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Research Article

Using Ammonia Nitrogen Excretion Rates as an Index for Evaluating Protein Quality of Prawns in Turbot (*Psetta maeotica*) Nutrition

Murat YİĞİT*, Sebahattin ERGÜN
Department of Aquaculture, Faculty of Fisheries, Çanakkale Onsekiz Mart University, 17100 Çanakkale - TURKEY
Ali TÜRKER, Burcu KARAALİ
Department of Aquaculture, Faculty of Fisheries, Ondokuz Mayıs University, 57000 Sinop - TURKEY
Sabri BİLGİN
Department of Fisheries Technology, Faculty of Fisheries, Ondokuz Mayıs University, 57000 Sinop - TURKEY

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Abstract: Total ammonia nitrogen excretion rates were measured in Black Sea turbot (averaging 88.8 g) to compare the protein quality of prawns, the natural food of turbot, with that of the protein source commonly used in the feed industry, i.e. anchovy meal. Two different prawn species (Baltic prawn, *Palaemon adspersus*, and rockpool prawn, *Palaemon elegans*) were offered to fish as wet feed at 17.5 ± 0.5 ºC and a salinity of 17 ppt. Furthermore, for determination of the effects of wet feed and dry feed on the peak times of ammonia nitrogen excretion rates, another experimental group of fish was offered a commercial dry diet with anchovy meal as a single protein source. The ammonia nitrogen excretion rate in both groups fed prawns peaked 3 h after feeding, while the peak of the excretion rate of fish fed the dry diet was delayed up to 6 h after feeding. Cumulative ammonia nitrogen excretion rates as well as the excretion as a proportion of ingested nitrogen were significantly lower ($P < 0.05$) in fish fed prawns than those in fish fed the dry diet. Significantly lower excretion levels in the prawn groups might be a reflection of the protein quality of these species, which may be higher than that of the anchovy meal for turbot nutrition.

Key Words: Black Sea turbot, Baltic prawn, rockpool prawn, protein quality, ammonia nitrogen excretion

Kalkan Balığı (*Psetta maeotica*) Beslenmesinde Karideslerin Protein Kalitesinin Belirlenmesi Amacıyla Amonyak Nitrojen Boşaltım Oranlarının Indeks Olarak Kullanımı

Özet: Kalkan balığının doğal besinini oluşturan karideslerin protein kalitesi ile yem sektöründe yaygın kullanılan hamsi ununun protein kalitesinin karşılaştırılması amacıyla, Karadeniz kalkan balıklarında (ortalama 88,8 g) total amonyak nitrojen boşaltım oranları belirlenmiştir. Balıklar, 17,5 ± 0,5 ºC ve ‰ 17 tuzluluk ortamında iki farklı karides türü (Baltık karidesi, *Palaemon adspersus* ve rockpool karides, *Palaemon elegans*) ile yemlenmiştir. Ayrıca taze yem ile kuru yemlerin amonyak nitrojen boşaltım oranlarının maksimuma ulaşma süreleri üzerine etkilerinin de belirlenmesi amacıyla, diğer bir deneme grubuna, protein kaynağı olarak hamsi unu içeren kuru yem verilmiştir. Her iki karides grubundaki amonyak nitrojen boşaltım oranları, yemlemeden üç saat sonra en üst düzeye ulaşırken, kuru yemle beslenen balıklardaki boşaltım oranının maksimuma ulaşma süresi altı saate kadar uzamıştır. Kümülatif amonyak nitrojen boşaltım oranları ve boşaltım miktarlarının tüketilen nitrojene oranı, karides ile beslenen balıklarda kuru yemle beslenen gruptaki oranlardan istatistiksel olarak daha düşük ($P < 0,05$) kaydedilmiştir. Karides gruplarındaki boşaltım oranlarının önemli derecede düşük olması ile, kalkan beslenmesinde bu türlerin protein kalitesinin hamsi ununa oranla daha yüksek olduğu sonucuna varılabilir.

Anahtar Sözcükler: Karadeniz kalkan balığı, Baltık karidesi, rockpool karidesi, protein kalitesi, amonyak nitrojen boşaltımı

Introduction

An experimental period of 2-3 months is usually necessary for growth studies to determine the protein and amino acid requirements of fish. These long-term growth studies for the optimization of the feed rations of alternative fish species for aquaculture might be considered a waste of time. Obviously, reliable data on weight gain and feed efficiency may not be gathered in a

* E-mail: muratyigit@comu.edu.tr

short time-frame, but metabolite indices such as plasma urea nitrogen (PUN) have been used as criteria in the determination of the dietary requirement for protein and amino acids (1). Brown and Cline (2) have reported in pigs and Taylor et al. (3) in humans that PUN concentrations increase with increasing protein intake, but decrease with improving protein quality. A direct relation between protein intake and ammonia excretion has been reported in fish (4). A close relation between protein quality and ammonia excretion in fish diets was reported by Robaina et al. (5) and Médale et al. (6); more ammonia was excreted, as with PUN in mammals, following consumption of feeds with low quality protein. Ammonia excretion rate was suggested as an index for evaluating dietary protein utilization and the quality of protein in rainbow trout (7) and in Black Sea turbot (8).

The turbot has often been divided into 2 subspecies, *Psetta maxima maxima* and *Psetta maxima maeotica*, the latter having been referred to as the Black Sea representative and an endemic subspecies (9). The Black Sea turbot (*Psetta maeotica*, also called *Scophthalmus maeoticus* P.) is a fairly new candidate for aquaculture in Turkey (8). Wild stocks of Black Sea turbot prey mainly on whiting, goby, anchovy, striped mullet, green crab and prawns such as the brown shrimp, Baltic prawn and rockpool prawn (Dr. S. Ateş and Dr. N. Samsun, Çanakkale Onsekiz Mart Univ. and Ondokuz Mayıs Univ., Turkey, respectively, personal communications). The protein sources whiting, goby and anchovy were previously studied in our laboratory (8). The present study was undertaken to evaluate the protein sources of Baltic and rockpool prawn for Black Sea turbot nutrition by using ammonia nitrogen excretion rates as an index and compare with a dry diet containing anchovy meal as the protein source, in order to see which best meets the nutritional requirements of this species.

Materials and Methods

Experimental fish were obtained from the Japan International Cooperation Agency (JICA) and the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey, and transported to our facilities at the University of Ondokuz Mayıs, Faculty of Fisheries in Sinop, Turkey, in October 2002. The fish were randomly distributed into 6 identical 50 l rectangular polypropylene tanks (filled with 45 l of water) and maintained for 2 months prior to the experiment. Each group of fish consisted of 14 individuals (7 fish per tank with 2 replicate tanks per treatment). In a flow-through system, seawater (salinity 17 ppt) was supplied to the tanks at a flow rate of 750 ml/min. Water temperature was maintained at 17.5 ± 0.5 °C during the course of the experiment. Each tank was supplied with an air-stone. Fish were fed a control diet containing 45% crude protein, 23% crude lipid, 21% NFE (gross energy of 23.5 kJ/g diet) at satiation level once a day during acclimation to the experimental site.

The prawns (Baltic prawn, *Palaemon adspersus* (Rathke, 1837), and rockpool prawn, *Palaemon elegans* (Rathke, 1843)) chosen as the feed for turbot were frozen 2 h after being collected by small hand net from a shallow shelf in Sinop bay, Turkey, and kept at a temperature of –20 °C until being used. Frozen prawns were thawed for 1 h before feeding.

Protein, lipid, ash and moisture content of the feeds were determined by following the Association of Official Analytical Chemists methods described by Williams (10), and all analyses were performed in triplicate. Nitrogen-free extract (NFE) was determined by the difference [NFE = 100 - (% protein + % fat + % ash)]. The nutritional compositions of the feeds are shown in Table 1. Gross energy (GE) of the diets was estimated using energy values of 23.6 kJ/g for protein, 39.5 kJ/g for lipid and 17.2 kJ/g for NFE.

Prior to the experiment, the experimental groups were fed once daily, at 09.00 h, with an extruded dry diet (control treatment, group 1), Baltic prawn (group 2) and rockpool prawn (group 3), and acclimated to the experimental feeds for 14 days. During this period, the size of the maximum ad libitum ration was determined for each experimental feed group by feeding the fish to satiation. During feeding, particular attention was given to ingestion of the feeds offered. If some remained uneaten, these feed particles were collected, counted and deducted from the amount of feed distributed. Therefore, the amount of feed offered and feed intake were equal. Feeding activity was monitored carefully to ensure the even distribution of the feed offered among all experimental fish in each tank. It was observed that fish in the experimental groups consumed 0.88%, 2.55% and 2.29% of their weight, respectively. On day 14, food was withheld for 3 days and on day 4 at 09.00 h the control diet and the experimental feeds of Baltic prawn and rockpool prawn were given to each group at the levels of 0.88%, 2.55% and 2.29% of their weight, respectively.

Table 1. Nutritional compositions (dry basis %, except for moisture) of the experimental feeds.

	Group 1	Group 2	Group 3
Moisture	8.44 ± 0.12	77.84 ± 0.10	77.66 ± 0.07
Crude lipid	23.36 ± 0.10	4.20 ± 0.05	2.72 ± 0.10
Crude ash	10.30 ± 0.02	24.72 ± 0.24	23.51 ± 0.20
Crude protein	45.28 ± 0.40	63.31 ± 0.44	68.64 ± 0.05
N-free extracts[1]	21.06 ± 0.53	7.77 ± 0.25	5.13 ± 0.35
GE (kJ/g)[2]	23.49 ± 0.04	17.92 ± 0.08	18.14 ± 0.01
P/E (mg/kJ)	19.27 ± 0.13	35.33 ± 0.09	37.83 ± 0.04

Group 1 = extruded dry diet (control treatment), Group 2 = Baltic prawn, Group 3 = rockpool prawn.
[1] Nitrogen-free extracts, calculated from = 100 - (CP + CL + CA)
[2] Gross energy, calculated from nutritive values of 23.6 kJ/g protein, 39.5 kJ/g lipid and 17 kJ/g NFE
P/E = mg protein/kJ energy

After completion of feeding activity (within 15 min), fish in each group were transferred into other, identical tanks, filled with 45 l of well-aerated seawater beforehand and the incoming water flow was stopped. Water samples were taken every hour over 6 h and total ammonia (NH_4^+ and NH_3) concentration was analyzed by the Nessler method with a HANNA C200 portable spectrophotometer (HANNA Instruments, Co., Italy) as described by Yigit et al. (8). At this stage, after completion of sampling (for 6 h), fish in each group were weighed individually (Table 2) in order to prevent any stress prior to the sampling procedure. The ammonia-N excretion rate was calculated by determining the ammonia produced in each vessel after each sampling period using the following formula for a static system (11):

$$A = [(N_2\text{-}N_1) \times V_2] / W / T_{2\text{-}1}$$

where A = ammonia excretion rate (µg total NH_3-N per g wet weight per hour), N_1 = ammonia concentration at time 1 (µg total NH_3-N ml^{-1}), N_2 = ammonia concentration at time 2 (µg total NH_3-N ml^{-1}), V_2 = volume of the medium at time 2 (ml), W = wet weight of the fish (g) and $T_{2\text{-}1}$ = time interval between samplings 1 and 2 (hours).

All results are expressed as mean ± SD. Statistical analyses were conducted using SPSS 10.0 for Windows. One-way ANOVA was used for nitrogen intake (NI), ammonia-N excretion (ammonia-NE) rate and the proportions of ammonia-N excretion to nitrogen intake (NE/NI). Significant ANOVA were followed by a post-hoc multiple comparison test (Duncan's new multiple range test). Differences were considered significant at $P < 0.05$.

Table 2. Average individual weights, total weights and number of fish used in the experiment (data are means ± SD, average individual weights of fish were not significantly different, at 5% level.

Group	Fish weight (g)	Total weight (g)	Number of fish (n)
Group 1	89.43 ± 1.01[a]	626.00 ± 7.07	14
Group 2	88.21 ± 1.52[a]	617.50 ± 10.61	14
Group 3	88.79 ± 1.72[a]	621.50 ± 12.02	14

Group 1 = extruded dry diet (control treatment),
Group 2 = Baltic prawn,
Group 3 = rockpool prawn.

Results

The feeding rates in the experimental groups were 0.88%, 2.55% and 2.29%, respectively; however, the nitrogen intake levels of turbot fed the control diet (group 1), Baltic prawn (group 2) and rockpool prawn (group 3) were similar (58.21, 57.21 and 56.22 mg-N/100 g fish, respectively). There were no significant differences ($P > 0.05$) between the nitrogen intake values obtained in all experimental groups. The ratios of P/E were 19.27, 35.33 and 37.83 mg protein/kJ for the control diet, Baltic prawn and rockpool prawn, respectively. Table 3 shows the ammonia nitrogen excretion (ammonia-NE) for 6 h in relation to the levels of ingested nitrogen. Ammonia-NE rate and the excretion as a proportion of ingested nitrogen (NE/NI) were significantly higher ($P < 0.05$) in group 1 (4.19 mg-

Table 3. Proportions of the rates of ammonia-N excretion to nitrogen intake when fed the experimental feeds (figures in a row with different superscripts are significantly different from each other ($P < 0.05$).

Groups	Feeding Rate (%)	Nitrogen Intake (mg-N/100 g fish)	Ammonia-NE1 (mg-N/100 g fish/6 h)	NE / NI2 (%)
Group 1	0.88 ± 0.10^{b}	58.21 ± 6.40^{a}	4.19 ± 0.23^{b}	7.22 ± 0.39^{b}
Group 2	2.55 ± 0.13^{a}	57.21 ± 2.72^{a}	3.06 ± 0.05^{a}	5.35 ± 0.33^{a}
Group 3	2.29 ± 0.13^{a}	56.22 ± 2.88^{a}	3.17 ± 0.05^{a}	5.65 ± 0.39^{a}

Group 1 = extruded dry diet (control treatment), Group 2 = Baltic prawn, Group 3 = rockpool prawn.
[1] ammonia nitrogen excretion
[2] (ammonia-N excretion for 6 h/nitrogen intake) x 100

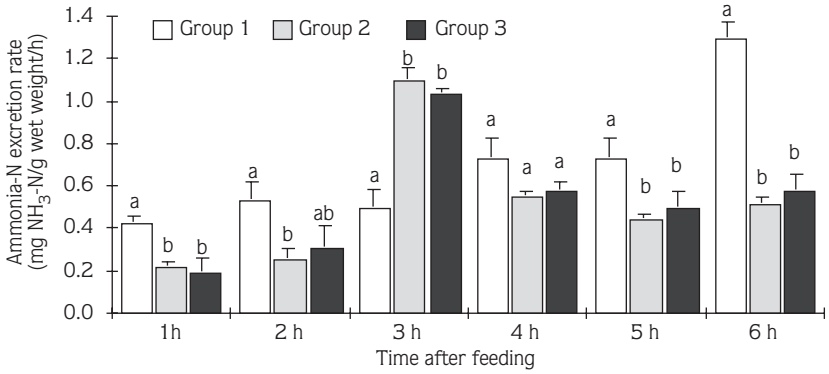

Figure. Diurnal changes in turbot fed the experimental feeds (Group 1 = extruded dry diet (control treatment), Group 2 = Baltic prawn, Group 3 = rockpool prawn). Data are means with SD (n = 2 replicates). Figures in each time interval with different superscripts are significantly different from each other ($P < 0.05$).

N/100 g fish/6 h and 7.22%, respectively) than in groups 2 and 3 (3.06 mg-N/100 g fish/6 h and 5.35%; 3.17 mg-N/100 g fish/6 h and 5.65%, respectively). The rate of ammonia-NE and the NE/NI ratio found in groups 2 and 3 were not significantly different ($P > 0.05$).

Diurnal changes in the rates of ammonia-NE of turbot over 6 h are shown in the Figure and post-prandial peak times of ammonia-NE rates for different fish species in relation to feed types offered are presented in Table 4. Immediately after feeding, the rates of ammonia-NE of fish in group 1 were 2-fold higher ($P < 0.05$) than those of the fish in groups 2 and 3. However, there was no significant difference between fish in groups 2 and 3 ($P > 0.05$) 1 h after feeding. In groups 2 and 3, the excretion rates reached a peak 3 h after feeding, and dropped thereafter to a level double the rates recorded for the first 2 h. These rates continued until the end of the 6 h period. In contrast to groups 2 and 3, the ammonia-NE rates in group 1 during the initial 0-3 h period were almost constant and were not significantly different ($P > 0.05$), and showed an increase at 4-5 h, but reached a peak 6 h after feeding.

Discussion

Total ammonia-NE rates were affected by the protein sources as there were significant differences in the hourly ammonia excretion patterns of fish fed the experimental diets. The hourly pattern of ammonia-NE of fed fish, i.e. rapid increase and gradual decline, was reported in a previous study carried out with young turbot in our laboratory (8). Similar results were also recorded by

Table 4. Post-prandial peak-times of ammonia-N excretion rates for different fish species in relation to feed types (fresh food or dry diets) offered.

Fish species	Feed type	Peak-time (h)	Reference
Black Sea turbot (*S. maeoticus*)	Fresh anchovy	2	(8)
Black Sea turbot (*S. maeoticus*)	Fresh whiting	2	(8)
Black Sea turbot (*S. maeoticus*)	Fresh goby	2	(8)
Sea bass (*L. calcarifer*)	Dry feed	3	(11)
Gilthead seabream (*S. aurata*)	Dry feed	0-3	(15)
Atlantic turbot (*S. maximus*)	Dry feed	6	(16,18)
Atlantic turbot (*S. maximus*)	Dry feed	5-8	(17)
Atlantic turbot (*S. maximus*)	Dry feed	6-8	(19)
Japanese flounder (*P. olivaceus*)	Dry feed	3-6	(20)
Coregonid (*C. shinzi palea*)	Artemia (fresh)	2	(21)
Coregonid (*C. shinzi palea*)	Dry feed	5-8	(21)
Black Sea turbot (*P. maeotica*)	Baltic prawn (fresh)	3	present study
Black Sea turbot (*P. maeotica*)	Rockpl. prawn (fresh)	3	present study
Black Sea turbot (P. *maeotica*)	Dry feed	6	present study

Kikuchi et al. (12,13) and Kikuchi (14) with Japanese flounder, by Almendras (11) with sea bass and by Porter et al. (15) with gilthead sea bream.

In the present study, the ammonia-NE rates in fish fed on prawns reached a peak 3 h after feeding, while the excretion rates in fish fed the control diet reached a peak 6 h after feeding. There are some discrepancies between the reported peak times of ammonia excretion in several studies. Dosdat et al. (16) reported a peak around 6 h post-feeding when fed 100% and 84% of the ad libitum ration in Atlantic turbot. Similarly, Dosdat et al. (17) observed one peak for 100 g Atlantic turbot fed once daily and the maximum post-prandial hourly excretion rate appeared 5-8 h after feeding. Pichavant et al. (18) also reported a peak of hourly ammonia excretion in juvenile Atlantic turbot 6 h after feed intake. Burel et al. (19) noted an increase in ammonia excretion in juvenile Atlantic turbot 4 h after food intake but they reported a maximum value 8 h after feeding at 11 ºC, and 6 h after feeding at 20 ºC. Maximum ammonia excretion rates in Japanese flounder were reported to occur 3-6 h after feeding (20). Almendras (11) found that ammonia excretion rates of the sea bass, *Lates calcarifer*, rose to a significant level over pre-feeding values just half an hour after feeding in both freshwater and seawater, and both groups showed also the same peak of ammonia excretion rates 3 h after feeding. However, in the case of gilthead seabream (*Sparus aurata*), the excretion rate reached a peak immediately after feeding (15). These discrepancies are probably due to different fish species, diet quality or feeding conditions, which may affect the diurnal pattern of ammonia excretion in fed fish. Furthermore, Dabrowski and Kaushik (21) reported an early peak in ammonia excretion in coregonid (*Coregonus schinzi palea* Cuv. et Val.) only 2 h after feeding with *Artemia*, whereas the peak value of fish fed dry diets was delayed up to 5-6 or 8 h after feeding, which is in agreement with the findings of the present study. Yigit et al. (8) observed a peak of hourly ammonia excretion in 74 g Black Sea turbot at 2 h after feeding with fresh anchovy. However, in the present study, the ammonia-NE rates in fish fed the control diet with anchovy meal as the protein source reached a peak 6 h after feeding. This result might be explained by the findings reported by Dabrowski and Kaushik (21) as mentioned above. However, considering the cumulative ammonia excretion rates over the 6 h period and the ratio of ammonia-NE to nitrogen intake (NE/NI) in fish fed the dry diet with anchovy meal, the recorded excretion rate (4.19 mg-N/100 g fish) and the NE/NI ratio (7.22%) in the present study are in agreement with the findings in Yigit et al. (8), where the cumulative ammonia excretion rate was 4.48 mg-N/100 g fish/6 h and the NE/NI ratio was 7.52% in Black Sea turbot after feeding with fresh anchovy.

In the present study, the significantly lower rates of ammonia-NE in fish fed on prawns may indicate that the protein utilization in these groups is much better than in those fed the control diet with anchovy meal as the protein source. Beamish and Thomas (22) and Arzel et al. (23) reported that ammonia excretion as a percentage of ingested nitrogen depended on the composition and quality of the diet. This supports the finding in the present study that the utilization of protein was improved and less protein was wasted as ammonia-NE when fish were fed with prawns.

In the present study, the results of the whole body prawn analyses show P/E ratios of 35.33 and 37.83 mg protein/kJ for Baltic prawn and rockpool prawn, respectively, while a lower P/E ratio of 19.27 mg protein/kJ was recorded for the control diet. The P/E ratios reported for both prawn species, offered as feed to the experimental groups with significantly lower ammonia excretion rates in the present study, fall within the range of the values (30-40 mg protein/kJ) reported by Bromley (24) for the best growth in Atlantic turbot, and are very similar to the value (35 mg protein/kJ) reported by Yigit et al. (8) for a good quality feed in Black Sea turbot nutrition.

From the results obtained in the present study, it can be concluded that both the Baltic, and rockpool prawn are highly suitable protein sources for turbot diets. However, the market price of prawn meal is generally higher than that of the common fish meal; this might restrict the use of prawn meal as a single protein source when formulating diets. Thus, further studies are suggested for investigating the use of diets with several replacement levels of prawn meal with fish meal.

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