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# Effect of ration level on non-fecal nitrogen excretion of juvenile yellowtail (*Seriola quinqueradiata*)

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**Abstract:** Post-feeding non-fecal nitrogen excretion was determined for juvenile yellowtail in a closed tank system. The highest ammonia excretion value was noticeable 2 hr post-feeding, while the urea excretion rate was highest at 4 hr post-feeding. The ammonia excretion rate of fish fed the lowest ration (1.4%bw) was a half of that of fish fed the highest ration (4.1% bw). The equivalent energy value from ammonia was in the range of 8.7 to 18.0 (KJ/kg bw/day) under present feeding condition.

**Keywords:** Yellowtail, non-fecal nitrogen excretion, ammonia, urea

## INTRODUCTION

The main end product of protein metabolism in almost all teleosts studied so far is ammonia (Smith, 1929; Fromm, 1963) and usually represents 75-90% of the nitrogen excretes (Wood, 1958). One of the research interests on nitrogen excretion measurement in fish is nutritional energetics. Nitrogen excretion increases as fish ingest low quality proteins (i.e., imbalance amino acid profile) compared to good quality ones. Therefore, nitrogen excretion is considered as "loss" of ingested nitrogen which is not used for growth and maintenance in fish.

The post-feeding changes in ammonia excretion has been studied (Brett and Zala, 1975; Kaushik and Dabrowski 1983; Kaushik 1980l; Rychly and Marina, 1977; Kikuchi et al 1991; Harris and Probyn 1996; Chakraborty et al 1992; Ogino et al 1973; Ming 1985), yet no such research has been reported on yellowtail, *Seriola quinquearadiata*. The purpose of this study is to investigate the post-feeding nitrogen excretion pattern of yellowtail with different ration levels and to investigate the endogenous nitrogen loss from non-fecal origin.

## MATERIALS AND METHODS

*Fish and diet*

The experiment was carried out at the Usa Marine Biological Center, Kochi University. Juvenile yellowtail (*Seriola quinqueradiata*) was used in the experiments. Juvenile yellowtail from natural stock were obtained from a commercial fish farmer in Usa, Kochi. These fish were kept indoor facility at natural light cycle. These fish were fed a commercial feed (Marubeni Co. Kobe, Japan) for 2 weeks to acclimate them to the experimental conditions. The experimental diets are shown in Table 1. A fish meal based control diet and protein-free diet (PFD) were formulated. These diets were made by thoroughly mixing the dry ingredients with oil and then added with cold water. The mixture was then passed through a pelleter and the resulting noodle-like materials were cut into appropriate size. These diets were stored in -20℃ freezer untill used.

**Table 1.** Composition of the experimental diets

| Ingredient (g/100g) | Control | Protein-free |
|---|---|---|
| Brown fish meal | 70.0 | - |
| α-corn starch | 8.5 | 38.5 |
| Dextrin | - | 17.5 |
| Pollock liver oil | 9.0 | 20.0 |
| Vitamin mix*1 | 3.0 | 3.0 |
| Mineral mix*1 | 2.5 | 2.5 |
| CMC-Na | 2.5 | 2.5 |
| Guargum | 0.5 | 0.5 |
| α-cellulose | 4.0 | 15.0 |
| Feeding stimulants*2 | - | 0.5 |
| Proximate composition (%)*3 | | |
| Crude protein | 52.2 | 0.8 |
| Crude lipid | 15.9 | 19.4 |
| Crude sugar | 10.9 | 44.6 |
| Crude ash | 11.9 | 2.2 |

*1 Shimeno et al. (1992)
*2 Takeda et al. (1981)
*3 Dry matter basis

*Nitrogen excretion*

Yellowtail with average body weight of 31.7g were stock into 6 aquaria with 10 fish each. Daily ration levels were planned for 1, 2, 2.5, 3, 4 % of body weight and satiation. The fish were fed two times daily (8:00 and 16:00). The PFD was fed to the separate group of fish until satiation in order to determine endogenous nitrogen excretion level. Fish were fed for 9 days. In the 10th day, they were fed at a half of the daily ration at 8:00 only and one fish in each group was placed in 50L capacity of an Artemia hatching tank (Earth Co., Japan). Water was filled 30L in the tank. At 2, 4, 8, 12, 18, and 24 hours, half of the water (15L) was exchanged with new water. The ammonia concentration was measured before and after these water exchange. The water sample was taken 500ml. For each experimental group, excretion measurement was conducted three times and these results expressed as an average. A 24h urea excretion pattern was separately measured only for the fish fed PFD and control diet both at satiation feeding. The water temperature during the experiment was in the range of 22-25℃.

*Analytical methods*

Ammonia and urea concentrations were determined by phenolhypochlorite reaction (Strickland and parsons, 1972). Water sample was filtered with 0.45 μm filter paper. For the ammonia-N determination, 10ml of water sample was analyzed for ammonia-N by adding with 0.4ml of 10% phenol-ethyl alcohol solution (95%), 0.4ml of 0.5% sodium nitroprusside solution, and 1.0ml of alkali hypochlorite solution, respectively, then incubating at room temperature for 1 hr, and recorded the absorbance at 640nm by means of a spectrophotometer (Hitachi U-1000). For the urea, 10ml of seawater sample was hydrolyzed by addition of 1.0ml of 0.1% urease solution, incubated at 50℃ for 20min, then analyzed as ammonia-N as the same procedure described above. For each sample, duplicate measurements were carried out. Total ammonia-N concentration in the rearing water was calculated by using following formula (Ming, 1985),

mg NH3-N / h = V x ( [NH3-N]ti - [NH3-N]t0)

where, [NH3-N]ti = ammonia-N concentration (mg/l) at time ti; t0 = beginning of the measurement after water exchange; t1 = end of the same measurement period (h) just before water exchange. V = total volume (liters) of water in the aquarium.

## RESULTS

The originally planned daily ration levels were 1, 2, 2.5, 3, 4% of body weight and satiation though, the overall ration levels during the feeding experiment shifted to 1.4, 2.0, 2.2, 2.5, 3.2 and 4%. The ammonia nitrogen excretion patterns of yellowtail is shown in Fig. 1. For each ration level, a single peak was noticeable at 2 hrs post-feeding. Although amplitude of the peak was not function of the ration level, the satiation feeding group was the highest among the group examined. All peak values became fairy constant after 8-12 h post-feeding. The PFD-fed fish also had a single peak at 2 hrs post-feeding. The time of appearance for the peak of PFD-fed fish synchronized to other control diet-fed groups, nevertheless nitrogen intake of PFD-fed fish was virtually zero. We suspected this peak was induced by stress, because stress is known as one of the factors that increase ammonia excretion rate (Smith, 1971). It is probable that fish had gotten stress when they were moved into the Artemia tank. Since this procedure was applied to all experimental fish, all ammonia excretion values may have been overestimated. Therefore, stress related ammonia excretion levels were estimated by subtracting an estimated basal level from actually measured levels (Table2). For the basal level estimation, the values from 4-8h, 8-12h, 12-16h and 18-24h were taken as the average and the estimated value becomes 9.5 (mg NH3-N/kg bw/h). This value was converted to daily excretion value multiplied by 24h and it resulted in 226.8 mg $NH_3$-N/kg bw/day. The difference of this value (226.8) and the actually measured daily excretion value (309.7) assumed to be explained by stress (82.9). In order to correct this stress effect, daily ammonia excretion values are subtracted by 82.9. These corrected values and their equivalent energy values are listed in Table 3. The difference between un-corrected and corrected values are in the range of 10-20%.

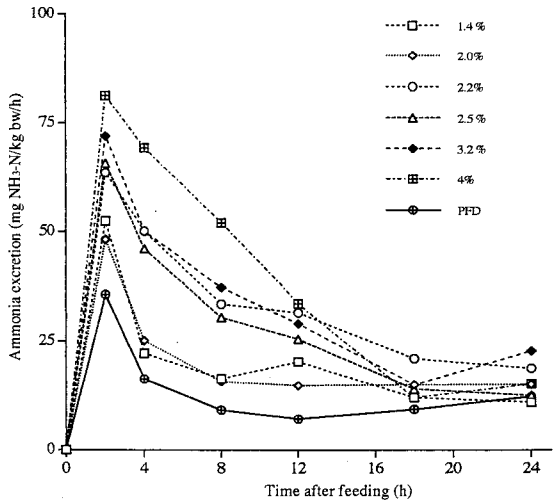


**Fig. 1.** Mean daily variation in ammonia-N excretion in yellowtail fed different feeding levels of control diet and satiation feeding of protain-free diet.

**Table 2.** Estimation in PFD-fed fish for ammonia excretion value explained by stress

| Time post-feeding (h) | Ammonia excretion (mg $NH_3$-N /kg bw) *1 | Ammonia excretion (mg $NH_3$-N /kg bw/h) | Estimated basal value *2 (mg $NH_3$-N /kg bw/h) | Estimated ammonia excretion value explained by stress |
|---|---|---|---|---|
| 0-2 | 69.4 | 34.7 | - | - |
| 2-4 | 35.7 | 17.9 | - | - |
| 4-8 | 37.4 | 9.4 | | - |
| 8-12 | 41.5 | 10.4 | 9.5 | - |
| 12-18 | 55.2 | 9.2 | | - |
| 18-24 | 70.5 | 8.8 | | - |
| Daily excretion (mg $NH_3$-N/kg bw/day) | 309.7 (=sum of 0-24 above) | - | 226.8 (=9.5 x 24) | 82.9 (=309.7-226.8) |

*1 Average of triplicated experiments

*2 Mean of 4-8, 8-12, 12-16 and 18-24 values in the ammonia excretion

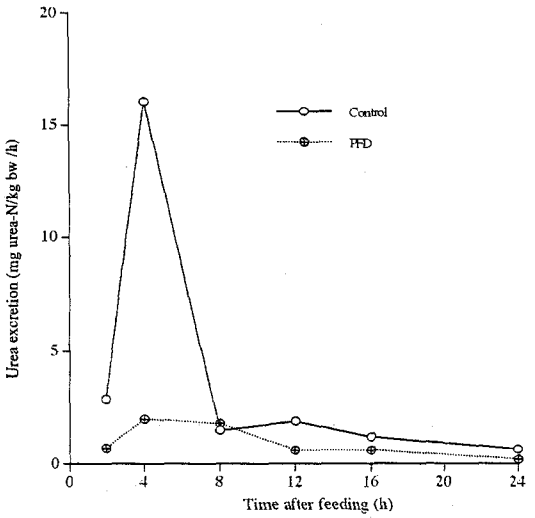


**Fig. 2.** Mean daily variation in urea-N excretion in yellowtail satiatory fed either control of protein-free diet.

The urea excretion was only measured in PFD- and control diet-fed fish both at satiation feeding level (Fig. 2). The both group of fish had peaks at 4 hrs post-feeding, which was slower appearance than the ammonia excretion peaks. The peak value of control diet-fed fish was higher than that of PFD-fed fish. A daily urea excretion was estimated based on the excretion pattern shown in Fig. 2, and these values were 25.3 in PFD-fed fish and 61.2 in control diet-fed fish, respectively. The concomitant measurement of ammonia in these groups were 334.5 in PFD and 585.4 in control diet. Thus the daily urea excretion was about 10% of the ammonia excretion in both groups.

## DISCUSSION

In the present study, a single peak was noticeable in ammonia excretion at 2 hrs post-feeding in this study (Fig. 1), while two peaks were observed in flounder (Dosdat, 1995; Kikuchi et

al., 1991). The discrepancy of excretion patterns in two fish species may be related to the differences in food passing time. The food passing time of warm water fish (e.g., yellowtail) is faster than that of cold water fish (e.g., flatfish). The slower passing time might result in discontinuous ammonia excretion.

Single peak was observed in daily ammonia excretion of fish fed PFD at 2 hrs post-feeding, while stable excretion patterns has been reported by other researchers (Rychly, 1977; Kikuchi et al., 1991; Dosdat, 1995). We suspected the peak seen in our study may be attributed to stress, so recalculation was made (Table 2, Table 3). Based on this recalculation, it was found out that about 10-20% of ammonia excretion was overestimated. In order to minimize stress effect during fish transfer, Kaushik and Dabrowski (1983) used anesthesia (ethylene glycol monophenyl ether, 0.3ml/L) before moving and measuring ammonia of fish. This approach may worthwhile to apply for yellowtail as well.

**Table 3.** Ammonia excretion value for yellowtail at different feeding levels

| Ration level (%) | Measured value (mg-N/kg bw/day) | Corrected value*1 (mg-N/kg bw/day) | Equiv. energy value*2 (KJ/kg bw/day) |
|---|---|---|---|
| 1.4 | 432.9 | 350.0 | 8.7 |
| 2.0 | 449.5 | 366.6 | 9.1 |
| 2.2 | 723.7 | 640.8 | 15.9 |
| 2.5 | 603.8 | 520.9 | 13.0 |
| 3.2 | 737.0 | 654.1 | 16.3 |
| 4.1 | 808.1 | 725.2 | 18.0 |

*1 Measured value - estimated ammonia excretion value explained by stress (see Table 2)
*2 Corrected value x caloric value of ammonia (=5.95KJ/g)

In determining an endogenous ammonia excretion, it is a general practice to use starved fish instead of protein free-diet fed fish because fish generally refuse intake of protein-free diet. In the present study, however, inclusion of feeding stimulants to the protein-free diet made it possible to have voluntary intake of the protein-free diet in yellowtail. This approach should be useful for estimating nutritive qualities of protein and feed, since precise endogenous nitrogen excretion measurement is necessary for that purpose.

Urea excretion peak was noticeable at 4 hrs post-feeding. This finding is interesting in two points. The first point is earlier study of Brett and Zala (1975) on salmonid and other studies on fresh water fish did not follow a definite pattern in urea excretion, while in the present study as well as studies conducted in flatfish, these sea water fish species showed post-feeding urea excretion peak. Different nitrogen excretion mechanism may exist in fresh water and sea water fish. The second point is the slower appearance of urea excretion peak compared to the ammonia peak. Such time difference of two nitrogen metabolites was also observed in flatfish (Kikuchi et al., 1991). It has been proposed that there are three possible pathways for the formation of urea (ornithine-urea cycle, catabolism of arginine and purine breakdown) and the latter seems to be the main source of urea synthesized by teleosts (Kaushik and Cowey, 1991). The time require for the urea synthesis may take longer than that for deamination. The daily urea excretion rate was estimated to be about 10% of ammonia excretion rate in the present study and it has been reported as 5-15% of total nitrogen excretion (Kaushik and Dabrowski, 1983). Therefore, the percentage of urea excretion in the total non-fecal nitrogen can be neglected. However, the significant post-feeding excretion patterns were noticeable in the

present study as welll as other workers (Kikuchi et al., 1991; Dosdat, 1995), suggest that urea excretion pattern may be an important indication for protein nutrition such as dietary protein quality in marine fish species.

## REFERENCES


BRETT, J. R., and C. A., ZALA, 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhyncus nerka*) under controlled conditions. *J. Fish. Res. Board Can.* **32**: 2479-2486.

CHAKRABORTY, S. C., L. G., ROSSAND and B., ROSS, 1992. The effect of dietary protein level and ration level on excretion of ammonia in common carp (*Cyprinus carpio*). *Comp. Biochem. Physiol.*, **103 A**: 801-808.

DOSDAT, A., R. METAILLER, N. TETU, F. SERVAIS, H. CHARTOIS, C. HUELVAN and E. DESBRUYERES, 1995. Nitrogenous excretion in juvenile turbot, *Scophthalmus maximus* (L.), under controlled conditions. *Aquaculture Res.*, **26**: *639*-650.

FROMM, P. O., 1963. Studies on renal and extra-renal excretion in a freshwater teleost, *Salmo gairdneri Comp. Biochem. Physiol.* **10**: 121-128.

HARRIS, S. A., and T. PROBYN, 1996. Nitrogen excretion and absorption efficiencies of white steenbrass, *Lithognathus lithognathus* Cuvier (Sparidae), under experimental culture conditions. *Aquaculture Res.*, **27**: 43-56.

KAUSHIK, S. J., 1980. Influence of nutritional status on the daily patterns of nitrogen excretion in the carp (*Cyprinus carpio* L.) and rainbow trout (*Salmo gairdneri*). *Reprod. Nutr. Develop.*, **20**: 1751-1765.

KAUSHIK, S. J., and C. B. COWEY, 1991. Dietary factors affecting nitrogen excretion by fish. *In* C. B. Cowey and C. Y. Cho (*eds.*), Nutritional Strategies and Aquaculture Waste. Fish Nutrition Research Laboratory, *University of Guelph, Guelph, Ontario*, pp. 3-19.

KAUSHIK, S. J., and K. DABROWSKI, 1983. Postprandial metabolic changes in larval and juvenile carp (*Cyprinus carpio*). *Reprod. Nutr. Develop.*, **23**: 223-234.

KIKUCHI, K., S. TAKEDA, H. HONDA, and M. KIYONO, 1991. Effect of feeding on nitrogen excretion of Japanese flounder *Paralichthys olivaceus*. *Nippon Suisan Gakkaishi*, **57**: 2059-2064.

MING, F. W., 1985. Ammonia excretion rates as an index for comparing efficiency of dietary protein utilization among rainbow trout (*Salmo gairdneri*) of different strains. *Aquaculture*, **46**: 27-35.

OGINO, T., J. KAKINO, and M-S. CHEN, 1973. Protein nutrition in fish- II . Determination of metabolic fecal nitrogen and endogenous nitrogen excretion of carp. *Nippon Suisan Gakkaishi*, **39**: 519-523.

RYCHLY, J. and B. MARINA. 1977. The ammonia excretion of trout during a 24-hour period. *Aquaculture*, **11**: 173-178.

SHIMENO, S., H. HOSOKAWA, H. MORIE, M. TAKEDA, and M. UKAWA. 1992. Inclusion of defated soybean meal to diet for young yellowtail. *Suisanzoshoku*, **40**: 51-56. (in Japanese).

SMITH, H. W., 1929. The excretion of ammonia and urea by the gills of fish. *J. Biol. Chem.*, **81**: 727-742.

SMITH, R. R., 1971. A method for determining the metabolizable energy for fish feeds. *Prog. Fish-Cult*, **33**: 132-136.

STRICKLAND, J. D. H. and T. R. PARSONS, T. R. 1972. A practical handbook of seawater analysis. *Fish. Res. Bd. Canada.*, **167**: 87-93.

TAKEDA, M., 1981. Application of feeding stimulants for capture and culture fish. *In* Japanese Society of Scientific Fisheries (*Ed*), Chemical Sense of Fish and Feeding Stimulants. Koseisya-Koseikaku, Tokyo, pp. 109-118. (in Japanese).

WOOD, J. D., 1958. Nitrogen excretion in some marine teleosts. *Can. J. Biochem. Physiol.*, **36**: 1237-1242.


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