Effects of Feeding Stimulants, and Diet pH on the Growth of Black Sea Turbot, *Psetta maxima*

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Abstract

Feeding and growth studies were conducted to define the basal nutritional requirements of juvenile Black Sea turbot (formerly *Scophthalmus maeoticus* or *Psetta maeotica*). First, diets with different feeding stimulants were tested on *P. maxima*. Inosine, alanine, and glutamic acid were effective feeding stimulants. A combination of 0.9% L-glutamic acid and 0.1% inosine had the same effect as 0.27% inosine. Second, it was found that the nutritional value of diets was significantly affected by the pH of the diet; the optimum for turbot was pH 7.1-7.5.

Key Words: fish nutrition.

Introduction

The 'Kalkan' or Black Sea turbot *Psetta maxima* (formerly called *Scophthalmus maeoticus* or *Psetta maeotica*) is a major target species of fisheries in the Black Sea (Moteki *et al*., 2001; Kohno *et al*., 2001, Suzuki *et al*., 2001). A decrease in the annual catch in the Black Sea has led to efforts to manage the species properly (Turkish fisheries statistics, 1967-1998). Interest in Kalkan aquaculture has developed in recent years, but the technology and basic information are still insufficient to establish commercial-scale farming of Kalkan in Turkey (the Black Sea area). Thus the Fish Culture Development Project in the Black Sea was started between the Japan International Cooperation Agency and the Central Fisheries Research Institute in Trabzon, Turkey. Moreover, farm production of the turbot is being intensified to diversify the market, which has recently been oversupplied with sea bass *Dicentrarchus labrax* and gilthead sea bream *Sparus aurata.*

Remarkable differences in habitat and morphology between the turbot *Psetta maxima* in the Atlantic and that in the Black Sea make it likely that the nutritional requirements vary between the two stocks. The nutritional requirements of the Atlantic stock have been well documented (Cowey *et al*., 1975; Day and González, 2000 and others). However, there has been no nutritional study for the Black Sea stock. The present paper defines the basal nutritional requirements of juvenile Kalkan, and summarizes studies on feed development conducted by the Fish Culture Development Project since 2000.

Feed Stimulants

Two feeding trials were carried out to test diets with different feed stimulants for juvenile Kalkan. The test diets were prepared as follows. Casein and other powdered ingredients were mixed. Fish oil was added to the mixture, then 1.3% sodium peroxide solution. The pH of the test diets ranged from 6.6 to 6.9 (Horiba pH meter D-22). The resulting dough was kept in the freezer for an hour, and then passed through a meat chopper twice to obtain pellets 3.5 mm in diameter. The moisture pellets were packed in plastic bags with oxygen absorber (Ageless, Mitsubishi Kasei Co., Ltd., Japan) and stored at -40ºC until use. The diets were highly stable in sea water.

In Trial 1, five test diets were formulated (Table 1) with added glycine (diet A1), taurine (diet A2), alanine (diet A3), or a mixture of alanine, glutamic acid and aspartic acid (diet A4) at a level of 1%. In the basal diet A5, no amino acid was added.

In Trial 2, five test diets were formulated (Table 2) with added inosine (diet B1), glutamic acid (diet B2), a combination of inosine and glutamic acid (diet B3), or combination of ribonucleic acid and alanine (diet B4). Diet A3 with alanine served as the control.

Each feeding trial was conducted in 15 fiberglass tanks with 150 liters sea water. In Trial 1, 20 turbot (30.8±1.8 g) stocked in each tank, and in Trial 2, 10 turbot (146.2±19.6 g). Each test diet was fed to fish in three replicate tanks. The water temperature was 18.1±0.5ºC in Trial 1 and 12.9±0.4˚C in Trial 2.

The Kalkan were fed until satiation at 08.30 h, 11.00 h, and 15.00 h. The satiation point was observed carefully to prevent waste of feed. At satiation, the Kalkan ceased active feeding at the surface and sank to the tank bottom to rest. The water flow to the tank was 1.8 liters/min and a photoperiod of 12 h light and 12 h dark was maintained.

The attractiveness of the test diets was measured as the feed intake rate, expressed as a percentage of the body weight gain:

Table 1. Composition of test diets used in feeding stimulant Trial-I.

Ingredient	Test diets				
	A-1	A-2	A-3	A-4	A-5
Non-essential amino acid					
Glycine	1.0				
L-taurine		1.0			
DL-α-alanine			1.0	0.3	
L-glutamic acid				0.3	
L-aspartic acid				0.4	
Casein (vitamin free)	55.0	55.0	55.0	55.0	55.0
β-corn starch	15.0	15.0	15.0	15.0	15.0
Fish oil [1]	8.0	8.0	8.0	8.0	8.0
Vitamin mixture [2]	4.0	4.0	4.0	4.0	4.0
Mineral mixture [2]	4.0	4.0	4.0	4.0	4.0
Guar gum	4.0	4.0	4.0	4.0	4.0
Cellulose	9.0	9.0	9.0	9.0	10.0
Total	100.0	100.0	100.0	100.0	100.0
1.30 % NaOH solution	122	122	122	122	122
pH	6.71	6.67	6.87	6.60	6.75

[1] Ω feed oil, Riken Vitamin, Tokyo, Japan
[2] See Alam *et al*., (2001)

Table 2. Composition of test diets used in feeding stimulant Trial-II.

Ingredient	Test diets				
	B-1	B-2	B-3	B-4	A-3
Dispensable chemical					
Inosine	0.27		0.10		
L-glutamic acid		1.00	0.90		
Ribonucleic acid				0.024	
DL-α-alanine				0.976	1.00
Basal components [1]	90.00	90.00	90.00	90.00	90.00
Cellulose	9.73	9.00	9.00	9.00	9.00
Total	100.00	100.00	100.00	100.00	100.00
1.34 % NaOH solution	125	125	125	125	125
PH	6.77	6.75	6.76	6.94	6.81

[1] Basal components is consisted of casein, β-corn starch, fish oil, vitamin mixture, mineral mixture and guar gum. See Table 1 for details.

Feed intake rate (%) = 100 x F / ((W_0+W)/2 x (N_0+N)/2) x D

where;

F : total amount of feed intake (dry basis, g)
W_0 : mean body weight at start (g)
W : mean body weight at end (g)
N_0 : number of fish at start
N : number of fish at end
D : duration of feeding trial (d)

Mortality was not significantly different among fish fed the different diets (Tables 3 and 4). In Trial 1, the highest feed intake was obtained with diet A3 and A4 (1.03-1.04%), significantly different from the basal diet A5 (0.90%). Feed intake was not different among diets A1, A2 and A5. The results indicated that alanine or mixture of alanine, glutamic acid and aspartic acid enhanced the palatability of the diet for juvenile Kalkan.

In Trial 2, the highest feed intake of 0.63% was obtained with diet B3 containing inosine and glutamic acid, followed by 0.60% with diet B1 containing inosine. Both these diets were better than B2, B4, and A3. The results showed that inosine and L-glutamic acid were more effective than alanine as feeding stimulants for Kalkan.

Amino acids such as alanine, glycine and taurine, and betaine are known to be feeding stimulants for fish and crustaceans (Deshimaru and Yone, 1978; Mackie and Mitchell, 1982; Métailler *et al*., 1983; Kanazawa, 2000). Mackie and Adrine (1977) identified inosine as a specific feeding stimulant for turbot, effective at a concentration of 0.27% of dry diet. The present study confirmed inosine as effective feeding stimulant for Kalkan, even at a concentration of 0.1% in combination with 0.9% glutamic acid.

Table 3. Percentage survival and feed intake rate (FIR) of young Black Sea turbot fed diets containing different non-essential amino acid for 10 days. Values are means of three replicate groups. Means with different letter in the same column differ significantly (P<0.05).

	Test diets					
	A-1	A-2	A-3	A-4	A-5	Pooled SEM
Survival (%)	100	98.3	100	100	100	
FIR	0.97abc	0.92bc	1.04a	1.03ab	0.90c	0.036

1 FIR = 400 x total feed intake in dry basis / (W_0+W) (N_0+N) x D.
W_0: mean body weight at start, w : mean body at end.
N_0: number of fish at start, n : number of fish at end.
D: duration of feeding trial = 10

Table 4. Percentage survival and feed intake rate (FIR) of young Black Sea turbot fed diets containing different feeding activator for 10 days. Values are means of three replicate groups. Means with different letter in the same column differ significantly (P<0.05).

	Test diets					
	B-1	B-2	B-3	B-4	A-3	Pooled SEM
Survival (%)	100	100	100	100	100	-
FIR (%)	0.60a	0.53ab	0.62a	0.56ab	0.49b	0.015

Table 5. Percentage survival, final body weight (FBW), feed efficiency (FE), feed intake rate (FIR) and specific growth rate (SGR) of young Black Sea turbot fed diets with varying dietary pH for 15 days. Values are means of three replicate groups. Means with different letter in the same column differ significantly (P<0.05).

Dietary pH	Survival (%)	FBW (g)	FE[1]	FIR[2] (%)	SGR[3] (%)
4.10	100	47.7b	94b	1.30b	1.77b
5.55	100	51.0ab	108ab	1.46ab	2.30a
6.94	100	51.5a	118a	1.58a	2.71a
8.54	100	51.1ab	115a	1.51a	2.51a
Pooled SEM	-	14.97	232	0.039	0.133

[1]Feed efficiency = 100 x weight gain (g)/total feed intake in dry basis (g).
[2]Feed intake rate = 400 x total feed intake in dry basis / (w0+wt) (N0+Nt) x DD.
W_0: mean body weight at start, Wt : mean body at end.
N_0: number of fish at start, Nt : number of fish at end.
[3]Specific growth rate = 100 x [ln (mean final weight) - ln (mean initial weight)]/DD.

Diet pH

Four test diets with pH 4.0, 5.5, 7.0 and 8.5 were prepared with Kalkan meal as protein source. The diets contained the following ingredients (in g/100 g dry matter): defatted whole Kalkan meal 66; β-corn starch 15; cod liver oil 8; DL-α-alanine 1; guar gum 4; vitamin mixture 4: mineral mixture 2. The vitamin and mineral mixture was according to Alam *et al*., (2001). The pH of each test diet was adjusted with 4N hydrochloric acid and 4N sodium hydroxide, during dough making and confirmed after palletized by pH meter. The diets were mixed and pelletized as described earlier, and kept in –40 °C until use.

Defatted whole Kalkan meal was prepared as follows. Male Kalkan from the wild were boiled whole for 15 minutes, dried in a forced-air oven, then ground into a fine powder. The powdered meal was defatted in absolute ethanol and eluted for 30 min at a temperature of 25°C. For second and third elution, the powdered meal was boiled in absolute ethanol for 15 min at a temperature of 75°C. Defatted whole meal was placed on an open air for 48 h to evaporate the ethanol and then dried at 50°C in a constant temperature oven.

The four diets were tested with three replicates each in 12 fiberglass tanks with 150 liters seawater at 17.9±0.4°C. Each tank had 15 Kalkan (50.4±2.4 g).

Feed intake rate, mean specific growth rate, and mean feed efficiency all significantly increased with diet pH between pH 4 and pH 7 and slightly declined at pH 8.5 (Figure 1). The optimum diet pH for Kalkan was estimated by orthogonal polynomial analysis. The highest feed intake rate was at pH 7.1, the highest specific growth rate at pH 7.4, and the highest feed efficiency at pH 7.5. Thus, diets for the Black Sea turbot must be at the optimal pH range of 7.2 to 7.4.

It had earlier been shown that the nutritional

value of diets for juvenile yellowtail varied with pH (Deshimaru *et al*., 1982) and that the growth of juvenile Japanese flounder was slightly improved by adjusting the diet pH between 6.5 and 7 (Kuroki and Deshimaru, 1984).

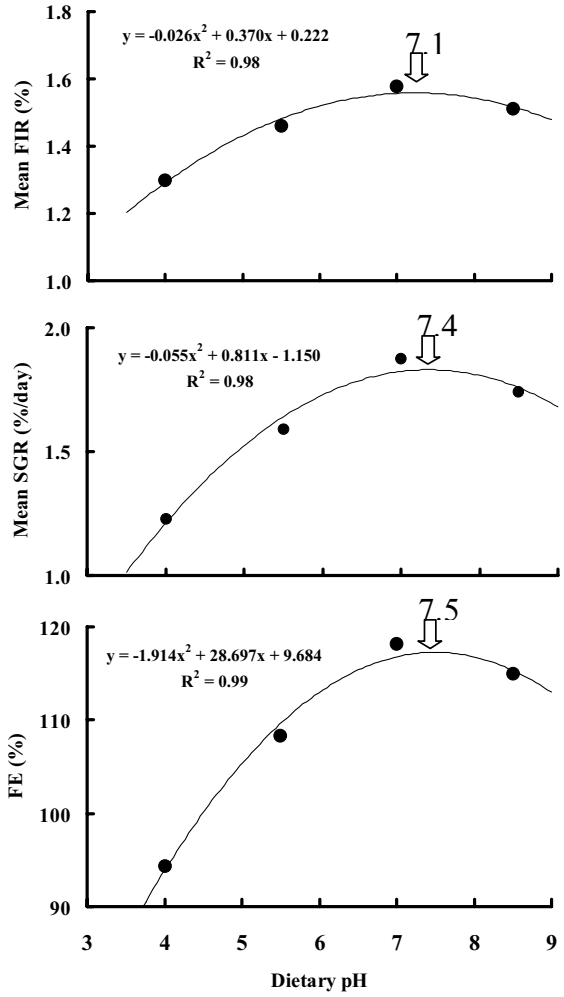

Figure 1. Relationship between dietary pH and mean feed intake rate (FIR, A), mean specific growth rate (SGR, B) and mean feed efficiency (FE, C).

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