Effects of Heat Stress and Dietary Tryptophan on Performance and Plasma Amino Acid Concentrations of Broiler Chickens

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ABSTRACT : Two experiments were conducted to investigate the impact of high temperature and dietary tryptophan (Trp) on performance, selected organ weights and plasma free amino acid (AA) concentrations in broiler chickens. In Experiment 1, exposure to 27-33°C of chickens for 2 weeks from 2 weeks of age did not affect growth and plasma free AA concentration except for a decrease in the concentration of plasma tyrosine (Tyr). In Experiment 2, 2-week-old birds were allocated to one of three temperature treatments; 24°C (control), 36°C (heat stress, HS) and 24°C pair-fed (24PF) for 2 weeks and fed on diets containing 50, 100 and 300% of NRC requirement for Trp. Heat stress caused a reduction of weight gain and feed intake irrespective of dietary Trp levels compared with control counterparts, while feeding of 300% Trp diet did not attenuate the reduced performance by HS exposure. In groups fed the 100% Trp diets, plasma aromatic AA (AAA) and Tyr concentrations were decreased in the HS birds compared with the 24PF group. Plasma concentrations in most of AA groups were increased by HS in chickens fed the 50% Trp diet, while those were not changed by HS in chickens fed the 300% Trp diet, compared with 24PF counterparts. The plasma Trp/LNAA (LNAA=large neutral AAs, which are comprised of BCAA, AAA and Trp) ratio was increased by HS in chickens fed the 100% Trp diet, while it was decreased in chickens fed on 50% Trp diet as compared with 24PF group. From these results, it is suggested that performance and plasma amino acid profile deranged by heat stress are modulated, at least, to be relieved from the heat stress by feeding 50% Trp diet but not at all by feeding 300% Trp diet. The involvement of altered plasma AA profiles, in particular plasma Tyr concentrations and Trp/LNAA ratio, is discussed in association with the performance characteristics of HS chickens. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 2 : 247-253)***

Key Words : Environmental Temperature, Tryptophan, Plasma Amino Acid Concentration, Broiler

INTRODUCTION

High ambient temperature constitutes a significant hindrance to poultry production in the tropical world. Thermal stress exerts its deleterious effects on feed intake and body weight gain (Howlider and Rose, 1987; MacLeod and Hocking 1993; Geraert et al., 1996a), as well as on carcass yield and mortality rates (Smith, 1993). Several research efforts have been made to overcome the negative effects of heat stress on chickens by manipulating the breeding programs, as well as the housing and nutrition of birds raised under high temperature environments (Howlider and Rose, 1987). With regards to nutritional manipulations in heat stressed birds, research has been conducted on energy, protein, fat, mineral and vitamin concentrations in chicken diets (Smith and Teeter, 1987; Sonaiya, 1989; Balnave and Oliva, 1990). Even though heat stress alters amino acid (AA) nutrition and metabolism, relatively few AAs, namely methionine (Met), lysine (Lys) and arginine (Arg), have been studied thus far in birds reared at high environmental temperature (Balnave and Oliva, 1990; Han and Baker, 1993; Brake et al., 1998). Geraert et al. (1996b) reported certain changes in plasma AA profile in chronically heat-stressed broiler chickens.

The physiological and biochemical changes induced in chickens by heat exposure may be modulated by tryptophan (Trp), because Trp has been shown to be involved in the stress responses. Immobilization stress increased brain levels of Trp and 5-hydroxyindoleacetic acid (HIAA), a metabolite of serotonin, while it increased plasma Trp concentrations in rats (Chaouloff et al., 1989). Acute exposure to cold stress decreased plasma Trp and tyrosine (Tyr) concentrations in human (Francesconi et al., 1972). Adeola and Ball (1992) reported that dietary manipulation of Trp and tyrosine (Tyr) might offer a practical means of reducing stress responses in swine. In broiler breeder males, increased levels of dietary Trp weakened aggressive behavior (Shea et al., 1990). In contrast, our previous findings showed that plasma Trp concentration increased but plasma Tyr concentration decreased; those changes consequently increased Trp/large neutral AA (LNAA) ratio in acutely heat-stressed broiler chickens (Tabiri et al., 2000). Trp regulates lipid and protein metabolism (Akiba et al., 1992; Lin et al., 1988), and influences feed intake (Rosebrough, 1996), all of which are altered in heat stressed chickens. From an aspect to investigate and address the problem of heat stress in broilers, however, there is less information available on the effects of dietary excessive- or deficient-Trp level on performance and plasma AA profile in heat-stressed broiler chickens.

The present experiments were therefore conducted to study the effect of heat stress and dietary Trp levels on performance, selected organ weights and plasma free AA concentrations of broilers reared in a high temperature

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environment.

MATERIALS AND METHODS

Animals and diets

One-day-old male broiler chicks (Ross strain), vaccinated for infectious bronchitis, were obtained from a commercial hatchery and kept in electrically heated brooders. Chicks had free access to water and commercial starter diet, which contained 3,200 kcal ME/kg and 22% CP, prior to the commencement of experiments at 11 days of age.

In Experiment 1, birds were divided into two groups of 12 birds (6 replicates with 2 birds/cage) at 11 days of age and transferred to cages in the experimental room for three days' adaptation to the new environment. After the adaptation period one group of birds was maintained at 24°C for 14 days and the other reared in a room with temperature cycling through the range 27-33°C. Body weight and feed intake were recorded weekly. An experimental diet (table 1) formulated to contain 20% CP, 3,200 kcal ME/kg and 100% of the NRC requirement for Trp (NRC, 1994) was given to all birds. At the end of feeding experiment, blood samples were taken for the determination of plasma AA concentration.

In Experiment 2, 90 birds (11 days of age) of similar body weight were transferred to cages in the experimental room for adaptation to the new chamber and experimental diets. At 14 days of age, 30 birds were reared at 24°C and formed the control group, another group of 30 birds was housed at 36°C (HS, heat stress), while a third group of 30 birds was housed at 24°C and pair-fed on the same amount of feed as that consumed by the HS group (24PF, 24°C pair-fed). Chicks in the control and HS groups were fed experimental diets *ad libitum* for the 14-day experimental period. The 24PF chickens were fed three times over the course of each day and received the equivalent amount in total of feed consumed the previous day by the HS group. The experimental diets were formulated to contain 50, 100 and 300% of the NRC requirement for Trp (table 1). Ten birds (5 replicates with 2 birds/cage) in each group were assigned to each of diets with different Trp level. In addition to the measurements made in Experiment 1, weights of the bursa of Fabricius and spleen were evaluated.

Table 1. Composition of experimental diets (%)

Ingredient	Experimental diet 50% Trp	100% Trp	300% Trp
L-Tryptophan	0	0.10	0.50
L-Glutamic acid	0.50	0.40	0
Constant ingrediets			
Yellow corn	--------	15.00	--------
Rice		30.00	
Corn starch		14.07	
Soybean meal (46.2% CP)		6.79	
Soybean oil		2.40	
Glucose		10.00	
Cellulose		2.08	
Amino acid mixture†		14.52	
Sodium chloride		0.44	
Potassium carbonate		0.12	
Calcium phosphate, dibasic		2.27	
Calcium carbonate		1.14	
Trace mineral mixture*		0.40	
Vitamin mixture*	-------	0.27	--------
Calculated analysis			
Metabolizable energy (kcal/kg)	3,200	3,200	3,200
Protein (%)	20.0	20.0	20.0
Tryptophan (%)	0.10	0.20	0.60

† The amino acid mixture provided (% diet): L-arginine 0.99, L-lysine-HCl 0.75, DL-methionine 0.67, glycine 3.79, L-histidine 0.18, L-isoleucine 0.49, L-leucine 0.60, L-phenylalanine 0.38, L-tyrosine 0.40, L-threonine 0.53, L-valine 0.52, L-glutamic acid, 5.22.

* See Akiba and Matsumoto (1978).

Measurements

At the end of each experiment, blood samples were obtained from a wing vein into heparinised syringes. Prior to blood sampling in Experiment 2, birds were subjected to 2 h of fasting followed by 2 h of refeeding in order to remove the effect of differences in feed intake on plasma AA concentrations. Birds were then killed by cervical dislocation. The bursa of Fabricius and spleen were excised and weighed in Experiment 2. Blood samples were centrifuged at 500×g for 10 min. Deproteinization was achieved by vortexing 0.5 ml of plasma with an equal volume of perchloric acid (10%) followed by centrifugation at 15,000×g for 20 min.

Plasma free amino acid analysis

Plasma free AA concentration was measured by reversed phase high-performance liquid chromatography (Shimadzu Model SCL-6A, Kyoto, Japan) using a column filled with octadecylsilica (ODS). The solvent system consisted of an aqueous buffer (0.14 M sodium acetate) titrated to pH 5.4 with glacial acetic acid and 60% acetonitrile in water. The separation procedure is principally based on the method previously developed (Bidlingmeyer et al., 1984) with some modifications. Large neutral amino acids (LNAA) were the sum of branched chain AA (BCAA; valine+leucine+isoleucine), aromatic AA (AAA; phenylalanine+tyrosine) and Trp.

Statistical analysis

A SAS application package (SAS, 1982 version, SAS Institute, Cary, NC, USA) was used for statistical calculations. Data in Experiment 1 were subjected to Student's t-test analyses. A three (dietary treatment) by three (temperature treatment) factorial statistical test was applied to analyze the data in Experiment 2, with the calculation carried out using the general linear model of SAS.

RESULTS

Experiment 1

Compared with the control 24°C group the high temperature (27-33°C) cycling protocol reduced body weight slightly but not significantly during the 14 d experimental period (data not shown). As shown in table 2, most of the plasma AA concentrations were unaffected by HS treatment. However, the plasma Tyr concentration was significantly decreased by the 27-33°C cycling protocol compared with the 24°C control group.

Experiment 2

Average values for weight gain, feed intake and gain/feed ratio in Experiment 2 are presented in table 3. HS significantly reduced body weight gain and feed intake of birds fed on 50, 100 and 300% Trp diets compared with their control groups, while there were no differences between the HS and the 24PF groups. Within each temperature treatment group, birds fed on the 50% Trp diet showed significant reduction in weight gain, feed intake and gain/feed ratio compared with those fed on the 100 and 300% Trp diets. The feeding of birds on the 300% Trp diet decreased feed intake slightly, but not significantly, compared with birds fed on the 100% Trp diet. In chickens fed on the 100 and 300% Trp diets, reductions in body weight gain in HS groups were proportional to reductions in feed intake, thus negating any significant effect of HS on gain/feed ratio.

Table 2. Effect of heat stress on plasma amino acid concentrations in broiler chickens (Experiment 1)

Amino acid	Plasma amino acid concentration (μmole/l)	
	24°C	27-33°C
Asp	99±13	81±7
Glu	228±16	183±6
Ser	481±29	537±43
His	192±8	165±12
Gly	843±30	745±40
Arg	472±29	490±15
Thr	589±35	584±66
Ala	497±29	427±45
Pro	351±18	295±21
Tyr	138±9[a]	103±1[b]
Val	177±6	159±7
Met	47±8	51±6
Cys	71±6	58±1
Ile	74±4	68±3
Leu	189±7	172±8
Phe	91±1	87±2
Trp	36±2	30±1
Lys	361±38	216±14
Trp/LNAA	0.054±0.003	0.051±0.004

[a,b] Means±SE (n=6) with different superscript letters are significantly different (p<0.05).

Relative weights (mg/100 g body weight) of the bursa of Fabricius in HS birds were significantly lower than control and 24PF groups when birds were fed on the 100 and 300% Trp diets, whereas no decrease was seen with HS when birds were fed on the 50% Trp diets (table 3). Relative spleen weights were reduced by HS irrespective of dietary Trp concentration used, although the reduction was least for the 50% Trp group.

The results in table 4 show that both HS treatment and dietary Trp altered the concentrations of several plasma free AA groups. A high temperature effect was found only for plasma AAA. In chickens fed the 100% Trp diets, essential AAs (EAAs) were decreased in the HS group compared with controls while they were comparable to the 24PF group. AAAs were decreased in the HS group relative to both the control and 24PF groups. In those birds fed the 50% Trp diets, plasma total AAs (TAAs), sulphur AAs (SAAs; Met+cysteine), BCAAs and AAAs were significantly increased by HS compared with their 24PF counterparts. In chickens fed the 300% Trp diet, TAAs and SAAs were increased in the HS group compared with controls, whereas they were comparable to the 24PF group.

A significant influence of dietary Trp level was found for plasma EAA, BCAA and AAA concentrations. In birds maintained at 24°C (control), TAA, EAA, BCAA and AAA concentrations were significantly decreased by the 300% Trp diet compared with those fed the 100% Trp diet. In the HS group, BCAA levels were increased in chickens fed the 50% Trp diet compared with those fed the 100% Trp diet.

As shown in table 5, plasma concentrations of several individual AAs were also modified by dietary Trp levels and HS. In chickens fed the 100% Trp diet, HS decreased Tyr concentrations compared with both the control and 24PF groups. In those fed on the 50% Trp diet, HS induced a significant rise in glutamic acid, serine, Arg, alanine, proline, Tyr, valine, Met, isoleucine, leucine and phenylalanine (Phe) levels compared with 24PF counterparts. Significant effects of dietary Trp on the

Table 3. Effects of heat stress and dietary tryptophan on performance and organ weights in broiler chickens (Experiment 2)

Dietary Trp (% NRC)	Treatment[1] Control	HS	24PF	SEM	ANOVA Treatment	Trp	Treatment×Trp
Body weight gain[2] (g/14 d)							
50	$261^{a,y}$	$177^{b,y}$	$136^{c,y}$				
100	$728^{a,x}$	$445^{b,x}$	$470^{b,x}$	27	*	*	*
300	$683^{a,x}$	$420^{b,x}$	$438^{b,x}$				
Feed intake[3] (g/14 d)							
50	$783^{a,y}$	$503^{b,y}$	$503^{b,y}$				
100	$1,198^{a,x}$	$841^{b,x}$	$841^{b,x}$	29	*	*	NS
300	$1,091^{a,x}$	$777^{b,x}$	$777^{b,x}$				
Gain/feed[3]							
50	$0.34^{a,y}$	$0.34^{a,y}$	$0.27^{b,y}$				
100	0.60^{x}	0.53^{x}	0.56^{x}	0.03	*	*	NS
300	0.62^{x}	0.54^{x}	0.56^{x}				
Bursa of fabricius[2] (mg/100 g body weight)							
50	323^{x}	280^{x}	308				
100	$244^{a,y}$	$118^{b,y}$	265^{a}	27	*	*	NS
300	$259^{a,x,y}$	$115^{b,y}$	312^{a}				
Spleen[2] (mg/100 g body weight)							
50	154^{a}	$90^{b,x}$	151^{a}				
100	136^{a}	$65^{b,y}$	119^{a}	14	*	NS	*
300	132^{a}	$82^{b,x,y}$	125^{a}				

[1] Abbreviations: Control, reared at 24°C; subjected to heat stress (36°C); 24PF, reared at 24°C and pair-fed on amount of feed consumed by HS group. [2] Mean of 10 observations. [3] Mean of 5 observations.

[a,b,c] Within treatments, means with different superscripts are significantly different ($p<0.05$).

[x,y] Within dietary Trp levels, means with different superscript are significantly different ($p<0.05$).

* $p<0.05$. NS: Not significant.

plasma amino acid profile were observed for histidine, threonine, Tyr, Phe, Trp and Lys concentrations.

The plasma Trp/LNAA ratio in the HS group was higher than that in the 24PF group in chickens fed the 100% Trp diet, whereas in chickens fed the 50% Trp diet it was lower than that in the 24PF group.

DISCUSSION

Exposure of chicks to 36°C depressed growth rate, feed intake and gain/feed ratio at all dietary Trp levels compared with their respective controls, but not when compared with 24PF groups (Experiment 2). The inverse relationship of weight gain with HS in the present experiment is in agreement with findings reported in other studies on chickens (Zuprizal et al., 1993; Mendes et al., 1997). In contrast, there was no significant effect of temperature on growth rate in chicks exposed to a cycling temperature of 27-33°C (Experiment 1). This observation could imply that the temperatures employed in the cycling temperature protocol (27-33°C) were not high enough to impact appreciably on performance for 2 weeks from 2 weeks of age in broiler chickens, because the sensitivity of chickens to HS has been shown to be dependent on the age of birds (Geraert et al., 1996b).

The dramatic reduction of feed intake and body weight gain of birds fed on low Trp diet was consistent with the finding of other workers (Lin et al., 1988; Mench, 1991). On the other hand, feeding the 300% Trp diet, a Trp-excessive diet, tended to decrease feed intake in every temperature treatment. The inverse effects of excessive Trp intake on performance are in good agreement with findings showing that excessive dietary Trp and intraperitoneal administration of Trp reduced feed intake in chickens (Pinchasov et al., 1996; Rosebrough, 1996). The present study, hence, showed that feeding a Trp-excessive diet is not beneficial in the attenuation of heat stress in broiler chickens.

In relation to the present study it should be emphasized that body weight gain and gain/feed ratio in chicks fed the 50% Trp diet in the HS group were greater than that seen for the 24PF group, while those parameters in chicks fed the 100 and 300% Trp diets responded inversely. Supplemental dietary Trp reduced feed intake and growth in chickens (Rosebrough et al., 1996). These findings could indicate that lowering of the dietary Trp concentration below the NRC (1994) requirement, resulting in a low plasma Trp/LNAA ratio, is advantageous when chickens are subjected to heat exposure. This may be partly substantiated by results observed in the control group (24°C) that the

Table 4. Effects of heat stress and dietary tryptophan on plasma amino acid concentrations in broiler chickens (Experiment 2)

Amino acid Group[2]	Dietary Trp (% NRC)	Plasma amino acid concentration[3] (µmole/l)			SEM	ANOVA		
		Control[1]	HS	24PF		Treatment	Trp	Treatment×Trp
TAA	50	$4,050^{a,b,x}$	$5,310^{a}$	$3,260^{b,y}$				
	100	$4,420^{x}$	3,620	$4,110^{x}$	260	NS	NS	*
	300	$3,040^{b,y}$	$4,110^{a}$	$3,890^{a,b,x,y}$				
EAA	50	$1,680^{a,b,x}$	$1,820^{a,x}$	$1,260^{b}$				
	100	$1,720^{a,x}$	$1,130^{b,y}$	$1,260^{b}$	109	NS	*	NS
	300	$960^{b,y}$	$1,200^{a,y}$	$1,090^{a}$				
SAA	50	$164^{a,x}$	168^{a}	$86^{b,y}$				
	100	$146^{x,y}$	135	$128^{x,y}$	13	NS	NS	*
	300	$110^{b,y}$	185^{a}	$163^{a,x}$				
BCAA	50	$301^{a,x}$	$355^{a,x}$	183^{b}				
	100	$297^{a,x}$	$226^{b,y}$	225^{b}	20	NS	*	*
	300	183^{y}	217^{y}	227				
AAA	50	$180^{a,y}$	235^{a}	$94^{b,z}$				
	100	$268^{a,x}$	215^{b}	$267^{a,x}$	15	*	*	*
	300	170^{y}	205	170^{y}				

[1] Abbreviations: See table 3.

[2] Abbreviations: TAA, total amino acids; EAA, essential amino acids; SAA, sulfur amino acids; BCAA, branched chain amino acids; AAA, aromatic amino acids.

[3] Mean of 6 observations.

[a,b,c] Within treatments, means with different superscript letters were significantly different ($p<0.05$).

[x,y,z] Within dietary Trp levels, means with different superscript letters were significantly different ($p<0.05$).

$p<0.05$. NS: Not significant.

decrease in the feed intake together with a small decrease in weight gain observed in chickens fed the 300% Trp diet was associated with a rise in the plasma Trp/LNAA ratio. It has been proposed that plasma Trp/LNAA may modulate feed intake (Peng et al., 1973; Asheley and Anderson, 1985). It is, however, relevant that the plasma Trp/LNAA ratio is not essentially the sole factor determining resistance of chickens to HS, because the plasma Trp/LNAA ratio was not changed by HS when chickens were fed a Trp-excessive diet (300% of NRC requirement).

Average weights of the bursa of Fabricius, which is involved in immune function, were decreased by HS compared with control and 24PF groups in chickens fed the 100 and 300% Trp diets, whereas spleen weights were decreased by HS treatment in chickens fed on diets containing any of the Trp levels used. It has been noted that the spleen, thymus and bursa of Fabricius become smaller during prolonged exposure of chickens to high temperature (Williamson et al., 1985). Our data may support the observation that, in general, HS impairs immune function in chickens. However, in the 50% Trp diet-fed chickens, changes in bursa of Fabricius and spleen weights by HS were not found or were less pronounced compared with 100 and 300% Trp diets-fed chickens. It is, therefore, suggested that the feeding of chicks on a low Trp diet could, in part, feasibly lessen the impairment of immune function imposed by HS in broiler chickens.

Exposure of chickens to 36°C for 14 days in the present study lowered the concentration of plasma free AA groups, in particular EAA, BCAA and AAA, in chickens fed the 100% Trp diet (a standard diet containing 100% of the NRC requirement for Trp). These findings substantiate previous evidence that plasma free AA concentrations, particularly BCAA, threonine, Lys and glutamine concentrations, were decreased in heat-exposed chickens at 4 weeks of age (Geraert et al., 1996b). In both the 50% and 100% Trp groups plasma Trp concentrations tended to increase by HS, which is in agreement with findings in a previous report by us that acute heat stress significantly increased plasma Trp concentrations in chickens (Tabiri et al., 2000). The significant decrease of plasma Tyr concentration found in HS birds compared with 24PF birds in the 100 % Trp group (Experiment 2) was consistent with the decrease seen with the 27-33°C temperature cycling protocol treatment in Experiment 1. Tyr is synthesized from Phe and subsequently metabolized through transamination catalyzed by Tyr aminotransferase in the liver. Plasma Phe concentrations in the HS groups tended to increase compared with the 24PF group in Experiment 2 although hepatic Tyr aminotransferase activity was not evaluated in the present study. In the 50% Trp group, however, the plasma Tyr concentration was increased by HS compared with the 24PF group. The decrease and increase of plasma Tyr concentrations in the 100% and 50% Trp groups,

Table 5. Effect of heat stress and dietary tryptophan on plasma amino acids concentrations (Experiment 2)

Amino Acid	Plasma amino acid concentration (µmole/l)[2]										ANOVA		
	50% Trp diet			100% Trp diet			300% Trp diet			SEM	Treatment	Trp	Treatment ×Trp
	Control[1]	HS	24PF	Control	HS	24PF	Control	HS	24PF				
Asp	120^{a}	93^{ab}	75^{b}	140^{a}	111^{b}	126^{ab}	104	92	103	7	*	*	NS
Glu	88b	170^{a}	68^{b}	110	100	106	82	147	100	14	*	NS	NS
Ser	711^{b}	1.018^{a}	611^{b}	806	817	838	611^{b}	860^{a}	906^{a}	35	*	NS	*
His	105	136	126	173	137	158	103^{b}	166^{a}	145^{a}	9	NS	*	*
Gly	544^{b}	923^{a}	655^{ab}	544	454	669	537^{b}	828^{a}	689^{a}	62	*	NS	NS
Arg	$187^{a,b}$	344^{a}	111^{b}	483^{a}	215^{b}	264^{b}	192	244	137	48	*	NS	NS
Thr	828	761	630	688^{a}	403^{b}	$536^{a,b}$	390	414	489	45	NS	*	NS
Ala	315^{b}	620^{a}	232^{b}	390	457	450	282	409	391	48	*	NS	NS
Pro	213^{a}	266^{a}	148^{b}	257	193	248	191^{b}	$221^{a,b}$	265^{a}	14	NS	NS	*
Tyr	128^{a}	162^{a}	66^{b}	198^{a}	147^{b}	212^{a}	126	139	128	12	NS	*	*
Val	157^{a}	162^{a}	101^{b}	152	$117^{a,b}$	111^{b}	89	112	109	7	NS	*	*
Met	69^{a}	93^{a}	37^{b}	50	38	39	49^{b}	82^{a}	$59^{a,b}$	6	*	NS	*
Cys	95^{a}	75^{ab}	50^{b}	96	97	89	61^{b}	104^{a}	103^{a}	6	NS	NS	*
Ile	74^{a}	80^{a}	45^{b}	64^{a}	46^{b}	46^{b}	44	39	53	5	*	NS	*
Leu	69^{b}	133^{a}	37^{b}	80	62	68	50	61	65	13	NS	NS	*
Phe	52^{a}	73^{a}	28^{b}	70^{a}	$68^{a,b}$	55^{b}	44^{b}	66^{a}	42^{a}	5	*	*	NS
Trp	12	17	12	25	34	25	40	43	46	2	NS	*	NS
Lys	284	204	230	$99^{a,b}$	127^{a}	66^{b}	44^{b}	88^{a}	$55^{a,b}$	18	NS	*	NS
Trp/LNAA	0.024^{b}	0.028^{b}	0.041^{a}	0.042^{b}	0.072^{a}	0.048^{b}	0.104	0.092	0.104	0.005	NS	*	NS

[1] Abbreviations: See table 3. [2] Mean of 6 observations.

[a,b] Within treatments, means with different superscript letters are significantly different ($p<0.05$).

* $p<0.05$. NS: Not significant.

respectively, appeared to lead to an increase and decrease in the Trp/LNAA ratio, respectively, since Tyr played a major part in the LNAA concentrations. The inclusion of Tyr and Tyr-metabolizing enzyme and thereby determination of Tyr requirements as well as Trp for heat exposed broilers requires further investigation.

With the exception of Phe, no significant influence of HS compared with 24PF treatment was observed in plasma AA concentrations in chickens fed the 300% Trp diet. In contrast, in chickens fed the 50% Trp diet, plasma AA groups, together with several individual AAs in the HS group, were higher than those in the 24PF group. The observed changes in plasma free AA concentrations induced by HS may represent alterations in amino acid utilization in these birds which in turn may change their requirement for amino acids compared with those kept at 24°C. The plasma EAA, SAA, BCAA and AAA concentrations in chickens fed the 50% Trp diet under HS became close to those in control chickens fed the 100% Trp diet compared with those in chickens fed the 100 and 300% Trp diets under heat stress conditions. It seems inferable therefore that if plasma AA concentrations in chickens fed a 100% Trp diet under a controlled environment (24°C) are within normal concentrations, then feeding of chickens with a low Trp diet instead of a standard or high Trp diet has the potential to normalize the concentration differences caused by HS.

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