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

Summary 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 example, in dairy cow, the nutritional status is usually monitored using the lactating quality and quantity as a criterion because the genetic diversity of cows is relatively large and the lactating periods are not the same among individual cows. For appropriate nutritional management, the estimation of nutritional requirements are important. But, especially avian, many of species except poultry were not estimated the nutritional requirements. 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. Because nutritional requirements were influenced by genetic diversity, environment, life stage, and stress, it is necessary to analyze these factors. Blood sampling and retention of animals in experiment were stress full for animals. Therefore, it is necessary to develop a new and noninvasive method.

The final amino acid product levels in the urine are noninvasive parameters which can be used to estimate the protein and amino acids requirements. With this criterion, it is also possible to test the same animals repeatedly. Therefore, it is useful for monitoring the nutritional condition. However, the method for protein and amino acid requirements using the taurine excretion as a criterion is not available for carnivorous animals.

Therefore, the present study was conducted to judge whether creatinine excretion will be criterion for estimation of nutritional condition of protein and amino acids 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.

1. Study of creatinine excretion as a criterion

Creatinine is one of the final amino acid products in the urine. Creatinine has a precursor, creatine which is synthesized from the following three amino acids: arginine, glycine, and methionine. These amino acids are essential amino acids for poultry. Methionine, in particular, is a primary source of methyl groups, and it is liable to be first

limited amino acid. Chamruspollert *et al.* (2002b) reported that dietary arginine and methionine levels influenced muscle creatine levels in broiler chicks. Furthermore, Chamruspollert *et al.* (2002a) suggested that muscle creatine levels can be used as a criterion to assess arginine requirement. Creatinine is non-enzymatically converted from creatine and excreted in the urine without any resorption. It indicated that creatinine excretion might respond to muscle creatine levels or creatine synthesis. Thus, cratinine excretion might reflect dietary it's prescursors levels.

In order to judge whether creatinine excretion will be criterion for estimation of amino acids and protein nutritional condition or not, at first, the responses of creatinine excretion to dietary methionine and arginine were made sure in experiment 1 and 2.

1-1. Effects of dietary methionine and arginine levels on the urinary creatine excretion in broiler chicks

In experiment 1 and 2, 8-day-old Chunky broilers chicks were used. The chicks were assigned to three dietary groups, with five chicks each, and were fed an experimental diet for 7 days. The experimental diets mainly consisted of corn and soybean meal, and contained deficient, adequate, or excessive methionine and arginine levels in experiments 1 and 2, 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.

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.

1-2. Effects of dietary protein levels on the urinary creatinine excretion in broiler chicks

In experiment 1 and 2, 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 three levels of protein (deficient, adequate, or excessive) in experiment 3, and two levels of arginine (adequate and excessive) and two levels of methionine (deficient and adequate) in experiment 4. Excreta and collect the livers were collected in the same method as experiment 1.

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 this 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.

2. Study of feeding habit

Because the carnivorous birds were restricted on the experiments, little is known about nutritional and metabolic studies in carnivorous birds. Many species of the carnivorous birds are regarded as endangered or near threatened species, and they were restricted by law. In addition, usually carnivorous birds are weak from stress, and have high palatability for foods. Hence the conventional methods to estimate protein and amino acid requirements are not available for carnivorous birds.

Therefore, in order to make sure the response of creatinine excretion to methionine and protein regardless feeding habit, experiment 5 and 6 were conducted using scops owls as carnivorous birds.

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. These results showed the 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, cretatinine excretion would be useful parameter for estimating the protein and amino acid nutritional condition regardless of feeding habit.