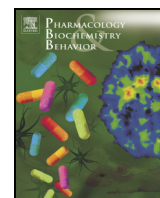


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Lauroyl-L-aspartate decreased food intake and body temperature in neonatal chicks

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ABSTRACT

We hypothesized that the effects of L- and D-amino acids might be influenced when conjugated with fatty acid. Thus, the effects of oral administration of lauroyl-L-aspartate (Lau-L-Asp) as well as lauroyl-D-aspartate (Lau-D-Asp) were examined. In **Experiment 1**, oral administration of both Lau-L-Asp and Lau-D-Asp decreased food intake while L- or D-Asp did not influence food intake. Interestingly, only Lau-L-Asp decreased body temperature. **Experiment 2** was conducted to determine whether non-conjugated mixture of L-Asp plus lauric acid has same effects under *ad libitum* feeding conditions. Lau-L-Asp decreased food intake and body temperature, but L-Asp plus lauric acid did not show any effect studied. In **Experiment 3**, we found that Lau-L-Asp declined food intake as well as time-dependently suppressed the body temperature in fasted chicks. However, L-Asp plus lauric acid did not show any effect. These results suggest that Lau-L-Asp may exert anorexigenic and hypothermic actions in chicks.

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1. Introduction

The regulation of food intake in poultry has been a focus of research interest in recent decades (Kuenzel, 1994; Richards, 2003). It has been reported that, unlike mammals, glucose does not play a major role for inducing satiety in birds (Smith et al., 1975; Gentle, 1976). One of the alternatives for such a role is performed by lipids. It has been reported that certain fatty acids such as the lauric acid, a medium-chain fatty acid or its chemically conjugated product with amino acids such as ethyl sodium-lauroyl-L-arginate hydrochloride (LAE), sodium lauroyl glutamate are being developed as a novel preservative in selected foods (Schwartz et al., 1997; Ruckman et al., 2004; Hawkins et al., 2009). Evans and Lepkovsky (1932) fed glyceryllaurate to rats at a 60% level in a synthetic diet and concluded this drug as a nontoxic one. Similar effects were found when rats were fed with mono-, di-, or triacylglyceroles at high levels in a synthetic diet for 70 days (Mattson et al., 1951). The test compound attributed no histological evidence to toxicity in rats. However, Cave (1982) observed that substitution of corn oil by lauric acid decreased food intake in broiler chick at the high level (30 g/kg) but not at the low level (10 g/kg). The reason for these different responses is likely to be the level of lauric acid in the diet. Furthermore, Little et al. (2005) administered lauric acid to healthy man and concluded that it has no effect on appetite, namely hunger, desire to eat, fullness or prospective consumption.

Since there has been existing conflicts regarding the hazard of adding synthetic lauric acid-emulsifier to food, the studies on the effect of feeding lauric acid emulsifiers have been investigated more thoroughly. Harris et al. (1951a,b) studied the effects of feeding sorbitan monolaurate, polyoxyethylene monolaurate, and polyoxyethylene monostearate in rats at 25% of their diet and in hamsters at 5 and 15% levels. They reported severe diarrhea, decreased weight gains, and increased mortality at all feeding levels and noted pathological conditions in rats fed the lauric acid emulsifiers. In non-mammalian vertebrates including chickens, however, there was no study to investigate the effect of lauric emulsifier on food intake. As most of the lauric emulsifiers are anorexigenic in mammals, it is possible that such compound may also inhibit feeding behavior in chicks. As for chemical properties, while lauric acid is a powder, lauroyl-L-aspartate potassium salt (Lau-L-Asp) and lauroyl-D-aspartate potassium salt (Lau-D-Asp) are solutions where Lau-L-Asp contains 28% solid at pH 7.7 and Lau-D-Asp contain 29% solid at pH 7.7. These solutions are new antimicrobial agents for using as cosmetic ingredients.

Several papers have discussed about the effect of amino acid supplementation on food intake or muscle metabolism. Asp occupies unique position in the intermediary metabolism, particularly in the mitochondria, where they play important roles in nitrogen and energy metabolism (Stegink, 1976). In avian species, similar to mammalian species, the L- and D-Asp have been shown to exist in the various brain regions of chickens (Neidle and Dunlop, 1990) and pigeon (Kera et al., 1996). It was reported that Asp is released from many tissues through non-classical neurotransmitter releasing pathways (D'Aniello, 2007; Hagberg et al., 1985; Homma, 2007), and in some cases can be enhanced by alterations of metabolism (Monda et al., 2003;

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Gundersen et al., 2001). It is well documented that brain neurochemical systems may regulate food intake, and a number of neurotransmitters contribute to this behavior. For example, intracerebroventricular (i.c.v.) administration or local administration of glutamate or the glutamate agonist, *N*-methyl-D-aspartate (NMDA), induced hyperphagia in mammals (Reddy et al., 1986; Ritter and Stone, 1987; Wandji et al., 1989).

Recently, we showed that oral administration of D-Asp but not L-Asp suppressed food intake in layer chicks (Erwan et al., 2013). Additionally, we demonstrated that central administration of both L- or D-Asp could induce a sedative effect, while the mechanism for hypnosis in neonatal chicks may be different for L-Asp in comparison with D-Asp (Erwan et al., 2012). Despite these existing evidences, it remains to be determined whether a chemical conjugate of L- or D-Asp with lipid, namely Lau-L-Asp or Lau-D-Asp could affect food intake. Moreover, recent reports on the presence of D-amino acids in diverse foods and the possibility of toxic effects of certain D-amino acids raises interest to investigate the effect of D-amino acids (Harper et al., 1970; Maruyama et al., 1972; Erwan et al., 2013). In this study, we therefore investigated the effect of Lau-L-Asp or Lau-D-Asp on food intake in chicks. Furthermore, we also investigated whether oral administration of these chemical conjugates affects body temperature of chicks to estimate the involvement of these chemicals in thermoregulation.

2. Materials and methods

2.1. Animals and drugs

One day-old layer chicks (Julia) 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 chicks) 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. 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. The number of animals used in each group was kept to the minimum ($n = 5–8$) that would still ensure adequate statistical power. This study was performed in accordance with the guidelines for animal experiments carried out in the Faculty of Agriculture and in 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). Lau-L-Asp and Lau-D-Asp were supplied by Asahi KASEI, Kawasaki, Japan.

2.2. Oral administration of the drugs

Following an acclimatization period, chicks were randomly selected and divided into four groups. The chicks were reared individually in experimental cages and had *ad libitum* access to diet during the whole experimental period. On the day of the experiment, each chick (5 days old) was orally administered drug solutions by the elastic plastic needle on small syringe for treatment groups and distilled water for control group. Chicks received oral administration at a dose of 6 mmol/kg body weight of L-Asp for 120 min. The criterion of dose selection was based on our preliminary findings in which L-Asp and D-Asp (3.75 mmol/kg body weight) caused no significant effect on food intake. **Experiment 1** was conducted to examine whether oral administration of the treatments namely, L-Asp, D-Asp, Lau-L-Asp and Lau-D-Asp affects the food intake, and body temperature of chicks under an *ad libitum* feeding condition. The chicks were provided *ad libitum* diets during the experimental period for 2 h immediately after the treatment. Food intake (at 30, 60 and 120 min) was determined by measuring the reduction in the amount of food consumed from a pre-weighed feeder. The weight of the feeder was measured using an electric digital balance.

At the end of the experiments, chicks were decapitated following anesthesia with isoflurane (Mylan Inc., Japan). **Experiment 2** was conducted to determine whether any different effect occurs between the oral injection of the conjugate Lau-L-Asp and non-chemically bonded mixture of lauric acid plus L-Asp on food intake and body temperature under an *ad libitum* feeding condition. **Experiment 3** was conducted similar to second experiment except chicks were fasted for 3 h prior to the drug administrations.

2.3. Measurement of body temperature

Body temperature of chicks was measured at 30, 60 and 120 min after treatments using a digital thermometer with an accuracy of ± 0.1 °C (Thermalert TH-5, Physitemp Instruments Inc., USA), by inserting the thermistor probe in the cloaca to a depth of 1–2 cm.

2.4. Statistical analysis

For food intake and body temperature parameters, a repeated-measures two-way ANOVA was applied. Significant differences were denoted as $P < 0.05$. Values were presented as means ± S.E.M. Statistical analysis was made using the commercially available package SAS (SAS Institute and Version 9.1. SAS Institute Inc., 2002). All data in each group were first subjected to a Thompson rejection test to eliminate outliers ($P < 0.01$), and the remaining data were used for the analysis among groups.

3. Results

3.1. Experiment 1: effects of oral administration of L- or D-Asp and Lau-L- or -D-Asp on food intake and body temperature

Fig. 1A shows the effects of oral administration of the drugs on food intake. Oral administration of L- or D-Asp did not affect food intake, while both Lau-L-Asp and Lau-D-Asp significantly ($F[4, 32] = 20.42$, $P < 0.0001$) reduced food intake and showed significant effect of the time ($F[2, 8] = 62.42$, $P < 0.0001$) and interaction between the treatment and time ($F[8, 64] = 8.54$, $P < 0.0001$), indicating that the Lau-L-Asp and Lau-D-Asp persistently reduced food intake with the progress of the experimental time. **Fig. 1B** shows the effect of oral administration of the drugs on body temperature during 2 h of the experimental period. A significant effect of Lau-L-Asp ($F[4, 29] = 11.07$, $P < 0.0001$) and time ($F[2, 8] = 4.35$, $P < 0.05$) were detected on body temperature. A significant interaction effect between Lau-L-Asp treatment and time was detected ($F[8, 58] = 4.26$, $P < 0.001$), indicating that the Lau-L-Asp persistently kept reduced body temperature with the advancement of time while reverse was true for the control chicks.

3.2. Experiment 2: effects of oral administration of L-Asp, lauric acid, lauric acid plus L-Asp and Lau-L-Asp on food intake and body temperature in *ad libitum* fed chicks

Fig. 2A shows the effects of oral administration of the drugs on food intake.

Oral administration of L-Asp, lauric acid or lauric acid plus L-Asp did not affect food intake, while Lau-L-Asp significantly ($F[4, 30] = 4.54$, $P < 0.01$) decreased food intake and also showed a significant effect of the time ($F[2, 8] = 93.53$, $P < 0.0001$) and interaction between the treatment and time ($F[8, 60] = 5.17$, $P < 0.0001$), implying that the effect of Lau-L-Asp, the time period of the experiment and their interaction caused constantly lower food intake. **Fig. 2B** shows the effect of oral administration of the drugs on body temperature during 2 h of the experimental period. Only Lau-L-Asp significantly decreased body temperature ($F[4, 28] = 12.25$, $P < 0.0001$).

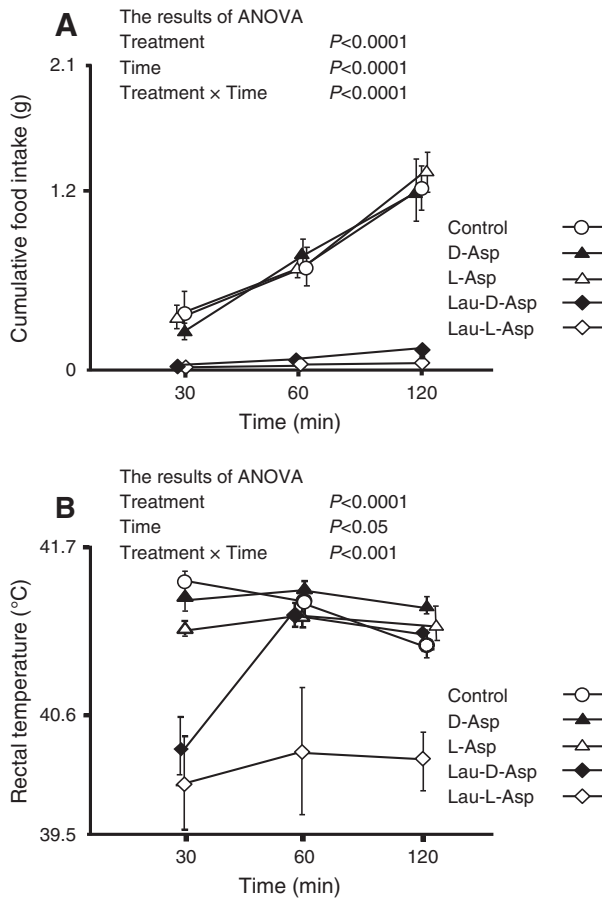


Fig. 1. The effects of oral administration of control, L-aspartate (L-Asp), D-aspartate (D-Asp), lauroyl-L-aspartate (Lau-L-Asp), lauroyl-D-aspartate (Lau-D-Asp) in chicks on food intake (A), and body temperature (B). The number of chicks used in each group ranged between 5 and 8. Values are means \pm S.E.M.

3.3. Experiment 3: effects of oral administration of L-Asp, lauric acid, lauric acid plus L-Asp and Lau-L-Asp on food intake, and body temperature in fasted chicks

Fig. 3A shows the effects of oral administration of the drugs on food intake. While oral administration of L-Asp, lauric acid or lauric acid plus L-Asp did not affect food intake, Lau-L-Asp significantly decreased food intake ($F[4, 27] = 9.88, P < 0.0001$) and also showed significant effects of time ($F[2, 8] = 57.98, P < 0.0001$) and interaction effect between the treatment and time ($F[8, 54] = 3.48, P < 0.005$), denoting that the effect of Lau-L-Asp on food intake is interminable within the experimental time and their interaction effect also caused a reduced food intake while antithesis was valid for control group. Fig. 3B shows the effect of oral administration of the drugs on body temperature during 2 h of the experimental period. Only Lau-L-Asp significantly decreased body temperature ($F[4, 27] = 12.80, P < 0.0001$). Significant effects of time ($F[2, 8] = 7.39, P < 0.005$) and interaction ($F[8, 54] = 11.33, P < 0.0001$) between Lau-L-Asp and time were also detected, designating that the body temperature was kept lower in the Lau-L-Asp treated groups than in the control groups and it declined gradually with the progress of time in the treated groups.

4. Discussion

The present study clearly revealed that oral administration of both Lau-D-Asp and Lau-L-Asp (6 mmol/kg) consistently depressed food intake (Figs. 1A, 2A, 3A). However, only Lau-L-Asp decreased body

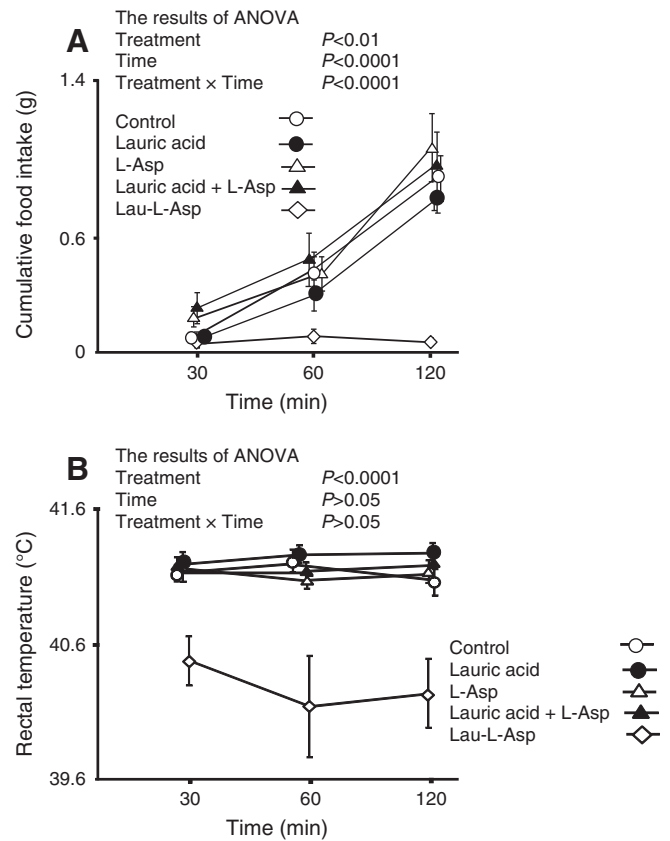


Fig. 2. The effects of oral administration of control, L-aspartate (L-Asp), lauric acid, lauric acid + L-Asp, lauroyl-L-aspartate (Lau-L-Asp) in *ad libitum* fed chicks on food intake (A), and body temperature (B). The number of chicks used in each group ranged between 5 and 8. Values are means \pm S.E.M.

temperature. We previously revealed that there was no significant effect of D-Asp on food intake when orally administered at the rate of 7.5 mmol/kg or lower than this amount, but at 15 mmol/kg showed a significant effect on food intake suppression (Erwan et al., 2013). Neither L-Asp nor D-Asp showed any effect on food intake in the current experiment which is inconsistent with our previous findings (Erwan et al., 2013). It is possible that the differences in dose caused this variation in response to food intake between the previous and present study. The dose of D-Asp used in the present study (6 mmol/kg) which was lower than those of the previous study (15 mmol/kg).

Since Lau-L-Asp and Lau-D-Asp caused to decrease food intake in the current study, it seems to be relevant with the following reports which indicate that lauric acid emulsifiers markedly reduced food consumption. For instance, Harris et al. (1951a,b) demonstrated that severe diarrhea, decreased weight gains, pathological conditions and increased mortality were occurred after feeding the emulsifiers of lauric acid in rats at 25% and in hamster at 5% and 15% of their diets. Krehl et al. (1955) concluded that there were low palatability of food when included polyoxyethylene mono- and di-laurates and other polyoxyethylene esters in the diets of rats and cats. Eagle and Poling (1956) and Poling et al. (1956) revealed the decreased of growth rate and increased the mortality in rats, hamsters and rabbits fed polyoxyethylene-20 sorbitan mono-laurate from 5 to 25% of their feeding levels. Fitzhugh et al. (1960) demonstrated the decreasing body weight and toxicity in rats fed sorbitan mono-laurate (Span 20) and polyoxyethylene-20 monolaurate (G-2129) with lauric acid as emulsifiers. Ruckman et al. (2004) further observed that an LAE, lowered body weight and food consumption during the first week in rats when received 1143 and 1286 mg/kg/day. In light with the information mentioned above, it seemed that the reduced food intake

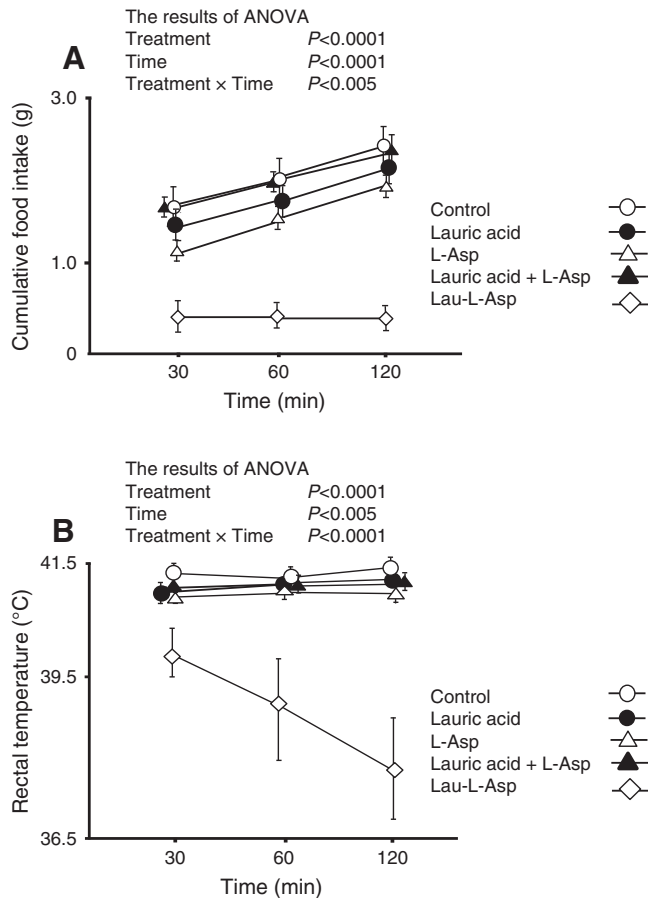


Fig. 3. The effects of oral administration of control, L-aspartate (L-Asp), lauric acid, lauric acid + L-Asp, lauroyl-L-aspartate (Lau-L-Asp) in fasted chicks on food intake (A), and body temperature (B). The number of chicks used in each group ranged between 5 and 8. Values are means \pm S.E.M.

due to the oral administration of Lau-L-Asp or Lau-D-Asp could be explained, *i.e.*, the anorexigenic effect of Lau-L-Asp and Lau D-Asp might be due to the emulsifier effect of lauric acid moiety with L-Asp or D-Asp.

To our knowledge, we are first to find out a fatty acid emulsifier that could be considered as a potential hypothermic agent. We have shown that oral administration of Lau-L-Asp, but not Lau-D-Asp, decreased body temperature (Fig. 1B). It seems to be an established fact that the metabolism and utilization of L- and D-Asp is different. For example, we revealed that there was a difference in the effect between L-Asp and D-Asp on stress response and food intake (Erwan et al., 2012, 2013) as well as on body temperature (*unpublished data*). These data raised the possibility of having different mechanism between the two isomers. In addition, the different effects on body temperature between Lau-L-Asp and Lau-D-Asp indicate that these two chemicals may work in different metabolic pathways for thermoregulation. Previous report revealed that the injection of D-Asp but not L-Asp released arginine vasotocin (AVT) in rats (Koyuncuoğlu et al., 1984). In addition, hypothermic actions of exogenous AVT were observed in sheep (Cooper et al., 1979; Ruwe et al., 1983) and rabbits (Naylor et al., 1985). Some reports have demonstrated that oral administration of D-Asp decreased body temperature in rats (Koyuncuoğlu et al., 1982; Koyuncuoğlu and Berkman, 1982). Thus, we could assume that the chemical conjugation of D-Asp with lauric acid (Lau-D-Asp) inhibited its potential in reducing body temperature compared to Lau-L-Asp. Further study focusing on AVT release and thermoregulation in relation with Lau-D-Asp and Lau-L-Asp in chicks is necessary to understand the mechanism of actions of these drugs in thermoregulation. It is well

known that the global surface temperature is increasing (IPCC, 2007) and high ambient temperature (HT) is a serious concern for birds as birds in general have a greater challenge than mammals in maintaining homeothermic body temperature (Ensminger et al., 1990; Chowdhury et al., 2012). Thus our current findings would help to think about some drug development to mitigate HT related problems.

We further examined separately the effect of the lauric acid, non-conjugated mixture of L-Asp plus lauric acid or conjugated Lau-L-Asp in Experiment 2. Neither L-Asp/lauric acid nor L-Asp plus lauric acid was associated with the decreasing food intake and body temperature. However, as observed in the Experiment 1, the form of Lau-L-Asp strongly depressed food intake and body temperature. Even though the doses of all treatments were similar but either oral administration of lauric acid or L-Asp plus lauric acid was not significant on food intake. These findings indicate that only when L- or D-Asp has emulsifier form; it may exhibit an anorexigenic effect. This result is consistent with the previous reports. Little et al. (2005) reported that there was no effect of lauric acid on appetite and food consumption in rats. Similar results revealed that there was no different digestibility in animals when fed either lauric acid or lauroyl glyceroles (Carrol, 1958; Deuel et al., 1941; Hoagland and Snider, 1943; Thomasson, 1956).

In Experiment 3, we confirmed more constant effects of Lau-L-Asp, on suppressing food intake and body temperature in fasted chicks (Fig. 3A,B). We cannot explain why oral administered Lau-L-Asp decreased body temperature so sharply in Experiment 3. However, we can speculate that Lau-L-Asp may play more effective role in thermoregulation in fasted chicks. Further experimentation is needed to clarify the mechanism.

In conclusion, orally administered Lau-L-Asp and Lau-D-Asp had a depressive effect on food intake in neonatal chicks. Importantly, it was demonstrated for the first time that Lau-L-Asp would be a potential emulsifier for food intake regulation and thermoregulation in chicks. Further investigations on Lau-L-Asp and Lau-D-Asp in controlling thermoregulation in chicks would be necessary to justify the possibility of utilizing the drug to mitigate HT related problems in chicks.

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