COMPARATIVE SERUM BIOCHEMICAL PROFILE OF TWO BROILERS STRAINS SUPPLEMENTED WITH L-CARNITINE, ALPHA-TOCOPHEROL AND RAW GINGER UNDER HIGH AMBIENT TEMPERATURES

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ABSTRACT

The effect of dietary supplementation of alpha-tocopherol, L-carnitine and ginger on blood biochemical profile of Hubbard and Cobb under the summer season was evaluated. A total of three hundred and twenty broiler chicks (both strains) were divided into two groups and four subgroups. In each strain subgroup, one was kept as control while other sub groups were provided with alpha-tocopherol, ginger and L-carnitine in basal diets until sixth week. Average temperature and humidity were 31.89 °C and 58.26% respectively. Supplementation of the diet with L-carnitine, ginger and alpha-tocopherol significantly ($P<0.01$) decreased mean serum cholesterol, ALT, glucose, triglyceride, LDL and increased HDL serum protein. The results of alpha-tocopherol were comparatively better than the other two additives. It is concluded that supplementation of diets with ginger, alpha-tocopherol and L-carnitine improve broiler blood biochemical parameters. The blood biochemical parameters did not vary between the broiler strains.

Keywords: Cholesterol, glucose, liver health, antioxidants, heat stress.

INTRODUCTION

In the present time, a very high number of broiler farms exist in tropical and subtropical zones of the world and the majority are still operating under the open sided housing system (Khan *et al*., 2012a; Chand *et al*., 2018) that adversely affects the physiological biochemistry of broiler reared under high ambient temperature (Chand *et al*., 2018). In tropical and sub-tropical countries, the primary problem in summer seasons is heat stress (Chand *et al*., 2016). Since the poultry farmers cannot afford expensive artificial control shed, therefore, heat stress negatively affects the broiler production and physiology (Khan *et al*. 2011).

Blood is a reservoir of nutrient, metabolic waste, and hormonal exchange. Blood biochemical profile provides some important information about the health of animals. High temperature increases serum alanine amino transaminase (ALT) and aspartate amino transferase (AST) (Shah *et al*., 2016), glucose (Ihsanullah *et al*., 2017), cholesterol and triglyceride (Chand *et al*., 2018) in farm animals. Different methods have been reported to reduce the negative impact of heat stress in poultry (Khan *et al*., 2012a). Because of the high cost of cooling animal buildings, focus has been diverted mostly on the products of supplementation (Khan *et al*., 2011). Nutritional strategy during summer high temperature is based on diet balancing for protein, energy and electrolytes in order to cover the need in heat stressed birds (Khan *et al*., 2014).

Alpha-tocopherol is a vital component in poultry diets to reduce the oxidative and heat stress in poultry (Shah *et al*., 2016; Zia ur Rehman *et al*., 2017). Alpha-tocopherol supplementation significantly decreased serum ALT in broilers under high temperature (Shah *et al*., 2016), glucose (Habibian *et al*., 2014) and increases serum protein (Zia ur Rehman *et al*., 2017). Ginger (*Zingiber officinale*) is an herbal plant being widely used for a variety of pharmacological purposes (Khan *et al*., 2012b). Ginger is a source of some very important alkaloids such as general, gingerdione, gingerdiol and shogaols (Zia ur Rehman *et al*., 2017). These compounds have antimicrobial, antioxidative and other pharmacological uses (Rehman *et al*., 2011; Khan *et al.,* 2013). Ginger extract reduces serum ALT, AST and glucose, and increases serum protein in broiler (Rehman *et al*., 2011). Supplementation of L-carnitine decreases serum ALT and AST, cholesterol and triglyceride in broilers (Hassan *et al*., 2011). Similarly, blood cholesterol significantly decreased in broilers supplemented with L-carnitine (Zhang *et al*., 2010).

This study investigated the suitability and efficacy of ginger, L-carnitine and alpha-tocopherol on biochemical and lipid profile of two broiler strains exposed to summer high ambient temperature.

MATERIALS AND METHODS

Experimental plan and bird husbandry: A total of three hundred and twenty day-old broiler chicks of Hubbard and Cobb strains were obtained from the local hatchery. The chicks were divided into two groups randomly (160 chicks/group). Each strain was further divided into four subgroups (40 chicks/group) with four replicates (having 10 chicks per replicate). Each strain had a control while the other subgroups were provided with ginger, alpha-tocopherol and L-carnitine at the rate of 2%, 250 and 500 mg/kg respectively. The chicks were reared according to brooding protocol for the first 14 days. Sawdust was used as bedding material. On day 15, chicks were exposed to under natural summer months in the open sided house. The stocking density of birds was 1 square feet/bird. Birds had free access to feed and water throughout the experiment. A 24 hours light program was provided throughout the experiment. Synthetic alpha-tocopherol and L-carnitin were procured from Khyber Medicine, Karachi Market, Peshawar, Paksitan). Ginger was obtained from the vegetable market in fresh condition. It was sundried and then minced to powder. The powder was added into the feed at the given concentration of 2%. The supplementation was continued from day 15 to 42. Mortality was counted whenever it occurred.

Temperature and humidity data: The house temperature and humidity data was recorded four times a day throughout the experiment The mean values of the temperature and humidity are given in Table 1.

Serum biochemical profile: For determination of blood biochemical profile, samples were collected from randomly selected three birds per replicate on weekly basis. Blood samples were centrifuged at 2000 rpm for 10 minutes to separate serum and analyzed for AST, ALT, glucose, triglyceride, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and total protein with the help of commercial kits (RANDOX CA, USA) using a spectrophotometer (Microlab 300, Holland).

Statistical analysis: Data were analyzed by two factorial completely randomized design with four diets and two strain. The least significant test was used to compare the differences among treatment. All statistical analysis was carried out using statistical package SAS (SAS Institute, 1992) using completely randomized (CRD) design.

RESULTS

Mean serum AST decreased significantly (P<0.01) in birds fed with alpha-tocopherol supplementation on day 21, 28, 35 and 42 compared to the control (Table 3). Mean serum AST did not vary (P>0.05) between ginger and L-carnitine on day 28, 35, and 42 and between the two strain.

Mean serum ALT decreased significantly (P<0.01) in alpha-tocopherol supplemented group compared to the control (Table 4). Serum ALT did not vary significantly (P>0.05) between the control and the L-carnitine treated birds. On day 42, alpha-tocopherol supplementation decreased serum ALT compared to the ginger fed birds.

Throughout the experiment, decreased mean serum glucose (P<0.01) was recorded in the supplemented group compared to the control (Table 5). Significantly (P<0.01) low serum glucose in alpha-tocopherol supplemented birds was observed compared to L-carnitine on day 28 and 35.

Significantly (P<0.01) high serum protein in birds fed with alpha-tocopherol supplementation throughout the experiment was observed (Table 6). It did not vary (P>0.05) between ginger and L-carnitine supplemented birds throughout the experiment.

Significantly (P<0.01) low serum cholesterol in birds supplemented with L-carnitine was observed (Table 7). Ginger and alpha-tocopherol supplemented birds did not show any significant difference in the two strains during the experimental period.

The results indicated that mean serum triglyceride was significantly (P<0.01) low in birds supplemented with L-carnitine during the whole period of the experiment (Table 8). It did not vary between the alpha-tocopherol and L-carnitine supplemented birds except day 35.

The concentration of LDL was significantly (P<0.01) lower in the treatment groups on day 21 compared to the control (Table 9). At the other stages of the experiment, significantly (P<0.01) low LDL was recorded in the L-carnitine treated group compared to the control.

Serum HDL did not vary between ginger and L-carnitine supplemented birds on all recorded stages (days) of the experiment (Table 10). No significant (P>0.05) change was observed in the two strains during the experimental period.

Table 1. Mean temperature and relative humidity during the experimental period

Hour	Ambient temperature (°C)	Relative humidity (%)
08:00	31.66 ±0.94	51.65 ± 0.32
12:00	36.43±0.12	52.29 ±0.91
16:00	33.25±0.45	65.32 ±0.47
20:00	31.62±0.82	64.43 ±0.78
24:00	29.85±0.91	60.67 ±0.82
04:00	28.87±0.43	55.22 ±0.11

Table 2. Composition of basal diets.

Ingredients	Starter (%)	Finisher (%)
Maize	57.53	60.7
Soybean meal (CP 46%)	35.24	35.6
Vegetable oil	2.37	0.5
Limestone	1.4	1.4
Dicalcium phosphate	2.3	1.2
DL Methionine	0.2	0.15
Salt	0.46	0.15
Vitamin + trace mineral premix[1]	0.5	0.3
Total	100	100
Analyzed composition		
Protein (%)	23.12	22.00
Poultry ME (kcal/kg)	3000	2960
Calcium (%)	1.05	1.00
Available phosphorous (%)	0.5	0.45
Lysine (%)	1.30	1.3
Methionine (%)	0.55	0.65

The premix provided the following per kilogram of diet: pyridoxine, 1mg; folic acid, 0.4mg; molybdenum, 0.32mg; ethoxyquin, 25mg; choline chloride, 60mg; dI-α-tocopherol acetate; 4mg; iodine, 0.2mg; thiamine, 0.3mg; Ca pantothenate, 3mg; cyanocobalamin, 3μg; biotin, 0.02mg; Mn, 15mg; Zn, 10mg; iron, 4mg; Cu, 1mg; Co, 0.06mg; Se, 0.02mg; cholecalciferol, 0.018mg; *trans*-retinol, 0.66mg; menadione, 0.4mg; riboflavin, 1.6mg; niacin, 6mg.

Table 3. Mean serum AST (U/L) level of fast growing broiler strains during heat stress **fed on alpha-tocopherol, ginger and** L-carnitine.

	Diets				Strains		P-value		
							Diets	Strains	Diet × strain
	Control	Alpha-tocopherol	Ginger	L-carnitine	Hubbard	Cobb			
Day 21	188.8±4.35^{a}	161.3±1.2^{c}	191.3±1.5^{c}	170±1.97^{b}	172.5±2.34	173.1±1.35	0.0001	0.06	0.49
Day 28	241.3±10.94^{a}	198.8±6.79^{c}	200.0±7.28^{bc}	293.8±5.40^{b}	214.4±9.3	212.5±4.32	0.0008	0.72	0.28
Day 35	237.5±3.89^{a}	208.8±1.3^{c}	225.0±1.56^{bc}	226.2±2.00^{b}	208.8b±3.80	217.5a±2.25	0.000	0.14	0.60
Day 42	282.5±7.25^{a}	240±3.01^{c}	258.7±2.44^{bc}	262.5±5.10^{b}	268.8±5.49	258.1±4.34	0.0000	0.09	0.29

Means in the same row with different superscripts are significantly different (P<0.01)

Table 4. Mean serum ALT (U/L) level of fast growing broiler strains during heat stress **fed on alpha-tocopherol, ginger and** L-carnitine.

	Diets				Strains		P-value		
	Control	Alpha-tocopherol	Ginger	L-carnitine	Hubbard	Cobb	Diets	Strains	Diet × strain
Day 21	193.25±3.74[a]	172.13±4.38[c]	167.13±2.14[c]	193.25±3.74a	181±3.75	176.75±2.97	0.0000	0.19	0.36
Day 28	220.75±4.27[a]	192.25±4.13[c]	198.5±4.48[bc]	220.75±4.27a	205.81±4.83	203.94±2.93	0.0006	0.66	0.43
Day 35	238.38±2.29[a]	208.50±7.92[c]	214.75±2.78[bc]	238.38±2.29a	224.50±3.32	218.44±5.40	0.0008	0.20	0.06
Day 42	284.50±3.71[a]	233.38±4.91[c]	266.13±3.26[b]	284.50±3.71[a]	265.19±5.10	262.13±5.79	0.0000	0.43	0.74

Means in the same row with different superscripts are significantly different (P<0.01)

Table 5. Mean serum glucose concentration (mg/l) of fast growing broiler strains during heat stress **fed on alpha-tocopherol, ginger and L-carnitine.**

	Diets				Strains		P-value		
	Control	Alpha-tocopherol	Ginger	L-carnitine	Hubbard	Cobb	Diets	Strains	Diet × strain
Day 21	282±3.69[a]	241.38±7.15[b]	248.63±7.02[b]	257.25±5.42[b]	255.88±4.76	258.75±6.57	0.0007	0.64	0.46
Day 28	295.75±3.05[a]	249.63±7.49[c]	256.38±4.05[c]	276.87±3.76[b]	272.19±4.20	267.13±6.90	0.0000	0.30	0.36
Day 35	286.75±2.78[a]	248.25±5.60[c]	264.75±3.74[b]	275.13±4.77[b]	270.38 ±4.46	267.06±4.91	0.0000	0.40	0.12
Day 42	281.25±2.31[a]	236.0±4.54[c]	242.23±4.18[bc]	252.42±6.49[b]	246.54 ±2.33	258 ±3.26	0.0001	0.07	0.45

Means in the same row with different superscripts are significantly different at (P<0.01)

Table 6. Mean total serum protein (g/dl) of fast growing broiler strains during heat stress **fed on alpha-tocopherol, ginger and** L-carnitine.

	Diets				Strains		P-value		
	Control	Alpha-tocopherol	Ginger	L-carnitine	Hubbard	Cobb	Diets	Strains	Diet × strain
Day 21	2.79±0.06[c]	3.04±0.03[a]	2.92±0.02[b]	2.90±0.01[b]	2.90±0.023	2.92±0.042	0.0007	0.64	0.31
Day 28	3.04±0.03[c]	3.26±0.02[a]	3.17±0.04[b]	3.11±0.021[b]	3.13±0.01	3.16±0.03	0.0000	0.25	0.18
Day 35	3.07±0.02[c]	3.22±0.019[a]	3.18 ±0.01[ab]	3.14±0.01[b]	3.14±0.011	3.16±0.017	0.0001	0.25	0.84
Day 42	3.10±0.02[c]	3.31±0.02[a]	3.25±0.01[b]	3.21±0.02[b]	3.23±0.01	3.17±0.02	0.0000	0.95	0.14

Means in the same row with different superscripts are significantly different (P<0.01)

Table 7. Mean serum cholesterol (mg/dl) of fast growing broiler strains during heat stress **fed on alpha-tocopherol, ginger and L-carnitine.**

	Diets				Strains		P-value		
	Control	Alpha-tocopherol	Ginger	L-carnitine	Hubbard	Cobb	Diets	Strains	Diet × strain
Day 21	178.00±3.85[a]	159.75±3.56[b]	160.8±3.71[b]	137.62±5.21[c]	162.63±5.23	155.5±3.77	0.0000	0.07	0.12
Day 28	153.50±6.26[a]	135.87±2.54[b]	132.88±3.10[b]	119±3.39[c]	132.94±5.53	137.69±3.21	0.0000	0.13	0.10

Day 35	146.38±2.90[a]	137.75±2.41[b]	132.75±2.28[bc]	126.75±2.47[c]	136.37±2.70	132.37±2.35	0.0002	0.72	0.57
Day 42	144.75±2.29[a]	132.5±2.69[b]	135.13±2.78[b]	124±2.79[c]	135.56±2.60	132.63±2.60	0.0001	0.26	0.25

Means in the same row with different superscripts are significantly different (P<0.01)

Table 8. Mean serum triglyceride (mg/dl) of fast growing broiler strains during heat stress **fed on alpha-tocopherol, ginger and L-carnitine.**

							P-value		
	Diets				Strains		Diets	Strains	Diet × strain
	Control	Alpha-tocopherol	Ginger	L-carnitine	Hubbard	Cobb			
Day 21	155.37±3.37[a]	137±3.70[bc]	143.00±2.21[b]	131.13±2.09[c]	139.12±2.95	144.13 ±3.03	P<0.01	0.10	0.94
Day 28	150.6± 2.31[a]	126.8± 3.54[c]	136.7 ± 3.08[b]	12300± 1.70[c]	134.8± 3.40	131.75± 3.25	P<0.01	0.66	0.07
Day 35	141.2±1.81[a]	129.3±2.29[b]	134.13±1.49[b]	122.3±2.08[c]	133.31±1.98	130.2±2.36	P<0.01	0.11	0.30
Day 42	142.1±3.92[a]	124.8±1.18[bc]	131.7±2.82[b]	119.25±1.46[c]	128.3±2.38	130.6±3.18	P<0.01	0.41	0.84

Means in the same row with different superscripts are significantly different (P<0.01)

Table 9. Mean serum low density lipoprotein (mg/dl) of fast growing broiler strains during heat stress **fed on alpha-tocopherol, ginger and** L-carnitine.

							P-value		
	Diets				Strains		Diets	Strains	Diet × strain
	Control	Alpha-tocopherol	Ginger	L-carnitine	Hubbard	Cobb			
Day 21	189.75±4.61[a]	178.12±3.23[b]	175.63±2.38[b]	172.5±1.22[b]	177.25±1.88	180.75±3.28	P<0.01	0.27	0.40
Day 28	193.50±3.80[a]	171.38±2.7[bc]	177.88±4.67[b]	167.38±6.41[c]	175.50±3.30	179.56±3.63	P<0.01	0.22	0.10
Day 35	194.88±3.54[a]	176.88±1.86[bc]	183±3.89[b]	173.38±1.33[c]	183.88±3.30	180.19±2.29	P<0.01	0.19	0.28
Day 42	197.50±4.39[a]	185.50±1.78[b]	188.13±2.34[b]	177.00±3.73[c]	189.5±3.12	184.56 ±2.51	P<0.01	0.09	0.06

Means in the same row with different superscripts are significantly different (P<0.01)

Table 10. Mean serum high density lipoprotein (mg/dl) of fast growing broiler strains during heat stress **fed on alpha-tocopherol, ginger and** L-carnitine.

							P-value		
	Diets				Strains		Diets	Strains	Diet × strain
	Control	Alpha-tocopherol	Ginger	L-carnitine	Hubbard	Cobb			
Day 21	65.5±0.86[c]	73.75±0.59 [a]	72.12±0.66[ab]	70.37±1.32[b]	69.81±1.10	71.06±0.87	P<0.01	0.12	0.13
Day 28	63.87±1.00[c]	73.50±0.98 [a]	70.50±1.11[b]	68.25±1.16[b]	67.93±1.03	70.12±1.21	P<0.01	0.14	0.61
Day 35	65.12±1.27[c]	73.25 ±0.86[a]	71.00±0.80[ab]	69.5 ±0.92[b]	70.18 ±1.16	69.25 ±0.82	P<0.01	0.35	0.52
Day 42	66.25±0.95[c]	73.75±0.64[a]	71.12±3.60[ab]	70.00 ±1.10[b]	70.37 ±0.92	70.18 ±1.04	P<0.01	0.85	0.46

Means in the same row with different superscripts are significantly different (P<0.01)

DISCUSSION

The current study revealed that ginger, alpha-tocopherol and L-carnitine decreased serum liver enzymes in broiler. Higher level of serum AST and ALT is the result of liver disease probably due to the oxidative damage of the tissue (Khan *et al*., 2013). The increase in serum enzymes at high ambient temperature could be the reason of reactive oxygen species. Alteration in serum enzymes activity occurs due to unhealthy condition of the liver, consequently, more enzymes are secreted from the necrotic cells of the tissue (Khan *et al*., 2011). The decrease in liver enzymes in treated groups may be due to the strong antioxidant property of alpha-tocopherol, ginger and L-carnitine, which scavenge free radicals (Khan *et al*., 2011 & 2013) and maintain the function of antioxidant enzymes (Shah *et al*., 2016). Several authors reported that alpha-tocopherol supplementation significantly decreased liver enzymes in broilers exposed to high ambient temperature (Khan *et al*., 2013). Under optimum environmental conditions, significant decrease in serum AST of broiler chicks fed ginger supplemented diet has been reported (Rehman *et al*., 2007). Hassan *et al*. (2011) reported a significant decrease in serum AST of broiler in response to L-carnitine. The reduction in serum liver enzymes in response to ginger may be due to the positive effects of the several active compounds such as gingerdione, gingerdiol, shogaols and gingerol (Khan *et al*., 2012b).

The higher serum glucose level in the present study may be due to over production of adrenal hormones leading to increased serum glucose (Ihsanullah *et al*., 2017). The decrease in serum glucose level of heat stressed broilers may be due to the decrease synthesis and production of corticosterone by alpha-tocopherol (Khan *et al*., 2011). Previous studies reported that serum glucose is reduced in response to alpha-tocopherol in broiler (Habibian *et al.,* 2014). Under heat stress conditions, we could not find previous reports, however, lower serum glucose under thermoneutral condition have been documented in response to supplementation of ginger and L-carnitine (Saeid *et al*. 2010; Rehman *et al*. 2011).

Heat stress damages biomolecules (Khan *et al*., 2011). In addition, high ambient temperature reduces the digestibility of protein leading to low blood protein concentration (Zia ur Rehman *et al*. 2017). Our findings showed that supplementation of ginger, alpha-tocopherol and L-carnitine significantly reduced serum cholesterol in summer stressed broilers. L-carnitine was more effective than alpha-tocopherol and ginger in reducing serum cholesterol of heat stressed broilers. Increased cholesterol level under heat stress may be caused by hyperstimulation of the adrenal gland which produces adrenocarticotropin hormones (Ihsanullah *et al*., 2017) for which cholesterol acts as a precursor. Alpha-tocopherol supplementation in poultry decreased the production and synthesis of adrenocarticotropin hormones and corticosterone (Khan *et al*., 2013), consequently, serum cholesterol is decreased. β–hydroxy-b-methyleglutaryl coenzyme A (HMG-CoA) is involved in cholesterol synthesis and ginger is a good HMGR-inhibiting drug and hence reduces cholesterol synthesis (Khan *et al*., 2012b). The decreased serum cholesterol in L-carnitine supplemented birds may be due to its hyperactivity of lipid metabolism (Parizadian *et al*., 2011).

High ambient temperature releases corticosterone and increases plasma concentrations of glucocorticoids in birds (Khan *et al*., 2011). Alpha-tocopherol supplementation reduces the production of adrenocarticotropical hormones and corticosterone (Khan *et al*., 2013) and hence decreases the production of triglyceride. Decreased serum triglyceride level in broilers fed L-carnitine diet may probably be caused by increased transportation of fatty acids for oxidation (Parizadian *et al*., 2011). Supplementation of L-carnitine in diets accelerates the activity of lipase, thus leading to higher concentration of fatty acid by increasing hydrolysis of triglyceride to glycerol and fatty acid (Zhang *et al*., 2010).

L-carnitine decreases the activity and the rate of HMG-co reductase in the liver and may be responsible for lower serum LDL in L-carnitine supplemented group in the present study (Hassan *et al*., 2011). Alpha-tocopherol is the major antioxidant and protects lipoprotein from oxidative injuries and may be responsible for the increased HDL level in the alpha-tocopherol supplemented group. Our results are consistent with the results of Habibian *et al.* (2014), who found that alpha-tocopherol significantly increased serum HDL of broiler under heat stress. It is pertinent to note that with the increasing age, the birds response did not change significantly, probably the average temperature and humidity during the experimental period did not change.

Further, this is perhaps the first study which compares the effects of different antioxidants in heat stressed broiler strains. In the present study, alpha-tocopherol was found to be more effective compared to the other additives. Ginger was still better than L-carnitine. It is difficult to give reason why alpha-tocopherol was better than the two except saying that alpha-tocopherol is a better antioxidant than the two.

Conclusion: It is concluded that supplementation of diets with ginger, alpha-tocopherol and L-carnitine reduce the negative effects of heat stress and improved hepatic and lipid profile of summer stressed broilers. Moreover, in term of blood biochemical parameters, the two broiler strains respond equally to the heat stress and the treatments.

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