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Hepatic Expression of Genes Related to Fatty Acid Biosynthesis during Tropical Summer in Broilers: Effect of Organic Selenium Supplementation

Jayasri Kantheti[1], Padmaja Kondeti[1], Eswara Prasad Pagadala[1], Adilaxmamma Kaliki[1], Arunachalam Ravi[1], Punyakumari Bhupati[1], A.V. Siva Kumar[1]

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ABSTRACT

Background: The physiological responses during adaptation to summer in poultry could be related to modulation of energy metabolism. Enhanced deposition of fat in spite of reduced feed intake seems to have an advantage under hot conditions. Thus, the present study was conducted to investigate the seasonal status of *de novo* synthesis of fatty acids in broiler chicken (*Gallus gallus*) and its response to organic selenium supplementation.

Methods: The study was conducted in two phases, one during autumn and the other during summer, with a total of 300 birds as autumn control, summer control, group-I (0.3 ppm SeMet), group-II (0.6 ppm SeMet) and group-III (0.9 ppm SeMet) groups. Blood samples and hepatic tissues were collected at 21 and 42 d for further analysis.

Result: An elevation in serum lipid profile, glucose, cortisol and T_4 levels was observed during the summer while the T_3 concentration decreased compared to the autumn. The hepatic expression of Acetyl Co. A carboxylase α (ACCα) and Fatty acid synthase (FASN) was increased at 21 d, while it was decreased at 42 d during summer in broilers. Serum total cholesterol levels and LDL levels decreased with SeMet at 0.6 and 0.9 ppm levels. The expression of hepatic ACCα and FASN genes was decreased with selenium supplementation at higher levels. Increased serum total cholesterol levels were associated with lower *de novo* fatty acid synthesis at 42d during summer in broilers.

Key words: Acetyl Co. A Carboxylase α, Fatty acid synthase, Heat stress, L- selenomethionine, Lipid metabolism.

INTRODUCTION

In the majority of tropical countries where a severe summer prevails, heat stress (HS) is a serious threat to broiler (*Gallus gallus*) production. It is a major cause of loss of production and reduced profit in poultry production. Due to the changes in environmental temperatures, heat stress adaptation is essential for all forms of life. The cellular adaptations that take place during HS lead to various neuroendocrine, physiological and immunological alterations, including modulation of lipid and glucose metabolism. Heat stress-induced changes in the serum biochemical profile of broilers include increased serum glucose, triglyceride and cholesterol concentrations (Xie *et al*., 2015; Flees *et al*., 2017; Huang *et al*., 2018). Serum hormone concentrations such as cortisol / corticosterone, T_3 and T_4 have also been reported to change during heat stress in broilers (Sohail *et al*., 2010; Rajaei-Sharifabadi *et al*., 2017).

Previous studies revealed that the reduction in basal metabolism and physical activity during heat stress could be related to the increased fat deposition in broilers (Geraert *et al*., 1996a). An increase in the abdominal, subcutaneous and intermuscular fat proportions observed during chronic heat stress could be due to its effect on lipid metabolism in broilers (Howlider and Rose, 1987). Enhanced abdominal fat deposition seems to have an advantage in hot conditions. The more dietary energy stored as fat, the lower the heat produced and thus less heat needs to be dispersed (Lu *et al*., 2007; Zhang *et al*., 2012).

Analysis of the transcriptome and metabolome of liver tissue from heat-stressed broilers revealed a differential alteration in the expression of ACCα, Acyl-CoA synthetase (ACSF3), Stearoyl-CoA-9-desaturase (SCD) and FASN in broilers (Jastrebski *et al*., 2017; Lu *et al*., 2019). The differences could be related to the age of the animal, the model of heat stress (constant or cyclic), the method used to measure the fat index (abdominal fat was generally used as the single fatness index) and the breed (Smith, 1993).

[1]Department of Veterinary Biochemistry, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati-517 502, Andhra Pradesh, India.

Corresponding Author: Jayasri Kantheti, Department of Veterinary Biochemistry, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati-517 502, Andhra Pradesh, India. Email: jayasrikantheti9720@gmail.com

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Though selenium is commonly supplemented to poultry rations as a trace mineral with antioxidant potential, it has been found to influence metabolism under different conditions (Del Vesco *et al*., 2017; Misu *et al*., 2010). Thus, the present investigation was conducted to study the seasonal effect on lipid metabolism, including *de novo* synthesis of fatty acids during the tropical summer, as well as the effect of organic selenium on lipid metabolism at different levels of supplementation during tropical summer in broilers.

MATERIALS AND METHODS

Animal experiment

The present study was planned during the autumn (October-November, 2018) and summer (April- May, 2019) seasons for 300 commercial broiler chicks (Cobb 400) under a deep litter system. The experiment was carried out at College of Veterinary Science, S.V. Veterinary University, Tirupati andhra Pradesh. Two hundred and forty birds were divided into four groups with six replicates (10 birds in each) during the summer months to study the effect of selenium supplementation during summer. A group of 60 birds, divided into six replicates with 10 birds in each replicate, was reared during the autumn months to provide a thermoneutral control. The composition of the basal ration was kept uniform as per ICAR standards (2013) in both phases of the experiment (Table 1). L-selenomethionine (Excential Se 4000, ORFFA, Netherlands) was mixed in the basal ration at 7.5, 15 and 22.5 g/100 kg to get the concentrations of 0.3 ppm, 0.6 ppm and 0.9 ppm SeMet, respectively. The experimental rations given to different groups were autumn control (basal ration), summer control (basal ration), group-I (basal ration + 0.3 ppm L-selenomethionine), group-II (basal ration + 0.6 ppm L-selenomethionine) and group-III (basal ration + 0.9 ppm L-selenomethionine). All the other management conditions were kept uniform throughout the experiment. The temperature of the poultry shed was recorded using a digital thermometer and thermohumidity index values were calculated using the formula,

$$THI = 0.85\ Tmax + 0.15\ Tmin$$ (Lallo *et al*., 2018)

Where,

*T*max = Maximum daily temperature.

*T*min = Minimum daily temperature.

The blood samples and hepatic tissues were collected after the slaughter of a bird from each replicate at 21 and 42 d. The serum samples and hepatic tissues immersed in RNA later (Applied Biosystems, USA) were stored at -80°C until further analysis.

Analysis of the serum biochemical profile

The analysis of serum biochemical constituents was carried out using an automated biochemical analyzer (A 15 Biosystems, Netherlands) and estimated as glucose by the glucose oxidase method (Trinder, 1969), triglycerides by the glycerol phosphate oxidase/ peroxidase method (Fossati and Prencipe, 1982), total cholesterol by the cholesterol oxidase/peroxidase method (Allain *et al*., 1974), HDL by the direct detergent method (Warnick *et al*., 2001), The LDL concentration was calculated using Friedwald's formula (Friedewald *et al*., 1972). The concentrations of serum cortisol, T3 and T_4 were estimated using competitive ELISA kits (Calbiotech, Inc., USA).

Gene expression by the relative quantification method

Isolation of RNA

RNA was isolated from the liver tissue using an RNA isolation kit (Medox, India) and NanoDrop lite (Thermo Fischer, USA) was used to determine the purity of the RNA.

Table 1: Broiler ration formulated for summer and autumn phases of the experiment.

Feed ingredients (kg/100 kg)	Pre-starter	Starter	Finisher
Deoiled rice bran	4.6	3.0	3.5
Maize	47.5	50.4	55.6
Soybean meal	41.3	40.0	34.4
Ca	1.3	1.26	1.04
Methionine	0.11	0.08	0.04
Veg Oil	3.09	3.26	3.42
Trace mineral mix (with no selenium)	2.0	2.0	2.0
P dical phosphate	0.1	-	-
Chemical composition			
ME (kcal/kg)	3000	3050	3100
Crude protein (%)	22.00	21.50	19.50
Calcium (%)	1.00	0.95	0.85
Avail phosphorus (%)	0.45	0.40	0.38

Vitamin premix provided (per kilogram of diet) thiamine; 1: Pyridoxine; 2: Cyanocobalamine; 0.01; Niacin; 15: Pantothenic acid; 10: α-tocopherol; 10: Riboflavin; 10: Biotin; 0.08: Menadione; 2: Retinol acetate; 2.75: Cholecalciferol; 0.03: Choline; 650 mg/kg diet. Trace mineral mix (per kilogram of diet) copper, 7.5: Manganese, 50: Zinc, 45 mg/kg diet.

RNA samples having purity (absorbance at 260/280) in the range of 1.8-2.1 were only used for the expression studies.

Preparation of cDNA

cDNA was synthesized using the high-capacity reverse cDNA transcription kit (Applied Biosystems, USA) as per the manufacturer's instructions.

Real time polymerase chain reaction

The real-time quantification was carried out using the primer sequences for Acetyl Co. A Carboxylase α (ACCα), fatty acid synthase (FASN) and 18S rRNA, as mentioned in Table 2. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical evaluation

The collected data were statistically analysed using an independent sample t-test to compare autumn control and summer control and a one-way ANOVA (summer control with different selenium treatments), followed by Duncan's multiple comparisons test (SPSS version 20).

RESULTS AND DISCUSSION

Higher THI indices recorded during the summer season compared to those recorded during the autumn season reveal that the birds are exposed to heat stress during the summer (Fig 1; Jayasri *et al*., 2022b). The stress ranges for poultry have been reported previously based on THI: <27.8 as normal, 27.8-28.8 as moderate, 28.9-29.9 as severe and ≥30.0 as very severe (Lallo *et al.,* 2018).

An increase (P<0.01) in serum triglyceride, total cholesterol and LDL levels was observed during the summer compared to autumn (Table 3), which is in agreement with earlier reports (Xie *et al*., 2015). Hyperlipidemia observed during summer was associated with elevated cortisol levels in the present study (Table 4). The role of corticosteroids in hyperlipidemia during stress was reported earlier, where it was found to increase the triglycerides, total cholesterol, HDL and LDL fractions of cholesterol in serum during HS in broilers (Pulai *et al*., 1997; Eid *et al*., 2003). Stress-induced depression in insulin secretion might enhance the activity of lipolytic enzymes and be responsible for increased serum lipids (Ognik and Sembratowicz, 2012). A significant (P<0.05) increase in blood glucose levels (23% at 42 d) observed during summer (Table 4), is consistent with the previous findings (Ognik and Sembratowicz, 2012; Bai *et al*., 2019). Enhanced cellular energy demand during stress drives glucose output from the liver, resulting in increased blood glucose levels. Insulin resistance reported during HS could lead to a decrease in the utilization of glucose, further contributing to hyperglycemia (Hargreaves *et al*., 1996; Honda *et al*., 2007).

Table 2: Primer sequences of ACCα, FASN and 18SrRNA of chicken (Flees *et al*., 2017).

Gene	Orientation	5′to 3′sequence	Product size (bp)
ACCα	Forward	CAGGTATCGCATCACTATAGGTAACAA	74
	Reverse	GTGAGCGCAGAATAGAAGGATCA	
FASN	Forward	ACTGTGGGCTCCAAATCTTCA	70
	Reverse	CAAGGAGCCATCGTGTAAAGC	
18SrRNA	Forward	TCCCCTCCCGTTACTTGGAT	60
	Reverse	GCGCTCGTCGGCATGTA	

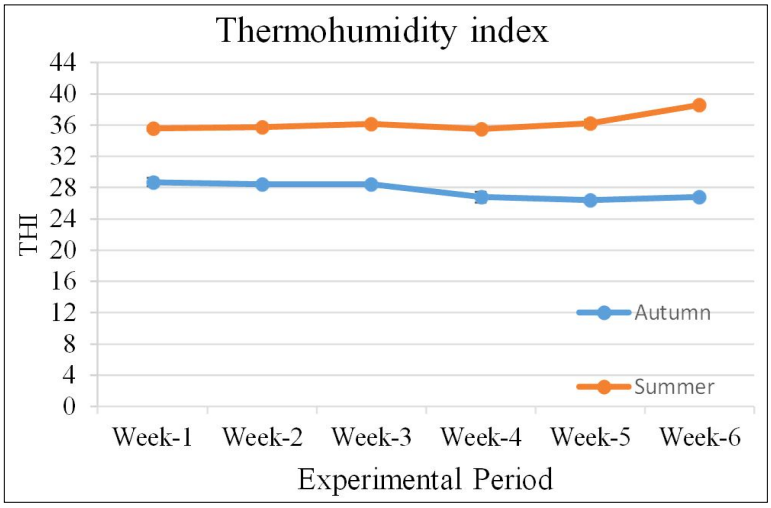

Fig 1: Thermo humidity indices recorded during autumn and summer.

Selenium supplementation resulted in a significant (P<0.01) reduction of serum TG levels at 21 d. SeMet at 0.3 ppm was found to be effective in reducing serum TC and LDL levels at 21 d, while 0.6 ppm was effective at 42 d (Table 3). The hypolipidemic effect of selenium at low doses on serum cholesterol was reported earlier in rats with induced hyperlipidemia (Sreekala and Indira, 2008; Zhang *et al*., 2018). SeMet supplementation at 0.9 ppm was found to increase serum TC and LDL levels. Similar to the present findings, an increase in plasma TC has been reported in chickens fed with supranutritional Se (3 ppm) under thermoneutral conditions (Huang *et al*., 2016). The association of high plasma selenium levels with hyperglycemia and hyperlipidemia was also revealed by the epidemiological data in humans (Steinbrenner *et al*., 2010).

Serum glucose concentration increased further with SeMet supplementation beyond 0.3 ppm at 21 d, whereas 0.6 ppm showed a hypoglycemic effect at 42 d when compared to the summer control (Table 4). Selenium is reported to influence glucose metabolism by improving insulin secretion and signaling (Fontenelle *et al*., 2018). However, Se in excess of the required amount has been shown to inhibit growth in chickens by increasing blood glucose levels (Xiang *et al*., 2017). The hyperglycemia observed with a higher selenium dose in the present study is supported by earlier reports of higher dietary selenium-induced hyperinsulinemia and insulin resistance resulting in hyperglycemia in different animal models like rats, pigs and chicken (Xu *et al*., 2017). The findings of the present study showed that higher blood glucose levels observed at 0.9 ppm SeMet were associated with poor growth performance (Table S1).

Serum cortisol and T_4 levels were significantly (P<0.05) increased while T_3 levels decreased at 42 d during summer (Table 4), which is in agreement with previous reports (Sohail *et al*., 2010, Rajaei Sharifabadi *et al*., 2017). Selenium supplementation showed a dose-dependent effect on cortisol levels, whereas it showed a plateau effect on serum T_3 levels at 42 days. Similar to the present findings, the ameliorative effect of 0.4 ppm selenium was reported earlier against the effect of oxidative stress on serum thyroxine levels in broilers (Fan *et al*., 2009).

Table 3: Effect of L-selenomethionine supplementation on serum lipid profile during summer (Mean±SE).

Treatment	Triglycerides** (mg/dl)		Total cholesterol **(mg/dl)		HDL* (mg/dl)		LDL* (mg/dl)	
	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
Autumn control	53.63^{y}±1.49	42.25^{y}±1.16	133.25^{y}±1.18	126.0^{y}±0.463	84.33^{y}±0.79	75.36±3.03	38.20^{y}±0.64	51.38^{y}±1.89
Summer control	93.38^{xa}±1.89	59.13^{x}±1.88	179.0^{xb}±3.71	174.5^{xa}±1.00	98.8^{xc}±1.39	82.45±5.49	61.53^{xab}±3.26	80.23^{xa}±5.28
Group-I(0.3 ppm)	66.88^{b}±1.17	60.88±1.17	174.88^{b}±2.83	156.5^{b}±2.05	112.99^{b}±8.62	85.95±1.43	48.51^{a}±10.1	58.38^{b}±2.96
Group-II (0.6 ppm)	67.88^{b}±1.88	57.00±2.12	229.63^{a}±4.25	143.5^{c}±2.76	137.24^{a}±2.94	87.99±3.09	78.81^{b}±5.34	44.11^{c}±5.54
Group-III(0.9 ppm)	69.25^{b}±0.94	56.88±1.22	223.63^{a}±3.44	159.75^{b}±2.76	140.21^{a}±2.43	87.04±2.8	69.56^{b}±4.62	62.45^{b}±3.54

$^{x, y}$ indicate significant difference between autumn and summer controls at ** (P<0.01), *(P≤0.05), ^{a,b,c,d} indicate significant differences among summer control, group I, II and III at **(P≤0.01), *(P≤0.05).

Table 4: Effect of L-selenomethionine supplementation on serum glucose and hormonal profile during summer (Mean±SE).

Treatment	Glucose** (mg/dl)		Cortisol** (ng/ml)		*T_3 (ng/dl)		*T_4 (µg/dl)	
	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
Autumn control	199.38±3.18	205.75^{y}±3.67	4.0±0.58	4.81^{y}±0.81	139.65^{x}±6.76	120.0^{x}±2.82	0.91^{y}±0.04	1.27^{y}±0.11
Summer control	196.50^{b}±4.1	252.0^{xab}±9.45	6.31±1.35	8.69^{xa}±0.28	73.74^{b}±2.49	88.75^{b}±2.80	1.27^{a}±0.17	2.12^{a}±0.13
Group-I (0.3 ppm)	201.63^{b}±6.35	246.5^{ab}±7.09	4.13±0.51	6.95^{a}±0.52	118.43^{a}±0.70	106.67^{a}±1.05	1.02^{ab}±0.04	1.35^{c}±0.07
Group-II (0.6 ppm)	235.38^{a}±3.36	237.50^{b}±9.1	3.79±0.58	5.00^{b}±0.08	121.25^{a}±0.82	108.33^{a}±1.40	1.02^{ab}±0.04	1.31^{c}±0.06
Group-III (0.9 ppm)	234.75^{a}±3.75	267.25^{a}±8.4	5.10±0.31	5.88^{b}±0.83	81.61^{b}±3.81	100.67^{a}±7.38	1.01^{b}±0.03	1.63^{b}±0.08

$^{x, y}$ indicate significant difference between autumn and summer controls at **(P<0.01), *(P≤0.05), ^{a,b,c,d} indicate significant differences among summer control, group I, II and III at **(P≤0.01), *(P≤0.05).

Table S1: Effect of L-selenomethionine supplementation on growth performance during summer (Mean±SE).

Treatment	Average feed consumption (g)	Body weight gain (g)	Feed conversion ratio
Autumn control	3440.71^{X}±17.41	2155.17^{X}±7.91	1.60^{Y}±0.003
Summer control	2538.86^{Ya}±51.36	1538.03^{Yab}±32.86	1.65^{Xa}±0.002
Group-I(0.3 ppm)	2569.64^{a}±8.04	1553.18^{ab}±7.00	1.65^{a}±0.003
Group-II (0.6 ppm)	2482.76^{ab}±14.62	1593.59^{a}±9.16	1.56^{c}±0.007
Group-III(0.9 ppm)	2348.26^{b}±38.12	1469.56^{b}±23.34	1.60^{b}±0.005

$^{X, Y}$ indicate significant differences between autumn control and summer control (P<0.01),
^{a,b,c,d} indicate significant differences among summer control, group I, II and III (P<0.01).

Table 5: Effect of L-selenomethionine on the expression of ACCα and FASN genes during summer (Mean±SE).

Treatment	ACCα		FASN	
	21 d	42 d	21 d	42 d
Autumn control	1.0^{x}	1.0^{y}	1.0^{x}	1.0^{y}
Summer control	4.90^{ya}±0.760	0.36^{xa}±0.046	2.25^{ya}±0.251	0.35^{xa}±0.068
Group-I(0.3 ppm)	0.62^{b}±0.064	0.31^{a}±0.003	0.60^{b}±0.017	0.42^{a}±0.012
Group-II (0.6 ppm)	0.26^{c}±0.078	0.18^{b}±0.009	0.24^{c}±0.003	0.25^{b}±0.024
Group-III(0.9 ppm)	0.22^{c}±0.062	0.16^{b}±0.012	0.26^{c}±0.030	0.24^{b}±0.021

$^{X, Y}$indicate significant differences between autumn and summer controls (P<0.01),

^{a,b,c,d}indicate significant differences among summer control, group- I, II and III (P<0.01).

The liver is the primary organ of lipogenesis and is responsive to HS in poultry (Flees *et al*., 2017). Several studies have reported that HS can enhance fat synthesis and deposition in broilers despite a substantial reduction in feed intake (Geraert *et al*., 1996b; Lu *et al*., 2007). The relative change observed in the expression of ACCα and FASN mRNA at 21 d was 4.90 and 2.25 fold, while it was 0.36 and 0.35 fold, respectively, at 42 d during summer when compared to autumn (Table 5). The enhanced expression of ACCα and FASN at 21 d is in agreement with previous findings where ACC expression in the liver was up-regulated during HS (8 hr./d for one week) in broilers (Jastrebski *et al*., 2017). However, such studies were conducted for a brief period of time, *i.e*., acute heat stress.

The decreased expression of ACCα and FASN mRNA at 42 d of summer is in concurrence with previous reports indicating adaptive responses of broilers to high temperatures (Flees *et al*., 2017; Lu *et al*., 2019). The reduced fatty acid synthesis at 42 d was associated with negative energy balance, as shown by poor feed consumption and an increase in the antioxidant activity and expression of PGC-1 in liver tissue (Jayasri *et al*., 2022a). Hepatic lipogenesis is highly responsive to changes in the diet, the energy status of the cell and the subsequent responses of key plasma metabolic hormones in broilers (Huang *et al*., 2008). Negative energy balance during HS (due to reduced feed intake and increased functioning of antioxidant machinery) could result in reduced serum glucagon levels, further contributing to a decrease in lipogenesis during chronic stress (Flees *et al*., 2017; Rix *et al*., 2019).

Prolonged heat stress along with the increased activity of antioxidant enzymes could result in impaired insulin signaling (Huang *et al*., 2018), which is further responsible for decreased expression of *de novo* lipogenic genes. Despite the decrease in lipogenesis, serum TG, TC and LDL concentrations increased at 42 d, which could be due to the release of intracellular storage lipids rather than *de novo* synthesis. This might be essential to meet the energy deficit created during summer, as evidenced by the elevated levels of PGC-1 (Jayasri *et al*., 2022a).

Selenium supplementation resulted in a decrease in the expression of ACCα and FASN both at 21 d and 42 d during the summer. A similar trend was reported earlier, where a significant reduction in abdominal fat content was observed upon selenium supplementation at 0.33 ppm in quails during HS (Del Vesco *et al*., 2017). The results of the present study are in agreement with a previous report where the suppression of hepatic *de novo* fatty acid synthesis due to dietary supplementation with Se and Mg at both high and low doses in high-fat-fed rats was observed (Zhang *et al*., 2018). Long-term selenium supplementation has also been shown to reduce hepatic steatosis in mice by reducing the mRNA levels of the ACC1 and FASN genes (Miyata *et al*., 2020). The role of selenoprotein P in reducing the activity of ACC was reported earlier in mouse hepatic tissues (Misu *et al*., 2010). Thus, the suppressive effect of selenium on *de novo* lipid metabolism during summer may be due to its effect on the cellular redox state, which is known to influence insulin secretion and signaling (Fontenelle *et al*., 2018).

CONCLUSION

The elevated serum TG, TC and LDL at 42 d during summer could be due to a negative energy-driven increase in lipolysis from storage lipids rather than from *de novo* synthesis of fats. Selenomethionine supplementation resulted in a decrease in serum total cholesterol and LDL concentrations during summer. The depressing effect of Se on blood glucose and the expression of hepatic ACCα and FASN genes could be due to its influence on insulin signaling in broilers. Supplementation of SeMet at 0.6 ppm was observed to have a better ameliorative effect compared to the other two levels after considering the growth performance of birds. However, further studies are necessary to provide a comprehensive view of seasonal variation in lipid metabolism. Further studies may be necessary to explore the seasonal effect on the lipid metabolism as well as the effect of excess selenium supplementation on it in broilers.

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Conflict of interest

I hereby declare on behalf of all the authors of the manuscript titled that there are no relevant financial or non-financial competing interests to report.

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