ヒラメの窒素排泄に与える摂餌の影響

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Effect of Feeding on Nitrogen Excretion of Japanese Flounder *Paralichthys olivaceus*

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Measurements of the rate of nitrogen excretion were carried out with fed and starved Japanese flounder of 163 to 575 g in body weight at 20°C. Ammonia-N and urea-N excretion of the starved fish were 0.20±0.05 mg-N/100 g/h and 0.04±0.02 mg-N/100 g/h, respectively. Immediately after feeding, the rate of ammonia-N excretion per hour of the flounder became 3 to 4 times that of the starved fish. These elevated rates continued for 12, 30 and more than 36 h, at 0.5, 1.0 and 1.5% rations, respectively. In contrast to ammonia-N excretion, the rate of urea-N excretion per hour of the fed fish showed a major peak during the period of 6–12 or 12–24 h after feeding.

In Japanese flounder fed on the pelleted diet containing 7.5% of nitrogen at average ration of 0.5%, it is estimated that 32% of the consumed nitrogen was excreted as ammonia-N, about 5% as urea-N and 8% as faeces-N within 24 h after feeding.

In fish culture with water recirculating system, the estimation of nitrogen excretion rate of fish is one of the most important factors governing the stocking fish density, because nitrogenous compounds are thought to be major excretory products harmful to fish. Many studies[1-13] have been conducted relating to the effects of temperature, body weight and feeding on the rates of nitrogen excretion or their daily patterns with various kinds of fish. For flatfish however, there are a few reports[14-17] on the nitrogen excretion.

Jobling,[16] studying on plaice *Pleuronectes platessa*, showed that the rate of nitrogen excretion was dependent upon both temperature and the amount of digestible nitrogen administered. Kikuchi *et al.*[17] reported the changes with growth in the rates of ammonia and urea excretion of starved Japanese flounder *Paralichthys olivaceus*.

In this study, effects of ration level on nitrogen excretion, especially ammonia, urea and faeces nitrogen, were measured with Japanese flounder under experimentally controlled conditions. Daily patterns of ammonia and urea excretion were also examined with fed and starved flounder.

Materials and Methods

Fish

In April 1988, juvenile flounder ranging from 1 to 5 g in body weight were transported from Hamaoka Onsui-Riyo Kenkyu Center, Shizuoka Pref., to our laboratory in Chiba Pref. The fish were reared in 1000 or 2000 *l* volume seawater recirculating aquaria for more than a year with a commercial diet for Japanese flounder (Higashimaru Foods Inc.), at 20°C under natural light conditions until the start of experiments.

The experiments were conducted between October 1989 and January 1990, using 40 fish ranging from 163 to 575 g in body weight (mean body weight; 400 g) under the condition of 12 h light and 12 h dark (fluorescent light, about 1000 *lx* at the water surface). Water temperature was kept at 20±1°C. As previously reported,[17] it is considered that the difference of body weight in this study does not affect the nitrogen excretion rates per unit weight of the flounder.

Starving Experiment

Each experimental fish, which had been starved for 72 h, was placed in a glass chamber of 20 *l* in capacity with a lid. The chamber contained 5 *l* of well aerated seawater. At 3, 6, 12 and 24 h after the commencement of experiment, 50 m*l* of seawater were sampled from the chamber, and duplicate measurements were carried out on ammonia and urea concentrations. At the end of 24 h period, another sample of 200 m*l* was analyzed for total dissolved nitrogen. The num-

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ber of fish used in the experiment was 17.

Feeding Experiment

Following the starving period of 72 h, fish were fed on a commercial diet for Japanese flounder (Higashimaru Foods Inc., P-6), at average rations of 0.5, 1.0 and 1.5% of their body weight. The diet contained 47% of crude protein (7.5% of nitrogen), 17% of crude fat and 18% of carbohydrate. Water content of the diet was 4 to 5% and the weight of the fish was measured as live weight. The numbers of fish used were 15 in the 0.5%, 13 in the 1.0% and 11 in the 1.5% ration level.

Each fish, immediately after feeding, was transferred to the glass chamber containing 10 or 15 *l* of well aerated seawater. Water volume varied dependent on body weight of fish and/or ration level. As in the starving experiments, water samples of 50 m*l* were taken from the chamber at 3, 6, 12 and 24 h after feeding, and then ammonia and urea concentrations were analyzed. At the end of 24 h period, the fish was transferred to another chamber containing the same volume of seawater, from which samplings for analysis of ammonia and urea concentrations were made at 30, 36 and 48 h after feeding.

At the end of 0–24 and 24–48 h periods, the whole volume of seawater of the chamber was filtered through Whatman GF/A filter in order to collect faeces, after the fish were removed from the chamber. The residue on the filter was subjected to analysis for faeces nitrogen.

Analysis

Ammonia and urea concentrations were determined by indophenol method[18] and diacethyl monoxime method,[18] respectively. Total dissolved nitrogen and faeces nitrogen were determined by Kjeldahl method with BÜCHI 425 digester and BÜCHI 321 distillation unit.

Results and Discussion

Nitrogen Excretion by Starved Fish

The rates of ammonia-N and urea-N excretion by starved fish are shown in Fig. 1. Ammonia-N excretion showed no significant differences during the periods of 0–3, 3–6 and 12–24 h, although the rate during the 6–12 h period was lower than the others (Mann-Whitney test, $P<0.05$). In urea-N excretion, the average rate during the 0–3 h period was about 4 times higher than the others.

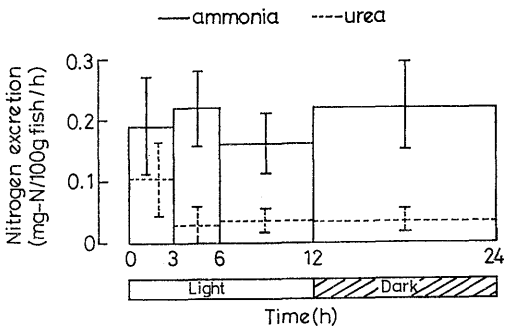

Fig. 1. Rates of ammonia-N and urea-N excretion of Japanese flounder after 72 h starvation.
Body weight: 163–575 g
The number of fish used was 17.
Photoperiod: 0–12 h; ligh, 12–24 h; dark.
Data represent means and standard deviations.

Urea-N excretion rates during the periods of 3–6, 6–12 and 12–24 h were in the same range. Photoperiod did not seem to affect the rates of ammonia-N and urea-N excretion. Ammonia-N and urea-N excretion in the 24 h experimental period were 0.20 ± 0.05 mg-N/100 g/h (4.83 ± 1.26 mg-N/100 g/day) and 0.04 ± 0.02 mg-N/100 g/h (0.99 ± 0.39 mg/100 g/day), respectively. No faeces were obtained. The rate of total dissolved nitrogen excretion in the 24 h period was 6.5 ± 2.8 mg-N/100 g/day. Therefore, ammonia-N and urea-N accounted for 74% and 15% of the total dissolved nitrogen excretion, respectively.

Diurnal Pattern of Ammonia Excretion by Fed Fish

The ammonia-N excretion by fed fish at three ration levels are shown in Fig. 2, together with the average rate of ammonia-N excretion of the starved fish above mentioned. Immediately after feeding, the rate of ammonia-N excretion of the flounder were 3 to 4 times that of the starved fish. The duration of these elevated rates continued for 12 to more than 36 h, depending on the ration level. The ammonia excretion rates of 0.5 and 1.0% rations dropped to the level of starved fish after the periods of 12–24 and 30–36 h, respectively, although the rate during the 24–30 h period of 0.5% ration was slightly higher ($P<0.05$). At 1.5% ration, however, the rate was still higher than the starved level even at the 36–48 h period ($P<0.05$). At 0.5% ration, the rate of ammonia-N excretion showed a single peak during the period of 3–6 h. At 1.0 and 1.5% rations, the highest rates, about 5 times the starved level, were observed during the 3–6 and

6–12 h periods, and these rates did not differ significantly between the ration levels.

The increase of the rate of ammonia-N excretion after feeding (exogenous excretion) is the resultant energy loss associated with the assimilation and deamination of dietary protein.[16] Effects of the ration level on the daily pattern of ammonia-N excretion were studied by Jobling[16] with plaice *Pleuronectes platessa*, and by Ramnarine *et al.*[12] with Atlantic cod *Gadus morhua*. In accordance with our results, they reported that the duration of elevated rate of ammonia-N excretion extended with inceasing ration level. With respect to the maximum rate of excretion, Jobling[16] suggested that the relationship between the maximum rate and the ration level seemed to be curvilinear, because the maximum rate showed no apparent increase at higher ration levels. Ramnarine *et al.*[12] also suggested existence of a maximum capability in the physiological mechanisms governing processes of protein assimilation and nitrogen excretion, and that the relationship between the ration level and its effect on the nitrogen excretion should be curvilinear.

In this study, the rate of ammonia-N excretion showed no significant increase between 1.0 and 1.5% ration levels. As was suggested in the above mentioned, it is considered that an upper limit exists in the rate of ammonia-N excretion of the Japanese flounder.

The maximum rate of ammonia-N excretion obtained in this study was about 5 times the rate

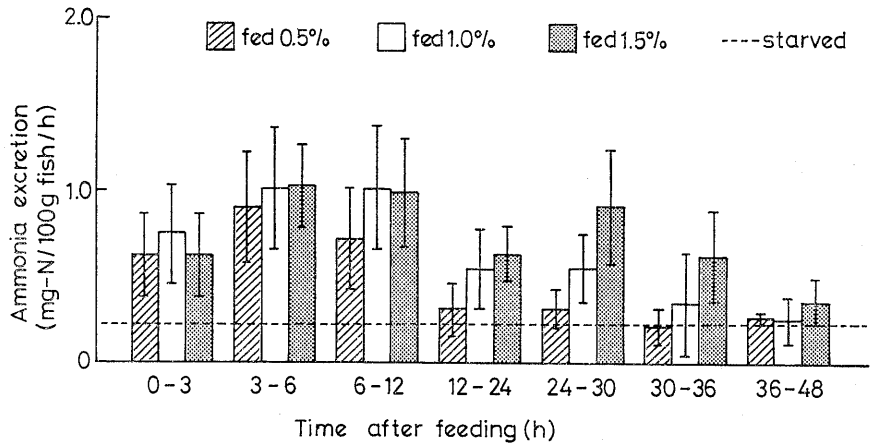

Fig. 2. Diurnal changes in rates of ammonia-N excretion of Japanese flounder fed average rations of 0.5, 1.0 and 1.5% of body weight.
Body weight: 189–575 g.
The numbers of fish used were 15 in the 0.5%, 13 in the 1.0% and 11 in the 1.5% ration level.
Photoperiod: 0–12 and 24–36 h; light, 12–24 and 36–48 h; dark.
Data represent means and standard deviations.

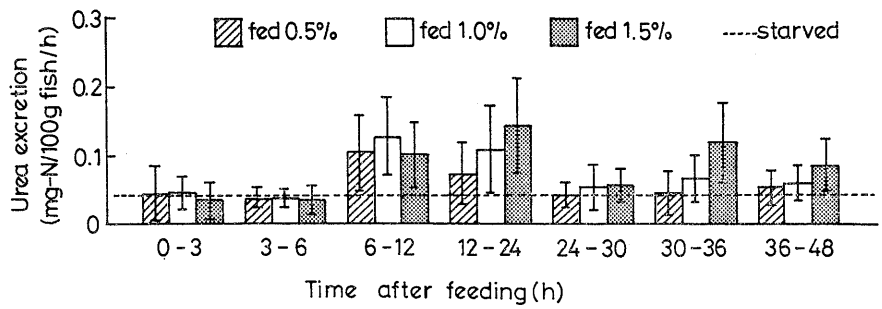

Fig. 3. Diurnal changes in rates of urea-N excretion of Japanese flounder fed average rations of 0.5, 1.0 and 1.5% of body weight.
Data represent means and standard deviations. Details are shown in Fig. 2.

of starved fish. This rise of 5 times is in agreement with the findings of Brett and Zala,[2] and Rychly and Marina,[3] but considerably lower than that of Jobling.[16]

Diurnal Pattern of Urea Excretion by Fed Fish

The urea-N excretion by fed fish at the three ration levels are shown in Fig. 3, together with the average rate of the starved fish. In contrast to the ammonia-N excretion, the rates of urea-N excretion during the initial 0–6 h period were almost constant at any ration level, and were not significantly different from the rate of starved fish. Urea-N excretion showed a peak during the period of 6–12 or 12–24 h after feeding, and it became 2 to 4 times the rate of starved fish. At rations of 0.5 and 1.0%, uera-N excretion rate did not show any considerable increase during the 24–48 h period after feeding. However, at 1.5% ration, another peak, about 3 times the rate of starved fish, was observed during the 30–36 h period.

Brett and Zala[2] studied the diurnal pattern of the urea-N excretion rate of Sockeye salmon *Oncorhynchus nerka*, and reported that the rate of urea-N excretion per hour by fed fish showed little fluctuation and was almost equal to the rate of starved fish. Tátrai[2] also studied the diurnal pattern of urea-N excretion for bream *Abramis brama*, and described that the urea-N excretion of feeding bream significantly surpassed (on average by 3–5 times) that of starved ones, but any fluctuations in the rate were not observed.

The result in this study was different from these two findings in that the rate of urea-N excretion showed fluctuations in Japanese flounder. It may well be that these differences would originate from the fish species, but there are no other reports to enable comparison.

Nitrogen Budget

Daily rates of ammonia-N, urea-N and faeces-N excretion at rations of 0.5, 1.0 and 1.5% of body weight are summarized in Table 1. As for ammonia-N and urea-N excretion, daily rates during the period of 24–48 h were significantly lower than those during the 0–24 h period ($P<0.05$), with the exception of urea-N at 1.5% ration level. During the period of 0–24 h, the rates of ammonia-N and urea-N excretion of 1.0% ration were higher than those of 0.5% ($P<0.05$), although there were not significant differences between 1.0% and 1.5% ration levels. During the 24–48 h period, however, both rates of 1.5% ration were significantly higher than those of 0.5 and 1.0% rations ($P<0.05$).

At 0.5% ration, the daily rate of urea-N excretion during the period of 24–48 h was not different from that of starved fish, while ammonia-N excretion rate was still higher ($P<0.05$). However, at 1.0 and 1.5% rations, ammonia-N and urea-N excretion rates during the 24–48 h periods were considerably higher than those of starved fish.

Average rates of faeces-N excretion during the 0–24 h period were 3.1, 3.1 and 3.4 mg-N/100 g/day for three ration levels, and no significant difference were observed among them. At 0.5% ration, daily rates of faeces-N excretion during the period of 24–48 h were not different from that of the 0–24 h period. However, at 1.0 and 1.5% rations, daily faeces-N excretion of the 24–48 h period were much higher than those during the 0–24 h period ($P<0.05$).

Proportions of the daily rates of ammonia-N, urea-N and faeces-N excretion to consumed

Table 1. Nitrogen excretion of Japanese flounder fed rations of 0.5, 1.0 and 1.5% of body weight

Ration level (%)	Consumed nitrogen (mg-N/100 g fish)	Time after feeding (h)	Nitrogen excretion (mg-N/100 g fish/day)			
			Ammonia	Urea	Faeces	Sum*
0.5±0.2	40.4±11.2	0→24	12.9±4.3	1.8±0.8	3.1±1.6	17.8±5.7
		24→48	6.5±1.7	1.2±0.5	3.2±1.7	10.9±3.0
1.0±0.2	72.3±14.4	0→24	18.0±4.4	2.3±1.0	3.1±2.2	23.4±4.5
		24→48	8.7±3.4	1.5±0.4	6.2±2.1	16.4±4.5
1.5±0.2	114.9±20.4	0→24	19.0±4.9	2.5±1.1	3.4±1.7	24.9±6.9
		24→48	13.6±4.8	2.1±0.8	5.5±2.7	21.2±5.5

* Sum of the daily rates of ammonia-N, urea-N and faeces-N excretion.
Body weight: 189–575 g. The numbers of fish used were 15 in the 0.5%, 13 in the 1.0% and 11 in the 1.5% ration level.
Data represent means and standard deviations.

Table 2. Proportions of excreted ammonia-N, urea-N and faeces-N to consumed nitrogen of Japanese flounder fed rations of 0.5, 1.0 and 1.5% of body weight

Ration level (%)	Consumed nitrogen (%)	Time after feeding (h)	Nitrogen excretion (%)			
			Ammonia	Urea	Faeces	Sum*
0.5±0.2	100	0→24	31.9	4.5	7.7	44.1
			(72.5)	(10.1)	(17.4)	(100)
		24→48	16.1	3.0	7.9	27.0
			(59.6)	(11.0)	(29.4)	(100)
1.0±0.2	100	0→24	24.9	3.2	4.3	32.4
			(76.9)	(9.8)	(13.3)	(100)
		24→48	12.0	2.1	8.6	22.7
			(53.0)	(9.2)	(37.8)	(100)
1.5±0.2	100	0→24	16.5	2.2	3.0	21.7
			(76.3)	(10.0)	(13.7)	(100)
		24→48	11.8	1.8	4.8	18.5
			(64.2)	(9.9)	(25.9)	(100)

* Sum of the daily rates of ammonia-N, urea-N and faeces-N excretion.
Figures in parentheses show proportions to the sum.
Details are shown in Table 1.

nitrogen and to sum of the daily rates of ammonia-N, urea-N and faeces-N excretion are shown in Table 2. Proportions of the daily rates of each substance to consumed nitrogen tended to decrease with increasing ration level. But the ration level did not affect relative importance of each substance in the sum. In the case of urea-N, proportions of daily excretion rates to sum of the excreted nitrogen were almost constant with the range of 9 to 11%. Those of ammonia-N during the initial 24 h period were in the range of 73 to 77%, and decreased to 53 to 64% in the subsequent 24 h period. In contrast to ammonia-N, faeces-N of the 0–24 h period were in the range of 13 to 17%, and increased to 26 to 38% during the 24–48 h period.

There is little information on the daily feeding rate of immature and mature Japanese flounder. Morizane and Takimoto[19] reported that the feeding rate per day (dry/wet) of the flounder of 120 to 130 g in body weight ranged from 0.45 to 0.93% (average; 0.62%), depending on the species of dietary fish. From the results of rearing experiments with the pelleted diet at 20°C, the daily feeding rate of the flounder of 550 g in mean body weight was 0.47%.* Therefore, average rations of 1.0 and 1.5% in this study are about 2 or 3 times higher than the rates in rearing experiments.

In this study, there were no significant differences in the daily rates of ammonia-N, urea-N and faeces-N excretion during the initial 24 h after feeding between 1.0 and 1.5% rations (Table 1). This result was similar to that in the maximum rate of diurnal pattern of ammonia-N excretion, therefore, it is considered that an upper limit in the nitrogen excretion rate exists on the daily basis as well as in the hourly basis.

With regard to the faeces-N excretion, at 0.5% ration, considerable amount of faeces-N excretion was observed during the 24–48 h period, while higher rate of ammonia-N excretion decreased to the starved level during the 12–24 h period after feeding. At 1.0% ration, that of ammonia-N decreased to the starved level during the 30–36 h period, faeces-N excretion during the 24–48 h period was about twice as much as that of the 0–24 h period. From these results, at 1.5% ration, in which higher rate of ammonia-N excretion continued more than 36 h after feeding, a large quantity of faeces-N would also be excreted after 48 h from feeding.

As shown in Fig. 2, higher rates of ammonia-N excretion, which indicate energy loss associated with the assimilation and deamination of dietary protein,[16] almost finished during the 12–24 h period for 0.5% ration, and during the 30–36 h period for 1.0% ration. Therefore, it is considered that the effects of feeding on the physiological mechanisms governing the nitrogen excretion nearly finished within a day for 0.5% ration, and within two days for 1.0% ration.

* H. Honda: unpublished data.

However, at 1.5% ration, the effects might continue more than 48 h after feeding, because of the higher rates of ammonia-N excretion during the 36–48 h period.

At 0.5% ration, about 32, 5 and 8% of the consumed nitrogen were excreted during the initial 24 h after feeding as ammonia-N, urea-N and faeces-N, respectively (Table 2). These values are similar to those for 1.0% ration with the exception of faeces-N, if the corresponding values for the initial and subsequent 24 h are summed for 1.0% ration. About 37% of the consumed nitrogen was excreted as ammonia-N, 5% as urea-N and 13% as faeces-N. It is estimated that Japanese flounder, fed on the diet containing 7.5% of nitrogen at 0.5 and 1.0% of their body weight, excrete about a half of the consumed nitrogen within 24 h for 0.5% ration, and within 48 h for 1.0% ration.

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