the chickens given dietary supplemental LPLs exhibited significantly lower serum concentration of total protein. Lysophospholipids can alter protein channel formation in the membrane by increasing ion exchanges (Maingret et al., 2000). This change increases the number and size of the membranous pores and improves the flux rate of macromolecules across the cell membrane consequently (Kelkar & Chattopadhyay, 2007; Lundbak et al., 2010). Both mechanisms can induce nutrient transport.

In this study, decreased serum concentration of uric acid was observed in chickens fed with VC-supplemented diet in both TN and HS groups. Activation of the hypothalamic-pituitary-adrenal cortical system occurs after stress exposure, so that the hypothalamus stimulates the pituitary to release adrenocorticotropic hormone (ACTH, Holmes & Phillips, 1976). Because of catabolic effect of ACTH, increasing concentrations of ACTH are parallel with yielding more glucose, uric acid, and triglycerides in the serum. Uric acid is a metabolite known as a major end product of nitrogen metabolism in broiler chickens, thus decreased uric acid concentration can be considered to determine amino acid utilization (Donsbough, Powell, Waguespack, Bidnerv, & Southern, 2010). According to Speranza et al. (2007), uric acid is a good indicator of stress. Decreased concentration of serum uric acid has also been shown in poultry fed with the VC-supplemented diets (Cinar et al., 2014; Khodadadi, Mousavinasab, Khamesipour, & Katsande, 2016; Saxena, Anand, Saxena, & Bajaj, 2009). Based on the in vitro and in vivo studies, it has been demonstrated that VC inhibits uric acid synthesis (Feigelson, 1952). Melegy et al. (2010) reported no significant effect of supplemental LPLs on serum concentration of uric acid. In contrast, Boontiam et al. (2017) showed linearly decreased the serum concentration of uric acid in response to increasing levels of supplemental LPLs in broilers' diet.

Decreased serum concentration of lactate was observed in birds within the TN group fed with the diets supplemented with LPLs and/or VC compared to the control diet. Lactate is the major substrate

used for glucose synthesis in isolated chicken hepatocytes, but pyruvate, glycerol, and some amino acids and lactate are converted to glucose in isolated chicken kidney tubules. In conditions such as increased protein catabolism and imbalance muscle activity and oxygen availability (high muscle activity and low oxygen availability), glucose is used for anaerobic muscles glycolysis, which in turn results in increased plasma concentration of lactate. It has been shown that reactive oxygen species affect the calcium release channel or Ca^{2+}-ATPase activity leading to overloading Ca^{2+} in muscles (Kaminishi, Matsuoka, Yanagishita, & Kako, 1989). In turn, Ca^{2+} overload enhances glycolysis through activating adenosine monophosphate-activated protein kinase, ending with increased lactate production (Lounsbury, Hu, & Ziegelstein, 2000).

4.3 | Thyroid hormones and enzymes activity

In this study, thyroid hormones were not significantly affected by dietary treatments, but a numerical increase in T3 concentration was observed in broilers fed with VC-supplemented diets in the HS group. Thyroid hormones play a central role in the regulation of the metabolic rate of birds, so thyroid gland hormones are important in adaptation to high ambient temperature (May, Deaton, Reece, & Branton, 1986). There is a reverse relationship between T3 plasma concentration and an increase in temperature (Sahin & Kucuk, 2003). Hypothyroidism and decrease in thyroid hormones have been shown in heat-stressed poultry (Yahav et al., 1996). Increased T3 level in chickens exposed to high ambient temperature and fed VC was detected by Sahin et al. (2003). This leads to the assumption that antioxidant supplementation may be desirable to reduce the negative effects of heat stress on the thyrotrophic axis.

A number of enzymes such as CPK and LDH are used as tools for differential diagnosis in the clinical biochemistry. The unusual appearance of CPK and LDH in the blood can give an indication of special muscle or organ damage, as the bulk of each is located in different tissues (Pech-Waffenschmidt, 1992). The elevated level of CPK enzyme in birds is most common with liver disease, and in birds with this condition, rise and fall in this enzyme occurs more quickly than aspartate aminotransferase levels (Pech-Waffenschmidt, 1992). In this study, numerical decreased activities of CPK and LDH were observed in broilers fed with the LPLs-supplemented diets in the HS group or fed with the VC-supplemented diet in the TN and HS groups. It supports that VC and LPLs may act to decrease the harmful effects of high temperature in broilers.

4.4 | Tissue and serum MDA content and antioxidant status

In this study, activity of liver antioxidant enzymes (SOD and GSH-Px) and total antioxidant status of blood were considered to evaluate oxidative stability. Also, malondialdehyde concentration in liver, breast It supports that VC and LPLs may act to decrease the harmful effects of high temperature in broilers, and blood was measured as a secondary lipid peroxidation product.

Oxidative stress has been associated with the increased production of free radicals and changes to the scavenging capacity of antioxidant systems. Antioxidant components can support antioxidant system through direct scavenging of free radicals of oxygen produced in stress conditions and/or prevent the formation of reactive oxygen species by inhibiting enzymes or chelating trace metals (Thring, Hili, & Naughton, 2011), antioxidant enzymes activation, and pro-oxidant enzymes (such as NADPH oxidase and lipoxygenase) inhibition (Schewe, Steffen, & Sie, 2008).

Significant increased TAS and numerical decreased MDA were detected in serum of birds fed with LPLs-supplemented diets within the TN group. Based on our knowledge, few studies in the literature investigate the effects of LPLs on oxidative stability of poultry, but some studies indicate that phospholipids may have antioxidant effects (Cui, 2015; Yoon & Min, 1987). Phospholipid can bind pro-oxidative metals through negative charges presented on the phosphate head group and inhibit lipid oxidation (Cui, 2015). Yoon and Min (1987) found that phospholipids acted as antioxidants as they chelated iron. Lysophospholipids might have positive effects on inflammation and indirectly combat oxidative stress. A relationship between inflammation and oxidative stress has previously been reviewed (Castellani, Balza, & Rubartelli, 2014; Mittal, Siddiqui, Tran, Reddy, & Malik, 2014). Inflammatory cytokine (IL-6) increased expression of NADPH oxidase 4 and produced reactive oxygen species (Li et al., 2015). Inflammation can cause oxidative stress, which in turn induces activation of multiple pathways like the reactive species hydrogen peroxide by activation of transcription factor NF-κB (Anderson, Staal, Gitler, Herzenberg, & Herzenberg, 1994; Floh'e, Brigelius-Floh'e, Saliou, Traber, & Packer, 1997). If oxidative stress is the primary event, inflammation will eventually develop (Vaziri & Rodr'ıguez-Iturbe, 2006). Treede et al. (2007) described the anti-inflammatory properties of LPLs molecularly. The exogenous addition of all phosphatidylcholine species significantly inhibited TNF-α-induced proinflammatory signalling and reduced the expression levels of IL-8, ICAM-1, IP-10, MCP-1, TNF-α (Treede et al., 2009). Moolenaar (2000) and Sturm et al. (2002) reported that lysophosphatidic acid and lysophosphatidylethanolamine significantly reduced the degree of inflammation. Based on the study by Boontiam et al. (2017), the addition of supplemental lysophospholipid to low-energy and nitrogenous broilers diet alleviated inflammation and decreased interleukin-1 level.

In the present experiment, decreased concentrations of MDA (as a lipid peroxidation product) in breast muscle were observed in chickens fed with the VC-supplemented diet in the HS groups. Similarly, dietary vitamin C supplementation caused a decrease in MDA value (Sahin, Kucuk, Sahin, & Sari, 2002; Sahin et al., 2003).

5 | CONCLUSIONS

In this study, the observed decrease in serum total protein levels in chickens fed with the LPL-supplemented diet in the HS group can in part explain the effect of LPLs on nutrient transport and

macronutrient absorption. Also, LPLs improved oxidative stability and increased the antioxidant capacity of the serum. In addition, vitamin C modified stress and reduced lipid peroxidation via affecting serum uric acid concentration and breast muscle MDA levels.

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