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Short Communication

AMMONIA NITROGEN EXCRETION RATE - AN INDEX FOR EVALUATING PROTEIN QUALITY OF THREE FEED FISHES FOR THE BLACK SEA TURBOT

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Abstract

Total ammonia nitrogen excretion rates were measured in young Black Sea turbot (74.79 g avg wt) reared in brackish water (17 ppt salinity) at 11±0.3°C. Duplicate groups of turbot were fed a fixed quantity of teleosts, either anchovy (*Engraulis encrasicolus*), goby (*Gobius sp.*) or whiting (*Merlangius merlangus*). Feeds were offered to the turbot as wet feed. Cumulative ammonia-N excretion over a 6-hour period was significantly lower ($p<0.05$) in fish fed goby (3.64±0.27 mg-N/100 g fish) and whiting (3.83±0.13 mg-N/100 g fish) than that in fish fed anchovy (4.48±0.06 mg-N/100 g fish). The ammonia-N excretion rate in all groups peaked two hours after feeding, but the peak value in the group fed anchovy was significantly higher ($p<0.05$) than in the other two groups. No differences were observed among diets in samples taken 3, 4, 5 and 6 hours after feeding. Significantly lower excretion levels in the goby and whiting groups could be related to the protein quality of these species which may be higher than that of the anchovy for turbot nutrition.

Introduction

The Atlantic turbot *(Scopththalmus maximus* L., also called *Psette maxima* R.) is of great aquacultural interest in Europe (Person-Le Ruyet, 1993) and its production has gradually increased throughout recent years. Due to the high commercial value of this species, its biology and especially its nutritional requirements have been well studied by many workers (Dosdat et al., 1995, 1996; Burel et al., 1996; Person-Le Ruyet et al., 1997; Pichavant et al., 1998, 2000; Chereguini et al., 2001; Person-Le Ruyet et al., 2002). However, little informa-

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tion is available (Moteki et al., 2001; Sahin, 2001) on the Black Sea turbot, kalkan *(Scophthalmus maeoticus* P., also called *Psetta maeotica),* a new candidate for aquaculture in Turkey.

Turbot has often been divided into two subspecies, *Psetta maxima maxima* and *Psetta maxima maeotica*, the latter being referred to as the Black Sea representative and an endemic subspecies (Nielsen, 1986). Both *Psetta maxima* (Rafinesque, 1810) and *Scophthalmus maximus* (Linnaeus, 1758) refer to the same turbot (Person-Le Ruyet et al., 1997, 2002). The Black Sea turbot is known as *Psetta maeotica* or *Scophthalmus maeoticus* (Pallas, 1811; Slastenenko, 1955-1956).

Studies of protein and amino acid requirements of fish are usually conducted for an experimental period of 8-12 weeks. One reason for this long duration is that statistical differences in the commonly measured growth criteria may not become apparent until late in the study. Other responses such as feed intake and feed efficiency are not reliable criteria because of the difficulty in collecting accurate data (Lovell, 1989). In contrast, nutritional studies on livestock commonly use experimental periods of 1-4 weeks. Obviously, reliable data on weight gain, feed intake, and feed efficiency may not be gathered in such a short time frame, but metabolite indices such as plasma urea nitrogen (PUN) have been used as additional criteria to determine the dietary requirements for protein and amino acids (Lewis, 1992). It has been reported in rats (Eggum, 1970), pigs (Brown and Cline, 1974), and humans (Taylor et al., 1974) that PUN concentrations increase as the protein intake increases, but decrease as the protein quality improves. A direct relation between protein intake and ammonia excretion was found in fish (Savitz, 1971; Kaushik, 1980; Kaushik and Cowey, 1991; Ballestrazzi et al., 1994; Médale et al., 1995; Robaina et al., 1999). The ammonia excretion rate was suggested as an index for comparing the efficiency of dietary protein utilization among three strains of rainbow trout (*Oncorhynchus mykiss*; Ming, 1985) and in carp (Eid and Matty, 1989). Ammonia excretion is related to protein quality in fish diets, as was shown by Robaina et al. (1995) and Médale et al. (1998); more ammonia was excreted, as with PUN in mammals, following consumption of feeds with low quality protein.

The less protein that is catabolized, the more nitrogen is accumulated in the fish body, indicating that the dietary protein is used for growth, not as an energy source (Yigit, 2001). Besides the high production costs associated with inadequate diets, water quality may deteriorate due to wasted feed and excretion through gills and kidneys (Yigit et al., 2002).

The objective of the present study was to evaluate three protein sources to see which best meets the nutritional requirements of young Black Sea turbot. Natural stocks of turbot prey mainly on whiting (*Merlangus merlangius*), goby (*Gobius sp.*), striped mullet (*Mullus barbatus*), anchovy (*Engraulis encrasicolus*), crabs (*Carcinus sp.*) and brown shrimp (*Crangon crangon*; Zengin, 2000). Three protein sources were chosen for this study: anchovy, goby and whiting.

Materials and Methods

Forty-eight hatchery-reared young turbot (74.79 g avg wt), obtained from the Japan International Cooperation Agency (JICA) and the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey, were transported to the facilities of the Faculty of Fisheries, University of Ondokuz Mayis in Sinop, Turkey. The fish were randomly distributed into six identical 50-l rectangular polypropylene tanks filled with 45 l water (eight fish per tank with two replicate tanks per treatment) for adaptation to experimental conditions. In a flow-through system, sea water (temperature 11±0.3°C, salinity 17 ppt) was supplied to the tanks at a rate of 0.7 l/min. Each tank was supplied with an air-stone. Fish were exposed to a natural light regime (11light/13dark hours) and fed a commercial diet containing 55% crude protein, 16% crude lipid, 9% NFE, 21 kJ gross energy/g feed and 26.19 mg protein/kJ to satiation once a day for one month.

For the next 12 days (the acclimation period), fish were fed either anchovy, goby or whiting. The feed fish were frozen within two hours after being caught in the fishing grounds of Sinop, Turkey (42º N) and kept at a temperature of -20°C for one day. Following a thawing period of one hour, the heads and caudal fins were removed and the remainder (not gutted) was cut into small identical pieces. These were again frozen and kept at -20°C until use. The frozen pieces of fish were thawed for one hour before feeding. The feed compositions and their chemical and energy contents are shown in Table 1. All groups were fed once a day at 09:00. Since the fish consumed 1.90% of their weight during a 20 min period, the maximum rate for once-a-day feeding, this rate was used during the subsequent ammonia excretion trial. Feeding was monitored carefully to ensure even distribution of the feed among all the fish in the tank.

After the 12 day acclimation period, food was withheld for three days. On the next day (the day of the experiment), anchovy, goby or whiting were given to the treatment groups at a level of 1.90% of their weight at 09:00. Feeding was completed within 20 minutes, then the water in the tanks was replaced with new water and the incoming water flow was stopped. Water samples were taken every hour for six hours and total ammonia (NH_4^+ and NH_3) concentrations were analyzed by the Nessler method with a HANNA C200 portable spectrophotometer (HANNA Instruments, Co., Italy). The ammonia-N excretion rate was calculated by determining the ammonia produced in each tank after each sampling using the following formula for a static system: $A = [(N_2-N_1) \times V_2]/B/T_{2-1}$, where A = ammonia excretion rate (µg total NH_3-N/g wet weight/hour); N_1 = ammonia concentration at time 1 (µg total NH_3-N/ml); N_2 = ammonia concentration at time 2 (µg total NH_3-N/ml); V_2 = volume of the medium at time 2 (ml); B = wet weight of the fish (g) and T_{2-1} = time interval between samplings 1 and 2 (hours).

Results are expressed as means±standard deviations. Statistical analyses were conducted using SPSS 10.0 for Windows. One-way ANOVA was used for nitrogen intake, ammonia-N excretion rate and the ratio of ammonia-N excretion to nitrogen intake. Significant ANOVA findings were followed by a post-hoc multiple comparison test (Duncan's new multiple range test; General Linear Model – Univariate procedure). Differences were considered significant at $p<0.05$. Prior to analysis by the ANOVA and post-hoc multiple comparison tests, data expressed in percent were arcsinus transformed.

Table 1. Proximate composition (% dry basis), gross energy and P:E ratios of the experimental feeds.

	Anchovy	*Goby*	*Whiting*
Dry matter	20.12 ± 0.35	19.51 ± 0.14	17.86 ± 0.0
Crude protein	73.84 ± 0.32	79.11 ± 0.44	75.92 ± 0.52
Crude lipid	10.79 ± 0.20	7.61 ± 0.04	7.27 ± 0.07
Crude ash	11.20 ± 0.41	10.29 ± 1.42	12.01 ± 0.74
NFE[1]	4.17 ± 0.11	2.99 ± 0.94	4.80 ± 1.33
Gross energy (kJ/g)[2]	22.40 ± 0.14	22.19 ± 0.28	21.60 ± 0.08
Protein to energy ratio (mg/kJ)	32.97 ± 0.06	35.66 ± 0.25	35.14 ± 0.37

[1] Nitrogen free extracts, calculated as 100 - (crude protein + crude lipid + crude ash).
[2] Calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid and 17 kJ/g NFE.

Results

The nitrogen intakes of turbot fed anchovy, goby or whiting were 59.61, 63.27 and 55.63 mg N/100 g fish, respectively (Table 2). There were no significant differences ($p>0.05$) between nitrogen intake values. The total ammonia-N excreted was significantly higher in the anchovy group but there was no significant difference in ammonia-N excretion between the goby and whiting groups. The total ammonia-N excreted relative to the ingested nitrogen was significantly higher ($p<0.05$) in turbot fed anchovy (7.52%) than in fish fed goby (5.75%) while neither group differed from the group fed whiting (6.90%).

The ammonia-N excretion patterns for each treatment during the 6-hour trial are shown in Fig.1. The amount of ammonia-N excreted in the first hour was similar in all groups. Two hours after feeding, the excretion rate of fish fed anchovy was 2.5 times higher than the level one hour after feeding, while the goby and whiting excretion rates after two hours were twice as high as the one hour post-feeding levels.

Discussion

As far as we know, this is the first attempt to study the nutritional aspects of the Black Sea turbot in relation to nitrogen excretion rates. In the present study, total ammonia-N excretion rates were affected by the protein source as there were significant differences in the hourly ammonia excretion patterns of the fish fed the experimental feeds.

The increase of ammonia-N excretion after feeding (exogenous excretion) is the resultant energy loss associated with the assimilation and deamination of dietary protein (Jobling, 1981). It was reported that fish size and feed consumption are important factors determining the nitrogen excretion rate in bream, *Abramis brama* (Tatrai, 1986) and Japanese flounder, *Paralichthys olivaceus* (Kikuchi et al., 1990, 1991, 1992). Cai et al. (1996) did not find a linear increase in ammonia excretion with the increasing dietary protein intake in rainbow trout fed 35%, 40% or 45% dietary protein. Nitrogen excretion rates were reported to be directly influenced by nitrogen consumption in bluegills, *Lepomis macrochirus*, fed high protein mealworms (Savitz, 1971), common carp, *Cyprinus carpio,* and rainbow trout, *Oncorhynchus mykiss,* fed 55% protein diets (Kaushik, 1980) and sea bass, *Dicentrarchus labrax,* fed diets with 40%, 44% and 49% protein (Ballestrazzi et al., 1994). It was reported that the excretion profile in turbot is a function of the ingested nitrogen and the higher the level of ingested nitrogen, the more ammonia is excreted (Dosdat et al., 1995).

In the present study, the same feeding level was used in all groups to avoid the effect

Table 2. Nitrogen intake, ammonia-N excretion ratio and ratio of ammonia-N excretion to nitrogen intake in tanks where turbot were fed one of three kinds of fish at 1.90% of their body weight.

Group	*Fish weight (g)*	*Nitrogen intake (mg-N/100gfish)*	*Ammonia-N excretion (mg-N/100gfish/6h)*	*NE/NI* (%)*
Anchovy	74.19±0.27	59.61±2.66	4.48±0.06 [a]	7.52±0.44 [a]
Goby	74.63±0.88	63.27±3.08	3.64±0.27 [b]	5.75±0.14 [b]
Whiting	75.56±0.97	55.63±4.10	3.83±0.13 [b]	6.90±0.74 [ab]

Values within columns sharing the same letter do not differ significantly ($p<0.05$).
* NE/NI = (ammonia-N excretion for 6 hours/nitrogen intake) x 100

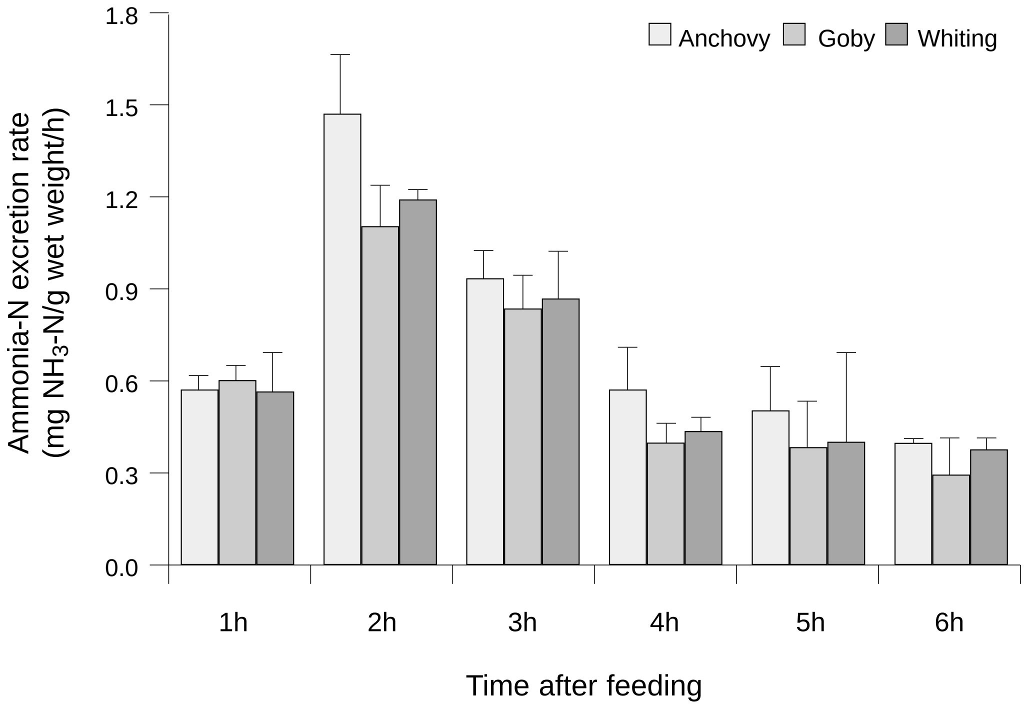

Fig. 1. Hourly ammonia-N excretion rate for six hours after feeding turbot either anchovy, goby or whiting. Data are means with standard deviations (n = 2 replicates).

of feed intake on ammonia-N excretion, and nitrogen intake did not significantly differ among dietary treatments. Despite the same level of nitrogen intake, ammonia excretion levels differed significantly, showing that the ammonia excretion rates might be affected by the source of protein (i.e., protein quality) fed to the fish. The protein utilization improved and less protein was excreted as ammonia-N when the fish were fed goby or whiting. This finding is in agreement with Beamish and Thomas (1984), Eid and Matty (1989), Kaushik and Cowey (1991), Forsberg and Summerfelt (1992), Arzel et al. (1994) and Ballestrazzi et al. (1994), who also investigated the effects of the quality of ingested protein on excretion and reported that the ammonia excretion as a percent of ingested nitrogen depends on the composition and quality of the diet.

In the present study, the maximum excretion rates for all dietary treatments were observed two hours after feeding. There are some discrepancies between reported peak times of ammonia excretion amongst studies. Dosdat et al. (1995) investigated the influence of ration size on total ammonia-N excretion in Atlantic turbot for 24 hours and noticed a peak about six hours after feeding when the fish were fed 100% and 84% of the *ad libitum* ration. Similarly, Dosdat et al. (1996) observed a peak for 100 g Atlantic turbot fed once a day about 5-8 hours after feeding. Pichavant et al. (2000) also reported a peak six hours after feed intake. But Burel et al. (1996) noticed an increase of ammonia excretion in juvenile Atlantic turbot four hours after food intake and a maximum value eight hours after feeding at 11ºC, and six hours after feeding at 20ºC.

Maximum ammonia excretion rates in Japanese flounder were reported to occur 3-6 hours after feeding (Kikuchi et al., 1995), while Yigit (2001) reported maximum ammonia excretion rates in Japanese flounder 0-2 hours after feeding. The cumulative rates of ammonia-N excretion six hours after feeding with goby and whiting in the present study were lower than the excretion rates reported by Kikuchi et al. (1995) and Yigit (2001) for Japanese flounder.

Bromley (1980) reported the best protein to energy (P:E) ratio for growth in Atlantic turbot as 30-40 mg protein/kJ. For Japanese flounder, Kikuchi et al. (2000) and Yigit (2001) reported 28 and 24 mg protein/kJ, respectively. In the present study, the P:E ratios for anchovy, goby and whiting were 32.97, 35.66 and 35.14 mg protein/kJ , respectively. The ratios for the goby and whiting (the treatments with the lower ammonia excretion rates) fell within the range reported by Bromley (1980) as being best for Atlantic turbot. These values are higher than those reported for pelagic fish species in the water column such as arctic charr, *Salvelinus alpinus* (19 mg protein/kJ; Jobling and Wandsvik, 1983), cod, *Gadus morhua* (17 mg protein/kJ; Jobling et al., 1991) and rainbow trout, *Oncorhynchus mykiss* (22 mg protein/kJ; Yigit et al., 2002). It may be that the higher P:E ratios optimize growth in flatfish because of their limited ability to utilize dietary fat.

In conclusion, the rates of ammonia-N excretion per fish weight for six hours after feeding differed considerably with the protein source; the lower excretion levels in the groups fed goby and whiting indicate significantly improved utilization of the ingested protein and show that the protein quality of goby and whiting is much higher than that of anchovy. These findings suggest that using white fishmeal instead of the currently used brown fishmeal in diets for turbot may lead to more effective and less polluting diets.

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References

Arzel J., Martinez-Lopez F.X., Métailler R., Stephan G., Viau M., Gandemer G. and J. Guillaume, 1994. Effect of dietary lipid on growth performance and body composition of brown trout (*Salmo trutta*) reared in seawater. *Aquaculture*, 123:361-375.

Ballestrazzi R., Lanari D., D'Agaro E. and A. Mion, 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax*). *Aquaculture*, 127:197-206.

Beamish F.W. and E. Thomas, 1984. Effects of dietary protein and lipid on nitrogen losses in rainbow trout, *Salmo gairdneri*. *Aquaculture*, 41:359-371.

Bromley P.J., 1980. Effect of dietary protein, lipid and energy content on the growth of turbot (*Scophthalmus maximus* L.). *Aquaculture*, 19:359-369.

Brown J.A. and T.R. Cline, 1974. Urea excretion in the pig: an indicator of protein quality and amino acid requirements. *J. Nutr.*, 104:542-545.

Burel C., Person-Le Ruyet J., Gaumet F., Le Roux A., Severe A. and G. Boeuf, 1996. Effects of temperature on growth and metabolism in juvenile turbot. *J. Fish Biol*., 49:678-692.

Cai Y., Wermerskirchen J. and I.R. Adelman, 1996. Ammonia excretion rate indicates dietary protein adequacy for fish. *Prog. Fish-Cult*., 58:124-127.

Chereguini O., de la Banda I.G., Rasines I. and A. Fernandez, 2001. Larval growth of turbot, *Scophthalmus maximus* (L.) produced with fresh and cryopreserved sperm. *Aquacult. Res.*, 32:133-143.

Dosdat A., Metailler R., Tetu N., Servais F., Chartois H., Huelvan C. and E. Desbruyeres, 1995. Nitrogenous excretion in juvenile turbot, *Scophthalmus maximus* (L.), under controlled conditions. *Aquacult. Res.*, 26:639-650.

Dosdat A., Servais F., Metailler R., Huelvan C. and E. Desbruyeres, 1996. Comparison of nitrogenous losses in five teleost fish species. *Aquaculture*, 141:107-127.

Eggum B.O., 1970. Blood urea measurements as a technique for assessing protein quality. *Brit. J. Nutr.*, 24:983-988.

Eid A. and A.J. Matty, 1989. Ammonia excretion rate as index for protein quality evaluation for carp diets. *Istanbul Univ. J. Aquat. Prod.*, 3:37-44.

Forsberg J.A. and R.C. Summerfelt, 1992. Ammonia excretion by fingerling walleyes fed two formulated diets. *Prog. Fish-Cult.*, 54:45-48.

Jobling M., 1981. Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice *Pleuronectes platessa* L. *J. Fish Biol.*, 18:87-96.

Jobling M. and A. Wandsvik, 1983. Quantitative protein requirements of Arctic charr, *Salvelinus alpinus* (L). *J. Fish Biol.*, 22:705-712.

Jobling M., Knudsen R., Pedersen P.S. and J. Dos Santos, 1991. Effects of dietary composition and energy content on the nutritional energetics of cod, *Gadus morhua*. *Aquaculture*, 92:243-257.

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

Kaushik S.J. and C.B. Cowey, 1991. Ammoniogenesis and dietary factors affecting nitrogen excretion. pp. 3-19. In: C.B. Cowey and C.Y. Cho (eds.). *Nutritional Strategies and Aquaculture Waste.* Univ. Guelf, Ontario, Canada.

Kikuchi K., Takeda S., Honda H. and M. Kiyono, 1990. Oxygen consumption and nitrogenous excretion of starved Japanese flounder *Paralichthys olivaceus*. *Nippon Suisan Gakkaishi*, 56:1891.

Kikuchi K., Takeda S., Honda H. and M. Kiyono, 1991. Effect of feeding on nitrogen excretion of juvenile and young Japanese flounder. *Nippon Suisan Gakkaishi*, 57:2059-2064.

Kikuchi K., Honda H. and M. Kiyono, 1992. Effect of dietary protein level on growth and body composition of Japanese flounder, *Paralichthys olivaceus*. *Suisanzoshoku*, 40: 335-340.

Kikuchi K., Sato T., Iwata N., Sakaguchi I. and Y. Deguchi, 1995. Effects of temperature on nitrogenous excretion of Japanese flounder. *Fish. Sci.*, 61:604-607.

Kikuchi K., Sugita H. and T. Watanabe, 2000. Effect of dietary protein and lipid levels on growth and body composition of Japanese flounder. *Suisanzoshoku*, 48:537-543.

Lewis A.J., 1992. Determination of the amino acid requirements of animals. pp. 67-85. In: S.L. Nissen (eds.). *Modern Methods in Protein Nutrition and Metabolism*. Academic Press, New York.

Lovell R.T., 1989. *Nutrition and Feeding of Fish.* Van Nostrad Reinhold, New York.

Médale F., Brauge C., Vallée F. and S.J. Kaushik, 1995. Effects of dietary protein/energy ratio, ration size, dietary energy source and water temperature on nitrogen excretion in rainbow trout. *Water Sci. Technol.*, 31:185-194.

Médale F., Boujard T., Vallée F., Blanc D., Mambrini M., Roem A. and S.J. Kaushik, 1998. Voluntary feed intake, nitrogen and phosphorus losses in rainbow trout (*Oncorhynchus mykiss*) fed increasing dietary levels of soy protein concentrate. *Aquat. Living Resour.*, 11:239-246.

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

Moteki M., Yoseda K., Sahin T., Ustundag C. and H. Kohno, 2001. Transition from endogenous to exogenous nutritional sources in larval Black Sea turbot *Psetta maxima*. *Fish. Sci.*, 67:571-578.

Nielsen J.G., 1986. Scophthalmidae. pp. 1287-1293. In: P.J.P. Whitehead, M.L. Bauchot, J.C. Hureau, J. Nielsen, E. Tortonese (eds.). *Fishes of the North-Eastern Atlantic and Mediterranean*, Vol. III. UNESCO, Paris.

Person-Le Ruyet J., 1993. L'élevage du turbot en Europe. *La Pisciculture Francaise,* 112:5-22.

Person-Le Ruyet J., Delbard C., Chartois H. and H. Le Delliou, 1997. Toxicity of ammonia to turbot juveniles: 1. effects on survival, growth and food utilization. *Aquat. Living Resour.*, 10:307-314.

Person-Le Ruyet J., Pichavant K., Vacher C., Le Bayon N., Sévère A. and G. Boeuf, 2002. Effects of O_2 supersaturation on metabolism and growth in juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture*, 205:373-383.

Pichavant K., Person-Le Ruyet J., Sévère A., Le Roux A., Quéméner L. and G. Boeuf, 1998. Capacités adaptatives du turbot (*Psetta maxima*) juvénile à la photopériode. *Bull. Fr. Pêche Piscic.*, 350-351:265-277.

Pichavant K., Person-Le Ruyet J., Le Bayon N., Sévère A., Le Roux A., Quéméner L., Maxime V., Nonnotte G. and G. Boeuf, 2000. Effects of hypoxia on growth and metabolism of juvenile turbot. *Aquaculture*, 188:103-114.

Robaina L., Izquierdo M.S., Moyano F.J., Socorro J., Vergara J.M., Montero D. and H. Fernández-Palacios, 1995. Soybean and lupin seed meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional and histological implications. *Aquaculture*, 130:219-233.

Robaina L., Corraze G., Aguirre P., Blanc D., Melcion J.P. and S. Kaushik, 1999. Digestibility, postprandial ammonia excretion and selected plasma metabolites in European sea bass (*Dicentrarchus labrax*) fed pelleted or extruded diets with or without wheat gluten. *Aquaculture*, 179:45-56.

Sahin T., 2001. Larval rearing of the Black Sea turbot, *Scophthalmus maximus* (Linnaeus, 1758), under laboratory conditions. *Turk. J. Zool.*, 25:447-452.

Savitz J., 1971. Nitrogen excretion and protein consumption of the bluegill sunfish (*Lepomis macrochirus*). *J. Fish. Res. Board Can.*, 28:449-451.

Slastenenko E., 1955-6. *Karadeniz Havzasi Balíklari. The Fishes of the Black Sea Basin.* Et ve Balik. Kurumu Umum Müdürlügü Yayinlari, Istanbul.

Tatrai I., 1986. Influence of temperature, rate of feeding and body weight on nitrogen metabolism of bream *Abramis brama* L. *Comp. Biochem. Physiol.*, 83A:543-547.

Taylor Y.S.M., Scrimshaw N.S. and V.R. Young, 1974. The relationship between serum urea levels and dietary utilization in young men. *Brit. J. Nutr.*, 32:407-411.

Yigit M., 2001. p. 72. In: *Effects of Dietary Protein and Energy Levels on Growth, Body Composition, Digestion Efficiency and Nitrogen Excretion of Juvenile Japanese Flounder, Paralichthys Olivaceus.* Ph.D. thesis. Ondokuz Mayis Univ., Inst. Sci., Samsun, Turkey (in Turkish).

Yigit M., Yardim Ö. and S. Koshio, 2002. The protein sparing effects of high lipid levels in diets for rainbow trout (*Oncorhynchus mykiss*, W. 1792) with special reference to reduction of total nitrogen excretion. *Israeli J. Aquacult.-Bamidgeh,* 54:79-88.

Zengin M., 2000. *The Bioecology and Population Parameters of the Turbot (Scophthalmus Maeoticus, Pallas, 1811) in the Turkish coast of the eastern Black Sea.* Ph.D. thesis. Technical Univ. Karadeniz, Inst. Sci., Trabzon, Turkey (in Turkish).