# Oral administration of D-aspartate, but not L-aspartate, depresses rectal temperature and alters plasma metabolites in chicks

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## ABSTRACT

Aims: L-Aspartate (L-Asp) and D-aspartate (D-Asp) are physiologically important amino acids in mammals and birds. However, the functions of these amino acids have not yet been fully understood. In this study, we therefore examined the effects of L-Asp and D-Asp in terms of regulating body temperature, plasma metabolites and catecholamines in chicks.
Main methods: Chicks were first orally administered with different doses (0, 3.75, 7.5 and 15 mmol/kg body weight) of L- or D-Asp to monitor the effects of these amino acids on rectal temperature during 120 min of the experimental period.
Key findings: Oral administration of D-Asp, but not of L-Asp, linearly decreased the rectal temperature in chicks. Importantly, orally administered D-Asp led to a significant reduction in body temperature in chicks even under high ambient temperature (HT) conditions. However, centrally administered D-Asp did not significantly influence the body temperature in chicks. As for plasma metabolites and catecholamines, orally administered D-Asp led to decreased triacylglycerol and uric acid concentrations and increased glucose and chlorine concentrations but did not alter plasma catecholamines.
Significance: These results suggest that oral administration of D-Asp may play a potent role in reducing body temperature under both normal and HT conditions. The alteration of plasma metabolites further indicates that D-Asp may contribute to the regulation of metabolic activity in chicks.

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## Introduction

Amino acids can exist in two enantiomers—L-amino acids and D-amino acids. Aspartate (Asp), one of the non-essential amino acids, plays various roles not only as a constituent of protein but also as a bioactive molecule. Asp occupies a unique position in intermediary metabolic activity, particularly in the mitochondria, where it plays important roles in nitrogen and energy metabolism (Steglink, 1976). L- and D-Asp have been shown to exist in various brain regions of chickens (Neidle and Dunlop, 1990) and pigeons (Kera et al., 1996). It has been reported that the injection of D-Asp released the antidiuretic hormone arginine vasopressin (AVP) in rats (Koyuncuoğlu et al., 1984). However, little is known about the function of D-Asp during early development when its level is physiologically high (Errico et al., 2008). In

mammals, it has been shown that oral administration of D-Asp, but not of L-Asp, decreased rectal temperature (Koyuncuoğlu et al., 1982a; Koyuncuoğlu and Berkman, 1982) and plasma triacylglycerol (TG) in rats (Koyuncuoğlu et al., 1982b). In addition, Monda et al. (2003) suggested the possibility of an association between Asp and interscapular brown adipose tissue that regulates thermogenesis in rats. Furthermore, we have demonstrated elsewhere that intracerebroventicular (i.c.v.) injection of either L-Asp or D-Asp clearly attenuates stress responses in chicks (Yamane et al., 2009; Erwan et al., 2012). Thus, L- or D-Asp may act as functional amino acids in chicks.

Various nutritional strategies have been used to overcome the adverse effects of heat stress in poultry (Austic, 1985; Leeson, 1986; Teeter and Belay, 1996; Yahav, 2000). These effects cause a reduction in egg size, in laying percentage, in egg shell durability, and in body weight gain, and increase mortality in layers (Sterling et al., 2003; Lin et al., 2004; Franco-Jimenez and Beck, 2007). Nutrients, especially amino acids, are widely used as anti-stress agents with regard to psychological and physiological stress (Yamane et al., 2009; Hamasu et al., 2010; Erwan et al., 2012). Amino acid supplementation, in particular that involving essential amino acids, has been performed in an

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attempt to overcome heat-stress problems in birds (Mendes et al., 1997; Rose and Salah Uddin, 1997; Daghir et al., 2003).

To our knowledge, there have been no reports regarding the effect of L- or D-Asp on thermogulation in chicks. Therefore, the first purpose of this study was to examine the effect of orally administered L- and D-Asp on rectal temperature and plasma metabolites in chicks. The second purpose was to investigate the effect of orally administered D-Asp on the regulation of body temperature and plasma catecholamines under high ambient temperature (HT) conditions. Since the effects of the cat-echolamines on body temperature differ among species (Andersson et al., 1966; Findlay and Robertshaw, 1967; Cooper et al., 1965; Marley and Stephenson, 1975; Takahashi et al., 2005), we examined the norepinephrine (NE) and epinephrine (E) concentrations in chicks under HT. The third aim was to analyze the central effect of D-Asp on thermoregulation. Thus, i.c.v. administration of D-Asp was carried out to investigate its effect on rectal temperature in chicks.

## Materials and methods

### Animals and drugs

One-day-old male layer chicks (Julia) (*Gallus gallus domesticus*) were purchased from a local hatchery (Murata hatchery, Fukuoka, Japan) and housed in a wire-meshed cage (50 × 35 × 33 cm) in a group (20–25 birds) at a constant temperature of 30 ± 1 °C and with continuous light. Chicks were all of the same age and were housed without any adult present. Food (AX, Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water were provided ad libitum. One day before the experiment, chicks (4 days old) were reared individually and assigned for treatment and control groups on the basis of their body weight in order to produce uniform groups. This study was performed in accor-dance with the guidelines for animal experiments carried out in the Fac-ulty of Agriculture and on the Graduate Course of Kyushu University, and adhered to Law no. 105 and Notification no. 6 of the government.

L- and D-Asp were purchased from Wako Pure Chemical Industries (Osaka, Japan).

### Administration of L- or D-Asp

Following an acclimatization period with individual rearing, chicks were randomly selected and divided into four groups in Experiments 1 and 2, each group consisting of 8 chicks. The birds were provided with *ad libitum* access to the diet during the whole experimental period. On the day of the experiment, each chick (5 days old) was orally admin-istered either a solution of L-Asp (Experiment 1) or D-Asp (Experiment 2) for the treatment groups, or distilled water (DW) for the control group, via an elastic plastic needle on a small syringe. Finkelstein et al. (1983) determined the plasma L-Asp concentration at 0, 15, 30, 45, 60, and 120 min after applying different doses of L-Asp (0, 1.88, 3.76, 4.89, 5.64 and 7.52 mmol/kg body weight). The plasma L-Asp concen-tration was minimally influenced by less than 3.76 mmol/kg body weight (Finkelstein et al., 1983). Thus, in the present study, for oral ad-ministration of L-Asp or D-Asp in Experiments 1 and 2, we used 3.75, 7.5 and 15.0 mmol/kg body weight as the low, medium and high levels, respectively.

In Experiments 3 and 4, following the habituation period with indi-vidual rearing, the birds (at 6 days old) were randomly selected and di-vided into groups. All the birds were provided with *ad libitum* access to the diet during the whole experimental period. In Experiment 3, two groups of chicks were orally injected with DW and then exposed to ei-ther HT (35 °C; 120 min) (n = 6) or the control thermoneutral temper-ature (CT, 30 °C; 120 min) (n = 8). A third group of chicks (n = 8) was orally administered with D-Asp (15 mmol/10 ml/kg body weight) and then exposed to HT (35 °C; 120 min). In Experiment 4, the D-Asp was administered by i.c.v. injection via a microsyringe into the left lateral ventricle of the chicks, according to the method of Davis et al. (1979).

The dose of D-Asp for i.c.v. was based on the findings of our previous re-port (Erwan et al., 2012). In brief, chicks were i.c.v. injected either with D-Asp (1.68 μmol/10 μl) as the treatment or with the same volume of saline as the control. All the experiments were done during the day time (09:00–17:00). Two groups of chicks were i.c.v. injected with saline and then exposed to either HT (35 °C; 180 min) (n = 7) in a chamber (Sanyo Electric Co. Ltd., Japan; catalog number: Sanyo MIR-253) or CT (30 °C; 180 min) (n = 8) by being placed in their cages on similar racks. A third group of chicks (n = 8) was i.c.v. admin-istered with D-Asp and then exposed to HT (35 °C; 180 min).

At the end of the experiments, birds were decapitated following anesthesia with isoflurane (Mylan Inc., Japan). Blood samples were col-lected in heparinized tubes and centrifuged at 10,000 ×g at 4 °C for 4 min. Plasma was stored at −80 °C until assay. TG, glucose (Glu), total cholesterol (TCHO), total protein (TP), uric acid (UA), calcium (Ca), aspartate aminotransferase (AAT), magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl) and amylase (Amyl) in the plasma were measured in Experiments 1 and 2. Plasma NE and E levels were in-vestigated in Experiment 3.

### Measurement of rectal temperature

The rectal temperature of chicks was measured with a digital ther-mometer with an accuracy of ± 0.1 °C (Thermalert TH-5, Physitemp In-struments Inc., USA) by inserting the thermistor probe into the cloaca to a depth of 1–2 cm. Rectal temperature was measured at 30-, 60- and 120-min after oral injection and at 30-, 120- and 180-min after i.c.v. injection.

### Analysis of plasma metabolites

The plasma metabolites, including TG, Glu, TCHO, TP, UA, Ca, AAT, Mg, Na, K, Cl and Amyl, were measured with Fuji Drychem-7000 (Fuji Medical System, Co. Ltd., Tokyo, Japan).

### Analysis of plasma NE and E

Plasma NE and E were analyzed by means of the alumina absorption method (as described by Takahashi et al., 2005). Briefly, plasma was diluted with Tris buffer (pH 7.8) and 30 mg of alumina was added to absorb NE and E from the plasma. After removal of the liquid phase, the alumina was rinsed with ultrapure water and transferred to centrifuge-filtration units (Ultra Free C3-GV, Millipore, Bedford, MA, USA). Alumina-absorbed NE and E were separated by adding 2% acetic acid solution, and then the solution was filtrated at 4 °C, 2000 ×g, for 4 min. The 30 μl filtrate was injected into an HPLC system (Eicom, Kyoto, Japan) with an ODS column (SC-5ODS, Eicom, Kyoto, Japan) for measurement of NE and E. The mobile phase consisted of 1 M phospho-ric acid buffer (pH 6.0), 3 mM sodium octanesulfonate, and 134 μM EDTA. NE and E were detected using an electrochemical detector (ECD-300, Eicom, Kyoto, Japan) at an applied potential of 450 mV. Dihydroxybenzlamine was used to confirm the accuracy of the NE and E extraction. The external standard was used to identify peaks eluting in the chromatogram according to retention time and conformation. The system's detection limit for NE and E was 0.1 pg/sample.

### Statistical analysis

For the rectal temperature, a repeated-measures two-way analysis of variance (ANOVA) was applied for Experiments 1–4. In Experiments 1 and 2, plasma metabolites were statistically analyzed by one-way ANOVA and regression equations. Plasma NE and E were statistically an-alyzed by one-way ANOVA, and the Tukey test was done as a post hoc test. Significant differences were denoted as $P < 0.05$. Values were pre-sented as means ± S.E.M. Statistical analysis was carried out using the commercially available package SAS (Version 9.1, SAS Institute, Cary,

U.S.A., 2002). All data in each group were first subjected to a Thompson rejection test as described by Kobayashi and Pillai (2013) to eliminate outliers ($P < 0.01$), and the remaining data were used for the analysis among groups.

## Results

**Experiment 1.** *Effects of the orally administered L-Asp on rectal temperature and plasma metabolites*

As shown in Fig. 1, rectal temperatures of chicks did not change significantly following oral administration of L-Asp ($F_{(3,24)} = 0.31$, $P > 0.05$). Table 1 shows the effect of oral administration of several doses of L-Asp on the concentration of plasma metabolites. There was no significant effect of L-Asp on the concentration of plasma metabolites except for increasing the TCHO concentration ($F_{(3,26)} = 3.15$, $P < 0.05$). However, no correlation was found between TCHO and levels of L-Asp.

**Experiment 2.** *Effects of the orally administered D-Asp on rectal temperature and plasma metabolites*

Changes in the rectal temperatures following the oral administration of the different doses of D-Asp are shown in Fig. 2. D-Asp significantly ($F_{(3,18)} = 4.00$, $P < 0.05$) decreased rectal temperature and also showed a significant effect of time ($F_{(2,6)} = 41.39$, $P < 0.0001$) and an interaction between dose and time ($F_{(6,36)} = 5.05$, $P < 0.001$), implying that the effect of the treatment was to consistently reduce the rectal temperature as the experimental time progressed. Table 2 shows the changes in plasma metabolites following the oral administration of D-Asp. A significant ($P < 0.05$) negative correlation was detected between the administered doses of D-Asp and the plasma concentration of TG (55.4 [SE 6.0] − 1.6 [SE 0.7] X, $R^2 = 0.161$). Plasma TG concentration showed a significant decrease, by 7.5 mmol/kg body weight D-Asp, compared with the control ($F_{(3,27)} = 3.78$, $P < 0.05$). In addition, a significant ($P < 0.05$) negative correlation was found between the administered doses of D-Asp and the plasma concentration of UA (9.50 [SE 0.6] − 0.14 [S E0.1] X, $R^2 = 0.131$). On the other hand, a significant ($P < 0.05$) positive correlation was observed between the administered doses of D-Asp and the plasma concentration of Glu (248 [SE 4.7] + 1.4 [SE 0.5] X, $R^2 = 0.204$). Furthermore, a significant ($P < 0.001$) positive correlation was detected between the administered doses of D-Asp and the plasma concentration of Cl (98.4 [SE 1.1] + 0.5 [SE 0.13] X, $R^2 = 0.341$). The values
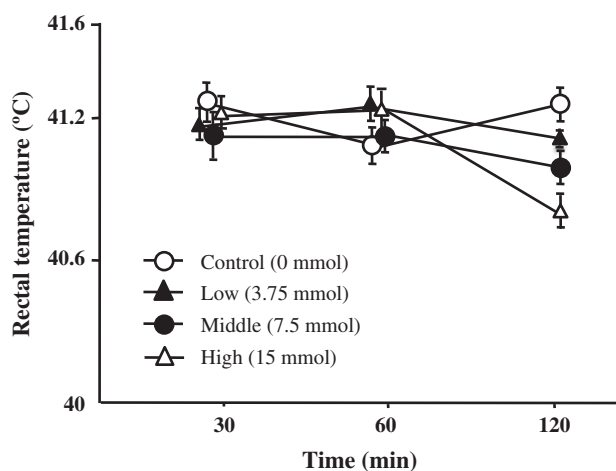
of TP, TCHO, Amyl, Ca, AAT, Mg, Na and K showed no significant changes in any of the treated groups.

**Experiment 3.** *Effects of the orally administered D-Asp on rectal temperature and plasma catecholamines in HT-exposed chicks*

Fig. 3 shows the changes in rectal temperature in response to D-Asp under HT in comparison with the changes in rectal temperature in response to DW under CT and HT (control birds). D-Asp significantly reduced rectal temperature ($F_{(2,19)} = 11.64$, $P < 0.001$) and also showed a significant effect of time ($F_{(2,4)} = 7.75$, $P < 0.005$) and an interaction between treatment and time ($F_{(4,38)} = 3.97$, $P < 0.01$), suggesting that the effect of D-Asp on declining body temperature became increasingly prominent as the treatment time progressed. In control birds, exposure to HT significantly increased rectal temperature at 30 and 60 min ($P < 0.05$). The rectal temperature of chicks administered with D-Asp and exposed to HT was lowered at 60 and 120 min compared with the control birds exposed to HT ($P < 0.01$). The rectal temperature of birds administered with D-Asp under HT at 120 min was even lower than that of the control birds under CT ($P < 0.01$). This denotes that the suppressive effect on rectal temperature of a single oral dose of D-Asp was lasting within the experimental time; the interaction between D-Asp and time was characterized by a reduction in rectal temperature, while the opposite was the case for the control groups.

Fig. 4 shows the plasma concentrations of NE and E at the end of the total treatment period of 120 min. NE concentrations did not differ according to whether the treatment was HT or D-Asp ($F_{(2,18)} = 0.97$, $P > 0.05$). D-Asp administration tended to increase plasma E concentration, but this effect was not significant ($F_{(2,18)} = 2.07$, $P > 0.05$).

**Experiment 4.** *Effects of the i.c.v. administration of D-Asp on rectal temperature in HT-exposed chicks*

The i.c.v. injection of D-Asp had no effect ($F_{(2,15)} = 1.18$, $P > 0.05$) on body temperature, while body temperature significantly ($F_{(2,4)} = 20.96$, $P < 0.0001$) increased as time progressed (Fig. 5). No significant interactions between the treatment and time ($F_{(4,30)} = 0.44$, $P > 0.05$) were observed.

## Discussion

A variety of data derived from studies involving rats have shown that L-Asp is a neurotransmitter in the central nervous system. L-Asp is not only concentrated in nerve endings (Gundersen et al., 1991), but is also found localized and accumulated in common synaptic vesicles (Gundersen et al., 1998; Fleck et al., 2001). However, the role of L-Asp has been questioned as the information was limited in avian species (Bungo et al., 2002). Thus, this study was focused on examining the effects of L-Asp and its enantiomer D-Asp on the regulation of rectal temperature and plasma metabolites in chicks. Furthermore, the concentrations of the plasma catecholamines were analyzed to clarify their physiological relevance to D-Asp and thermoregulation in birds. In the current study, L-Asp was not found to influence rectal temperature. The results of the current experiments confirmed previous findings which showed that oral administration of L-Asp did not decrease rectal temperature in rats (Koyuncuoğlu et al., 1982a; Koyuncuoğlu and Berkman, 1982). However, we recently found that when L-Asp was chemically conjugated with lauric acid, a medium-chain fatty acid (Lauroyl-L-Asp), it had the potential to decrease body temperature (Erwan et al., 2013a). Thus, the chemical modification of L-Asp assists in its development of new functions.

Investigation of plasma metabolites in birds and mammals enables metabolic alterations to be determined that are due to the effects of several factors, including physiological state, pharmacological condition, age, husbandry condition, and genetic type (Meluzzi et al., 1991; Harr, 2002; Gayathri et al., 2004; Juráni et al., 2004; Alonso-Alvarez, 2005; Yanni et al., 2005, 2010). In the present study, we therefore examined



**Fig. 1.** Effect of L-Asp administration on rectal temperatures in chicks during 120 min of the experimental period. The number of chicks used in each group ranged between 5 and 8. Values are means ± S.E.M.

**Table 1**
Effects of oral administration of several doses of L-Asp on plasma metabolites in chicks.

| | L-Asp (mmol) | | | |
|---|---|---|---|---|
| Items | 0 | 3.75 | 7.5 | 15 |
| Glucose (mg/dl) | 248 ± 4 | 252 ± 5 | 245 ± 4 | 254 ± 4 |
| Total cholesterol (mg/dl) | 159 ± 5[ab] | 145 ± 4[b] | 170 ± 8[a] | 151 ± 7[ab] |
| Amylase (IU) | 1,346 ± 93 | 1,233 ± 147 | 1,279 ± 82 | 1,306 ± 227 |
| Total protein (g/dl) | 2.82 ± 0.05 | 2.74 ± 0.07 | 3.09 ± 0.12 | 2.89 ± 0.12 |
| Calcium (mg/dl) | 12.6 ± 0.1 | 12.7 ± 0.1 | 12.9 ± 0.3 | 12.6 ± 0.1 |
| Aspartate aminotransferase (IU) | 151 ± 4 | 156 ± 5 | 155 ± 7 | 136 ± 3 |
| Triacylgcerol (mg/dl) | 78 ± 9 | 52 ± 7 | 72 ± 9 | 48 ± 8 |
| Uric acid (mg/dl) | 10.0 ± 0.6 | 10.0 ± 0.5 | 10.7 ± 1.0 | 10.2 ± 0.3 |
| Magnesium (mg/dl) | 2.30 ± 0.06 | 2.29 ± 0.07 | 2.23 ± 0.05 | 2.18 ± 0.04 |
| Sodium (mEq/l) | 140 ± 0 | 140 ± 1 | 140 ± 0 | 141 ± 1 |
| Potassium (mEq/l) | 5.4 ± 0.1 | 5.4 ± 0.1 | 5.5 ± 0.3 | 5.4 ± 0.3 |
| Chlorine (mEq/l) | 100 ± 1 | 101 ± 1 | 99 ± 1 | 103 ± 2 |

Means with different superscripts were significantly different at $P < 0.05$.
Values are means ± SEM. The number of samples used for analysis was 5–8.

the concentrations of TG, TCHO, TP, UA, Ca, AAT, Amyl, Mg, Na, K and Cl to estimate whether the physiological condition of chicks was changed as a result of the oral administration of L- or D-Asp. In the current experiment, when L-Asp was administered, no significant changes were observed in plasma Glu, Amyl, TP, Ca, AAT, TG, UA, Mg, Na, K or Cl. Tada et al. (2008) found that subchronic oral administration of L-Asp with a dietary concentration of up to 5.0% had no influence on serum Glu, Na, K, or Cl in rats. In the present experiment the findings of no significant changes in AAT or UA were similar to the previous findings of Schieber et al. (1997), who demonstrated that there was no change in serum AAT or UA in chicks following supplementation of L-Asp during the 28 days of the experimental period. Furthermore, Delaney et al. (2008) and Karaman et al. (2011) also confirmed that oral administration of L-Asp or N-acetyl-L-aspartic acid, an N-acetylated derivative of L-Asp, had no influence on TG, Glu, TP, Na, K or Cl. The results of the present study and previous studies indicate that short-term or even long-term administration of L-Asp or its derivative may not alter the plasma metabolites in chicks and rats.

The effects of L-Asp on lipid metabolism remain unclear. It has been demonstrated that orally administered L-Asp and L-glutamate inhibited fatty streak initiation in cholesterol-fed rabbits (Yanni et al., 2003). Yanni et al. (2003) indicated that when rabbits were fed with a diet of cholesterol plus corn oil or supplemented with Asp and glutamate for 4 weeks, serum levels of TCHO, TG, low-density lipoprotein cholesterol and apolipoprotein B were not altered, but they found that these two amino acids increased high-density lipoprotein cholesterol. However, the underlying mechanisms were not elucidated. The present findings contrasted with previous findings in rats where L-Asp, given for 90 days, caused a significant decrease in plasma TCHO concentration (Tada et al., 2008). These discrepancies in the TCHO concentration in response to L-Asp may be due to variations in experimental period, differences in doses, or species differences. Further experimentation would be needed to clarify the factors involved in this disparity.

In Experiment 2, it was clearly observed that orally administered D-Asp decreased rectal temperature. D-Asp has previously been found to be an agent causing body temperature to decrease in rats (Koyuncuoğlu et al., 1982a; Koyuncuoğlu and Berkman, 1982). Although in the present study we were not able to determine the weight of feces or the volume of urine, it has been suggested elsewhere that the administration of D-Asp decreases rectal temperature and consequently decreases fecal weight as well as volume of urine (Koyuncuoğlu et al., 1982b). In addition, Koyuncuoğlu et al. (1982b) revealed that food intake and rectal temperature significantly decreased when rats received D-Asp or D-Asp plus L-Asp in a 1:1 ratio. Since the analgesia is produced by D-amino acids which are naloxone reversible, the decrease in rectal temperature may be caused by D-Asp (Koyuncuoğlu et al., 1982a). We reported that central administration of both L- and D-Asp induced sedative effects under an acutely stressful condition in chicks (Erwan et al., 2012). Recently, we have further found that central injection of L-Asp can induce sedative and hypnotic effects to attenuate stress behavior through the N-methyl-D-aspartate (NMDA) receptor, but the attenuation of stress behavior by D-Asp might be via the simultaneous involvement in this process of other receptors besides the NMDA receptor (Erwan et al., 2014). However, the question of whether the oral administration of D-Asp may work on other glutamatergic receptors in the central nervous system that are responsible for sedation in chicks has not yet been studied. Further research is needed to answer this question. Furthermore, several factors may contribute to the changes in rectal temperature induced by D-Asp. For example, nitric oxide causes vasodilatation in chicks (Hamal et al., 2010), and ghrelin induces the inhibition of food intake as well as decreasing the respiratory quotient in chicks (Saito et al., 2002; Geelissen et al., 2006). However, the question of whether D-Asp could modulate these factors has not yet been clarified. Additional experiments are needed to determine the relationships between D-Asp and other factors.

In the current study, plasma TG and UA were found to significantly decrease with the administration of D-Asp in comparison with the control chicks. This finding is consistent with the previous report by Kanjanapruthipong and Thaboot (2006), where it was revealed that decreased rectal temperature was associated with decreasing levels of β-hydroxybutyrate and serum UA in cows. It was further reported that D-Asp decreased food intake and TG in rats under free or restricted
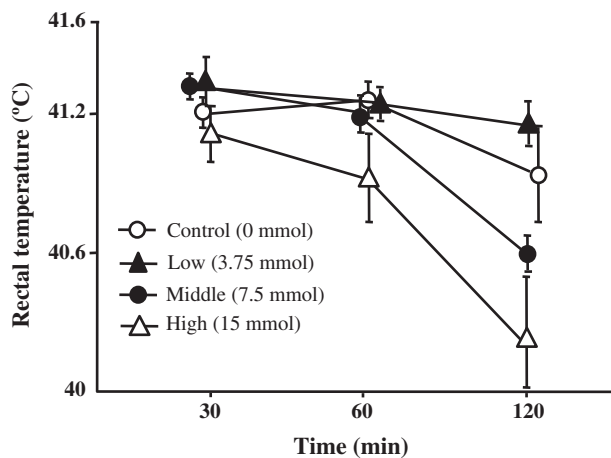


Fig. 2. Effect of D-Asp administration on rectal temperatures in chicks during 120 min of the experimental period. The number of chicks used in each group ranged between 5 and 6. Values are means ± S.E.M.

**Table 2**
Effects of oral administration of several doses of D-Asp on plasma metabolites in chicks.

| | D-Asp (mmol) | | | |
|---|---|---|---|---|
| Items | 0 | 3.75 | 7.5 | 15 |
| Glucose (mg/dl) | $253 \pm 6^{ab}$ | $251 \pm 6^{b}$ | $249 \pm 2^{b}$ | $274 \pm 7^{a}$ |
| Total cholesterol (mg/dl) | $166 \pm 7$ | $161 \pm 9$ | $156 \pm 7$ | $161 \pm 5$ |
| Amylase (IU) | $900 \pm 90$ | $1,045 \pm 144$ | $880 \pm 74$ | $905 \pm 81$ |
| Total protein (g/dl) | $2.90 \pm 0.09^{a}$ | $2.76 \pm 0.09^{ab}$ | $2.59 \pm 0.06^{b}$ | $2.85 \pm 0.06^{ab}$ |
| Calcium (mg/dl) | $12.8 \pm 0.2$ | $12.2 \pm 0.2$ | $12.4 \pm 0.2$ | $12.6 \pm 0.3$ |
| Aspartate aminotransferase (IU) | $168 \pm 7$ | $153 \pm 6$ | $160 \pm 4$ | $162 \pm 8$ |
| Triacylglycerol (mg/dl) | $65 \pm 13^{a}$ | $42 \pm 5^{ab}$ | $34 \pm 3^{b}$ | $37 \pm 4^{ab}$ |
| Uric acid (mg/dl) | $9.8 \pm 1.0$ | $9.4 \pm 0.5$ | $7.3 \pm 0.5$ | $8.0 \pm 0.7$ |
| Magnesium (mg/dl) | $2.41 \pm 0.08$ | $2.30 \pm 0.09$ | $2.29 \pm 0.06$ | $2.36 \pm 0.05$ |
| Sodium (mEq/l) | $137 \pm 2^{ab}$ | $134 \pm 2^{b}$ | $137 \pm 1^{ab}$ | $141 \pm 1^{a}$ |
| Potassium (mEq/l) | $5.6 \pm 0.3$ | $5.4 \pm 0.2$ | $5.3 \pm 0.2$ | $5.7 \pm 0.3$ |
| Chlorine (mEq/l) | $98 \pm 2^{b}$ | $100 \pm 2^{ab}$ | $102 \pm 1^{ab}$ | $106 \pm 1^{a}$ |

Means with different superscripts were significantly different at $P < 0.05$.
Values are means $\pm$ SEM. The number of samples used for analysis was 6–8.

feeding conditions (Koyuncuoğlu et al., 1982b). Our previous study showed that oral administration of D-Asp decreased food intake (Erwan et al., 2013b), and the current results demonstrated that plasma TG concentration decreased in chicks as a result of administration of D-Asp. In another previous study we showed that i.c.v. administration of L-Asp, a precursor to D-Asp, increased plasma corticosterone concentration in chicks (Yamane et al., 2009). In addition, Marley and Stephenson (1975) revealed that hypothermia was associated with increased blood Glu concentration in chicks. Our planned future study will clarify the hormonal relationships and D-Asp-induced changes in plasma metabolites.

In Experiment 3, we confirmed a further constant reduction in rectal temperature following orally administered D-Asp in HT-exposed chicks. This finding raised the possibility that D-Asp may be a useful agent for reducing body temperature during summer heat stress. It is well documented that the global surface temperature is increasing (IPCC, 2013), and HT is a serious concern for birds in general as they have more difficulty than mammals in maintaining homeothermic body temperature (Ensminger et al., 1990; Chowdhury et al., 2012, 2014). Thus, our current findings might encourage others to think about the development of a drug to mitigate HT-related problems. In terms of plasma catecholamines (NE and E), no significant changes were observed, which is a similar finding to that of our previous report where we showed that the i.c.v. or oral administration of L- or D-Asp did not alter brain catecholamine levels in chicks (Erwan et al., 2012, 2013b). Furthermore, Andersson et al. (1966) and Findlay and Robertshaw (1967) reported

that the injection of NE and E did not alter rectal temperature in the goat (*Capra hircus*) or the ox (*Bos taurus*), respectively. Contrary to these findings, Cooper et al. (1965) and Bligh (1966) revealed that i.c.v. administration of NE caused an increase in rectal temperature in rabbits and sheep. It has also been reported that central injection of NE lowered the body temperature at 75 min after injection in 2–3-
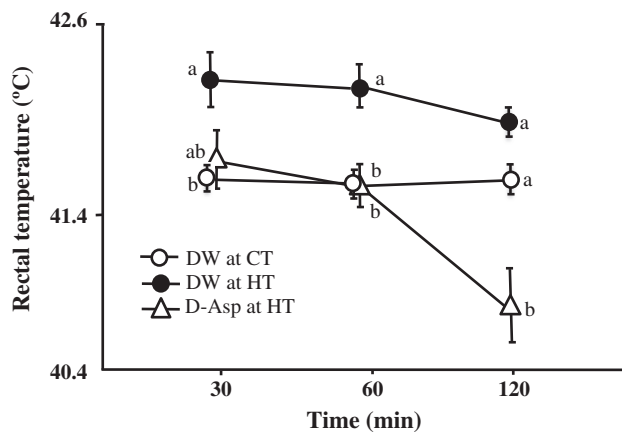


**Fig. 3.** Effect of oral administration of D-Asp on rectal temperatures under high ambient temperature (HT) in chicks during 120 min of the experimental period. The number of chicks used in each group ranged between 6 and 8. Values are means ± S.E.M. CT, control thermoneutral temperature.
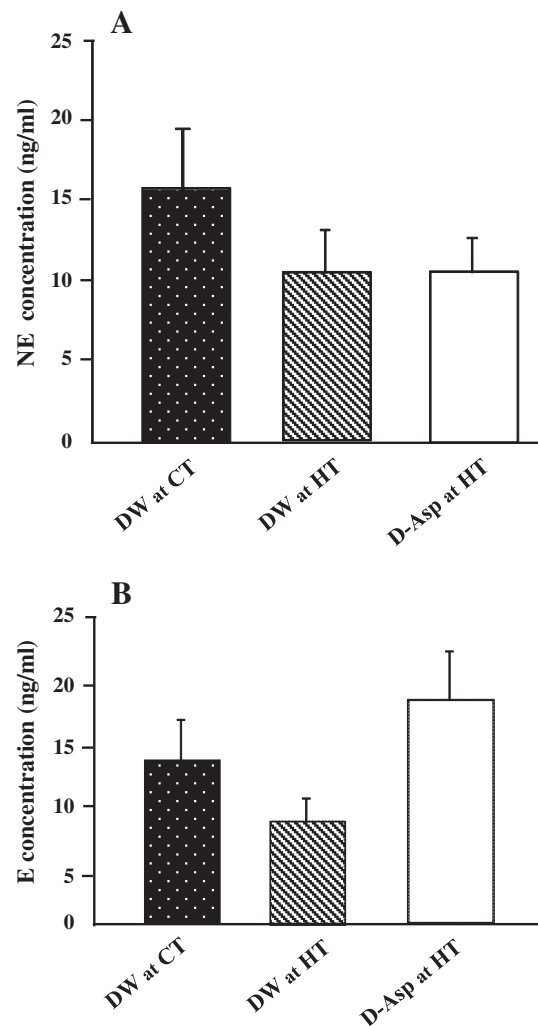


**Fig. 4.** Effect of oral administration of D-Asp on plasma norephinephrine (A) and epinephrine (B). The number of chicks used in each group ranged between 6 and 8. Values are means ± S.E.M. HT, high ambient temperature; CT, control thermoneutral temperature.
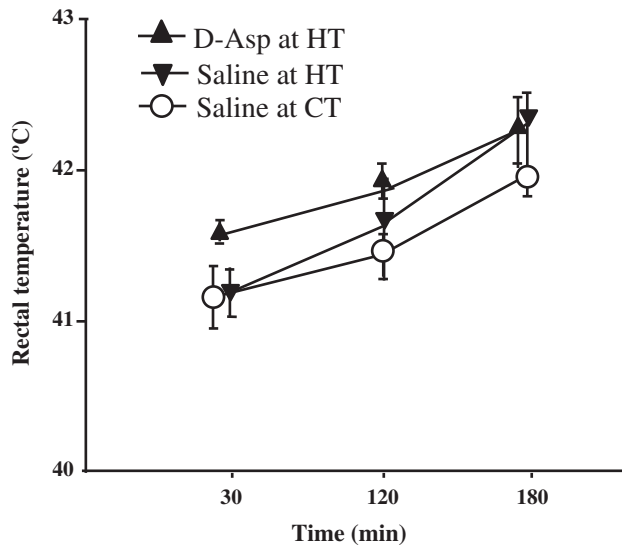
**Fig. 5.** Effect of intracerebroventricular (i.c.v.) injection of D-Asp on rectal temperatures under high ambient temperature (HT) and control thermoneutral temperature (CT) in chicks during 180 min of the experimental period. The number of chicks used in each group ranged between 5 and 6. Values are means ± S.E.M.

week-old chicks at a thermoneutral temperature and that body temperature returned to initial levels within 3–4 h (Marley and Stephenson, 1975). In the case of young chicks, Takahashi et al. (2005) demonstrated that i.c.v. injection of E did not significantly influence body temperature. Taken together, these results show that the body-temperature-lowering effect of D-Asp may not be related to that of E.

In Experiment 4, we demonstrated that i.c.v. injection of D-Asp definitely did not lead to a reduction in body temperature under HT, unlike the effect of oral injection of D-Asp under HT (Fig. 5). Previous reports revealed that the injection of D-Asp released arginine vasopressin (AVP) in rats (Koyuncuoğlu et al., 1984; Okuno et al., 1965). In birds, the AVP homologous nonapeptide is arginine vasotocin (AVT) (Acher et al., 1970; Sawyer et al., 1961). AVT, a neurohypophyseal hormone in non-mammalian vertebrates, is involved in water balance in birds, acting in a way that is similar to the function of AVP in mammals (Stallone and Braun, 1986). Tachibana et al. (2004) demonstrated that i.c.v. injection of AVT increased the rectal temperature in chicks. However, central injection of D-Asp did not influence the body temperature in the present study. Thus, we can speculate at least that central D-Asp would not be able to stimulate the release of AVT in young chicks. On the other hand, body temperature showed an increasing tendency to rise following i.c.v. injection, as shown in Fig. 5; this may be due to the minimal amount of stress that is induced by i.c.v. injection. However, we have previously confirmed that feeding behavior (Furuse et al., 1999) and plasma corticosterone concentration (Koutoku et al., 2005) were not affected by the same process of i.c.v. injection.

## Conclusion

Orally administered D-Asp, but not orally administered L-Asp, was found to cause the suppression of body temperature in neonatal chicks, along with the alteration of some plasma metabolites—namely, TG, UA, Glu and Cl. Interestingly, orally administered D-Asp strongly suppressed body temperature in HT-exposed chicks. These results suggest that D-Asp could be a useful amino acid for mitigating heat-stress problems in the poultry industry if it is provided through water/feed. A future study will focus on elucidating the mechanisms of the central and peripheral actions of D-Asp in thermoregulation.

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