Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Apr - Jun 2017 / v.19 / n.2 / 289-296
http://dx.doi.org/10.1590/1806-9061-2016-0404

The Effects of Different Amount of Protein and Vitamin E Supplementation in Rations on Lipid and Antioxidant Metabolism of Broilers Exposed to Heat Stress

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■Keywords

Heat stress, Broiler, Protein, Vitamin E, Oxidative stress.

Submitted: October/2016
Approved: February/2017

ABSTRACT

Heat stress, causes economic losses and has negative effects on both broiler husbandry and animal welfare. Nutritional strategies are applied for minimizing the negative effects of heat stress. In the present study, at the finishing period (24-39 days of age) of heat stress, the effects of diet involving 21% and 19% proteins and vitamin E on lipid metabolism and antioxidant mechanism of action, aimed to be identified. This study was carried out in six groups as: HPC (24°C heat + 21% crude protein (CP)), HPS (34°C heat + 21% CP), LPC (24°C heat + 19% CP), LPS (34°C heat + 19% CP), HPSVE (34°C heat + 21% CP + Vitamin E) and LPSVE (34°C heat + 19% CP + vitamin E) groups. Superficial pectoral muscles (breast) and liver tissues were used for oxidative stress and antioxidant defence determinations. Triglyceride and cholesterol levels have also been determined in blood serums. During the research, it is found that heat stress increased serum triglyceride and cholesterol levels, where Vitamin E has recovered triglyceride levels limitedly and cholesterol levels significantly. It is also observed that the adverse effect of high temperature was directly related to oxidative stress. Protein levels and vitamin supplementation relatively ameliorated these adverse effects, suggesting the tissue specificity. Consequently, the importance of feeding strategies such as the presence of Vitamin E and protein ratios on broiler nutrition in heat stress was established.

INTRODUCTION

Broilers were very sensitive to the environmental temperature changes due to their rapid growth and hyperthermic characteristics. High environmental temperature lead to economic losses as well as adverse effects of animal welfare through decreasing feed consumption, restricting growth and increasing death rate in the flock (Syafwan *et al*., 2011). Animals exposed to stress, use the nutrients that improve performance, to be able to adapt to stress. The apportionment that goes to feed the body, is directed as follows; reproduction (30%), growth (30%), health (10%), and survival (30%) while during stress only 80% can be directed to health and 20% to survival (Siegel & Gross, 2014).

The most important precautions to be taken for minimizing or eliminating the negative effects of heat stress are: developing refractory breeds, regulating the growing conditions and feeding strategies. Nutritional strategies, regulating the feed and additives are applied to improve their performance on animals exposed to stress (Syafwan *et al.*, 2011; Dalólio *et al.*, 2015). These nutritional strategies aimed to get the maximum performance and affecting the metabolism at least from heat stress. Protein ratio of the diet plays a major role on bird performance and carcass quality. A positively relation is known between the protein ratio of diet and animal growth (Temim *et al.*, 2000b; Furlan *et al.*, 2004;

Gonzalez-Esquerra & Leeson, 2005; Faria *et al.*, 2007; Syafwan *et al.*, 2011). However, we have not found many reports about how the protein ratio of the diet affects the antioxidant metabolism at heat stress.

Free radicals formed as a result of metabolic activities, increases with high environmental temperature. Heat stress is characterized by reduced antioxidant status in poultry, resulting in increased oxidative stress. Antioxidants for the removal of free radicals, require more regular rations. One of the most widely used antioxidant is vitamin E for the removal of these free radicals (Maritim *et al.*, 2003; Habibian *et al.*, 2016).

Vitamin E is a major chain-breaking antioxidant in biological systems. An optimum response with supplementation of vitamin E in feed has been found to improve feed intake, body weight gain, feed efficiency, egg production and quality, nutrient digestibility, immune response and antioxidant status in poultry birds. Also, Vitamin E is a vitamin that cannot synthesize in the metabolism of poultry. Therefore, in order to satisfy the needs of the animals, it should be added to the diets (Khan *et al.*, 2011; Habibian *et al.*, 2016).

In the present study, lipid and antioxidant metabolism of broilers fed with 19% and 21% protein were determined in primarily comfort (24°C) and heat stress (34°C) conditions. Then, the effects of Vitamin E added to the diet of both proteins on these metabolism were investigated.

MATERIAL AND METHODS

Animals and experimental design

During the research a total of 60 male broilers (Ross 308) were divided into 6 treatment groups. Ingredients and chemical compositions of the basal diet were formulated according to NRC (NRC, 1984) guideline (Table 1). Chemical analyses of the diets were run using references of AOAC (AOAC, 1984).

This study was carried out in six groups as High protein control (HPC), High protein stress (HPS), Low protein control (LPC), Low protein stress (LPS), High protein stress and Vitamin E (HPSVE) and Low protein stress and Vitamin E (HPSVE). In the present study, until 10 days of age all groups were fed on a ration containing 23% of CP. Starting from the 11th day to the end of the trial until 35 days of age, the HPC, HPS and HPSVE groups were fed on a ration containing 21% of CP. On the other hand, the CP content of the ration fed to the LPC, LPS and LPSVE groups was 21% between days 11-25 and 19% between days 25-35 and the trial ended when the animals were 35-days-old. Finally, the HPSVE and the LPSVE groups were fed on a basal ration containing 21% and 19 % of CP respectively, and 150 mg/kg ration of Vitamin E and was exposed to an environmental temperature of 34°C. In the present study, feeds of animal origin were not used as protein or energy sources.

Table 1 – Ingredients of crude nutrient proportions in the basal ration

	Crude protein		
Ingredients	23%	21%	19%
Corn	56.99	58.74	64.16
Corn glutein	20.00	20.00	20.00
Wheat short*	7.00	7.00	7.00
Soybean oil	0.78	3.72	3.22
Soybean meal	11.53	7.14	1.99
Calcium carbonate	1.36	1.23	1.18
Dicalciumphosphate	1.06	0.91	1.00
L-lysine	0.40	0.42	0.56
Salt	0.26	0.27	0.27
Vitamin-mineral premix**	0.20	0.20	0.25
Toxin binder	0.10	0.10	0.10
Anticoccidial	0.10	0.10	0.10
Sodium bicarbonate	0.10	0.09	0.09
Growth factor	0.05	0.05	0.05
Phyzyme XP TPT	0.03	0.03	0.03
DL-Methionine	0.04	-	-
Nutritional levels			
Metabolic energy kcal/kg	3000	3200	3200
Ether extract	3.46	6.37	5.98
Crude fiber	2.79	2.68	2.64
Methionine	0.5	0.43	0.41
lysine	1.10	1.0	1.0
Calcium	1.00	0.90	0.90
Phosphorus	0.7	0.65	0.65

*the additives (ascorbic acid, alfa lipoic acid) were added in place of wheat short

**the vitamin-mineral premix provides the following (per kg): all-trans-retinyl acetate 1.8 mg; all-rac-α-tocopherol acetate 1.25 mg; menadione sodium bisulphate 1.1 mg; riboflavin 4.4 mg; thiamine (thiamine mononitrate) 1.1 mg; vitamin B6 2.2 mg; niacin 35 mg; Ca-pantothenate 10 mg; vitamin B12 0.02 mg; folic acid 0.55 mg; d-biotin 0.1 mg; choline chloride 175 mg; manganese (from manganese oxide) 40 mg; iron (from iron sulphate) 12.5 mg; zinc (from zinc oxide) 25 mg; copper (from copper sulphate) 3.5 mg; iodine (from potassium iodide) 0.3 mg; selenium (from sodium selenite) 0.15 mg.

HPC: 24°C heat + 21% CP (After 11th day)

HPS: 34°C heat + 21% CP (After 11th day)

LPC: 24°C heat + 21% CP (between 11-24 days)+ 19% CP (After 25th day)

LPS: 34°C heat + 21% CP (between 11-24 days)+ 19% CP (After 25th day)

HPSVE: 34°C heat + 21% CP + Vitamin E (After 11th day)

LPSVE: 34°C heat + 21% CP (between 11-24 days)+19%CP+vitamin E (After 25th day)

Until the 14th day of the trial, all animals were raised at comfort temperatures (the temperature was gradually decreased from 36°C to 24°C). From the 15th day onwards till the end of the trial, the temperature to which the animals were exposed was 24°C for the HPC and LPC groups, and 34°C for the HPS, HPSVE, LPS and LPSVE groups, between 08:^{00}-6:^{00} h. All groups were reared on a lighting cycle of 17 h light/day.

Biochemical Analyses

Male broilers grow up faster than female broilers. Therefore, it can be thought that the experiment, six male broilers selected randomly from every group were decapitated, and the analysis was made on their breast (superficial pectoral) muscles and liver tissues. Breast muscle and liver tissues were homogenized by using liquid nitrogen and then stored at -80 °C until the biochemical investigations was made.

Biochemical parameters such as triglyceride and cholesterol in the serum were analyzed immediately through an automatic analyzer (Cobas 6000 analyzer, Roche) with commercial test kits.

Superoxide dismutase (SOD) and Catalase (CAT) enzyme activities and the amounts of Glutathione (GSH) and Lipid Peroxidation (LPO) in the tissues were determined. To prepare the tissue homogenates, the muscles tissues were grounded with liquid nitrogen in a mortar. A 0.5 g of tissue was then treated with 4.5 ml of appropriate buffer (SOD: pH 7.4/0.2 mM Tris–HCl buffer, CAT: pH 7/50 mM phosphate buffer, GSH: pH 7.4/50 mM Tris–HCl buffer, LPO: %10 KCl solution). The mixtures were homogenized on ice using an ultra-turrax homogenizer for 15 min. Homogenates were filtered and centrifuged at 4 °C. Then, these supernatants were used for biochemical measurements. All biochemical measurements were carried out using a UV–Vis spectrophotometer.

Determination of LPO

The level of LPO in tissues was determined by estimating malondialdehyde (MDA) using the thiobarbituric acid test (Ohkawa *et al.*, 1979). The muscles were scraped, weighed and homogenized in 10 ml of 100 g/l KCl. The homogenate (0.5 ml) was added with a solution containing 0.2 ml of 80 g/l sodium laurylsulphate, 1.5 ml of 200 g/l acetic acid, 1.5 ml of 8 g/l 2-thiobarbiturate and 0.3 ml of distilled water. The mixture was incubated at 98 °C for 1 h. Upon cooling, 5 ml of n-butanol: pyridine (15:l) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of supernatant was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane. The recovery rate was over 99%. The results were expressed as nmol MDA/g tissue.

SOD activity

SOD Activity was measured according to Sun *et al.* (1988). SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which reacts with nitro blue tetrazolium (NBT) to form formazan. SOD activity was then measured at 560 nm by the degree of inhibition of this reaction, and was expressed as mmol/min/mg of tissue.

Total GSH

The amount of GSH in the tissues was measured according to the method described by Sedlak & Lindsay (1968), with some modifications. The muscle´s tissues were homogenised in 2 ml of 50 mM Tris–HCl buffer containing 20 mM EDTA, at pH 7.5. After adding 2 ml ethanol (to precipitate the proteins), the homogenate was centrifuged at 3000 g for 40 min at 4 °C. The supernatant was used to determine GSH level using 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB). The absorbance was measured at 412 nm. Following the GSH level of the muscles was expressed as nmol/g tissue.

CAT activity

Decomposition of H_2O_2 in the presence of CAT was followed at 240 nm (Aebi, 1984). The CAT activity was defined as the amount of enzyme required to decompose 1 mmol of H_2O_2 per minute, at 25 °C at pH 7.8. Results were expressed as mmol/min/mg tissue.

Statistical analyses

All statistical analyses have been made with the SPSS 20 package software (SPSS, 2011). All values measured were tested with One-way ANOVA. Differences between the groups were determined by the Duncan Multiple Comparison Test with the $p<0.05$ value for significance.

RESULTS

LPO amounts of liver and breast tissues were shown in the Table 2 and 3 respectively. Difference of protein concentrations causes interesting results. Although LPO levels are decreased in breast tissues depending on the protein concentration ($p<0.05$), contrarily LPO wasn´t affected by protein amounts of the diet in liver.

Heat stress lead to increase LPO ratio of liver tissues only in low protein fed broilers where high protein

Table 2 – Effects of dietary protein levels and Vit E on antioxidant system of liver tissues of broilers exposed to heat stress.

	LPO (nmol MDA/mg tissue)	SOD (mmol/min/mg tissue)	GSH (nmol/mg tissue)	CAT (mmol/min/mg tissue)
HPC	0.913±0.101^{a,b}	0.171±0.006^{b}	0.160±0.008^{b}	2.612±0.053^{b}
LPC	0.754±0.093^{b}	0.188±0.005^{b}	0.219±0.055^{a}	2.073±0.064^{c,d}
HPS	1.153±0.112^{a}	0.181±0.006^{b}	0.149±0.010^{b}	2.183±0.048^{c}
LPS	1.062±0.119^{a}	0.179±0.013^{b}	0.126±0.008^{b,c}	1.974±0.043^{d}
HPSVE	0.811±0.054^{b}	0.258±0.009^{a}	0.084±0.001^{c}	2.493±0.071^{b}
LPSVE	0.320±0.025^{c}	0.233±0.013^{a}	0.088±0.001^{c}	3.579±0.105^{a}

The results were considered significant when $p<0.05$. Figures having the similar letters are not statistically significant ($p>0.05$). Results are given as means ± Standard Error. The number of broilers (N) is 6.

Table 3 – Effects of dietary protein levels and Vit E on antioxidant system of breast tissues of broilers exposed to heat stress.

	LPO (nmol MDA/mg tissue)	SOD (mmol/min/mg tissue)	GSH (nmol/mg tissue)	CAT (mmol/min/mg tissue)
HPC	0.514±0.101^{b}	0.220±0.010^{b}	0.155±0.005^{c}	0.353±0.046^{c}
LPC	1.073±0.096^{a}	0.314±0.012^{a}	0.154±0.006^{c}	0.643±0.038^{a}
HPS	1.084±0.116^{a}	0.153±0.011^{c}	0.250±0.015^{a}	0.203±0.008^{d}
LPS	1.279±0.099^{a}	0.237±0.005^{b}	0.205±0.008^{b}	0.454±0.021^{b}
HPSVE	0.685±0.075^{b}	0.248±0.008^{b}	0.122±0.003^{d}	0.585±0.026^{a}
LPSVE	0.251±0.015^{c}	0.238±0.011^{b}	0.152±0.001^{c}	0.608±0.014^{a}

The results were considered significant when $p<0.05$. Figures having the similar letters are not statistically significant ($p>0.05$). Results are given as means ± Standard Error. The number of broilers (N) is 6.

fed animals were not affected ($p>0.05$). On the other hand in breast meat, heat stress increase LPO levels only in high protein fed animals where low protein fed animals already have a sign of higher LPO ratio. Vitamin E decreased LPO levels, increased by heat stress in both liver and breast tissues. Interestingly this decrease is much more in low protein fed broilers than that of the high ones.

CAT activities of liver and breast tissues were shown in Table 2 and 3 respectively. Protein concentrations cause very significant changes on CAT activity in breast muscle and liver tissues. CAT activity is increased in proportion to the protein concentration in liver. Contrarily, increasing protein concentration decrease CAT activity in breast ($p<0.05$).

Stress inhibits CAT activity in both breast muscle and liver tissues ($p<0.05$). However, a limited decrease is not statistically significant, only in low protein fed groups of liver tissues ($p>0.05$). On the other hand in both liver and breast muscle tissues, Vitamin E enhanced the CAT activity, which is decreased by stress ($p<0.05$).

Table 2 and 3 show SOD activities of liver and breast tissues respectively. SOD activity is decreased inversely proportional to the protein concentration in breast tissues ($p<0.05$). However, protein concentration does not affect SOD in liver tissues ($p>0.05$). Stress decreased SOD activity only in the breast where liver tissues were not affected. SOD activity is found higher in liver tissues of Vitamin E fed broilers ($p<0.05$). On the other hand, Vitamin E has no effects on decreased SOD activity in LPSVE group ($p>0.05$). But Vitamin E increased SOD activity in HPSVE group ($p<0.05$).

Table 2 and 3 respectively show the GSH levels of liver and breast tissues. Heat stress caused increasing GSH levels in proportion to the protein concentration in breast tissues ($p<0.05$). In contrast, this stress aimed to decrease GSH in liver. But this decrease is not statistically significant in high protein fed animals ($p>0.05$). Vit E also aimed to decrease GSH levels under stressed animals. However, this decrease is not statistically significant in liver tissues ($p>0.05$).

Heat stress enhanced the triglyceride amounts where Vitamin E decreased in a limited range which was not statistically significant (Table 4). Likewise, stress caused an increase on cholesterol levels. The increase in high protein fed animals is significant while low protein fed is not. Adding Vitamin E to the diet caused signs of liver cholesterol levels on animals under stress (Table 4).

DISCUSSION

Today heat stress is one of the most significant problems encountered in poultry breeding and responsible for economic loses. Animals under stress use the consumed nutrients to ensure the adaptation of stress instead of improving their metabolic parameters. Heat stress adversely affects the physiological and metabolic events of the organism. In hot environments,

Table 4 – Effects of dietary protein levels and Vit E on lipid levels of blood serum of broilers exposed to heat stress.

	Triglyceride (mg/dl)	Cholesterol (mg/dl)
HPC	116.860±8.316[b]	142.140±9.473[b]
LPC	119.710±6.015[b]	142.290±7.286[b]
HPS	137.860±5.161[a]	155.140±6.864[a]
LPS	138.290±9.446[a]	148.860±4.595[a,b]
HPSVE	135.860±10.703[a]	140.121±7.017[b]
LPSVE	132.570±6.561[a,b]	132.710±4.010[c]

The results were considered significant when $p<0.05$. Figures having the similar letters are not statistically significant ($p>0.05$). Results are given as means ± Standard Error. The number of broilers (N) is 6.

the chemical composition of chickens is changed and carcass quality is decreased by heat stress (Gu *et al.*, 2008). Many possibilities were suggested to explain the negative effects on performances. The oxidative damage of tissues induced by heat stress is one of the possible reasons of the increase of the production of free radicals, which converts the balance between oxidants and antioxidants, in favour of the oxidants (free radicals) (Maritim *et al.*, 2003; Syafwan *et al.*, 2011; Habibian *et al.*, 2016; Tufarelli *et al.*, 2016).

Various nutritional strategies have been suggested to overcome the negative impact of heat stress in broilers. Especially recent researches have focused on dietary protein levels, and one other favorite way is adding antioxidants to the ration (Temim *et al.*, 2000a; Temim *et al.*, 2000b; Gonzalez-Esquerra & Leeson, 2006; Syafwan *et al.*, 2011). Therefore, we have focused on; how antioxidants system affected from protein level and vitamin content, which has to be, organized to resolve or to minimize the negative effects of stress.

Although free radicals directly affect several molecule and compounds, these radicals primarily damage the membrane proteins by causing lipid peroxidation (LPO) in membranes through attacking the unsaturated fatty acids. The damage to membrane proteins decreases the membrane permeability, the activities of enzymes and receptors and the activation of cells. Thus, LPO is accepted as the indicator of oxidative stress following with autocatalytic chain reactions, as collectively leading to damages in many biological structures (Davies & Goldberg, 1987; Halici *et al.*, 2012; Habibian *et al.*, 2016). It is known that heat exposure reduces body protein deposition and increases body fat deposition (Faria *et al.*, 2007). Thus, it is expected that heat stress should increase LPO levels and Vitamin E to restore them. However, in our research (Table 2 and 3), heat stress lead to increase LPO ratio of liver tissues only in low protein fed broilers (LPS group) where high protein fed animals (HPS group) was not affected. On the other hand, in breast meat, heat stress increased LPO levels not only in high protein fed animals (HPS group) but also low protein fed animals (LPC and LPS groups) already had a sign of higher LPO ratio. Gonzalez-Esquerra & Leeson (2006) declared heat stress has lipogenic effect on birds, and thigh muscles have greater capacity for lipid oxidation.

On the other hand, we have found that low protein (LPC group) in liver reduce the LPO levels, likewise that of the high protein (HPC group) done in breast, which means that sensitivity of tissues against oxidative stress is different. Therefore, the antioxidant response changes according to tissues. Actually, our findings support this idea. Unlike to liver tissues, SOD and CAT activities of low protein fed animals (LPC and LPS groups) are significantly higher than high protein fed animals (HPC and HPS groups) in breast tissues. Increasing activities of any enzyme can be related with an enhanced substrate production during the metabolic processes. Indeed, the accumulation of the substrates of SOD and CAT enzymes leads to increase the activities, which might be responsible for an increased LPO following the heat stress (Halici *et al.*, 2012). Vitamin E decreased LPO levels, increased by heat stress; in both liver and breast tissues are already expected. Interestingly this decrease is much more in low protein fed broilers (LPSVE groups) than that of the high ones (HPSVE groups) (Table 2 and 3).

Despite we have not found many reports, our findings are also consistent with previous studies about heat stress lead to increase LPO levels in liver (Ramnath *et al.*, 2008; Tan *et al.*, 2010) and in meat tissues (Imik *et al.*, 2010; Halici *et al.*, 2012). Temim *et al.* (2000a) carried out a research that animals fed with diets containing 19% and 24% protein, at 22°C and 32°C. In this study, Temim *et al.* (2000a) found that heat stress adversely affects the conversion metabolism of proteins to the muscles, and increasing protein percentage of the diet partially prevented this negative effect.

In a hot environmental, chickens grow and lay by exerting an effort to maintain their body temperature within a normal range, to cope with stress responses and to ensure their visceral organs function under a heavier heat burden (Lin *et al.*, 2006). Therefore, metabolic effects of heat stress may differ from tissue to tissue. Heat stress tended to decrease SOD and CAT activities, which means that the defence system is deactivated by heat stress. On the other hand, neither protein concentration nor heat stress has any

effect on SOD activity in liver. As expected, Vitamin E tried to recover SOD activities. However, Vitamin E did not show any effect in breast tissues of low protein fed animals (LPSVE group). Similarly, stress tended to decrease CAT activity and Vitamin E aimed to raise more (Table 2 and 3). Our findings are also similar to previous studies showing the activities of CAT and SOD were different in liver and breast tissues (Ozturk-Urek *et al.*, 2001; Ramnath *et al.*, 2008; Yardibi & Hosturk, 2008; Tan *et al.*, 2010; Moshera & Seliem, 2011).

We use GSH as a marker used to understand how defence system acts directly, and ROS amount indirectly. When the GSH levels are insufficient, peroxides may accumulate therein causing several damages. Organisms use the GSH to eliminate several peroxides and other radicals. Heat stress raised GSH levels in proportion to the protein concentration in breast tissues (Table 3). In contrast, this stress aimed to reduce GSH in liver. But this decrease is not statistically significant in high protein fed animals (Table 2). Both protein synthesis and breakdown plays critical role on GSH production. When cysteine, glutamic acid, and glycine amino acids levels are inadequate, organism will not be sufficient to produce GSH. Birds exposed to heat stress, aimed to increase their plasma volume in order to reduce the amount of heat to be dissipated. This may reduce the levels of some blood components, such as amino acids that partially elucidates the lower plasma amino acids values reported in birds at high temperatures. Similar events could also reflect significant changes in muscle protein turnover taking place during hyperthermia (Geraert *et al.*, 1996; Gonzalez-Esquerra & Leeson, 2006). Therefore, protein turnover has great importance on GSH levels. On the other hand, protein turnover differs from tissue to tissue.

Biomolecules such as GSH, vitamin E and α-lipoic acid are used in a circle for regeneration of each other. Thus, under stressed animals, metabolism may prefer to consume GSH in donating one electron to Vitamin E. An optimum response with supplementation of vitamin E is, as important as protein ratio, in feed has been found to improve feed intake, body weight gain, feed efficiency, egg production and quality, nutrient digestibility, immune response and antioxidant status in poultry birds.

Animals under stress, several changes occur in some metabolic parameters to ensure the adaptation of stress. Some of these varying parameters are; plasma concentrations of glucose, total protein, triglyceride, cholesterol, non-esterified fatty acids and VLDL (Siegel & Gross, 2014; Imik *et al.*, 2009). Serum triglyceride and cholesterol levels were showed in Table 4. Parameters return to normal levels when animals adapted to stress. In the present study, the protein ratio of diet did not cause any difference on cholesterol and triglyceride amounts. On the other hand, heat stress enhance triglyceride amounts while Vitamin E tended to decrease them. Heat stress caused an increase only in the high protein feed group (HPS group) where low protein relatively protected from enhanced cholesterol levels (LPS group). Also the increase of cholesterol in LPS group is not significant as the decrease caused by Vitamin E on triglyceride amounts. Our findings are also similar to previous studies showing that heat stress inclined to enhance the cholesterol and triglyceride amounts while Vitamin E tended to decrease them (Azzi & Stocker, 2000; Bayraktar *et al.*, 2011).

The increasing ratio of poultry production in tropical and subtropical regions makes it necessary to reconsider the selection strategies of today's commercial breeding programmes. Of these, the most promising strategies are generally focused on modifying protein levels as well as vitamin and mineral supplements to enhance the thermotolerance. When defining protein levels of a diet, one has to consider not only technical and economic aspects but also effects of such diets on the metabolism in broilers. In present study, it was observed that both antioxidant metabolism of liver and breast muscle was markedly different. Additionally, we tried to determine the diet protein rates and vitamin E efficacies on antioxidant and lipid metabolism by examining liver and breast tissues with serum of chickens which are exposed to heat stress. The extensive research is needed for clarifying in this subject. Finally, as well as tissue antioxidant system and serum triglyceride levels, the chickens' adaptation capability against to long-term heat stress is shown in present study.

ACKNOWLEDGEMENTS

This study was approved from Ataturk University Faculty of Veterinary Medicine ethic committee with the Decision No: 2007/5f. The research was performed at the research and application centre of Veterinary Medicine Faculty at Ataturk University.

We also thank Ataturk University Scientific Research Unit for their kind project supports (BAP 2007-189).

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