

**Studies on noninvasive methods to estimate nutritional
condition of protein and amino acids for animals**

Abstract of Doctoral Thesis

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In animals which have small genetic diversity, like as experimental animals and commercial chickens, same nutritional management is usually carried out for all population in the same age or life period. On the other hand, these had large genetic diversity, like as dairy cow, pets, and wild life animals, require order made nutritional management. For appropriate nutritional management, the estimation of nutritional requirements are important. While energy requirement for maintenance was able to estimate by the body weight using metabolic body size, protein metabolism differs among the feeding habits. Therefore, protein and amino acid requirement have to estimate experimentally. Blood sampling and retention of animals in experiment were stress full for animals. Therefore, it is necessary to develop a new and noninvasive method.

Therefore, the present study was conducted to judge whether creatinine excretion will be criterion for estimation of nutritional condition of amino acids and protein or not regardless of feeding habit.

Housing, handling, feeding, and killing procedures were in accordance with policies of Nippon veterinary and life science university committee on laboratory animal care.

In experiment 1, 2, 3 and 4, 8-day-old Chunky broilers chicks were used. The chicks were assigned to three or four dietary groups, with five chicks each, and were fed an experimental diet for 7 days. The experimental diets contained deficient, adequate, or excessive methionine, arginine, or protein levels in experiments 1, 2 and 3, and two levels of arginine (adequate and excessive) and two levels of methionine (deficient and adequate) in experiment 4, respectively. Excreta were collected for the last 3 days of the feeding trial, and chicks were terminated by dislocation of the neck at the end of the feeding trial to collect their livers. Creatinine concentration in the excreta and hepatic L-arginine-glycine amidinotransferase (AGAT) activities were determined.

In Experiments 5 and 6, 4 adult Eurasian scops owls (*Otus scops*) were allocated to 4 dietary methionine or protein levels x 4 periods recommended by Latin square experimental design, respectively. Each period was consisted of the acclimatizing 3 days and the experimental 4 days, and excreta was collected for last 24 hr. Experimental diets used were neonatal mice (*Mus musculus*) containing capsule of crystalline amino acids mixture (in experiment 5) or casein and lard (in experiment 6) in abdomen. Total dietary methionine levels were 0.22%, 0.35%, 0.60%, and 0.72% in experiment 5. Total

protein: energy ratio were 0.113, 0.125, 0.138, 0.15 in experiment 6.

Urinary creatinine levels increased with increasing both dietary methionine and arginine levels from deficient to adequate recommended by Japanese feeding standard ($P < 0.05$), and then remained constant in experiments 1 and 2, respectively. The hepatic AGAT activity decreased when both dietary creatinine precursors levels were increased from deficient to adequate levels ($p < 0.05$), and then remained constant. These results suggested that creatinine excretion was changed with both increasing dietary methionine and arginine, dose-dependently.

In experiment 3, urinary creatinine levels decreased with increasing protein levels from deficient to adequate ($P < 0.05$), and then turned to increase. The hepatic AGAT activity was same response as creatinine concentration. In experiment 4, urinary creatinine concentration was higher at excess arginine levels and deficient methionine levels than other dietary groups ($p < 0.05$). The hepatic AGAT activity was decreased at excess arginine diets ($p < 0.05$). In these results, excess dietary arginine levels promoted creatine synthesis, and increased creatinine excretion regardless of methionine deficient. These results suggested that creatinine excretion would be useful parameter for estimating the protein and amino acids requirements.

In experiment 5 and 6, urinary creatinine excretion of scops owls were similar response of broilers ($p < 0.05$). The facts suggest that creatinine excretion will be criterion for estimation of protein and amino acid requirement for carnivorous birds.

In conclusion, creatinine excretion would be useful parameter for estimating the protein and amino acid nutritional condition regardless of feeding habit.