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論文題名　: 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）

区　　分　：甲

論　文　内　容　の　要　旨

High ambient temperature (HT) associated with global warming is a rising environmental challenge for animal welfare. Nutrients play vital roles to support physiological functions under HT. Development of nutritional biotechnologies is very important to face the increasing challenge of HT. Thermal manipulation (TM) of incubation temperature causes metabolic alterations and contributes to develop thermotolerant chickens. In this study, fertilized chunky broiler 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 the changes of amino acid concentrations during embryogenesis following TM treatment. It was found that TM resulted in the alteration of amino acid metabolism and reduced several free amino acid concentrations, including leucine (Leu), phenylalanine and lysine (Lys), in the embryonic brain and liver, which may provide important information to select suitable amino acids for *in ovo* administration to develop thermotolerance. Then 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 dissolved into sterile water with 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 or L-Val or combined BCAAs, *in ovo* administration significantly declined body temperature at hatching in broiler. Therefore, amino acid of L-Leu was focused for further study. The *in ovo* administration processes and dose of L-Leu was same as mentioned above. The metabolic activity (O_2 consumption, CO_2 production, respiratory quotient and heat production (HP)) and embryonic plasma metabolites (glucose, triacylglycerol (TG), non-esterified fatty acid (NEFA) and ammonia) were measured on ED 14 and 19. It was further confirmed that L-Leu *in ovo* administration stimulated embryonic metabolic activity and lipid metabolism during embryogenesis, which showed sexual differences in reducing body temperature at hatching. After hatching, the post-hatched broiler chicks were subjected to sexing, and male and females were reared separately with free access to food and water under control thermoneutral temperature (CT) of 30 ± 1°C during day 1–4 and 28 ± 1°C during day 5–10. Body weight and body temperature were recorded daily under CT. It was found that the daily body temperature and body weight were not affected by L-Leu *in ovo* administration until 9- or 10- day old in both male and female chicks. Both sexes of chicks were exposed to heat challenge (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 heat exposure, the blood, liver and brain (diencephalon) were collected for further analysis, including plasma metabolites, amino acids concentrations and mRNA gene expressions. Interestingly, at 9- or 10- day old, 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 heat stress (35 ± 1 °C, 180 min). Further analysis suggested that L-Leu *in ovo* administration modified the

metabolisms of amino acids under heat stress in male broiler chicks, particularly increased plasma and diencephalon Lys and declined hepatic and diencephalon arginine, as well as attenuated mRNA expressions of heat shock protein (HSP) -70 and -90 under heat stress. These results indicated that L-Leu *in ovo* administration could be able to afford thermotolerance in broilers and it may serve as a new bio-technology for developing thermotolerant broilers in future.