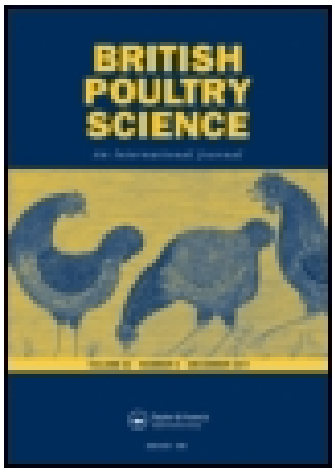

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Changes in free amino acid concentrations in the blood, brain and muscle of heat-exposed chicks

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Changes in free amino acid concentrations in the blood, brain and muscle of heat-exposed chicks

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Abstract 1. An experiment was conducted to analyse the changes in free amino acid concentrations in the blood, brain and muscle of chicks in response to 15 or 30 min exposure to high ambient temperature (HT).
2. Food intake and body weight were not affected, while rectal temperature was significantly increased by short-term HT exposure.
3. Several free amino acid concentrations increased in the blood, brain and muscle even with short-term HT, whereas levels of a few amino acids declined significantly. As well as the nonessential amino acids, essential amino acids also significantly increased with exposure to HT.
4. 3-Methylhistidine, a marker of proteolysis, significantly declined in the muscle of HT chicks, implying a reduction of protein breakdown under HT.
5. These results indicate that alteration of protein metabolism may occur in chicks even with short-term heat exposure.

INTRODUCTION

Amino acids play various important roles, not only as constituents of proteins but also as regulators of many physiological and/or pharmacological functions. In particular, much attention has been paid to the regulation of physiology and behaviour, including stress responses (Asechi *et al.*, 2006; Hamasu *et al.*, 2009*a*; 2009*b*; Kurauchi *et al.*, 2010; Kurata *et al.*, 2011; Erwan *et al.*, 2014). Therefore, relationships between stress and amino acid metabolism may partly explain the physiological state of the individual. In general, high ambient temperature (HT) may induce stress to birds and interfere with the maintenance of homeothermic body temperature as they lack sweat glands and have relatively high body temperatures (around 41.5°C), relying on evaporative cooling (panting) to keep them cool (Marder and Arad, 1989). The exposure of birds to HT can lead to an increase in deep body temperature (rectal temperature) (Yahav and Hurwitz, 1996; Chowdhury *et al.*, 2012*a*, 2012*b*). When the heat increment of the birds exceeds the heat dissipation, HT can cause stress (Soleimani *et al.*, 2010). This heat stress has a detrimental effect on food intake and body weight gain, which ultimately causes low production efficiency in chickens and, at worst, increases mortality (Lin *et al.*, 2006; Daghir, 2008; Azad *et al.*, 2010). Behavioural, physiological and molecular adjustments occur when chickens are exposed to heat stress (Etches *et al.*, 1995). In our previous report, it was shown that young chicks, when exposed to HT, reduced their food intake and that the expression of a food intake-regulating hypothalamic neuropeptide mRNA increased (Chowdhury *et al.*, 2012*b*).

Balnave and Oliva (1991) showed that the absorption of arginine, an essential amino acid in chickens, decreased significantly during HT.

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Supplementation of amino acids, in particular essential amino acids, has been performed to overcome the problems caused by heat stress in birds (Mendes *et al.*, 1997; Rose and Uddin, 1997; Brake *et al.*, 1998; Daghir *et al.*, 2003; Willemsen *et al.*, 2011; Dai *et al.*, 2012). However, knowledge of the overall changes in the metabolism of amino acids when exposed to stress is helpful for proper amino acid supplementation. For instance, acute isolation stress reduced brain proline in chicks and its administration attenuated the stress response (Hamasu *et al.*, 2009*a*). Hamasu *et al.* (2009*a*) also found that even 30 min of restraint with isolation-induced stress reduced several free amino acid concentrations in the brain of neonatal chicks. To the best of our knowledge, there are no reports on the overall amino acid metabolism in chickens at an early stage of HT exposure. Therefore, the aim of the present experiment was to investigate the effect of short-term HT on free amino acid concentrations in the blood plasma, brain and muscle of chickens as well as to examine plasma corticosterone and metabolites just after HT exposure.

MATERIALS AND METHODS

Experimental animals

One-d-old male layer chicks (Julia) (*Gallus gallus domesticus*) were purchased from a local hatchery (Murata hatchery, Fukuoka, Japan) and housed in groups in metal cages at a constant temperature of 30 ± 1°C under continuous light until they were 14 d old. Food (Commercial starter diet (metabolisable energy: 12.77 MJ/kg and protein: 24%; food ingredients: grain 61% (mainly maize), defatted meal 25% (soybean meal and maize gluten meal), fish meal 9%, rice bran 1% and others 4%; AX; Toyohashi Feed and Mills Co. Ltd., Aichi, Japan)) and water were available *ad libitum*. On the basis of our previous experiment, 14- or 21-d-old chicks were more responsive to HT in terms of food intake than neonatal chicks (Chowdhury *et al.*, 2012*b*). Thus, in the present study, we used 14-d-old chicks to examine the effect of HT (35°C) in comparison with that of a control thermoneutral temperature (CT; 30°C). Chicks were placed in individual cages (floor space: 15 cm × 28 cm; height: 13 cm) 24 h before the start of the experiment. A total of 30 chicks (n = 7 or 8 in each group) were exposed to HT or CT for either 15 or 30 min, with chicks allocated to groups according to the body mass so that the initial body mass was similar in the two groups. Chicks were given free access to water and feed during the exposure to HT or CT. This study was performed according to the guidelines for animal experiments in the Faculty of Agriculture of Kyushu University and complied with Law No. 105 and Notification No. 6 of the Japanese Government.

Experimental design

Body weight and rectal temperature were recorded initially. The HT chicks were then placed in their cages into a temperature-controlled chamber (Sanyo Electric Co. Ltd., Tokyo, Japan), whilst the control chicks (CT) were placed in their cages on similar racks as per the methods described previously (Chowdhury *et al.*, 2012*a*). Rectal temperature, food intake and body weight were measured in all 30 independent replicates in both groups at 15 or 30 min of HT or CT. The rectal temperature of chicks was measured using a digital thermometer with an accuracy of ± 0.1°C (Thermalert TH-5, Physitemp Instruments Inc., Clifton, NJ, USA), by inserting the thermistor probe in the cloaca to a depth of 1–2 cm. All birds were killed at the end of experiment of either 15 or 30 min HT or CT by exposure to isoflurane (Mylan Inc., Tokyo, Japan). The blood was immediately collected through the jugular vein into heparinised tubes and centrifuged at 4°C and 10 000*g* for 4 min. Then, the plasma was collected and stored at –80°C until the analysis of the concentrations of free amino acids, corticosterone and metabolites (glucose, total cholesterol, triacylglycerol and uric acid) was carried out. Plasma total protein, calcium and aspartate aminotransferase were also analysed. The brains were dissected, and the diencephalon, telencephalon, mesencephalon and cerebellum as well as about 50 mg of each type of breast muscle (*pectoralis* muscle and *supracoracoideus* muscle) were collected and stored in Eppendorf tubes. Then, these samples were frozen into liquid nitrogen and stored at –80°C in the deep freezer until their use.

Analysis of free amino acids

To investigate the effect of HT, free amino acid concentrations in the plasma, brain and breast muscle were analysed by HPLC. Free amino acid concentrations were analysed according to the method of Boogers *et al.* (2008) with some modifications. The brain tissue and breast muscle were homogenised in ice-cold 0.2 M perchloric acid solution containing 0.01 M ethylenediaminetetraacetic acid disodium salt (EDTA•2Na) and left for deproteinisation on ice. After 30 min, the homogenates were centrifuged at 0°C at 20 000*g* for 15 min. The supernatants were adjusted to pH 7 with 1 M sodium hydroxide. Plasma was deproteinised by filtration through a 10 000 dalton molecular weight cut-off filter (Millipore, Bedford, MA, USA) via centrifugation at 12 000*g* for 10 min at 4°C (MX-307, Tommy, Japan). Each 20-μl sample of

the brain tissue and breast muscle and a 10-µl sample of the plasma were dried under reduced pressure at −100 kPa (Centrifugal Vaporizer, CVE-200D, Eyela, Japan). The dried residues were dissolved in 10 µl of 1 M sodium acetate–methanol–triethylamine (2:2:1), re-dried under reduced pressure and then converted to their phenylthiocarbamoyl derivatives by dissolving in 20 µl of methanol–distilled water–triethylamine–phenylisothiocyanate (7:1:1:1) and allowing to react for 20 min at room temperature. The samples were dried again and dissolved in 200 µl of Pico-Tag Diluent (Waters, Milford, CT, USA). These diluted samples were filtered through a 0.20-µm filter (Millipore). The same methods were performed on standard solutions which were prepared by diluting a commercially available L-amino acid solution (type ANII, type B, L-asparagine, L-glutamine and L-tryptophan; Wako, Osaka, Japan) with distilled water. The solution containing the derivatives was applied to a Waters HPLC system (Pico-Tag free amino acid analysis column (3.9 mm × 300 mm), Alliance 2690 separation module, 2487 dual-wavelength UV detector and Millennium 32 chromatography manager; Waters). They were equilibrated with buffer A (70 mM sodium acetate adjusted to pH 6.45 with 10% acetic acid–acetonitrile, ratio 975:25) and eluted with a linear gradient of buffer B (water–acetonitrile–methanol (40:45:15) (0%, 3%, 6%, 9%, 40% and 100%)) at a flow rate of 1 ml/min at 46°C. The concentrations of free amino acids and dipeptides (phosphoserine, aspartic acid, glutamine, α-aminoadipic acid, hydroxyproline, serine, asparagine, glycine, glutamic acid, β-alanine, taurine, histidine, GABA, threonine, alanine, carnosine, arginine, proline, 1-methylhistidine, anserine, 3-methylhistidine, tyrosine, valine, methionine, cystathionine, isoleucine, leucine, phenylalanine, tryptophan, ornithine and lysine) were determined by the absorbance at a wavelength of 254 nm. The plasma amino acid concentrations were expressed as pmol/µl, and the amino acid concentrations in the brain and breast muscle were expressed as pmol/mg wet tissue.

Plasma levels of corticosterone and metabolites

Corticosterone concentrations were determined by an enzyme immunoassay kit (Enzo Life Sciences, Inc. Farmingdale, NY, USA) and expressed as ng/ml plasma. This assay had < 0.03% cross-reaction with pregnenolone, β-oestradiol, cortisone and 11-dehydrocorticosterone acetate. The intra- and inter-assay variations were 7.67% and 9.70%, respectively, and the sensitivity was 27.0 pg/ml according to the manufacturer's protocol. Plasma metabolites were measured with an automatic dry chemistry blood analyser (Fuji Dry-CChem 7000 V; Fuji Medical Systems, Tokyo, Japan). All the samples were assayed together and in a random sequence for each metabolite.

Statistical analyses

Data were analysed by factorial two-way analysis of variance with respect to HT and treatment time of chicks. Bonferroni's test was performed as a means separation test to compare HT and CT in terms of treatment time. Statements of significance were based on $P < 0.05$. Data were expressed as means ± SEM.

RESULTS

Rectal temperature, food intake and body weight

The changes in rectal temperature in chicks exposed to HT or CT for either 15 or 30 min are shown in Table 1. Rectal temperature increased significantly ($P < 0.05$) in the heat-treated group in comparison with the control group. Food intake and body weight were not significantly ($P > 0.05$) affected by the treatment (data not shown).

Free amino acid concentrations in the plasma

The changes in free amino acid concentrations in the plasma of chicks exposed to either HT or CT are shown in Table 2. Hydroxyproline, asparagine, glycine, 1-methylhistidine, methionine, tryptophan, ornithine and the peptide carnosine significantly ($P < 0.05$) increased following treatment; however, taurine significantly ($P < 0.05$) decreased. In addition, hydroxyproline, 1-methylhistidine and the peptide anserine significantly ($P < 0.05$) increased with the length of treatment.

Table 1. *Changes in rectal temperature in chicks exposed to heat treatment or control (HT or CT) for 15 or 30 min*

	CT		HT		*P*-value		
	15 min	30 min	15 min	30 min	Treatment	Time	Treatment × Time
Rectal temperature	0.050 ± 0.098	−0.029 ± 0.123	0.538 ± 0.081	0.629 ± 0.119	$P < 0.001$	NS	NS

The number of chicks used in each group was as follows: CT for 15 min, 8; CT for 30 min, 7; HT for 15 min, 8; HT for 30 min, 7. NS: Not significant. Values are means ± SEM in °C.

Table 2. *Free amino acids contents in plasma of chicks exposed to heat treatment or control (HT or CT) for 15 or 30 min*

	CT		HT		*P*-value		
Amino acids	15 min	30 min	15 min	30 min	Treatment	Time	Treatment × Time
Essential amino acids[1]							
Histidine	5.11 ± 0.23	4.69 ± 0.31	4.51 ± 0.39	4.35 ± 0.40	NS	NS	NS
Threonine	143 ± 8	138 ± 12	151 ± 12	153 ± 15	NS	NS	NS
Arginine	35.6 ± 3.3	34.4 ± 3.1	32.8 ± 2.5	34.7 ± 3.6	NS	NS	NS
Valine	35.6 ± 3.4	41.2 ± 5.4	41.5 ± 4.8	48.1 ± 5.8	NS	NS	NS
Methionine	10.2 ± 1.4	7.5 ± 1.2	20.2 ± 3.8	17.1 ± 4.3	$P < 0.05$	NS	NS
Isoleucine	28.6 ± 2.7	32.2 ± 2.6	34.4 ± 3.0	39.8 ± 4.1	NS	NS	NS
Leucine	27.8 ± 4.5	25.6 ± 4.7	35.3 ± 6.7	35.9 ± 8.0	NS	NS	NS
Phenylalanine	14.4 ± 1.7	15.5 ± 1.4	12.9 ± 0.6	14.8 ± 1.2	NS	NS	NS
Tryptophan	11.1 ± 0.8	11.0 ± 0.2	13.9 ± 1.5	14.0 ± 0.9	$P < 0.05$	NS	NS
Lysine	147 ± 15	147 ± 17	238 ± 45	166 ± 34	NS	NS	NS
Nonessential amino acids[1]							
Hydroxyproline	12.9 ± 0.4	15.5 ± 2.0	16.7 ± 1.2	22.1 ± 2.7	$P < 0.05$	$P < 0.05$	NS
Asparagine	18.2 ± 1.8	20.3 ± 2.0	26.2 ± 2.7	30.0 ± 4.8	$P < 0.05$	NS	NS
Glycine	34.5 ± 1.2	36.2 ± 2.3	49.0 ± 5.8	47.8 ± 5.8	$P < 0.05$	NS	NS
Taurine	18.2 ± 1.1	17.3 ± 0.6	15.8 ± 1.0	13.2 ± 1.2	$P < 0.05$	NS	NS
Carnosine	37.5 ± 7.8	46.7 ± 9.5	53.7 ± 6.8	70.2 ± 11.5	$P < 0.05$	NS	NS
1-Methylhistidine	2.98 ± 0.32	4.43 ± 0.48	4.12 ± 0.48	5.23 ± 0.43	$P < 0.05$	$P < 0.05$	NS
Anserine	5.08 ± 0.36	6.60 ± 0.23	6.23 ± 0.49	6.53 ± 0.30	NS	$P < 0.05$	NS
Ornithine	5.1 ± 0.4	5.3 ± 0.5	11.2 ± 1.9	12.4 ± 1.2	$P < 0.05$	NS	NS

The number of chicks used in each group was as follows: CT for 15 min, 8; CT for 30 min, 7; HT for 15 min, 8; HT for 30 min, 7. [1]All the essential amino acids were analysed and only significantly changed nonessential amino acids are shown. NS: Not significant. Values are means ± SEM in pmol/µl.

Free amino acid concentrations in the brain

The changes in free amino acid concentrations in the different brain segments of HT and CT chicks are shown in Tables 3–6. In the diencephalon, proline and cystathionine significantly ($P < 0.05$) increased following treatment. Although hydroxy-proline was not affected by treatment, it did significantly ($P < 0.05$) increase with the duration of the treatment. In addition, phosphoserine significantly ($P < 0.05$) decreased with the duration of treatment. On the other hand, a significant ($P < 0.05$) interaction between the time and treatment was detected for phosphoserine, glutamic acid and cystathionine, implying that the levels of these amino acids in the control did not change or only gradually decreased with the progress of time, but that the reverse was true for the HT group. In the telencephalon, threonine and isoleucine significantly ($P < 0.05$) increased in the HT chicks. Ornithine showed a significant interaction ($P < 0.05$) between treatment and time, whereby the level of ornithine gradually decreased as the treatment progressed; however, the inverse effect

Table 3. *Free amino acids contents in chick brain (diencephalon) exposed to heat treatment or control (HT or CT) for 15 or 30 min*

	CT		HT		*P*-value		
Amino acids	15 min	30 min	15 min	30 min	Treatment	Time	Treatment × Time
Essential amino acids[1]							
Histidine	150 ± 19	171 ± 24	191 ± 24	187 ± 21	NS	NS	NS
Threonine	1674 ± 509	1452 ± 540	1599 ± 414	1560 ± 711	NS	NS	NS
Arginine	504 ± 37	507 ± 42	622 ± 66	518 ± 48	NS	NS	NS
Valine	298 ± 19	264 ± 21	344 ± 37	302 ± 42	NS	NS	NS
Methionine	129 ± 10	118 ± 12	161 ± 22	141 ± 27	NS	NS	NS
Isoleucine	130 ± 6.5	123 ± 11	158 ± 20	142 ± 20	NS	NS	NS
Leucine	145 ± 5	143 ± 8	172 ± 19	155 ± 13	NS	NS	NS
Phenylalanine	169 ± 13	160 ± 12	202 ± 25	155 ± 17	NS	NS	NS
Lysine	1369 ± 213	1116 ± 255	1516 ± 239	1214 ± 382	NS	NS	NS
Nonessential amino acids[1]							
Phosphoserine	137 ± 9	137 ± 6	118 ± 5	136 ± 5	NS	$P < 0.05$	$P < 0.05$
Glutamic acid	9341 ± 557	8865 ± 393	8417 ± 268	9588 ± 356	NS	NS	$P < 0.05$
Hydroxyproline	249 ± 25	262 ± 33	270 ± 33	368 ± 46	NS	$P < 0.05$	NS
Proline	168 ± 13	161 ± 12	214 ± 23	196 ± 21	$P < 0.05$	NS	NS
Cystathionine	30 ± 1	30 ± 3	32 ± 1	38 ± 23	$P < 0.05$	NS	$P < 0.05$

The number of chicks used in each group was as follows: CT for 15 min, 8; CT for 30 min, 7; HT for 15 min, 8; HT for 30 min, 7. [1]All the essential amino acids were analysed and only significantly changed nonessential amino acids are shown. NS: Not significant. Values are means ± SEM in pmol/mg.

Table 4. *Free amino acids contents in chick brain (telencephalon) exposed to heat treatment or control (HT or CT) for 15 or 30 min*

	CT		HT		*P*-value		
Amino acids	15 min	30 min	15 min	30 min	Treatment	Time	Treatment × Time
Essential amino acids[1]							
Histidine	12.6 ± 1.3	14.8 ± 2.3	17.0 ± 2.1	13.6 ± 1.7	NS	NS	NS
Threonine	133 ± 12	125 ± 6	140 ± 6	169 ± 20	$P < 0.05$	NS	NS
Arginine	69.4 ± 5.4	76.0 ± 7.2	70.3 ± 7.9	70.0 ± 5.3	NS	NS	NS
Valine	50.2 ± 3.3	41.3 ± 1.1	45.8 ± 2.0	45.1 ± 4.3	NS	NS	NS
Methionine	42.4 ± 2.5	44.2 ± 4.5	40.4 ± 2.8	39.3 ± 4.7	NS	NS	NS
Isoleucine	16.3 ± 0.7	16.8 ± 0.6	19.7 ± 0.6	18.3 ± 1.9	$P < 0.05$	NS	NS
Leucine	44.7 ± 3.5	39.9 ± 5.6	47.2 ± 2.8	50.2 ± 5.3	NS	NS	NS
Phenylalanine	22.6 ± 2.1	23.0 ± 0.7	21.5 ± 1.5	19.5 ± 1.3	NS	NS	NS
Lysine	368 ± 39	338 ± 48	365 ± 30	195 ± 19	NS	NS	NS
Nonessential amino acids[1]							
Ornithine	10.8 ± 1.6	15.4 ± 2.0	16.1 ± 2.5	11.7 ± 1.6	NS	NS	$P < 0.05$

The number of chicks used in each group was as follows: CT for 15 min, 8; CT for 30 min, 7; HT for 15 min, 8; HT for 30 min, 7.
[1]All the essential amino acids were analysed and only significantly changed nonessential amino acids are shown. NS: Not significant. Values are means ± SEM in pmol/mg.

Table 5. *Free amino acids contents in chick brain (mesencephalon) exposed to heat treatment or control (HT or CT) for 15 or 30 min*

	CT		HT		*P*-value		
Amino acids	15 min	30 min	15 min	30 min	Treatment	Time	Treatment × Time
Essential amino acids[1]							
Histidine	59 ± 9	57 ± 4	91 ± 18	105 ± 27	$P < 0.05$	NS	NS
Threonine	652 ± 123	535 ± 107	710 ± 47	770 ± 86	NS	NS	NS
Arginine	335 ± 60	261 ± 43	408 ± 79	368 ± 83	NS	NS	NS
Valine	212 ± 44	167 ± 39	232 ± 19	191 ± 26	NS	NS	NS
Methionine	116 ± 24	114 ± 26	164 ± 13	138 ± 27	NS	NS	NS
Isoleucine	97 ± 22	79 ± 17	111 ± 8.5	91 ± 10	NS	NS	NS
Leucine	91 ± 20	73 ± 14	106 ± 9.2	86 ± 12	NS	NS	NS
Phenylalanine	120 ± 25	97 ± 21	131 ± 21	106 ± 19	NS	NS	NS
Lysine	1106 ± 271	944 ± 309	1062 ± 228	858 ± 210	NS	NS	NS
Nonessential amino acids[1]							
Proline	104 ± 19	85 ± 14	148 ± 21	136 ± 32	$P < 0.05$	NS	NS

The number of chicks used in each group was as follows: CT for 15 min, 8; CT for 30 min, 7; HT for 15 min, 8; HT for 30 min, 7. [1]All the essential amino acids were analysed and only significantly changed nonessential amino acids are shown. NS: Not significant. Values are means ± SEM in pmol/mg.

Table 6. *Free amino acids contents in chick brain (cerebellum) exposed to heat treatment or control (HT or CT) for 15 or 30 min*

	CT		HT		*P*-value		
Amino acids	15 min	30 min	15 min	30 min	Treatment	Time	Treatment × Time
Essential amino acids[1]							
Histidine	111 ± 26	115 ± 22	142 ± 26	132 ± 18	NS	NS	NS
Threonine	501 ± 34	528 ± 57	480 ± 17	616 ± 51	NS	NS	NS
Arginine	274 ± 19	297 ± 23	329 ± 38	294 ± 25	NS	NS	NS
Valine	179 ± 4	158 ± 5	170 ± 12	179 ± 12	NS	NS	NS
Methionine	81 ± 5	84 ± 9	109 ± 8	86 ± 6	$P < 0.05$	NS	NS
Isoleucine	72.5 ± 2.6	66.1 ± 2.3	76.8 ± 5.6	79.9 ± 9.1	NS	NS	NS
Leucine	144 ± 10	150 ± 10	163 ± 14	158 ± 13	NS	NS	NS
Phenylalanine	111 ± 5	110 ± 5	135 ± 3	106 ± 4	$P < 0.05$	$P < 0.05$	$P < 0.05$
Tryptophan	253 ± 10	254 ± 10	270 ± 18	246 ± 12	NS	NS	NS
Lysine	1782 ± 262	1738 ± 385	2285 ± 480	1862 ± 512	NS	NS	NS
Nonessential amino acids[1]							
Aspartic acid	1704 ± 35	1550 ± 39	1730 ± 86	1641 ± 47	NS	$P < 0.05$	NS
GABA	762 ± 16	753 ± 8.6	878 ± 55	804 ± 13	$P < 0.05$	NS	NS
Alanine	673 ± 15	606 ± 23	647 ± 39	693 ± 23	NS	NS	$P < 0.05$
Carnosine	50.6 ± 3.8	54.0 ± 2.9	65.1 ± 4.8	53.5 ± 1.2	NS	NS	$P < 0.05$
Cystathionine	47.8 ± 1.5	50.1 ± 3.2	47.2 ± 2.4	60.1 ± 2.8	NS	$P < 0.05$	$P < 0.05$

The number of chicks used in each group was as follows: CT for 15 min, 8; CT for 30 min, 7; HT for 15 min, 8; HT for 30 min, 7. [1]All the essential amino acids were analysed and only significantly changed nonessential amino acids are shown. NS: Not significant. Values are means ± SEM in pmol/mg.

was apparent in the control groups. In the mesencephalon, histidine and proline significantly ($P < 0.05$) increased following treatment. In the cerebellum, GABA, methionine and phenylalanine significantly ($P < 0.05$) increased following treatment. Furthermore, aspartic acid, cystathionine and phenylalanine significantly ($P < 0.05$) increased with the duration of the treatment, giving a hint that length of the experiment acted to increase these amino acids in the treated group, while almost no change was found in the control group. A significant ($P < 0.05$) interaction was detected between duration and treatment for alanine, carnosine, cystathionine and phenylalanine. The interaction effect between duration and treatment for carnosine and cystathionine suggests that the former declined but the latter increased as the treatment progressed, while no significant change occurred in the control groups. As for alanine, the concentration of free amino acids increased with treatment duration, while a counter change was found in the control groups. In the control group, no significant change occurred in the cystathionine; however, it increased in the HT chicks with the duration of treatment.

Free amino acid concentrations in the breast muscle

The concentrations of the free amino acids in the breast muscle (*pectoralis* muscle and *supracoracoideus* muscle) of HT or CT chicks are shown in Tables 7 and 8. Glutamic acid, asparagine, histidine, threonine, arginine, proline and cystathionine significantly ($P < 0.05$) increased following treatment in the *pectoralis* muscle. However, *β*-alanine and 3-methylhistidine significantly ($P < 0.05$) decreased. Furthermore, asparagine, histidine, threonine, proline and methionine significantly ($P < 0.05$) increased with the duration of treatment, while *β*-alanine significantly ($P < 0.05$) decreased, suggesting that several amino acids increased with treatment duration, although the reverse was true for *β*-alanine. Threonine showed a significant ($P < 0.05$) interaction between time and treatment, implying that the concentration of this free amino acid declined as the treatment progressed, while there was no change in the control group. In the *supracoracoideus* muscle, glycine significantly ($P < 0.05$) increased with heat treatment and a significant interaction between duration and treatment was also observed, suggesting that glycine increased with advancing treatment time, while the reverse was apparent in the control groups. In addition, histidine significantly ($P < 0.05$) declined as heat treatment progressed, while heat treatment initially showed a tendency to increase it at 15 min compared to the control.

Plasma corticosterone and metabolite concentrations

Plasma corticosterone concentration was not significantly ($P > 0.05$) affected by the treatment (ng/ml: CT, 15 min 3.65 ± 0.38; CT, 30 min 3.45 ± 0.50 and HT, 15 min 2.71 ± 0.37; HT, 30 min 2.32 ± 0.21). No plasma metabolite concentrations showed any significant changes as a

Table 7. *Free amino acids contents in breast muscle of chicks (pectoralis muscle) exposed to heat treatment or control (HT or CT) for 15 or 30 min*

	CT		HT		*P*-value		
Amino acids	15 min	30 min	15 min	30 min	Treatment	Time	Treatment × Time
Essential amino acids[1]							
Histidine	30 ± 3	77 ± 12	68 ± 9	102 ± 19	$P < 0.05$	$P < 0.05$	NS
Threonine	646 ± 50	646 ± 38	694 ± 54	965 ± 77	$P < 0.05$	$P < 0.05$	$P < 0.05$
Arginine	320 ± 17	308 ± 16	376 ± 34	400 ± 35	$P < 0.05$	NS	NS
Valine	271 ± 17	287 ± 54	269 ± 57	358 ± 74	NS	NS	NS
Methionine	147 ± 10	172 ± 16	161 ± 9	195 ± 20	NS	NS	NS
Isoleucine	120 ± 10	142 ± 37	131 ± 36	188 ± 53	NS	NS	NS
Leucine	202 ± 12	210 ± 34	225 ± 49	283 ± 57	NS	NS	NS
Phenylalanine	106 ± 7	105 ± 20	100 ± 15	139 ± 19	NS	NS	NS
Tryptophan	184 ± 12	185 ± 22	174 ± 14	198 ± 21	NS	NS	NS
Lysine	677 ± 142	444 ± 95	690 ± 111	510 ± 118	NS	NS	NS
Nonessential amino acids[1]							
Proline	359 ± 25	436 ± 26	420 ± 46	554 ± 60	$P < 0.05$	$P < 0.05$	NS
Glutamic acid	718 ± 33	648 ± 46	787 ± 42	816 ± 64	$P < 0.05$	NS	NS
Asparagine	248 ± 14	287 ± 15	282 ± 21	353 ± 39	$P < 0.05$	$P < 0.05$	NS
β-Alanine	163 ± 25	100 ± 2	114 ± 16	63 ± 1	$P < 0.05$	$P < 0.05$	NS
3-Methylhistidine	371 ± 29	387 ± 17	283 ± 49	322 ± 30	$P < 0.05$	NS	NS
Cystathionine	15 ± 2	29 ± 10	55 ± 19	60 ± 22	$P < 0.05$	NS	NS

The number of chicks used in each group was as follows: CT for 15 min, 8; CT for 30 min, 7; HT for 15 min, 8; HT for 30 min, 7. [1]All the essential amino acids were analysed and only significantly changed nonessential amino acids are shown. NS: Not significant. Values are means ± SEM in pmol/mg.

Table 8. *Free amino acids contents in breast muscle of chick (supracoracoideus muscle) exposed to heat treatment or control (HT or CT) for 15 or 30 min*

	CT		HT		*P*-value		
Amino acids	15 min	30 min	15 min	30 min	Treatment	Time	Treatment × Time
Essential amino acids[1]							
Histidine	681 ± 52	506 ± 47	816 ± 114	607 ± 104	NS	$P < 0.05$	NS
Threonine	238 ± 37	224 ± 35	324 ± 68	332 ± 60	NS	NS	NS
Arginine	3568 ± 583	3046 ± 408	2699 ± 516	2365 ± 376	NS	NS	NS
Valine	226 ± 38	177 ± 30	238 ± 52	264 ± 49	NS	NS	NS
Methionine	304 ± 47	273 ± 54	270 ± 52	336 ± 65	NS	NS	NS
Isoleucine	90 ± 10	63 ± 9.6	151 ± 37	94 ± 25	NS	NS	NS
Leucine	164 ± 13	154 ± 33	147 ± 26	201 ± 47	NS	NS	NS
Phenylalanine	133 ± 22	130 ± 31	101 ± 22	141 ± 33	NS	NS	NS
Tryptophan	4.54 ± 1.42	6.02 ± 2.20	4.86 ± 0.85	6.91 ± 1.54	NS	NS	NS
Lysine	523 ± 158	183 ± 20	613 ± 155	626 ± 154	NS	NS	NS
Nonessential amino acids[1]							
Glycine	449 ± 27	331 ± 51	410 ± 56	598 ± 39	$P < 0.05$	NS	$P < 0.05$

The number of chicks used in each group was as follows: CT for 15 min, 8; CT for 30 min, 7; HT for 15 min, 8; HT for 30 min, 7. [1]All the essential amino acids were analysed and only significantly changed nonessential amino acids are shown. NS: Not significant. Values are means ± SEM in pmol/mg.

result of the heat treatment, except total cholesterol, which showed a significant ($P < 0.05$) interaction between treatment and time, implying that the duration of treatment increased the plasma total cholesterol in the heat-treated group, while the total cholesterol of the control group chicks decreased with treatment duration (mg/dl: CT, 15 min 153 ± 6; CT, 30 min 135 ± 6 and HT, 15 min 146 ± 4; HT, 30 min 153 ± 8).

DISCUSSION

In this study, rectal temperature significantly increased following a short period of experimental HT, suggesting that the experimental temperature acted on the thermoregulatory mechanisms to increase sensible heat loss in the experimental chicks. However, we did not find any influence of this short period of HT on the level of food intake by chicks. In agreement with our previous report under similar HT conditions (Chowdhury *et al.*, 2012*a*), we also did not see any significant difference in food intake until 8 h of the heat treatment in chicks compared to the control group. Thus, the current experimental period for HT was too short to demonstrate its influence on food intake behaviour in chicks. Moreover, the plasma corticosterone concentration did not significantly change in chicks exposed to HT for either 15 or 30 min. Chowdhury *et al.* (2012*a*) reported that the plasma corticosterone concentration was not affected by HT during 48 h of heat treatment in young chicks. Furthermore, Lin *et al.* (2006) and Han *et al.* (2010) reported that HT did not affect plasma corticosterone concentrations. These findings are consistent with our current observations whereby we found that neither 15 nor 30 min of HT had a significant effect on plasma corticosterone concentrations in chicks. It is possible that our current experimental HT condition had less influence, or no influence, on the hypothalamic–pituitary–adrenal axis as also found in the above-mentioned reports. However, we could not preclude the possibility that the heat stress response was due to the activation of the sympathetic nervous system. Bottje and Harrison (1986) reported that after 50 min of heat treatment rectal temperature, respiratory rate and plasma epinephrine were significantly greater in cockerels than in control birds. Thus, further research is needed to measure plasma epinephrine and norepinephrine when chicks are exposed to a short period of HT.

In the current study, free amino acid concentrations in blood plasma, brain and breast muscles were influenced even by a short period of HT. In particular, several free amino acids showed an upward tendency in their changes, but few declined significantly. In the plasma, several free amino acids significantly increased with HT, namely glycine, carnosine, tryptophan, methionine, hydroxyproline, asparagine, ornithine and 1-methylhistidine. Apart from tryptophan and methionine, which are essential amino acids, levels of several nonessential amino acids increased in the plasma of the HT chicks. The reason for the increment is still unknown, but we suggest that the increased concentration of free amino acid was not the result of the increased rate of the body muscular protein breakdown as body weight was not changed by the treatment. In addition, however, these increased plasma amino acids may be derived from the liver or some other soft tissues which are more labile than myofibrillar proteins in muscles. Interestingly, the increment of free amino acids during short-time HT in the present study is almost reverse to our recent

findings (Chowdhury *et al.*, 2014) where most of the free amino acids were declined in chicks exposed to HT (35°C) for long time (24 or 48 h). For instance, tryptophan and ornithine declined in the plasma of HT-exposed chicks (Chowdhury *et al.*, 2014), whereas they increased in the current study. Therefore, the duration of exposure to HT may be an important factor for the alteration of free amino acids in the chicks. Furthermore, we could also predict that these increased free amino acids were not synthesised in the body because essential amino acids, which chickens cannot synthesise in sufficient quantity, were also increased by HT. Additionally, it could be assumed that the increase in the level of free amino acids is not due to the increased absorption of amino acids from the gut in the HT chicks because thermal stress disrupts epithelial homeostasis in the gut mucosa (Varedi *et al.*, 2001; Prosser *et al.*, 2004) and reduces intestinal absorptive capacity, as reported by Mitchell and Carlisle (1992). Soleimani *et al.* (2010) further reported that the gut amino acid flow in birds subjected to one week or 2 weeks of HT was significantly higher than it was in those under no heat exposure. However, in future, it would be interesting to study amino acid absorption from the gut in chickens subjected to acute heat stress.

Many free amino acids were also altered in the brain and breast muscle of heat-exposed chicks. Although most of the amino acids were nonessential, essential amino acids were also altered in HT chicks. Interestingly, most of the free amino acids in the brain and muscle are different from those of the plasma. Apart from proline and cystathionine, all the altered free amino acids in the various parts of the brain are different. These findings indicate that alteration of the free amino acid concentrations may be tissue specific due to the result of the metabolism of different amino acids or proteins in the tissues. However, we still do not know which precise factor(s) are affecting in HT chicks for alteration of amino acids and whether there is any tissue-specific function of this altered amino acid pool in HT chicks. In the breast muscle, 3-methyl histidine, the marker of proteolysis (Young and Munro, 1978; Nishizawa, 1983), concentration declined significantly ($P < 0.05$). Since the concentration of 3-methyl histidine decreased in the breast muscle of chicks, it could be assumed that the amount of protein degradation was also reduced in the breast muscle during HT. As there is always a balance between the synthesis and degradation of protein metabolism in any individual, it could be predicted that the synthesis of protein would also decline during HT and that the free amino acids pool could be increased in the tissue.

In conclusion, we found that concentrations of several free amino acids significantly changed in the blood, brain and muscle following short-term heat exposure in chicks. These results suggest that even a short period of 15 or 30 min of heat exposure may affect the metabolism of amino acids in young chicks.

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