Studies on the elucidation of mechanisms to afford thermotolerance by L-leucine in ovo administration in broiler chicks

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論　文　名	Studies on the elucidation of mechanisms to afford thermotolerance by L-leucine *in ovo* administration in broiler chicks （L-ロイシンの卵内投与によるブロイラーヒナへの熱耐性付与の機構解明に関する研究）
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論　文　審　査　の　結　果　の　要　旨

Heat stress is a rising environmental challenge for animal production. Metabolic regulation plays important roles to minimize stress. Thermal manipulation (TM) of egg incubation temperature causes metabolic alterations and contributes to develop thermotolerant chickens. In the present study, TM and *in ovo* technologies have been used for metabolic manipulation of embryos to develop heat stress resistant broiler chicks.

First, fertilized broiler (chunky) eggs were incubated under 37.6°C with approximate 60% relative humidity. During embryonic day (ED) 10 to 18, the embryos were exposed to TM (38.6°C, 6 h/d) or control (37.6°C) treatments to investigate changes in free amino acid concentrations of tissues due to the TM treatment. TM resulted in the alteration of amino acid concentrations in embryos. In particular, several free amino acid concentrations were reduced, including leucine (Leu), phenylalanine and lysine (Lys), in the embryonic brain and liver.

Second, L-Leu, associated with other branched chain amino acids (BCAAs), L-isoleucine (L-Ile), L-valine (L-Val) and combined BCAAs (L-Leu + L-Ile + L-Val), were *in ovo* administrated into eggs during embryogenesis on ED 7. Amino acids were administered at doses of 35, 21 and 29 μmol/500 μl/egg for L-Leu, L-Ile and L-Val, respectively, and the combined BCAAs group was the combination of the above doses. It was found that L-Leu, but not L-Ile, L-Val or combined BCAAs, *in ovo* administration significantly declined rectal temperature at hatching in broiler chicks.

Third, the metabolic activity (oxygen (O_2) consumption, carbon dioxide (CO_2) production, respiratory quotient and heat production (HP)) and plasma metabolites (glucose, triacylglycerol, non-esterified fatty acid and ammonia) were measured on ED 14 and 19 due to the effect of L-Leu *in ovo* administration. L-Leu *in ovo* administration stimulated embryonic metabolic activity and lipid metabolism, which showed sexual differences in lowering rectal temperature at hatching. Post-hatched male and females were reared separately with free access to food and water under control thermoneutral temperature (CT: 30 ± 1°C during day 1–4 and 28 ± 1°C during day 5–10). Body weight and rectal temperature were recorded daily under CT. It was found that the daily rectal temperature and body weight were not affected by L-Leu *in ovo* administration until 9- or 10-days old in both male and female chicks. Both sexes of chicks were exposed to high ambient temperature (HT: 35 ± 1°C; 180 min) or CT (28 ± 1°C; 180 min) at day 9 (male chicks) and day 10 (female chicks). At the end of the experiment, the blood, liver and brain (diencephalon) were

collected for further analysis of plasma metabolites, amino acids and mRNA expressions of heat-shock proteins (HSP). Interestingly, L-Leu *in ovo* administration significantly reduced the food intake, stimulated the lipid metabolism and improved thermotolerance in male, but not female broiler chicks under HT.

Fourth, L-Leu *in ovo* administration modified the metabolisms of amino acids under heat stress in male broiler chicks. In particular, plasma and diencephalic Lys concentrations were increased and hepatic and diencephalic arginine concentrations were declined. Moreover, the mRNA expressions of HSP-70 and -90 were attenuated in male chicks under heat stress.

In summary, this thesis provided that L-Leu *in ovo* administration could be able to afford thermotolerance in broiler chicks and gave a valuable achievement contributing to the development of avian nutrition and physiology. Therefore, this research deserves a Ph.D. (doctor of agriculture) degree.