Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 50, Number 4, p. 303-319, December 2022
DOI: 10.21608/zvjz.2022.156750.1188

RESEARCH ARTICLE

Dietary Alpha-Lipoic Acid Effects on The Mitigation of the Negative Impact of Heat Stress in Broilers.

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Article History: Received: 11/09/2022 Received in revised form: 29/11/2022 Accepted: 29/12/2022

Abstract

The goal of the current study was to assess how α-lipoic acid (ALA) mitigates the negative effects of heat stress (HS) on various biochemical parameters and antioxidant status. For 35 days, a total of 72 one-day-old chicks with an initial body weight average of 45±3 gm were divided into four groups: TNC: no supplements were provided in diet (control group); TN-ALA: 25 gm ALA /100 kg of feed was supplemented; HS-Control: no supplements were added, and the birds were exposed to heat stress; and (HS-ALA: 25 gm ALA/100 kg of diet was supplemented and the birds were exposed to heat stress). From the 21st day to the end of the experiment, heat stress groups were subjected to (40±5˚C) for eight hours each day (from 7 a.m. to 3 p.m.). Four healthy birds were randomly chosen from each group and slaughtered at the end of the trial for sampling and analysis. Body weight, body weight gain, and feed conversion ratio were not significantly increased by the addition of ALA, while feed intake was significantly raised. When compared with HS-Control group, the dietary addition of ALA considerably lowered the serum total protein and albumin. ALT and AST activities were increased by heat stress unlike with ALA treatment, ALT and AST considerably dropped. The level of serum uric acid and urea decreased while creatinine was not considerably impacted. The addition of ALA to the diet resulted in a considerable decrease in serum total lipids. Malondialdehyde (MDA) dropped noticeably while catalase enzyme activity was elevated. The glucagon like peptide-1 (*GLP-1*), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PGC-1*), superoxide dismutase-1(*SOD-1*) and mucin-2 (*MUC-2*) gene expression levels were boosted dramatically in groups supplemented with ALA. The findings indicated that a meal rich in ALA had an impact on some biochemical variables, improved the antioxidant status and boosted the level of genes expression (*GLP-1*, *PGC-1α*, *SOD-1* and *MUC-2*).

Keywords: Broilers, α-lipoic Acid, Thermal Stress, Gene Expression, Metabolic Parameters.

Introduction

"Climate change" refers to shifts in natural temperatures and geographical occurrences on Earth [1]. Greenhouse gas emissions are critical to the global energy balance [2]. As a result of human activities, CO2 concentrations in the atmosphere have increased by 40% in the last two centuries [3]. High ambient temperatures have a harmful influence on the animal's health and can be used to measure the impact of sensible and latent heat transfers between the animal and its surroundings [4]. The demand for cheap protein sources has increased during the past few decades. So, the consumption of poultry meat increased globally due to its

low cost and high protein content [5]. Birds are susceptible to heat stress because of their high rate of metabolism, lack of sweat glands, and heavy feather coating [6]. Heat stress impairs the growth performance by lowering the feed utilization, which leads to a decrease in body weight and body gain, increasing the feed conversion ratio and rate of mortality [7]. In laying hens, heat stress affects egg production and quality, and all of these lead to huge financial losses [8]. Also, the high ambient temperature elevated the birds' body temperatures, and birds physiologically adapted by increasing panting, which led to the impairment of acid-base balance [9]. Heat stress induces damage to liver and kidney tissues [10], so there are alterations in liver enzyme activities, kidney functions [11,12], and the immune system [13]. Thermal stress increased the concentration of corticosteroids in the bloodstream [14] while decreasing thyroid hormone activity [15]. A high level of corticosteroids is considered a causative agent of oxidative stress, which initiates lipid peroxidation [16]. Heat stress also induces oxidative stress, which interrupts the antioxidant defense system and oxidizes biomolecules such as fats, proteins, and DNA [17].

The temperature-sensing neurons are peripheral nerves that express thermotransient receptor potentials, which are ionic receptors that, by a not-yet-understood mechanism, respond to temperature [18]. One of the suggestions is that thermo TRP is expressed in the hypothalamus (especially the preoptic area, which has a responsibility to detect any differentiation in temperature) in response to temperature in brain tissues and surrounding blood vessels [19]. Behaviorally and in ways to adapt to the high ambient temperatures, birds use many methods to reduce the effect of heat stress. For example, birds raise their wings sometimes and approach cold surfaces sometimes (to transfer heat from the higher medium to the lower medium through radiation). Also, the evaporation process is one of the important ways to lose heat through panting, but this method is useless in the case of very hot and humid weather [20].

Based on the foregoing, it is very important to have inexpensive and natural solutions to solve the problem of heat stress in poultry in general, and attention is currently turning to antioxidants, which are used as natural additives that will reduce the severity of these damages [21].

Alpha-lipoic acid (thioctic acid or 1,2-dithiolane-3-pentanoic acid; molecular formula: $C_8H_{14}O_2S_2$) [22], which is considered a potent and universal antioxidant, is water and fat soluble, so it is easily absorbed and transformed through cell membranes [23].It has a metal chelating activity, converts and replenishes endogenous antioxidants like glutathione, Vitamin E, and Vitamin C, scavenges free radicals like reactive oxygen species and reactive nitrogen species. It also is involved in the metabolism of carbohydrates and energy and acts as a cofactor for the enzymes -ketoglutarate dehydrogenase and pyruvate dehydrogenase activity [24, 25].ALA improves liver parameters [26], stimulates the antioxidant status, positively modifies the lipid profile [27], and affects the expression levels of immune-related genes [28]. ALA improves broiler meat quality [29,30]and positively affects the growth performance parameters [31].

In this study, we tried to study the effect of alpha-lipoic acid on the gene expression of some genes that were isolated from the hypothalamus, like

GLP-1 and *PGC-1*; the liver, like *SOD-1*; and the intestine, like *MUC-2*.

glucagon-like peptide-1(*GLP-1*) is a gastrointestinal peptide that appears in the bloodstream in response to a meal [32]. *GLP-1* plays an important role in the stability of blood glucose levels, stimulates insulin secretion, and decreases glucagon production [33]. The peroxisome proliferator activated receptor gamma coactivator-1 alpha(*PGC-1α*), this gene is responsible for regulating cellular energy metabolism, as mentioned by Liang and Ward [34]. They also suggested that *PGC-1* participates in carbohydrate and lipid metabolism. The study reported that *PGC-1* has a significant role in the regulation of mitochondrial functions, especially the expression of antioxidant genes [35]. One of the anti-oxidative proteins and the primary line of antioxidant defense is superoxide dismutase-1(*SOD-1*) [36]. Mucin-2 (*MUC-2*) is a member of the mucin protein family; it is a highly molecular-weight glycoprotein produced by different types of epithelial tissues. Intestinal muc-2 composes the mucosa to protect the GIT from self-digestion and invasion by microorganisms [37].

In order to better understand how dietary alpha-lipoic acid affects blood chemistry, antioxidant status, and expression of previously mentioned genes in broilers under heat stress, this study set out to look at these topics.

Materials and Methods

The study's ethical statement

The use of animals in this investigation conforms to all applicable rules and moral standards. The scientific panel in the veterinary medicine faculty at Zagazig University has made certain the animals were kept in suitable conditions with access to veterinary services. All workers involved in animal care were adequately skilled in both the experimental process and the ethical treatment of the animals (ZU-IACUC/2/F/242/2022**)**.

Animals, housing, experimental design, and diet

This trial was conducted at the faculty of veterinary medicine's experimental unit at Zagazig University in Egypt. A total of 72 one-day-old chicks (cobb500) were purchased from a local hatchery. The birds were divided into four groups; each group contains 18 chicks with two replicates. The thermoneutral control (TNC) birds were fed only the basal diet. The thermoneutral α-lipoic acid group (TN-ALA) received a basal diet supplemented with 25 gm ALA per 100 kg diet [38]. The heat stress control group (HSC), birds of this group fed only the basal diet and subjected to HS. The heat stress α-lipoic acid (HS-ALA) group, birds of this group fed with basal diet provided by 25 gm/100 kg diet and subjected to HS. For five weeks, the birds were raised in wood chip floor pens. Full-time lightning programme. The experiment's pen temperature was set at 22±5˚C for the first three weeks. On the 22^{th} of the experiment, pens from the heat challenged groups were exposed to a temperature of 40±5˚C for 8 hours daily from 7 a.m. to 3 p.m. Food and water were supplied *ad libitum* for all groups. All birds were fed a diet suitable for their age (starter from 1–21 days and finisher from 22–35 days). The ration was provided by the Al-Eman Company for poultry and livestock rations. Table 1 demonstrates the diet composition ingredients.

Table 1: Composition of diet for starter (0-21 days) and finisher (21-35 days) period in broiler chickens reared under normal and heat stress.

Ingredients	Starter Diet (0-21 days).	Finisher Diet (22-35 days).
Corn, Grain	48.20	58.70
Wheat	8.00	7.50
Soybean meal (40% CP)	28.50	20.50
Protein Conc.	10.00	10.00
Vegetable Oil	4.00	2.50
Salt	1.00	0.50
Vitamin + Mineral	0.30	0.30
Composition:		
AME (kcal/kg)	3079.00	3102.60
Crude Protein	22.06	19.37
Lysine	1.21	1.03
Methionine + Cystine	0.82	0.75
Calcium	1.2	0.95
Phosphorus (%)	0.44	0.42

Supplied the following per kilogram of diet: Vit.A, 25000 IU; Vit.D, 5000 IU; Vit.E,12.5 IU; Vit.K,2.5 IU; Vit.B1,1mg; Vit.B2, 8 mg; Vit.B6, 3 mg; Vit.B12, 0.015 mg; Folic acid, 0.025 mg; Nicotinic acid, 17.5 mg; Calcium pantothenate, 12.5 mg; Fe, 80 mg; Cu, 10 mg; Mn, 80 mg; Se, 0.15 mg; I, 0.35 mg.

Chemicals used

Medications or supplements

α-Lipoic acid is a pale- and light-yellow powder with 100% purity, obtained from Atos Pharma-Bilbeis-Ash Sharqiyah-Egypt.

Chemicals for qRT-PCR

Chloroform HPLC grade, Isopropanol HPLC grade, and 70 % Ethanol HPLC grade (Sigma Aldrich), Qiazol (Qiagen; Germany), High-Capacity cDNA Reverse Transcription Kit cDNA Kit; (Applied Bio systems™, USA), and TOP real™ qPCR 2X PreMIX (SYBR Green with low ROX) (Cat.P725 or P750) (Enzynomics, Korea).

Inclusion and exclusion criteria

The animals used in this study were selected as healthy and free from any pathogenic disorders to minimise the possibility of inter-individual differences.

Growth performance

All birds were weighed at days 7, 14, 21, 28, and 35. Body weight gain was calculated weekly by subtracting the body weight between two successive weights. The amount of feed added to each pen was recorded, and the amount of feed left in the pen was weighed on a weekly basis to calculate the feed intake, subtracting the residual feed from the offered feed. The feed conversion ratio is calculated by

dividing the feed intake by the weekly body weight gain.

Blood samples

At the end of the investigation, four birds from each group were chosen at randomly. Wing vein blood was taken, placed in a tube for blood serum isolation, and allowed to clot. Serum is is separated by centrifugation at 3,000 rpm for 15 minutes. The serum is then kept at -20°C in 1.5 mL Eppendorf tubes until analysis.

Tissue samples

Tissue samples (hypothalamus, liver, and intestine) were collected immediately after the birds' slaughter and covered with 50 mg/1µl Qiazol and stored at -8˚C for total RNA extraction.

Biochemical analysis

According to Young [39] colorimetric techniques were used for the determination of total proteins, albumin, ALT, AST, uric acid and creatinine. The method described by Chabrol and Charonnat [40] was used to determine the total lipid concentration.Uric acid was measured using techniques from Fawcett and Scott [41]. The examination of the catalase enzyme can also be done using a colorimetric approach as mentioned by Aebi [42]. Hiroshi *et al.*[43] methodology was used for Malondialdehyde analysis.

RNA Extraction, cDNA synthesis and qRTC-PCR

Total RNA was isolated from the hypothalamus *(GLP-1 and PCG-1α),* liver *(SOD-1)* and intestine tissue *(MUC-2)* samples using the Qiazol (Qiagen, Germany) and the concentration was assessed by measuring the absorbance at 230-260 nm and the ratio accepted value for RNA quality fall in range of 1.8-2, values out the range were excluded. Total RNA was reverse transcripted with high-capacity reverse transcriptase cDNA synthesis kits (applied bio system, USA) [44, 45]. According to Livak and Schmittgen [46], the mRNA expression was evaluated using qPCR and the $^{2-\Delta\Delta}$Ct method. Then, according to the manufacturer's instructions, qPCR mixes were prepared on ice. They took place with the use of a Rotor Genes [47]. The primers were produced by Sangon Biotech using the Primer 5.0 software and are shown in Table 2.

Statistical analysis

Results were reported as mean ± SEM (Standard Error of Mean). In order to assess the influence of the five treatment groups on the different biochemical parameters, one-way analysis of variance (ANOVA) followed by Duncan multiple tests as post hoc test were used. Statistical significance was denoted by the value of $P < 0.05$. The Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY) and Graph Pad Prism 8.0.2 were used for ALL analyses and visualisations (Graph Pad Software, Inc.) [48].

Table 2: The primers sequence and parameters

Primers	Primers Forward	Primers Reverse	Product Length	Accession No.
GLP-1	GGCTGAAGAAATGGGCCGAA	TTGGCAGCCATATCATCCAGG	81	NM_001190165.5
PGC-1α	AGTAAGCTCTCAGAAACTTTGTTG	AAGGTTGAAACAGAAGCCGC	144	NM_001006457.2
SOD-1	TGATGACCTGGGTAGAGGGG	ACAACGGTTAGCACTTGGCT	104	NM_205064.2
MUC-2	TCACCCTGCATGGATACTTGCTCA	TGTCCATCTGCCTGAATCACAGGT	228	XM_040673077.2
ß-Actin	GTGGATCAGCAAGCAGGAGT	ATCCTGAGTCAAGCGCCAAA	182	NM_205518.2

Note, the abbreviations of the primer names are as the following: *GLP-1*: glucagon like peptide-1; *PGC-1α*: peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *SOD-1*: superoxide dismutase one; *MUC-2:* mucin-2.

Results and discussion

Growth performance

Effects of ALA supplementation on growth performance were displayed in Tables 3. The birds' growth performance parameters were negatively impacted by heat stress, as described by Lara and Rostagno [9]. The addition of ALA had no significant effects on the birds' body weight during the first three weeks of the experiment. On the fourth week of the experiment, heat stress did not significantly decrease the body weight, and on the other hand, the addition of ALA did not significantly elevate the body weight when compared with the control group. On the 5^{th} week of the experiment, heat-stressed birds had the lowest body weight, while the groups that received ALA showed the best body weight when compared with the control group. These outcomes were reached as a result of Wasti *et al.* [49],which showed a notable improvement in body weight after the addition of ALA. The results of Yoo *et al*. [50] showed an improvement in body weight with ALA supplementation. ALA supplementation had no effect on body weight gain during the first three weeks of the experiment. On the 4^{th} and 5^{th} weeks of the experiment, heat stress decreased the weight gain, and the addition of alpha-lipoic acid improved it. The results of Yoo *et al*. [50] and Zhang *et al*. [51] showed a significant good impact on body weight gain with ALA adding. During the first three weeks of the experiment, ALA supplementation significantly increased the feed intake when compared with the control groups. As a result of exposure to heat stress, birds reduced their feed utilization in an adaptation reaction to high temperatures on the 4^{th} and 5^{th} weeks. On the other hand, groups that were supplemented with ALA showed a significant elevation in feed intake in the same period.This study's findings were agreed upon with the results ofGuo *et al*. [31], while adding ALA to the broilers' diet caused a significant decrease in feed intake [51]. Yoo *et al*. [50] recorded that there are not significant changes in FI after ALA addition. The feed conversion rate of the birds was not affected by the addition of lipoic acid during the first three weeks of the experiment. High temperatures had a negative effect on the birds' feed conversion, as the temperature increased it non-significantly. The addition of lipoic acid moderated the increase in the feed conversion ratio caused by the heat, although this improvement was not significant.There were no differences in our results and those of Guo *et al*. [31], Yoo *et al*. [50], and Zhang *et al*. [51]. Murali *et al.* [52] reported that there are not significant changes in all growth performance parameters after ALA supplementation.

Table 3: Effects of dietary ALA on body weight, weight gain, feed intake and feed conversion ratio during the experiment in thermoneutral and heat stress groups.

Group	7 days	14 days	21 days	28 days	35 days
Body weight (g)					
Thermoneutral Control	202.50±9.68	481.25±9.66	913.75±31.32	1288.75±38.21	2012.50±42.70^{ab}
Thermoneutral ALA	203.75±2.39	487.50±7.77	925.00±47.87	1337.50±37.50	2132.50±13.31^{a}
Heat Stress Control	203.75±3.75	482.50±4.79	912.50±55.43	1212.50±62.50	1950.00±84.16^{b}
Heat Stress ALA	203.75±4.73	485.00±4.08	925.00±52.04	1221.25±12.64	2037.50±23.94^{ab}
Feed Intake (g)					
Thermoneutral Control	331.50±1.44^{b}	640.75±0.75^{b}	907.50±1.66^{b}	1071.75±1.38^{b}	
Thermoneutral ALA	362.75±1.31^{a}	672.75±0.85^{a}	942.50±1.04^{a}	1108.25±1.38^{a}	
Heat Stress Control	331.75±2.25^{b}	641.50±1.7^{b}	883.25±1.03^{c}	1022.25±1.97^{d}	
Heat Stress ALA	362.25±1.3^{a}	671.75±0.75^{a}	911.25±3.75^{b}	1040.00±0.71^{c}	
Feed conversion ratio (%)					
Thermoneutral Control	1.17±0.04	1.61±0.04	1.72±0.10	1.75±0.04^{bc}	
Thermoneutral ALA	1.16±0.05	1.55±0.08	1.69±0.02	1.67±0.08^{c}	
Heat Stress Control	1.17±0.04	1.62±0.02	1.81±0.02	1.97±0.05^{a}	
Heat Stress ALA	1.16±0.04	1.55±00.04	1.75±0.03	1.88±0.02^{ab}	
	7-14 days	14-21 days	21-28 days	28-35 days	
Body weight gain (g)					
Thermoneutral Control	283.75±8.26	471.25±20.85	627.50±15.48 ^{a}	742.50±32.56^{a}	
Thermoneutral ALA	285.00±5.40	493.75±42.59	660.00±16.33 ^{a}	772.50±31.26^{a}	
Heat Stress Control	282.50±5.95	467.50±21.26	508.75±15.60 ^{b}	621.25±11.97^{b}	
Heat Stress ALA	288.75±16.63	492.50±41.56	542.50±18.43 ^{b}	651.25±18.53^{b}	

$^{a, b, c}$ Means within the same column carrying different superscripts are significant different at (P value < 0.05).

3Biochemical parameters

Effects of alpha lipoic acid on liver functions

The results of serum total protein (g/dl), albumin (g/dl), ALT (U/L) and AST (U/L) in broiler chicks fed ALA under thermoneutral and heat stress is shown in Table 4. The serum total protein, albumin, ALT, and AST levels are all significantly raised by heat stress.Under thermoneutral or heat stress settings, the effects of ALA on serum total protein, albumin, ALT, and AST were significantly diminished. Our findings match the effects of HS on liver parameters discovered in another study[53], the findingsof Attia [54] were comparable with ours for ALT and AST but not for total proteinThe addition of 100 mg/kg of food to broilers given a diet high in animal fat had no appreciable effects on serum albumin but increased the level of total protein [55].Different types and doses of ALA were added to the broiler feed, and while this had no significant effects on serum total protein or albumin, it did reduce the activity of the liver enzymes ALT and AST [26]. According to our findings, feeding 250 mg of ALA/kg of diet to Japanese quails under HS reduced ALT and AST activity, but serum total protein and albumin levels did not alter significantly [56]. In contrast to the current investigation, heat-stressed broilers' ALT and AST activities did not significantly increase when 250 mg of ALA/kg/day was added to their feed [57].

Effects of alpha lipoic acid on total lipid

Table 4 shows the effects of dietary ALA administration on total lipids (mg/dl) in broilers reared under normal and thermal stress conditions. HS markedly increased serum total lipids. Dietary ALA non-significantly decreased the total lipid concentration in the TN-ALA and HS-ALA groups. Much research has been done on the negative effects of heat stress on the lipid profile. Chaturvedani *et al*. [6] reported a significant increase in serum cholesterol and triglycerides. Likewise, Mundim *et al*. [58] reported a considerable rise in serum cholesterol, triglycerides, LDL, and VLDL, while HDL recorded a non-significant increase. Various levels of alpha-lipoic acid reduced fat content [59]. Adding 100 mg ALA/kg to the broiler fed an animal fat diet reduced the serum triglycerides, cholesterol, LDL, and VLDL but augmented the HDL value [52]. The addition of 250 mg of ALA/kg diet to broilers exposed to heat stress showed a non-significant improvement in lipid profile parameters [57]. All parameters did not contrast with the previous study except cholesterol, which had a significant increase after the addition of 250 mg ALA to the basal diet of Japanese quail under HS conditions [56], and they hypothesized that the high cholesterol was caused by an increase in free radicals, a rise in cortisol, and changes in lipid metabolism.

Effects of alpha-lipoic acid on kidney functions

The effects of ALA addition to the broiler feed reared under normal and heat stress conditions on uric acid (mg/dl), urea (mg/dl), and creatinine (mg/dl) are presented in Table 4. In this study, heat stress considerably raised the serum uric acid and urea levels, but there was a non-significant difference in serum creatinine. The addition of ALA to the broiler diet had a non-significant reduction in uric acid and urea serum concentration under thermoneutral conditions, while it significantly dropped serum uric acid and urea under HS conditions. Either in

broilers reared under thermal stress or heat stress, there was a non-significant decrease in creatinine serum. Adding 300 mg/kg ALA to the basal diet of birds significantly dropped the uric acid creatinine [60]. Adding of 250mg ALA/kg/day to the broilers diet caused a non-significant reduction in serum uric acid levels [57]. These results could be explained by recalling the renoprotective and antioxidant properties of ALA, according to explanations of Kim *et al.* [26].

Oxidant /Antioxidant status

The addition ALA effects on catalase enzyme activity (U/ml) and malondialdehyde concentration (nmol/ml) in broilers under thermoneutral and thermal stress situations were presented in Table 5. Heat stress leads to a drop-in catalase activity and an excess of malondialdehyde concentration. Dietary ALA supplementation raised catalase activity and drops the MDA serum concentration. Many studies have shown that heat stress and oxidative stress have a negative impact on antioxidant status. Heat stress elevated the lipid peroxidation as a result of an increase in free radicals and ROS products [61], decreased activities of GSH-Px, catalase and SOD while MDA concentrations are noticeably increased [62].

In agreement with our findings, the addition of 500 mg ALA/kg diet increased the total antioxidant capacity, SOD and GSH-Px activities in plasma and liver while dropping the MDA concentrations [28]. ALA doses of 25, 75, and 150 mg/kg diet increased oxidative stability while decreasing lipid peroxidation [59]. Japanese quails were given a dietary ALA treatment of 250 mg/kg to the diet showed a significant elevation in catalase activity [63]

Table 4: Effects of dietary alpha lipoic acid on blood metabolites on 35 days of the experiment in thermoneutral and heat stress groups.

Group	Parameters							
	Total protein	Albumin	ALT	AST	Total lipid	Uric Acid	Urea	Creatinine
TNC group	3.05±0.08 ^{c}	1.15±0.09 ^{bc}	10.27±1.72 ^{b}	154.67±1.45 ^{c}	188.67±1.45 ^{b}	5.71±0.24 ^{c}	16.67±0.18 ^{c}	0.53±0.09 ^{ab}
TN-ALA group	2.47±0.08 ^{d}	0.99±0.01 ^{c}	8.65±1.54 ^{b}	150.67±1.45^{c}	179.33±0.67 ^{b}	5.52±0.22 ^{c}	16.07±0.18 ^{c}	0.43±0.09 ^{b}
HSC group	4.72±0.05 ^{a}	1.81±0.03 ^{a}	21.33±1.13 ^{a}	377.67±1.45^{a}	222.67±1.45 ^{a}	9.05±0.12^{a}	20.40±0.21 ^{a}	0.73±0.09 ^{a}
HS- ALA group	3.71±0.05 ^{b}	1.20±0.03 ^{b}	18.13±1.62 ^{a}	266.67±1.1.45 ^{b}	213.00±7.02 ^{a}	7.80±0.19^{b}	18.80±0.21 ^{b}	0.57±0.07 ^{ab}

$^{a-d}$ Means with different superscript letters within the same column are significantly different at (P value <0.05), in the determination of the interaction between the two factors (heat stress and treatments). ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 5: Effects of dietary -lipoic acid on serum oxidant /antioxidant status after 35 days in thermoneutral and heat stress groups.

Group	Parameter	
	Catalase	MDA
Thermoneutral Control	58.03±0.30 ^{b}	4.67±0.04 ^{c}
Thermoneutral ALA	60.93±0.30 ^{a}	4.65±0.04 ^{c}
Heat Stress Control	55.43±0.30 ^{c}	5.23±0.04 ^{a}
Heat Stress ALA	56.40±0.36 ^{c}	4.96±0.04 ^{b}

$^{a, b, c}$Means with different superscript letters within the same column are significantly different at (P value <0.05).
MDA: malondialdehyde.

Gene expression levels

The effects of dietary supplementation of ALA on the gene expression levels were presented in Table 6. Glucagon like peptide-1 *(GLP-1)* level expression is improved by the dietary addition of ALA either under normal or thermal stress conditions. In our study, the effects of ALA on serum glucose were not determined, but based on the results of previous studies, the addition of 250 mg of ALA to the basal diet of quails reared under high temperatures showed a significant decrease in glucose level [56]. On the other hand,Imık *et al*.[57] they reported a non-significant decrease in glucose concentration after ALA addition. The *PGC-1α* (peroxisome proliferator activated receptor gamma coactivator-1 alpha) expression level has been raised after the addition of ALA to the diet of broilers under thermoneutral or heat stress conditions. In the present study, confirmed the effects of this gene expression on total lipids, catalase enzyme activity, and MDA levels and the potential effect of ALA on its expression.

Table 6: Effects of dietary alpha lipoic acid on gene expression levels after 35 days in thermoneutral and heat stress groups.

Group	Parameters			
	Hypothalamus		Liver	Intestine
	GLP-1	*PGC-1α*	*SOD-1*	*MUC-2*
Thermoneutral Control	1.02±0.12 ^{c}	1.08±0.23 ^{b}	1.00±0.05 ^{b}	1.01±0.06 ^{b}
Thermoneutral ALA	1.97±0.09 ^{a}	1.82±0.08 ^{a}	1.79±0.18 ^{a}	1.63±0.01 ^{a}
Heat Stress Control	0.30±0.01 ^{d}	0.29±0.05 ^{c}	0.33±0.02 ^{c}	0.32±0.00 ^{c}
Heat Stress ALA	1.39±0.01 ^{b}	1.09±0.02 ^{b}	1.02±0.11 ^{b}	0.92±0.01 ^{b}

$^{a-d}$ Means with different superscript letters within the same column are significantly different at ($P<0.05$), in the determination of the interaction between the two factors (heat stress and treatments). *GLP-1*: glucagon like peptide-1; *PGC-1α:* peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *SOD-1:* superoxide dismutase one; *MUC-2:* mucin2.

In our study, adding dietary ALA to the broilers' diet had a significant increase in the expression level of *SOD-1* either under heat stress or thermoneutral conditions. The SOD enzyme activity in this trial was not estimated, but rather based on the findings of previous studies; the addition of ALA improved SOD activity. The addition of 500 mg of ALA/kg diet to oxidatively stressed broilers increased SOD activity in the liver and plasma [28]. The addition of 250 mg of ALA per kg of heat-stressed Japanese quail diet elevated activity of SOD [63].

Heat stress badly alters the expression of the *MUC-2* while the addition of ALA to the broiler diet under thermal and neutral conditions improves the expression of *MUC-2*. It's important to note that in the present study, the expression level of *PCG-1, SOD, and MUC2* in the HS-ALA group reaches that of the TNC group. Our findings are in line with the results of Wasti *et al.*[49] they found the addition of ALA mitigates the

bad effects of HS on the expression of *SOD-1* and *MUC-2*. Unfortunately, there are not enough previous reviews available on the effects of ALA on broiler chickens under heat stress, especially with regard to biochemical parameters. Most of the previous studies focused on studying the properties related to oxidative resistance and improving the status of antioxidants in living organisms.

Conclusion

Heat stress is a reaction between humans, animals, or plants and their surroundings. Heat stress affects growth performance, impaired liver and kidney functions, and antioxidant status. The addition of the potent antioxidant, alpha-lipoic acid, to the diet of heat-stressed broilers decreased total protein, albumin, ALT, AST, uric acid and urea. The serum creatinine was not affected by HS and ALA. Addition of ALA decreased MDA concentration and increased catalase activity. The expression levels of *GLP-1, PGC-1, SOD-1, and MUC-2* were improved. Dietary ALA is effective in the mitigation of the negative impact of heat stress.

Conflict of interest

There have been no conflicts of interes
t among the authors.

Acknowledgments

The authors want to offer sincere thanks to everyone who helped to perform and produce this work.

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الملخص العربي

آثار حمض ألفا ليبويك الغذائي على تخفيف التأثير السلبي للإجهاد الحراري في دجاج التسمين.

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تهدف هذه الدراسة إلى تقييم دور حمض الألفا ليبويك في تخفيف الاثار السلبية نتيجة للاجهاد الحراري على عدد من المعاملات البيوكيميائية و مضادات الأكسدة. في أول أيام الدراسة التي امتدت ل ٣٥ يوما تم تقسيم ٧٢ فرخا بعمر يوم و بوزن ٣±٤٥ جم إلى أربع مجموعات كالتالي : مجموعة محايدة : لم تتلق أي إضافات غذائية تحت الظروف الطبيعية من درجة الحرارة و الرطوبة ، مجموعة محايدة بإضافة غذائية: تمت إضافة 25 جم من حمض الالفا ليبويك لكل 100 كيلو من النظام الغذائي تحت الظروف الطبيعية من الحرارة و الرطوبة ، مجموعة الاجهاد الحراري: تعرضت الافراخ في هذه المجموعة إلى درجات حرارة عالية و لم تتلق أي إضافات غذائية ، مجموعة إجهاد حراري بإضافات غذائية : تعرضت أفراخ هذه المجموعة إلى درجات حرارة عالية و تمت إضافة ٢٥ جم من حمض الالفا ليبويك لكل 100 كيلو من النظام الغذائي . بداية من اليوم ال21 وحتى نهاية التجربة تم تعريض الأفراخ في المجموعات الحرارية إلى درجات حرارة (٤٠±٥ درجة مئوية) لمدة ثمان ساعات يوميا (من السابعة صباحا و حتى الثالثة عصرا). في نهاية التجربة تم اختيار أربعة طيور عشوائيا من كل مجموعة لأخذ العينات و لإجراء التحاليل .لم تؤدي إضافة حمض الألفا ليبويك إلى تغييرات ملحوظة في وزن الجسم و زيادة وزن الجسم و معدل التحويل الغذائي بينما تغيرت معدلات تناول العلف إيجابيا . ادت اضافة حمض الألفا ليبويك إلى انخفاض معدلات البروتين الكلي و الالبيومين و انزيمات الكبد و التي ارتفعت بفعل الاجهاد الحراري. انخفضت كل من قيم احماض اليوريك و اليوريا انخفاضا ملحوظا نتيجة لإضافة حمض الألفا ليبويك بينما لم تتأثر قيم الكرياتينين بهذه الإضافة . أدت إضافة حمض الألفا ليبويك إلى النظام الغذائي إلى تقليل مستويات الدهون الكلية في الدم . أيضا تحسنت حالة مضادات الأكسدة نتيجة لانخفاض قيم MDA و زيادة نشاط انزيم CAT . كما تم تعزيز مستويات التعبير الجيني للجلوكاجون مثيل الببتيد-1 (GLP-1) ، كما تم تعزيز مستويات التعبير الجيني لمستقبلات البيروكسيسوم المنشط بتكاثر البروكسيسوم 1 ألفا (PGC-1α) و فوق أكسيد ديسميوتاز-1 (SOD-1) و الميوسين-2 (MUC-2) في المجموعات التي تم إضافة حمض الألفا ليويك إليها.. أشارت النتائج إلى أن حمض الألفا ليبويك كان له تأثيرات إيجابية على بعض المعاملات البيوكيميائية و حالة مضادات الاكسدة و عززت التعبير الجيني لكل من الجينات (SOD-1,GLP-1,PCG-1α,MUC-2).