Oral Administration of Watermelon Rind Extract to Induce Hypothermia in Chicks

Linh T.N. Nguyen1, Hatem M. Eltahan1,#, Cuong V. Pham2, Guofeng Han1, Vishwajit S. Chowdhury2 and Mitsuhiro Furuse1

1 Laboratory of Regulation in Metabolism and Behavior, Graduate School of Bioresource and Bioenvironmental Science, Kyushu University, Fukuoka 819-0395, Japan
2 Laboratory of Stress Physiology and Metabolism, Division for Experimental Natural Science, Faculty of Arts and Science, Kyushu University, Fukuoka 819-0395, Japan
# Visiting Researcher from Animal Production Research Institute, Agriculture Research Center, Agriculture Ministry, and Division for Poultry Production, Faculty of Agriculture, Kafr-Elsheikh University, Egypt

Oral administration of L-citrulline (L-Cit) caused hypothermia, but L-Cit is not recommended in poultry diets in Japan. Watermelon is a natural source of L-Cit. The objective of this study is to examine the effect of watermelon waste, i.e., watermelon rind (WR) on the body temperature and plasma free amino acids of chicks. In Experiment 1, 14-day-old chicks were subjected to acute oral administration of WR extract (WRE) (2 ml) under control thermo-neutral temperature (CT). In Experiment 2, 15-day-old chicks were orally administered 1.6 ml of either WRE, low-dose L-Cit (7.5 mmol/10 ml), or high-dose L-Cit (15 mmol/10 ml) under CT. In both experiments, rectal temperature (RT) and plasma free amino acids were analyzed. In Experiment 3, after dual oral administration of (1.6 ml) WRE or L-Cit (15 mmol/10 ml), 15-day-old chicks were exposed to high ambient temperature (HT; 35±1°C, 2 h) to monitor changes in RT. Acute oral administration of WRE significantly reduced RT under CT. The degree of RT reduction by WRE was similar to that by high L-Cit. Moreover, RT was significantly low at HT owing to the oral administration of WRE. However, the reduced RT was difficult to explain by the content of Cit in WRE alone. In conclusion, WRE could be used as a dietary ingredient to reduce body temperature for imparting thermotolerance in chicks.

Key words: body temperature, chicks, plasma free amino acids, watermelon rind extract

J. Poult. Sci., 57: 37-44, 2020

Introduction

Heat stress induced by high ambient temperature is a serious concern in poultry farming. Heat stress increases body temperature and induces heat stress responses in chicks (Chowdhury et al., 2012; Ito et al., 2014). High ambient temperature can reduce food intake, food efficiency, and body weight gain in broilers (Howlider and Rose, 1987; Azad et al., 2010). Additionally, several free amino acids, including l-citrulline (l-Cit), were found to decline in the plasma of heat-exposed chicks (Chowdhury et al., 2014). Recently, it has been further found that oral administration of l-Cit can lower the body temperature of chicks (Chowdhury et al., 2015) and impart them with thermotolerance (Chowdhury et al., 2017). However, the use of synthetic l-Cit in poultry rations is still not approved in Japan (Food and Agricultural Materials Inspection Center, Japan, 1953).

Watermelon is a rich natural source of l-Cit, and interestingly, watermelon rind (WR), an agricultural waste product, contains a greater amount of l-Cit than its flesh (Rimando and Perkins-Veazie 2005; Tarazona-Diaz et al., 2011). In our recent study, dried WR powder was fed as a diet supplement to 3- to 15-day-old chicks to examine its effect on their rectal temperature (RT) and food intake. Although RT did not significantly change under control thermoneutral temperature (CT; 30±1°C), food intake and food efficiency were significantly reduced in chicks fed with WR. In conclusion, WR could be used as a dietary ingredient to reduce body temperature for imparting thermotolerance in chicks.
plasma t-Cit increased significantly (Nguyen et al., 2019). We attributed these effects to the fact that WR powder contains fiber that dilute the t-Cit concentration in the WR powder. In this study, therefore, we collected the juice of WR, i.e., WR extract (WRE), and examined its effect on the RT and plasma free amino acids of chicks orally administered WRE.

Materials and Methods

Animals

One-day-old male layer chicks (Julia strain; Gallus gallus domesticus) were obtained from a local hatchery (Murata Hatchery, Fukuoka, Japan) and housed together in metal cages (50 × 35 × 33 cm) in a group (14 birds) at a constant temperature of 30 ± 1°C with continuous light illumination. Food (adjust diets: metabolizable energy > 12.55 MJ/kg, protein > 23%; Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water were provided ad libitum during the experimental period. This study was performed in accordance with the guidelines of animal experiments and carried out in the Faculty of Agriculture, Kyushu University, and it adhered to Law No. 105 and Notification No. 6 of the Japanese government.

Preparation of WRE

Fresh watermelon was procured from Suika-no-Meisan (Kumamoto, Japan). The rind was separated from the flesh, and its juice was extracted with a commercial juicer (MJ-600 T; Panasonic, Kadoma, Japan). The juice was passed through four-layer gauze (FC Gauze, China), filtered, and its juice was extracted with a commercial juicer (MJ-600 T; Panasonic, Kadoma, Japan). The rind was separated from the flesh, the rind was dried in an oven (Matsui MFG Co., Ltd., Osaka, Japan) at 80°C for 30 min, and then filtered again through a 0.20-μm filter (Millipore, Bedford, MA, USA). Twenty μl of the supernatants were adjusted to pH = 7 with 1 M sodium hydroxide. Next, the WRE sample and 10 μl standard were dried under reduced pressure at −100 kPa (Centrifugal Vaporizer, CVE-200D, Eyela, Japan). The dried residue was dissolved in 400 μl of DDW, and then filtered again through a 0.20-μm filter (Millipore, Bedford, MA, USA). Twenty μl of the supernatants were adjusted to pH = 7 with 1 M sodium hydroxide. Next, the WRE sample and 10 μl standard were dried under reduced pressure. The dried residues were dissolved in 10 μl of 1 M sodium acetate-methanol-triethylamine (2:2:1), dried under reduced pressure, and then converted to their phenylthio carbamoyl derivatives by dissolving in 20 μl of methanol-distilled water-triethylamine-phenylisothiocyanate (7:1:1:1) and allowed to react for 20 min at room temperature. The sample and standard solutions 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 procedure was carried out on the standard solution, which was prepared by diluting a commercially available l-amino acid solution (type ANII, type B, l-asparagine (l-Asn), l-glutamine (l-Gln), and l-tryptophan; Wako, Osaka, Japan) in distilled water. The solution containing the derivatives was applied to a Waters HPLC system. 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 were determined by the absorbance at a wavelength of 254 nm. The concentrations of WRE amino acids are expressed as pmol/mg.

Free Amino Acids in Plasma

Amino acid concentrations in plasma samples were analyzed by HPLC. The concentrations of free amino acids were analyzed according to the method of Boogers et al. (2008) with some modifications. Plasma was deproteinized by filtration through a 10,000 Da molecular weight cut-off filter (Millipore) via centrifugation at 12,000 × g for 10 min at 4°C (MX-307; Tommy, Tokyo, Japan). Ten μl of plasma
samples and standard solution were dried under reduced pressure. The dried residues were dissolved in 10 μl of 1 M sodium acetate-methanol-triethylamine (2:2:1) and then analyzed by HPLC as described above for analysis of WRE. Plasma amino acid concentrations are presented as pmol/μl.

**Statistical Analysis**

Plasma free amino acids were analyzed by Student’s t-tests in Experiment 1 and by one-way ANOVA followed by Tukey-Kramer post-hoc test in Experiment 2. Changes in RT were analyzed by two-way ANOVA. When a significant interaction was detected, the t-test and Tukey-Kramer test were applied as a post-hoc test at each time point in Experiment 1 and in Experiments 2 and 3, respectively. Statistical analyses were performed using the Stat View Version 5.0 software (SAS Institute, Cary, NC, USA, 1998). Values are presented as mean±S.E.M.

**Results**

Free amino acid contents in WRE are shown in Table 1. Cit was the most abundant free amino acid present in WRE (6638 pmol/mg). In addition, the second most abundant free amino acid was arginine (Arg) at 1175 pmol/mg. The concentrations of other amino acids (β-alanine, valine, isoleucine, proline (Pro), γ-aminobutyric acid (GABA), alanine (Ala), Asn, Gln, leucine, aspartic acid (Asp), and glycine (Gly)) were lower than 1000 pmol/mg.

**Experiment 1. Effects of Single Oral Administration of 2 ml of WRE or DDW on RT and Plasma Free Amino Acids in 14-day-old Chicks**

Changes in RT following oral administration of WRE are shown in Fig. 1. Initial RT at 0 min in the control and WRE groups was 41.1±0.2°C and 41.1±0.2°C, respectively. WRE decreased the RT significantly at 30 and 60 min (P<0.05) following administration. Table 2 shows the effect of WRE on plasma free amino acids. Only the concentration of Cit increased significantly (P<0.001), whereas the concentrations of all other free amino acids (Asn, Ala, Gln, Gly, histidine, Pro, serine (Ser), and tyrosine) decreased significantly (P<0.05, P<0.01 or P<0.001).

| Amino acids | Content   |
|------------|-----------|
| Citrulline | 6638±571  |
| Arginine   | 1175±57   |
| Valine     | 692±31    |
| Isoleucine | 582±55    |
| Proline    | 486±55    |
| GABA       | 410±33    |
| Alanine    | 329±16    |
| Asparagine | 271±12    |
| Leucine    | 236±25    |
| Aspartic acid | 229±16  |
| Glycine    | 204±15    |
| Glutamine  | 193±14    |

Thirty-three samples were analyzed. GABA, γ-aminobutyric acid. Values are mean±S.E.M. in pmol/mg.
Experiment 2. Effects of Single Oral Administration of 1.6 ml of WRE, or Low- or High-dose l-Cit on RT and Plasma Free Amino Acids Under CT in 15-day-old Chicks

Fig. 2 shows changes in RT after oral administration of either WRE, low-dose l-Cit, or high-dose l-Cit. RT at the beginning of experiment in the control, WRE, low-dose l-Cit, and high-dose l-Cit groups was 41.3±0.1°C, 41.3±0.1°C, 41.2±0.1°C, and 41.2±0.2°C, respectively. There was a significant (P<0.01) decline in RT after oral administration of WRE, low-dose l-Cit, and high-dose l-Cit. We observed significant effects of time (P<0.0001) and significant interaction between treatment and time (P<0.0001), indicating that WRE and high-dose l-Cit consistently reduced RT as the experimental time progressed. Table 3 reveals the effect of orally administered WRE, low-dose l-Cit, and high-dose l-Cit on free amino acids. l-Cit significantly (P<0.001) increased the concentrations of Cit, Arg, ornithine (Orn), methionine, Ser, taurine, Asp, and GABA compared to those in the control and WRE groups. In contrast, WRE significantly (P<0.01 or P<0.001) decreased the amounts of almost all amino acids, including Cit, compared to those in the control and l-Cit groups.
Environmental temperature.

Perkins-Veazie (2005) and Tarazona-Díaz interaction between treatment and time (\(P_{RT} \); significant (\(P_{RT} < 0.0001 \)) was observed, which indicated that the effect of treatment progressed with time, although RT is dependent on the environmental temperature.

**Discussion**

In WRE, Cit content was higher than that of other free amino acids, in accordance with the reports by Rimando and Perkins-Veazie (2005) and Tarazona-Díaz et al. (2011). In a previous study, we found that WRE and WR powder contain Cit at 8.61 mmol/mg (1.51 mg/g) and 6.64 mmol/mg (1.12 mg/g), respectively. Other studies have reported higher concentration of Cit in WR than that reported by us; however, these previous studies analyzed other parts of watermelon and processed the watermelon differently. For instance, Rimando and Perkins-Veazie (2005) reported 24.7 mg Cit/g dry weight for WR processed by freeze-drying. Tarazona-Díaz et al. (2013) found 2.33 g Cit/l in fresh flesh juice after pasteurization at 80°C for 40 s followed by immediate cooling on ice to 8°C until use. Furthermore, the species and growth stages of the watermelon used may be different among the different studies, and it was difficult to determine these aspects based on the information reported in the papers.

The present study was based on our previous study of WR powder in chick diet (Nguyen et al., 2019) and WR juice administration (unpublished data). However, RT was not found to change significantly during the period. We assumed that because WR powder contained high fiber content of 14.5% and WR powder mash diet contained 0.002 mmol of Cit/mg of food, chicks consumed 0.03 and 0.04 mmol of l-Cit when they were 6- and 15-day-old, respectively (Nguyen et al., 2019). Further, a 2-ml dose of WR juice was calculated to contain high water content of 82.8–88.2% (Pansy and Thin, 2011) and low Cit content of 0.002 mmol. In addition, an effective l-Cit dose of 1.03 mmol has been reported to reduce RT in 6-day-old chicks upon acute oral administration (Chowdhury et al., 2015). Thus, in the present study, WRE was used owing to its low fiber content and high Cit concentration for examining the effect of natural Cit on the chick body.

In Experiment 1, RT decreased significantly (\(P < 0.05 \)) after oral administration of 2 ml of WRE. Oral administration of 2 ml was selected in accordance with a previous study (Do et al., 2017). Although 2 ml of WRE contained 0.004 mmol of l-Cit, which is lower than the effective l-Cit dose of 1.03 mmol (Chowdhury et al., 2015), administration of pure synthetic l-Cit (Chowdhury et al., 2015) and concomitant administration of Cit with other ingredients in the present study may have different effect on RT. In fact, acute or chronic administration of a medium containing l-Cit-producing live bacteria reduced RT and surface body temperature in chicks even though the l-Cit level was low (Tran et al., 2019). WRE contains several free amino acids, as shown in Table 1. These amino acids may interact with each other to decrease RT. The significant increase in plasma Cit concentration (\(P < 0.05 \)) after administration of 2 ml WRE may be caused by the presence of Cit in WRE. Other free amino acids were also reduced significantly (\(P < 0.05 \)) after oral administration of WRE. Under short-term

### Table 3. Effects of oral administration of 1.6 ml of WRE, low l-Cit, or high l-Cit on plasma free amino acid concentrations in 15-day-old chicks

| Amino acid     | Control | WRE | Low l-Cit | High l-Cit | \(P\) value |
|----------------|---------|-----|-----------|------------|-------------|
| Citrluline     | 347±33^a | 263±40^b | 450±48^b | 562±54^b | \(P < 0.001\) |
| Alanine        | 130±4^a  | 96±10^b | 141±3^a  | 141±11^a | \(P < 0.01\) |
| Arginine       | 344±49^ab | 283±35^b | 492±34^ab | 530±46^c | \(P < 0.001\) |
| Asparagine     | 228±12^a | 141±6^b | 227±14^a | 200±21^a | \(P < 0.001\) |
| Aspartic acid  | 33.0±2^ab | 26.6±3^a | 37.1±2^b | 40.9±4^b | \(P < 0.01\) |
| GABA           | 20±1^a   | 71±4^a | 2280±75^a | 4629±427^b | \(P < 0.001\) |
| Glutamine      | 848±36^a | 506±21^b | 734±31^ac | 655±41^c | \(P < 0.001\) |
| Glycine        | 248±16^a | 173±8^b | 270±15^a | 282±27^a | \(P < 0.01\) |
| Histidine      | 107±8^a  | 66±3^a | 103±6^a  | 89±6^b  | \(P < 0.001\) |
| Methionine     | 105±12^a | 67±4^b | 273±11^a | 471±55^b | \(P < 0.001\) |
| Ornithine      | 57±7^a   | 367±37^b | 124±7^b  | 133±15^b | \(P < 0.001\) |
| Proline        | 613±38^a | 357±33^b | 563±13^a | 549±62^a | \(P < 0.001\) |
| Serine         | 487±24^a | 325±22^b | 497±33^a | 455±22^a | \(P < 0.001\) |
| Taurine        | 55±8^a   | 61±7^a | 93±15^b  | 133±18^b | \(P < 0.01\) |
| Threonine      | 668±33^a | 513±28^b | 662±13^a | 701±56^a | \(P < 0.01\) |

Different superscripts indicate mean values that are significantly different at \(P < 0.05\). WRE, watermelon rind extract dissolved in a 0.25% methylcellulose solution at a ratio of 1:2 (wt/wt). Low L-Cit, 7.5 mmol/10 ml. High l-Cit, 15 mmol/10 ml. The number of samples used for analysis was 6 to 7. Values are mean±S.E.M. in pmol/ml.
heat stress, RT increases as the blood concentrations of several free amino acids increase (Ito et al., 2014). Conversely, some free amino acids in the brain of neonatal chicks subjected to either restraint with isolation-induced or fasting stress were found to decrease (Hamasu et al., 2009). In these cases, plasma amino acids are correlated with brain amino acids (Hamasu et al., unpublished data).

In Experiment 2, there was a similar effect of decreasing RT due to both WRE and high l-Cit under CT (Fig. 2). Because it is difficult to dissolve synthetic l-Cit in water, MC was used to prepare the suspension. Thus, MC was used as a control and for dissolving WRE in this experiment. High-dose l-Cit (15 mmol/10 ml) was used as an effective dose for regulating RT in chicks, in accordance with Chowdhury et al. (2015). The 1.6 ml dose was based on 10 ml/kg body weight of 15-day-old chicks. This l-Cit dose acted as a potential hypothermic agent to impart thermotolerance in the chicks (Chowdhury et al., 2017). Although l-Cit concentration in the high-dose l-Cit group (2.40 mmol) was approximately 470-fold higher than that in the WRE group (0.004 mmol), plasma Cit level in the WRE group was nearly half that in the high-dose l-Cit group (263 ± 40 and 562 ± 54 pmol/μl, respectively). Both groups showed the same effect in reducing RT even at 120 min after oral administration, implying that the nutrients in WRE and the high-dose l-Cit could be easily absorbed and were active, as mentioned above. In contrast, changes in plasma free amino acids after WRE injection showed an opposite trend to those after high-dose l-Cit injection (Table 3). High-dose l-Cit significantly (P < 0.05) increased plasma Cit concentration and led to a significant increase in the concentrations of Arg and Orn due to the urea cycle; Cit can bypass the liver and then be converted to Arg in the kidney (Windmueller and Spaeth, 1981). Birds lack carbamoyl phosphate synthetase, an enzyme of the urea cycle necessary for synthesizing l-Cit from l-Orn in the liver and kidney (Tamir and Ratner 1963). Therefore, birds cannot synthesize Cit or Arg, but can synthesize Orn from Arg (Suenaga et al., 2008). Additionally, oral administration of high-dose l-Cit may not induce toxic effects in chicks (Chowdhury et al., 2017). The content of other free amino acids would significantly increase owing to high l-Cit dose. Conversely, there was a significant decrease in the concentration of almost all plasma free amino acids in the WRE group, compared to that in the l-Cit and control groups. Moreover, Cit content in plasma did not significantly increase in the WRE groups compared to that in the control group, which may be partially due to the lower l-Cit in the 1.6 ml dose.

In Experiment 3, we investigated the effect of dual oral administration of WRE and l-Cit on the RT of chicks under HT after its thermotolerance effect was shown in normal condition. Under stress conditions, dual oral administration
of WRE significantly declined RT. Before this experiment, we examined the effect of single oral administration of WRE and l-Cit under HT, and did not observe any change in RT (result not shown). Chowdhury et al. (2017) found that dual, but not single, oral administration of l-Cit resulted in thermotolerance; thus, the chicks in this study were subjected to dual oral administration of WRE and l-Cit before being exposed to HT. Although Cit concentration in the WRE group was lower than that in the l-Cit group, RT declined significantly \((P<0.05)\) in the WRE group compared to that in the control group. These data suggested that unrevealed substances in WRE may be easily absorbed, and then may produce a strong effect on RT.

It is suggested that thermoregulation is caused by nitric oxide (NO) (De Luca et al., 1995; Gourine, 1995), which is synthesized from L-Arg (Wu et al., 1998). L-Cit is an endogenous precursor of L-Arg (Wu et al., 1998) and synthesis of NO is increased by supplementation of L-Cit (Sureda et al., 2009). However, Chowdhury et al. (2017) indicated that the RT of chicks was significantly reduced under CT and HT by dual high l-Cit administration under CT or HT condition. Because plasma NO\(_x\) (NO\(_2^+\)NO\(_3^-\)) concentration did not change significantly \((P>0.05)\), NO production may not be the main factor of thermotolerance in chicks owing to l-Cit-mediated hypothermia. This implies that the thermoregulatory effect of chicks was not influenced by production of NO following l-Cit oral supplementation. WRE decreased RT only because of l-Cit but also because of other chemicals present in addition to the amino acids mentioned above. First, we focused on l-Cit in WRE, but the body temperature-lowering effect of WRE could not be explained by l-Cit level in WRE. Tran et al. (2019) also indicated that even when l-Cit was present at a low concentration in a medium containing L-Cit-producing live bacteria, the medium reduced the RT and surface body temperature of chicks upon acute oral or chronic administration. Tarazona-Diaz et al. (2011) found that WR contains phenolic acids. Furthermore, WR contains significantly greater amounts of chemicals with free radical-scavenging activity, such as \(\beta\)-carotene, 4-hydroxybenzoic acid, vanillin, and coumaric acid (Han et al., 2013). Moreover, other substances in WRE, which act as antioxidants, may collaborate to impart thermotolerance in chicks after oral administration. For instance, lycopen, which is an antioxidant, could increase the animal parameter under HT (Sivakuma et al., 2010). WR in the present study contained lycopen at the level of 1.1 mg/100 g.

In conclusion, WRE induced immediate hypothermia not only under CT upon single administration but also under HT condition upon dual oral administration. WRE could combine with unknown substances that facilitate/replace l-Cit synthesis and decrease body temperature in chicks by regulating plasma free amino acids. Further research is required to elucidate which unknown substances contribute to the effects of WRE in imparting heat tolerance in young chicks under HT.

**Acknowledgments**

We thank the members from Can Tho University Improvement Project VN14-P6, supported by the Japanese ODA loan to LTNN, who came from the Department of Animal Sciences, College of Agriculture and Applied Biology, Can Tho University, Vietnam to study at the Kyushu University. The authors are very grateful to Mr. Junya Harada, Suika-no-Meisan, Ueki, Kumamoto (http://www.suika-meisan.com/) for the generous donation of fresh watermelons required for the study. This work was partly supported by the JSPS KAKENHI (Grant No. JP18 K19271 to VSC) and JSPS KAKENHI (Grant No. JP17H 01503 to MF).

**References**

Azad MA, Kikusato M, Maekawa T, Shirakawa H and Toyomizu M. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 155: 401–406. 2010.

Boogers I, Plugge W, Stokkermans YQ and Duchateau AL. Ultra-performance liquid chromatographic analysis of amino acids in protein hydrolysates using an automated pre-column derivatisation method. Journal of Chromatography A, 1189: 406–409. 2008.

Chowdhury VS, Han G, Bahry MA, Phuong VT, Phong HD, Yang H and Furuse M. L-citrulline acts as potential hypothermic agent to afford thermotolerance in chicks. Journal of Thermal Biology, 69: 163–170. 2017.

Chowdhury VS, Shigemura A, Erwa E, Ito K, Mohammad AB, Tran PV and Furuse M. Oral administration of L-citruline but not L-arginine or L-ornithine, acts as a hypothermic agent in chicks. Journal of Poultry Science, 52: 331–335. 2015.

Chowdhury VS, Tomonaga S, Ikegami T, Erwan E, Ito K, Cockrem JF and Furuse M. Oxidative damage and brain concentrations of free amino acid in chicks exposed to high ambient temperature. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 169: 70–76. 2014.

Chowdhury VS, Tomonaga S, Nishimura S, Tabata S and Furuse M. Physiological and behavioral responses of young chicks to high ambient temperature. Journal of Poultry Science, 49: 212–218. 2012.

De Luca B, Monda M and Sullo S. Changes in eating behavior and thermogenic activity following inhibition of nitric oxide formation. American Journal of Physiology, 268: 1533–1538. 1995.

Do PH, Tran PV, Bahry MA, Yang H, Han G, Tsuchiya A, Asami Y, Furuse M and Chowdhury VS. Oral administration of a medium containing both D-aspartate- producing live bacteria and D-aspartate reduces rectal temperature in chicks. British Poultry Science, 58: 569–577. 2017.

Food and Agricultural Materials Inspection Center. 1953. FAMIC, Japan; Act No. 35.

Furudate F and Meguro T. Content and composition of free amino acids in cabbage grown under various cultivation conditions [in Japanese]. Journal of Home Economics of Japan, 53: 199–203. 2002.

Gourine AV. Pharmacological evidence that nitric oxide can act as an endogenous antipyretic factor in endotoxin-induced fever in
rabbit. General Pharmacology, 26: 835–841. 1995.
Hamasu K, Haraguchi T, Kabuki Y, Adachi N, Tomonaga S, Sato H, Denbow DM and Furuse M. L-Proline is a sedative regulator of acute stress in the brain of neonatal chicks. Amino Acids, 37: 377–382. 2009.
Hanan MAA and Abdelrahman RA. Utilization of watermelon rinds and shartlyn melon peels as a natural source of dietary fiber and antioxidants in cake. Annals of Agricultural Science, 58: 83–95. 2013.
Howlilder MAR and Rose SP. Temperature and the growth of broilers. World’s Poultry Science Journal, 43: 228–237. 1987.
Ito K, Erwan E, Nagasawa M, Furuse M and Chowdhury VS. Changes in free amino acid concentrations in the blood, brain and muscle of heat-exposed chicks. British Poultry Science, 55: 644–652. 2014.
Nguyen TNL, Han G, Yang H, Ikeda H, Eltahan HM, Chowdhury VS and Furuse M. Dried watermelon rind mash diet increases plasma l-Citrulline level in chicks. Journal of Poultry Science, 56: 65–70. 2019.
Pansy KH and Thin TK. Preparation of beverages powder from fruits. Universities Research Journal, 4: 335–354. 2011.
Rimando AM and Perkins-Veazie P. Determination of citrulline in watermelon rind. Journal of Chromatography A, 1078: 196–200. 2005.
Sivakumar AVN, Singh G and Varshney VP. Antioxidants supplementation on acid base balance during heat stress in goats. Asian-Australasian Journal of Animal Science, 23: 1462–1468. 2010.
Suenaga R, Yamane H, Tomonaga S, Asechi M, Adachi N, Tsuneyoshi Y, Kurauchi I, Sato H, Denbow DM and Furuse M. Central l-arginine reduced stress responses are mediated by l-ornithine in neonatal chicks. Amino Acids, 35: 107–113. 2008.
Sureda A, Cordova A, Ferrer MD, Tauler P, Perez G, Tur JA and Pons A. Effects of l-citrulline oral supplementation on polymorphonuclear neutrophils oxidative burst and nitric oxide production after exercise. Free Radical Research, 43: 828–835. 2009.
Tamir H and Ratner S. Enzymes of arginine metabolism in chicks. Archives of Biochemistry and Biophysics, 102: 249–258. 1963.
Tarazona-Díaz MP, Alacid F, Carrasco M, Martínez I and Aguayo E. Watermelon juice: potential functional drink for sore muscle relief in athletes. Journal of Agricultural and Food Chemistry, 61: 7522–7528. 2013.
Tarazona-Díaz MP, Viegas J, Moldao-Martins M and Aguayo E. Bioactive compounds from flesh and by-product of fresh-cut watermelon cultivars. Journal of the Science of Food and Agriculture, 91: 805–812. 2011.
Tran PV, Do PH, Han G, Bahry MA, Yang H, Chowdhury VS and Furuse M. Oral administration of a medium containing l-citrulline-producing live bacteria reduces body temperature in chicks. Journal of Poultry Science, 56: 285–289. 2019.
Windmueller HG and Spaeth AE. Source and fate of circulating citrulline. American Journal of Physiology, 241: E473–E480. 1981.
Wu G, Sidney M and Morris Jr. Arginine metabolism: Nitric oxide and beyond. Biochemical Journal, 336: 1–17. 1998.