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Original Article

The effect of L-tryptophan on the food intake, rectal temperature, and blood metabolic parameters of 7-day-old chicks during feeding, fasting, and acute heat stress

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Abstract

Background: Exposure to a high ambient temperature (HT) can cause heat stress, which has a negative impact on physiological functions. L-tryptophan (L-Trp) as a precursor of serotonergic and kynurenine (Kyn) pathways, has a calmative effect during different stress statuses. **Aims:** This study was carried out to determine the influence of intraperitoneal injection of Trp on feeding behavior, rectal temperature, and some blood parameters in the heat stress condition. **Methods:** L-tryptophan (25 and 50 mg/kg body weight, BW) was administered intraperitoneally during either HT (39°C) or control temperature (CT; 31°C) for 5 h whilst fed or fasted in 7-day-old chicks. **Results:** L-tryptophan caused elevation in decreased food intake and significantly decreased rectal temperature during acute heat stress at the dose of 50 mg/kg BW. Rectal temperature reduced in the fasted state at the dose of 50 mg/kg BW, and at the dose of 25 mg/kg BW Trp in the fed state in comparison with the other experimental groups. Reduction of serum glucose, triglyceride, and corticosterone levels was seen during the fed state. L-tryptophan had a significant reducing effect on the serum corticosterone level in the fasted state in comparison with the fed state, and also revealed a significant decline at the dose of 25 mg/kg BW on the elevated serum corticosterone under heat stress. **Conclusion:** Administration of L-tryptophan leads to increase cumulative food intake and decrease rectal temperature during heat stress. Also, L-Trp causes to decline increased serum corticosterone level under heat stress and fasted state. These findings indicated the potential regulator role of Trp to modulate stress response in heat-exposed chicks.

Key words: Blood parameter, Food intake, Heat stress, L-tryptophan, Rectal temperature

Introduction

The activation of the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic nervous system (SNS), and other neuroendocrine pathways are responsible for the changes in the physiological and behavioral parameters. Some changes in the concentration of different free plasma amino acids have been reported in heat stress-exposed chicks (Ito *et al*., 2015; Chowdhury *et al*., 2017).

Heat stress can stimulate the formation of reactive oxygen species (ROS) and induce oxidative stress and lipid peroxidation, and change the metabolic profile of chickens as reflected in the decreased levels of plasma triglyceride, total cholesterol, uric acid, and thyroid hormones and the increased level of plasma glucose and corticosterone (Lin *et al*., 2006; Bahry *et al*., 2017).

Exposure to heat stress less than 30 min can significantly alter the free amino acids concentrations in the brain, muscle, and plasma of chickens (Ito *et al*., 2014). This change in the free amino acids during heat stress provided the basis for the use of amino acids supplementation to alleviate heat stress in chicks (Erwan *et al*., 2014; Chowdhury *et al*., 2017). Later, the use of essential and nonessential amino acids during systemic stress, such as heat stress, or mental stress, such as fear and anxiety, were shown to have promising effects on counteracting stress responses (Shea *et al*., 1991; Yoshida *et al*., 2015). Amino acids or their derivates have been administered orally, intraperitoneally, centrally or through feed to experimental animals and livestock (Erwan *et al*., 2014; Yoshida *et al*., 2015; Tran *et al*., 2016; Chowdhury *et al*., 2017).

There are many reports on the beneficial effects of amino acid supplementation on the physiological and mental parameters of birds during acute and cyclical-

chronic heat stress (Zhao *et al*., 2009; Bandeira *et al*., 2015).

L-tryptophan (L-Trp) is an essential aromatic amino acid and precursor of several bioactive molecules, including 5-hydroxytryptamine (5-HT, serotonin), melatonin, and kynurenine (Kyn). Most of Trp oxidative degradation is metabolized through the Kyn pathway in liver, and the remaining may be either metabolized through serotonin or converted into melatonin through the methoxyindole pathway (Mellor and Munn, 1999). Nicotinamide adenine dinucleotide (NAD+) production following Trp oxidation in the (Kyn) pathway is used to enhance electron transport chain efficiency and repair DNA damage following oxidative stress (Kujundžić and Lowenthal, 2008).

Monoamine neurotransmitter serotonin (5-HT) is involved in many physiological (mood, appetite, sleep, etc.) and pathological (anxiety and depression) conditions. Tryptophan supplementation in poultry, chicken, mice, rat, pig, and horse diets has been shown to have beneficial results (Yoshida *et al*., 2013; Bandeira *et al*., 2015; Yoshida *et al*., 2015). Also, a Trp-deficient diet was found to elevate plasma corticosterone levels, decline plasma serotonin level, and increase sensitivity to stress in rats (Tanke *et al*., 2008).

The oral, central, and dietary administration of Trp reduced stress induced by social isolation (Yoshida *et al*., 2015), high stocking density and aggressive behavior in ducks (Liu *et al*., 2015) and there was not complete finding in chicks. Accordingly, this experiment was designed to investigate the effects of the intraperitoneal injection of Trp on food intake, rectal temperature, and some blood parameters such as thyroid hormones, corticosterone, total antioxidant capacity (TAC), malondialdehyde (MDA) during exposed to either high temperature (HT) or control temperature (CT) for 5 h whilst fed or fasted in 7-day-old.

Materials and Methods

One-day-old male broiler chicks (Ross 308) (*Gallus gallus domesticus*) were purchased from a commercial hatchery (Mahan Chicken Production Complex, Kerman, Kerman Province, Iran) and housed in a group in metal temperature-controlled-chamber at a constant temperature of 31°C and 50% relative humidity (RH) under continuous light until 4-day-old. For each experiment, after 48 h of acclimation the chicks were isolated in individual plexiglas cages (floor space: 14 cm × 24 cm; height: 21 cm). *Ad libitum* nipple-water and a commercial diet (2850 kcal/kg metabolizable energy, 23% crude protein) (Table 1) were available during the experiment (Ashraf *et al*., 2013). Food was placed in the front part of the plexiglas cages so that the chicks could not scatter feed crumbs. This allows an exact measurement of food intake.

Experimental design

Four experiments were performed. Food and water were available *ad libitum* throughout the experimental period during experiment 1 (Exp. 1) and 2 (Exp. 2), whereas only water was available in experiment 3 (Exp. 3) and 4 (Exp. 4).

Table 1: Composition of diet (%)

Ration ingredient	(%)	Amino acid	(%)
Corn	53	Lysine	1.43
Soy been	39	Methionine	0.55
Oil	3.75	Met + Cys	1.05
Carbonate calcium	1.43	Threonine	0.95
Di-calcium phosphate	1.18	Valine	1.10
Vitamin permix	0.25	Leucine	1.53
Mineral permix	0.25	Iso-leucine	0.95
salt	0.32	Arginine	1.50
Lysine	0.32	Tryptophan	0.22
Methionine	0.24		
Energy (kcal/kg)	2850	Calcium	1.05
Protein	23	Phosphors	0.52

Met: Methionine, and Cys: Cysteine

Ninety-six chicks (8 chicks in each group, totally 24 chicks in each experiment) were used. For each experiment chicks were intraperitoneally injected either with 0, 25, and 50 mg/kg body weight (BW) L-Trp in 0.25 ml. Chicks were returned to their cages and the cages moved to a temperature-controlled chamber maintained at either HT (39°C) or control thermoneutral temperature (31°C). Chicks were kept for 5 h at these temperatures. Experiment 3 and 4 were conducted in the same way as Exp. 1 and 2 except the chicks had water but not food available during the 5 h treatment period. Chicks were exposed to either HT (39°C and 50% RH) or control thermoneutral temperature (31°C and 50% RH) for 5 h in temperature-controlled chambers.

The fasted group remained unfed during the experiment but the fed group had availability to feed during the experiment (for 5 h). Immediately before the start of the experiment, Trp was injected intraperitoneally by insulin syringe. The treatments were arranged in the following factorial arrangement:

1. Fasted and heat-exposed chicks (24 chicks)
2. Fed and heat-exposed chicks (24 chicks)
3. Fasted-CT chicks (24 chicks)
4. Fed-CT chicks (24 chicks)

In all above classified groups, intraperitoneal injection of L-Trp (0, 25, and 50 mg/kg BW) at the onset of treatment was done. Cumulative food intake was measured by subtracting the weight of the food remaining in the feeder at 1, 2, 3, and 4 h from the initial amount. Rectal temperature was recorded immediately before, 2 and 4 h after heat stress induction. Rectal temperature was measured by inserting a thermistor probe (contact-type digital thermometer, TES-1310, Taiwan- 0.1 decimal accuracy) in the cloaca to a depth of 2 cm. Cumulative feed intake was recorded every hour.

Five h after the onset of all experiments, chicks were anesthetized by exposure to isoflurane (Baxter International Inc., AErrane Isoflurane USP). The blood was immediately collected by cutting the jugular vein.

The serum was separated by centrifuging samples at 750 g for 15 min and freezing them at -20°C until biochemical analysis.

Preparation of drug

L-tryptophan was purchased from Bio-Basic Inc. (20 Konrad Cres, Markham Ontario, Canada). The white crystalline powder was dissolved in 0.9% saline and 0.1 N hydrochloric acid (9:1), 1 h before the intraperitoneal injection. Tryptophan was injected based on BW of chicken. 7-day-old chicks average BW was 75 g. Thus at the dose of 25 mg Trp/kg BW about 1.88 mg for each chick was dissolved in 0.25 ml (volume of intraperitoneal injection) HCl solution (for 24 chicks, 24 × 1.88 mg in 24 × 0.25 ml HCl solution). Finally in primary stock, each 0.25 ml of solution contained 1.88 mg Trp. At the dose of 50 mg Trp/kg BW two fold Trp concentration, according to above instruction primary stock was prepared.

Biochemical analysis

Serum biochemical parameters

Serum biochemical variables, including glucose, uric acid, cholesterol, and triglyceride, were measured using commercial kits (Pars Azmun Co., Tehran, Iran) and a biochemical autoanalyzer (Alpha Classic AT++, Sanjesh, Iran).

Serum triiodothyronine (T3) and thyroxin (T4)

Serum T3 and thyroxin T4 were measured using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (chicken ELISA kits; Shanghai Crystal Day Biotech, Shanghai, China). The sensitivity of T3 and T4 kits were 0.5 ng/ml and 0.4 µg/dL, respectively. The intra- and inter-assay coefficient of variation T3 kit were < 8% and <12%, respectively. The intra- and inter-assay coefficient of variation T4 kit were both < 15%.

Serum corticosterone

The serum corticosterone level was measured using a solid-phase sandwich ELISA (chicken ELISA kits; MyBioSource.com, USA). The sensitivity of the corticosterone kit was 1.0 ng/ml. The intra- and inter-assay coefficient of variation (CV) corticosterone kit were =4.2% and =6.5%, respectively.

Total antioxidant capacity

Serum TAC was measured using a commercial kit following the manufacturer's protocol (ZellBio GmbH, Germany) on the basis of the oxidation reduction colorimetric assay at a wavelength of 490 nm. This method can determine TAC with 0.1 mM sensitivity (100 µmol/L). The intra- and inter-assay CVs were below 3.4% and 4.2%, respectively.

Measurement of MDA

Malondialdehyde was measured using ZellBio GmbH kit (Germany) based on the reaction with thiobarbituric acid in an acidic condition and high temperature; the color complex was measured calorimetrically at 535 nm. The values were finally expressed as mmol/L.

Statistical analysis

Rectal temperature and feed intake data were analyzed by factorial three-way ANOVA using the (MIXED) procedure (the type of variance-covariance structure is compound symmetry) for repeated measures in time, with feeding statuses, environmental temperature, L-Trp doses. The effects of feeding statuses, L-Trp doses, and environmental temperature on blood metabolites were analyzed with the general linear model (GLM) procedure and using a factorial three-way design. All analyses were performed in SAS Institute, NC, USA (2001) (version 9.1, SAS Institute, Cary, USA, 2001) (Kaps and Lamberson, 2017). Normal distribution of data was performed in Univariate proc of SAS Institute, NC, USA (2001). Some of them without normal distribution converted to normal by logarithmic converter but real least squares means (LSM) were showed in manuscript. Least squares means were obtained Tukey adjustment. The level of significance was $P<0.05$. The treatment means were shown as (least squares means) LSM±SEM.

Results

Rectal temperature and food intake

Food intake was significantly reduced in heat stress group compared to that of the control group at 3 and 4 h after heat stress induction ($P<0.05$) (Fig. 1). There was no significant difference between the food intake in groups were treated with 25 and 50 mg/kg BW Trp under heat stress ($P>0.05$). The effects of the injection of 25 and 50 mg/kg BW Trp on the food intake of heat stress-exposed chicks were not significant compared to those of chicks exposed to CT. L-tryptophan (25 and 50 mg/kg BW) cause to increase food intake during heat stress so that food intake approximately was accreted to amount of CT group (Fig. 2). The results showed that 25 mg/kg BW significantly increased food intake in first h of experiment in compare with 50 mg/kg BW and control groups (Fig. 3). Briefly L-Trp compensated heat stress induced food intake reduction in chicks.

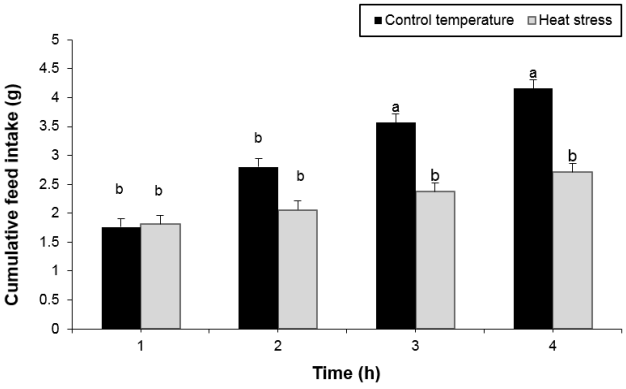

Fig. 1: The effects of heat stress on cumulative feed intake over different times. Bars (LSM±SEM) with different superscript are significantly different in each time ($P<0.001$)

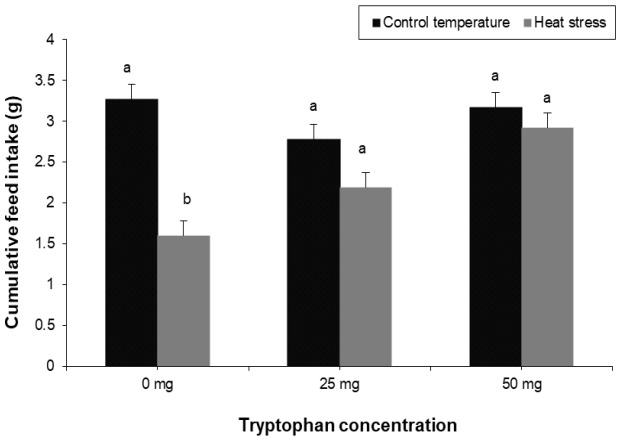

Fig. 2: The effects of heat stress on cumulative feed intake at different tryptophan concentration. Bars (LSM±SEM) with different superscript are significantly different in each concentration (P<0.001)

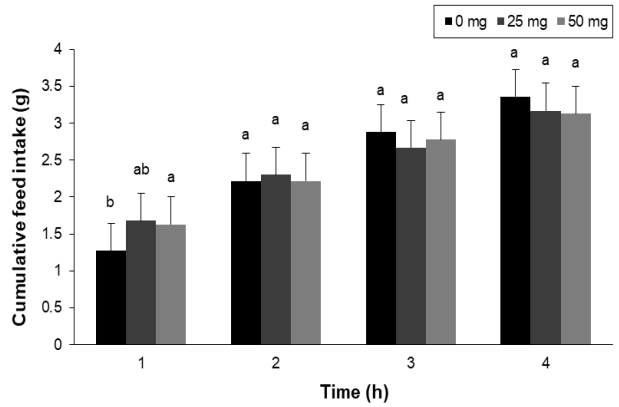

Fig. 3: The effects of various dosages of L-tryptophan on cumulative feed intake over different times. Bars (LSM±SEM) with different superscript are significantly different in each hour (P<0.001)

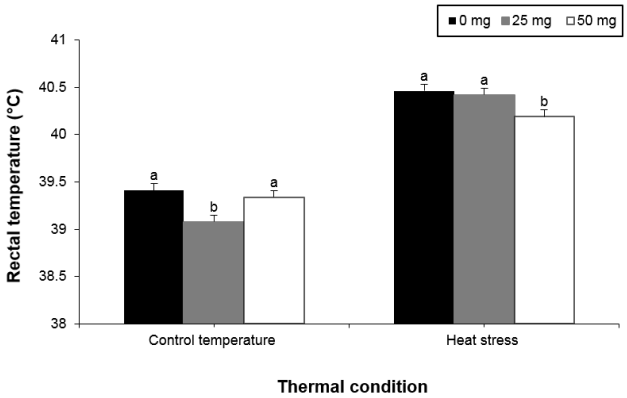

Fig. 4: The effects of various dosages of L-tryptophan on rectal temperature at different thermal conditions. Different superscript on bars (LSM±SEM) show significant differences in each thermal condition (P<0.01)

Rectal temperature was significantly affected by heat stress (P<0.0001), Trp injection (P<0.0001), feeding status (P<0.0001), hour (P<0.0001), and the interaction between them (P<0.0001). Rectal temperature significantly decreased in 50 mg/kg BW Trp injected compared to 25 mg/kg BW Trp injected group during heat stress whereas in thermoneutral temperature condition 25 mg/kg BW L-Trp cause to significant decline in rectal temperature (Fig. 4). The results showed the different effects of feeding status along with Trp treatment on rectal temperature. In fed state, 25 mg/kg BW Trp significantly decreased rectal temperature and in fasted state 50 mg/kg BW Trp reduced rectal temperature in comparison with other two groups. In other word in fed state, lesser dose of Trp was more effective (Fig. 5). Injection of Trp significantly reduced rectal temperature at 2 and 4 h after onset of experiment (Fig. 6).

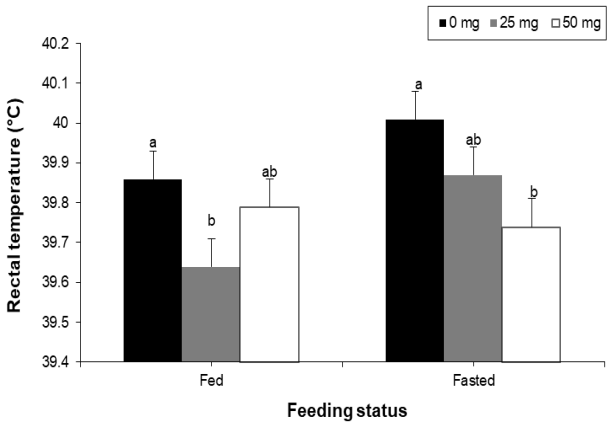

Fig. 5: The effects of various dosages of L-tryptophan on rectal temperature in different feeding statuses. Different superscript on bars (LSM±SEM) show significant differences (P<0.01)

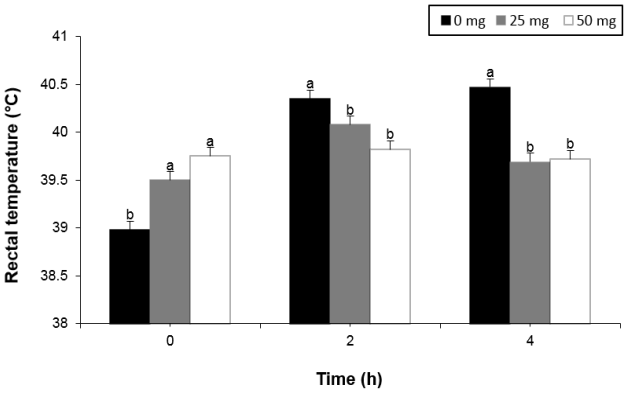

Fig. 6: The effects of various dosages of L-tryptophan on rectal temperature at different times. Different superscript on bars (LSM±SEM) show significant differences in each time (P<0.01)

Blood metabolite parameters

The effects of feeding state, heat stress, and Trp injection on blood metabolic parameters are presented in Tables 2 and 3, and Figs. 7, 8, 9, 10, and 11. In fed state significantly lower serum glucose, triglyceride and corticosterone levels compare with fasted state was seen (Table 3, Fig. 7) (P<0.05). Serum MDA, TAC and thyroid hormones were not affected by any dose of Trp, but serum glucose, corticosterone, and triglyceride concentrations significantly decreased in the fed state

compared to those of the fasted state (Fig. 7). Heat stress-exposed chicks showed a significant decrease in the serum triglyceride and cholesterol concentrations compared to those in the CT group (Fig. 8). 25 mg/kg BW L-Trp showed significant decreasing effect on blood corticosterone level (Fig. 9). Also, treatment with 25 and 50 mg/kg BW L-Trp in fasted state, significantly declined corticosterone level in comparison with control and fed state groups (Fig. 10).

During heat stress 25 mg/kg BW L-Trp significantly decreased corticosterone level in compare with the other groups (Fig. 11). Significant decrease in the serum uric acid level in fed state and CT group compared to that of fasted state exposed to the same thermal condition was observed. Also 50 mg/kg BW of L-Trp significantly decreased serum uric acid concentration (Tables 2 and 3).

Table 2: Main, and interaction effects of feeding, tryptophan, and temperature factors on blood variables of 7-day-old chick

Parameter	Temp	Feeding	Tryptophan	Temp × feeding	Temp × Tryptophan	Feeding × Tryptophan	Feeding × Tryptophan × Temp
Glucose (mg/dL)	NS	***	NS	***	***	NS	NS
TG (mg/dL)	***	***	NS	***	***	***	NS
Cholesterol (mg/dL)	**	NS	NS	NS	*	***	NS
Uric acid (mg/dL)	NS	NS	NS	***	NS	NS	NS
T3 (ng/ml)	NS	NS	NS	NS	NS	NS	NS
T4 (ng/ml)	NS	NS	NS	NS	NS	NS	NS
Corticosterone (ng/ml)	NS	**	*	***	***	***	NS
TAC (mmol/L)	NS	NS	NS	NS	NS	NS	NS
MDA (mmol/L)	NS	NS	NS	NS	NS	NS	NS

TG: Triglyceride, T3: Triiodothyronine, T4: Thtroxine, TAC: Total antioxidant capacity, MDA: Malondialdehyde, Temp: Temperature, and NS: Non-Significant. * P<0.05, ** P<0.01, and *** P<0.001

Table 3: LSM±SEM of blood variables of 7-day-old chicks in response to feeding status, thermal condition, and tryptophan administration

Groups	Glucose (mg/dL)	TG (mg/dL)	Cholesterol (mg/dL)	Uric acid (mg/dL)	T3 (ng/ml)	T4 (ng/ml)	Corticosterone (ng/ml)	TAC (mmol/L)	MDA (mmol/L)
Heat stress	200.85±8.35	28.76±2.27^{b}	98.33±4.35^{b}	6.19±0.30	1.44±0.004	9.57±0.034	49.80±0.49	1.01±0.012	7.97±0.029
Control temperature	182.07±8.35	44.62±2.29^{a}	113.88±4.40^{a}	6.26±0.30	1.44±0.004	9.57±0.035	48.72±0.49	1.01±0.012	7.98±0.030
Change in blood metabolites in response to thermal condition in chick									
Fasted state	208.6±8.44^{a}	45.86±2.29^{a}	107.15±4.40	6.44±0.30^{a}	1.44±0.004	9.56±0.035	50.18±0.49^{a}	1.01±0.012	7.96±0.030
Fed state	174.2±8.35^{b}	27.52±2.27^{b}	105.06±4.35	6.01±0.30^{b}	1.44±0.004	9.58±0.034	48.35±0.49^{b}	1.01±0.012	7.98±0.029
Change in blood metabolites in response to feeding status									
0 mg	183.7±10.4	37.9±2.8	109.6±5.3	6.5±0.37^{a}	1.4±0.005	9.5±0.04	48.8±0.6^{a}	1.01±0.01	7.97±0.036
25mg	195.9±10.4	34.4±2.8	108.8±5.3	6.6±0.37^{a}	1.4±0.005	9.5±0.04	46.4±0.6^{b}	1.00±0.01	7.99±0.036
50 mg	194.7±10.4	37.6±2.8	99.8±5.3	5.5±0.37^{b}	1.4±0.005	9.5±0.04	49.4±0.6^{a}	1.01±0.01	7.96±0.036
Change in blood metabolites in response to tryptophan administration									

LSM: Least squares means, SEM: Standard error mean, TG: Triglyceride, T3: Triiodothyronine, T4: Thyroxine, TAC: Total antioxidant capacity, and MDA: Malondialdehyde. Different superscript ($^{a, b}$) in column show significant difference P<0.01

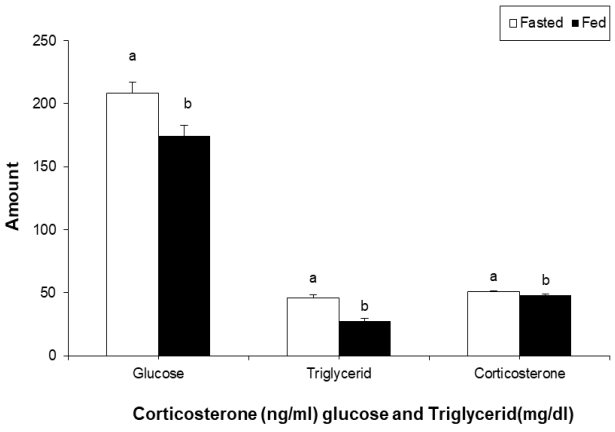

Fig. 7: The changes of blood parameters amounts (glucose (mg/dL), triglyceride (mg/dL), and corticosterone (ng/ml)) during fed or fasted status following of L-Tryptophan IP injection. Bars (LSM±SEM) with different superscript are significantly different (P<0.01)

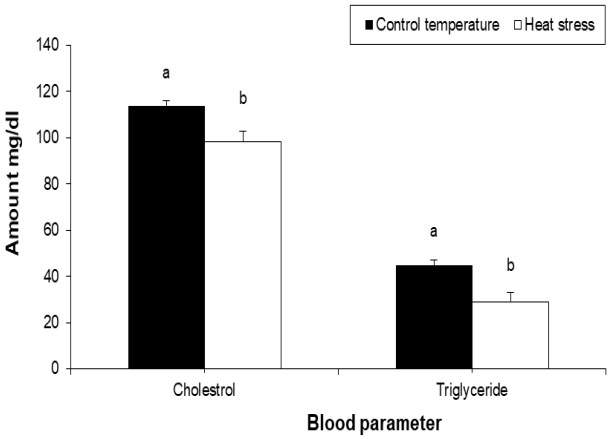

Fig. 8: The changes in the blood parameters amounts (cholesterol (mg/dL) and triglyceride (mg/dL)) during control temperature or heat stress status following of L-Tryptophan IP injection. Bars (LSM±SEM) with different superscript are significantly different (P<0.01)

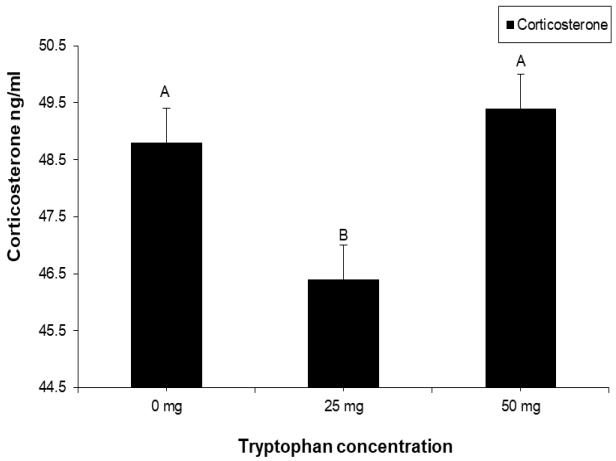

Fig. 9: The effects of tryptophan administration on the serum corticosterone amount. Different superscript on bars (LSM±SEM) show significant differences (P<0.01)

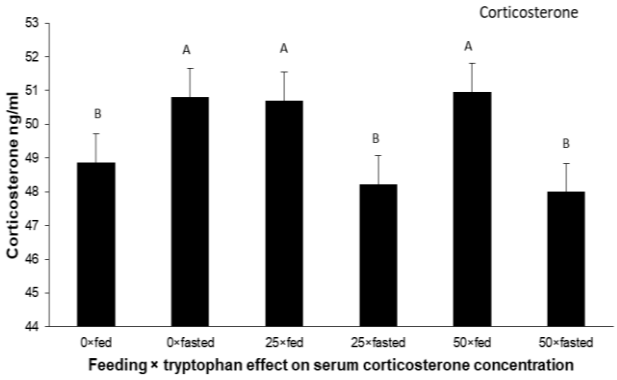

Fig. 10: The effects of feeding × L-tryptophan dosages on the serum corticosterone amounts. Different superscript on bars (LSM±SEM) show significant differences (P<0.01)

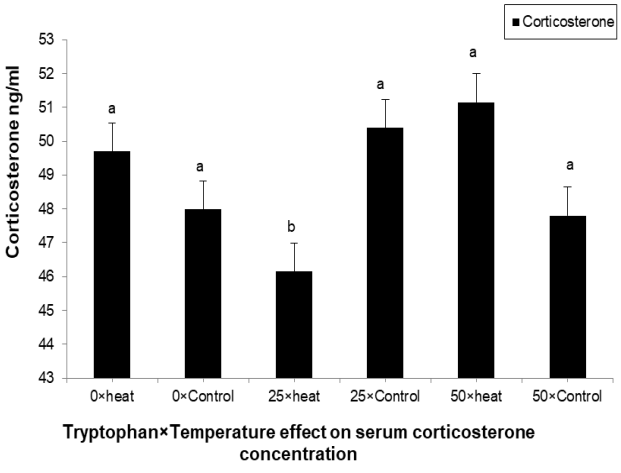

Fig. 11: The effects of L-tryptophan dosages × heat stress on the serum corticosterone amounts. Different superscript on bars (LSM±SEM) show significant differences (P<0.01)

Discussion

This experiment was designed to investigate the effect of L-Trp on the food intake, rectal temperature, and serum parameters of chickens in the fed and fasted states during 5 h heat stress. Cholesterol, triglyceride, glucose, and corticosterone were the main blood variables significantly influenced by feeding state, heat stress, and L-Trp treatment respectively.

TAC and MDA

The results showed that elevated rectal temperature caused by heat stress could induce some changes in the blood parameters but not in cases related to oxidative stress. The study in 5-week-old broiler chickens showed significantly higher plasma thiobarbituric acid reactive substances (TBARS) (an indicator of MDA) in the sixth but not the 3 h after exposure to heat stress (32°C) (Lin *et al*., 2006). In another investigation in chicks, treatment with vitamin C and chromium, those exposed to heat for 42 days showed higher serum MDA levels (Sahin *et al*., 2003). These findings show that oxidative stress induction during heat stress may depend on age, heat stress duration, and thermal acclimatization. Some studies have shown that intraperitoneal injection of Trp in mice can reduce pro-inflammatory cytokine (TNF-α), increase metanephrine (catecholamine), and improve wound healing (Bitzer-Quintero *et al*., 2010; Bandeira *et al*., 2015). Mentioned findings indicate the anti-inflammatory effects of Trp achieved by the repeated daily injection of Trp. Despite the antioxidant activity of Trp that was reported, the results of present study showed that there were not any significant changes in the blood MDA and TAC in the seven days old broiler chicks. Chowdhury *et al*. (2014) reported a significant brain MDA increase after 48 h of exposure to heat stress in chicks. Furthermore, for chicken plasma antioxidant capacity to change in response to heat stress, chickens should be exposed to chronic and cyclical thermal stress (Sahin *et al*., 2003; Lin *et al*., 2006). Based on this finding, it may be suggested that to investigate the antioxidant function of Trp, there should be a chronic and cyclical period of heat stress and several administrations of Trp.

Food intake and rectal temperature

In the present study, food intake in the heat stress condition was suppressed at 3 and 4 h after the onset of experiment. Treatment with 25 and 50 mg/kg BW L-Trp during heat stress cause the elevation in food intake. In agreement with our results in other investigations, heat stress significantly reduced food intake within 5 h compared to 2 h after hyperthermia induction in 14-day-old chicks (Ito *et al*., 2015). Researches have shown that 3-hour heat stress could significantly reduce food intake and elevate body temperature in the nine-day-old broiler chicks. However, in ovo injection of l-leucine during egg incubation significantly reduced body temperature in the second hour after heat stress (Han *et al*., 2018).

It has been reported that the inhibition of Trp hydroxylase, a rate-limiting enzyme in the serotonin pathway, by the intraperitoneal injection of p-chlorophenylalanine in mice resulted in a significant decrease in the hypothalamus, plasma serotonin

concentration, food intake, and BW and an increase in the expression of hypothalamic 5-HT2C receptors and proopiomelanocortin (Nonogaki *et al*., 2018). Based on these findings, it can be inferred that Trp administration before the induction of heat stress can prevent serotonin depletion and food intake decline in heat stress-exposed chicks.

In the present study, there was no significant difference in cumulative food intake from the second hour after the onset of heat stress condition to the end and there was only significantly increased food intake at first h after 25 mg/kg BW L-Trp administration. Also, Trp injection significantly reduced rectal temperature from the second hour. The effect of Trp on reducing rectal temperature was observed to increase as time lapsed. In line with our results, the oral administration of high amounts of D-aspartate significantly reduced rectal temperature in 6-day-old chicks during 2-hour exposure to heat stress (Erwan *et al*., 2014). Similarly, intraperitoneally injection of Trp in adult rats (200, 400, and 600 mg/kg BW) showed a significant reduction in the rectal temperature at 30-150 min after the administration of Trp. Also, it has been shown that mice injected with endotoxins developed severe hypothermia when received Trp; this effect could be due to the funneling of Trp into the serotonin pathway (Moon and Berry, 1968). Intubating White Leghorn cockerels with a Trp solution (1.25 g) significantly reduced their rectal temperature and food intake (Lacy *et al*., 1986). Contrary to the expectation, dietary Trp supplementation resulted in increased BW and food intake in broilers (Shan *et al*., 2003). The results of investigations showed that serotonin (5-HT) affected food intake differently in the fed- and fasted-chicks (Denbow *et al*., 1982). According to our results, Trp injection significantly decreased rectal temperature and increased food intake during acute heat stress. It means that Trp may activate the heat loss mechanism in chicks during acute heat stress.

Corticosterone and thyroid hormones

Based on the findings of the current study, 25 mg Trp significantly reduced the serum corticosterone level in heat-exposed chicks. In line with our results, the central injection of Trp (400 and 800 nmol) to 5-6 day-old chicks significantly counteracted corticotrophin releasing hormone (CRH)-augmented social isolation stress (Yoshida *et al*., 2015). Corticotrophin-releasing hormone is central in adjusting any stress-activated HPA axis. Tryptophan supplementation, in a dose-dependent manner, decreased the plasma cortisol level and increased intestinal enzymes in salmon fish (Mardones *et al*., 2018). These findings support the buffering effect of Trp on the HPA axis.

There are different reports about the HPA axis activation during heat stress. For instance, 14-day-old male layer chicks exposed to hyperthermia, induced by exposure to 35°C for 1 or 48 h, did not show any significant change in the plasma corticosterone compared to those chicks exposed to CT (Chowdhury *et al*., 2012). Similarly, cyclical heat stress did not cause any significant change in the plasma corticosterone levels of those broilers between 35 and 42 days of age (Sun *et al*., 2015). Conversely, 14-day-old chicks exposed to heat stress for 2 and 5 h showed significantly higher plasma corticosterone level in the fifth h of exposure (Ito *et al*., 2015). Considering the discrepancy in the blood corticosterone level during the induction of heat stress, it can be suggested that the level of blood corticosterone changes temporarily and may return to its previous level either immediately or in the long term depending on the birds age, heat stress duration, breed, gender, and experimental condition. Furthermore, it should be noted that glucocorticoid secretion has a diurnal rhythm. However, our results showed a significant increase in the serum corticosterone level during heat stress, but L-Trp significantly reduced elevated serum corticosterone level. The reported findings point to an inverse correlation between the serotonergic system and the HPA axis; thus, that over activity in one of them reduces the activity of the other one. For instance, Wang *et al*. (2014) reported that broilers with high levels of fearfulness, compared to those with low levels of fearfulness, had different expressions of hypothalamic genes in the serotonergic system and HPA axis. In addition, Ahmed *et al*. (2014a, b) showed that injecting egg with a high dose of corticosterone changed the expression of hypothalamic genes in the serotonergic system of the broiler. Moreover, Nakagawa *et al*. (2016) observed a significant reduction in the serotonin level in the dorsomedial hypothalamus and other brain parts of rats after chronic and acute heat stress (32°C).

Furthermore, thyroid hormones had no changes. Contrary to our results, five-week aged chickens showed a significantly decrease in the levels of plasma T3 after 6 h of exposure to heat stress compared to the control group. The levels of plasma T4, however, significantly increased in the sixth h post-heat exposure (Lin *et al*., 2006). The T3 level significantly decreased following 12-hour cyclical heat stress during the 21-35 days of production whereas the T4 level significantly increased (Piestun *et al*., 2008). Thyroid hormones have been shown to accelerate oxidative metabolism by increasing the cellular mitochondrial content, mitochondrial cytochrome content, and respiratory rate.

The metabolic rate is an age- or BW-related factor. The metabolic rate is higher in older chickens and this is reflected in the glucose turnover, lipid oxidation and deposition, and metabolic heat production (Buyse *et al*., 2004). Older chickens with higher body mass and tissue synthesis have more compromised blood metabolite parameters in response to environmental challenges (Govaerts *et al*., 2000). A reduction in the blood thyroid hormone concentrations reflects decreased metabolic rate and helps to reduce heat production. Thus, the non-significant changes in the thyroid hormones observed in our study might be related to the neonatal age of chicks.

Glucose, triglyceride, cholesterol, and uric acid

Stress conditions, such as heat stress and feed deprivation, are reflected in altered blood metabolite

variables, such as glucose, triglyceride, and somewhat cholesterol. Our experiment showed a significant decrease in the triglyceride and cholesterol levels of heat stress-exposed chickens compared to those of chickens in the CT group. Fasting significantly elevated blood glucose and triglyceride levels. There are many reports about the changes in blood metabolite during heat stress. Heat stress affects lipid metabolism in fasted and heat stress-exposed chickens. Triglyceride is used as an energy source to cope with reduced energy intake. Therefore, decreased serum triglyceride could be due to the contribution of triglyceride to energy supply during HT. In agreement with our results, the peripheral serotonin levels were reported to be associated with glucose and lipid metabolism; accordingly, some studies have introduced blood serotonin as a decreasing agent of blood lipid and glucose (Watanabe *et al*., 2010; El-Merahbi *et al*., 2015). In a relevant study supporting this role of serotonin, the intraperitoneal injection of serotonin to fasted mice resulted in significantly lower blood glucose, triglyceride, cholesterol levels in 30 min, but only the glucose level increased after 30 min (Watanabe *et al*., 2010). The injection of serotonin to diabetic rats decreased blood glucose concentration in a dose-dependent manner; also, selective and nonselective serotonin receptor antagonists abolished the decreasing effect of serotonin on glucose (Chi *et al*., 2007). Tryptophan is a serotonin precursor and its administration has been reported to result in elevated blood serotonin levels (Paredes *et al*., 2007; Bandeira *et al*., 2015). Based on the above findings, the decline in the glucose, triglyceride, and cholesterol content in fed and heat-exposed chicks can be attributed to the Trp administration.

The uric acid concentration was not affected by any of the experimental factors (Trp, feeding state, temperature), but the interaction between feeding state and temperature had a significant effect on the serum uric acid concentration. A significant decrease in the serum uric acid level in the fed state and CT group compared to that of fasted state exposed to the same thermal condition was observed. Also, 50 mg/kg BW of L-Trp significantly decreased serum uric acid concentration (Tables 2 and 3).

Therefore, it can be postulated that fasting for 5 h can reduce the serum uric acid concentration in neonatal chickens. Furthermore, Trp significantly reduced blood uric acid in humans with hyperuricemia (Oshima *et al*., 2019). In birds and reptiles, uric acid is the main pathway for removing the nitrogenous products of protein breakdown and amino acid deamination. When the activity of the gluconeogenesis pathway is increased to compensate for the increased energy demands, more protein and amino acids are consumed, leading to increased blood uric acid.

There are mixed findings regarding blood uric acid concentration during different stress conditions. Cyclical and chronic heat stress in broilers of 35-42 days of age (Sun *et al*., 2015) and 7-day cyclical heat stress in 24-week-old hens significantly elevated blood uric acid (Song *et al*., 2012a), but 6-hour acute heat stress did not change blood uric acid concentration (Lin *et al*., 2006). Compared to the control group, 7-day-old chicks exposed to heat stress did not show any significant change in the blood uric acid concentration (Moraes *et al*., 2004) 14-day-old chicks showed a significant decrease in blood uric acid within 48 h but not 1 h after heat stress (Chowdhury *et al*., 2012). Tryptophan-treated layer hens showed a significant reduction in blood uric acid and corticosterone levels (Khattak and Helmbrecht, 2018).

The gluconeogenesis pathway is activated by the glucocorticoids released in response to stressful conditions and its activity is continued by glycogenolysis, lipolysis, protein breakdown, and ketone body formation. Unchanged blood uric acid in our experiment could be attributed to the short duration of heat stress. This is a reasonable suggestion because previous studies have shown that uric acid concentration is not affected by short-term heat stress.

Tryptophan injection could be effective in improving chicken metabolism during stressful conditions, such as fasting and heat stress. The injection of Trp reduced both serum corticosterone and uric acid levels. Lin *et al*. (2004) reported significantly higher uric acid in chickens exposed to acute stress induced by glucocorticoids (Pan *et al*., 2018). Fasting 7-day-old chicks for 48 h significantly increased the uric acid concentration, but re-feeding restored its concentration back to its normal range (Song *et al*., 2012b). Accordingly, it can be stated that there is a relationship between amino acids and corticosterone secretion during stress conditions. It is better to investigate the effects of L-Trp in many different doses during stressful conditions. Sampling interval is also suggested to be decreased, and the other methods of injection such as intracerebroventricular injection is recommended.

Based on our results, it is concluded that L-Trp causes to increase in cumulative food intake and decreases rectal temperature during heat stress and reduces corticosterone production, uric acid formation in the seven-day-old chicks. Blood lipid profile was reduced in heat stress exposed chicks and similarly in the fed state by L-Trp administration. It can be concluded that intraperitoneal injection of Trp can manage stressful conditions in birds.

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Conflict of interest

There was not any conflict of interest in this paper.

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