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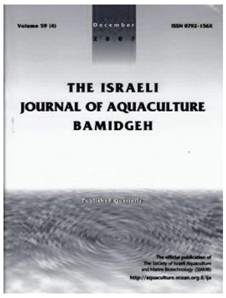

Effects of Feeding Frequency and Ration Level on Growth Performance and Non-Specific Immunity of Juvenile Turbot (*Scophthalmus maximus*) at Different Growth Stages

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Keywords: ration level; feeding frequency; growth; feed utilization

Abstract

Four growth trials were carried out to optimize a feeding regime for turbot in industrial aquaculture systems at 15±0.5°C. Six ration levels (50%, 60%, 70%, 80%, 90%, and 100%) of satiation were tested in trial I and five ration levels (60%, 70%, 80%, 90% and 100%) of satiation were tested in trial III. For optimal feeding frequency, turbot were fed to satiation for 1 meal/day, 2 meals/day, 3 meals/day in trial II, and 1 meal every other day, 1 meal/day, and 2 meals/day in trial IV. The average initial body weight of fish was 28.90±0.07g for trials I & II, and 181.75±1.05g for trials III & IV. Growth performance generally decreased and feed utilization increased with decreasing ration levels, while no significant differences were observed with small sized turbot fed to 80%, 90%, 100% levels of satiation, and with large sized turbot fed to 90% & 100% levels of satiation. There was no increase in size heterogeneity with decreasing ration levels. Ration levels significantly influenced water quality. There were no significant differences in growth and feed efficiency among turbot fed with different feeding frequencies. Feeding small size turbot once a day and large size turbot once every other day at a ration level of 80% satiation is suggested for better feed utilization and less waste output.

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Introduction

Turbot (*Scophthalmus maximus*) is a very valuable flatfish species in the family Scophthalmidae (order Pleuronectiformes). This species has been cultured in Europe since the 1970s and was introduced into China in 1990s. In recent years, China has become the leading turbot producer in the world with an annual amount of 50,000-60,000 tons. The technology involved in the culture of turbot is well established, with a complete farm-raising cycle in land-based facilities. Maximum yields are not the only criterion; attention is also focused on cost effectiveness and sustainability. Feed represents around 60% of costs in intensive aquaculture and efficient use is therefore a prime economic factor in turbot aquaculture.

Feeding frequency and ration levels play a determining role in regulating feed intake, growth, and wastage. Manipulating feeding strategies are key to the economic success and sustainability of fish production (Cho and Bureau, 2002). There are limited studies related to feeding strategies and growth performance of turbot. Feeding frequency was found to affect growth, feed utilization, and body composition of juvenile turbot at low (5-7.5°C) temperatures (Türker (2006). A factorial design of two feeding rates and three feeding frequencies was reported with juvenile black sea turbot (*psetta maxima*, linneaus, 1758) (Aydın et al. 2011). The present study was conducted to improve understanding of the effect of feeding frequency and ration levels on growth performance, feed utilization, waste production, and non-specific immunity of turbot (*Scophthalmus maximus*) at different growth stages.

Materials and Methods

Experimental design and maintenance of fish. The current study comprised four feeding trials (Trials I, II, III, and IV). Turbot were obtained from Yantai Tianyuan Aquatic Product Co., Ltd, Shandong, China and acclimated in a flow-through industrial rearing system for 2 weeks before onset of the trials. The average initial body weight of the fish was 28.90±0.07g for trials I & II, and 181.75±1.05g for trials III & IV. The fish were distributed randomly into fiberglass tanks (water volume 120 L in trials I, II, and 800 L in trials III, IV) with 20 fish per tank, and three replicate tanks for each treatment. Each tank was equipped with an air stone and supplied with 10 L/min of deep well seawater with a salinity of 17-18 g/L. During the experiment, water temperature was constant at 15±0.5°C; ammonia nitrogen was less than 0.1 mg/L; dissolved oxygen was above 5 mg/L and pH about 6.8.

Experimental conditions are shown in table 1. Trials I & III were carried out to investigate the optimum ration levels for turbot. Six ration levels were tested in trial I: 50%, 60%, 70%, 80%, 90% and 100% of satiation. Five ration levels were tested in trial III: 60%, 70%, 80%, 90% and 100% of satiation. Each trial had its own control and fish in the control group were hand-fed twice a day at 8:00 and 17:00 to apparent satiation (100% satiation), and daily feed intake was recorded. Ration levels of fish in the other experimental groups were determined based on the average feed consumption of fish in the control group. Trials II & IV were conducted to test feeding frequency; turbot were fed to satiation for 1 meal per day (8:00), 2 meals per day (8:00, 17:00), 3 meals per day (8:00, 12:00, 17:00) in trial II, and 1 meal every other day (8:00), 1 meal per day (8:00), and 2 meals a day (8:00, 17:00) in trial IV. Fish were offered commercial pellets (Fuzhou Haima Feed Co., Ltd) containing 46% protein, 15% lipid, 11% moisture and 13% ash. Particle size was 3 mm in trials I & II, and 9 mm in trials III & IV. All growth trials lasted for 45 days.

Table 1. Experimental conditions.

	Initial body weight (g)	*Nr tanks*	*Treatment*
I	28.90±0.07g	18	50%, 60%, 70%, 80%, 90% & 100% satiation
II	28.90±0.07g	9	1 meal/day, 2 meals/day, 3 meals/day
III	181.75±1.05g	15	60%, 70%, 80%, 90% & 100% satiation
IV	181.75±1.05g	9	1 meal every other day, 1 meal/day, 2 meals/day

Sampling. At the end of the trial, fish were starved for one day and then batch weighed. In trial I, ammonia nitrogen (NH_4-N), nitrite (NO_2-N), and phosphorus (P) concentrations in the water were tested in each tank 5 hours after feeding. The water flow was stopped before every water sampling. Concentration of ammonia nitrogen (NH_4-N) was measured with a Nessler reagent colorimetric determination kit. The concentration of nitrite (NO_2-N) was measured with naphthylethylenediamine spectrophotometry, and the phosphorus concentration was measured with the molybdenum blue spectrophotometric method in accordance with *Water and Wastewater Monitoring Analysis (State Environmental Protection Administration of China, 2002)*.

Analytical methods. The proximate composition of the experimental diets was determined by standard laboratory procedures of AOAC i.e. dry matter by drying at 105°C for five hours until constant weight was reached; crude protein (N*6.25) by the Kjeldhal method (UDK142 automatic distillation unit, VELP); crude lipid by petroleum ether extraction in a Soxtec™ System (Soxtec™ 2050 system, Foss Tecator, Hoganas, Sweden); ash by incineration in a muffle furnace at 550°C.

Statistical analysis. SPSS 16.0 for Windows software was used for data processing and statistical analysis. The homogeneity of variances was checked using the Levene's test. Data were compared by one-way ANOVA followed by Duncan's multiple range test. Differences between treatments were considered significant when P <0.05. All data are presented as means ± S.E.M (standard error of the mean) of three replicates.

Results

Growth and feed utilization of turbot at different ration levels. No mortality was observed in the four feeding experiments. Specific growth rate (SGR), final body weight, and feed efficiency (FE), were significantly affected by the ration levels (Tables 2 & 3). In trial I, small size turbot fed to 80%, 90% levels of satiation showed no significant differences in their final body weight and SGR compared to fish fed to satiation (P>0.05). Final body weight and SGR showed a linear decrease with decreasing ration levels from 70% to 50% satiation. In trial III, large size turbot fed at 90% satiation showed no significant differences in final body weight and SGR compared to fish fed to satiation (P>0.05). The growth of the fish fed at 80% and 70% satiation was significantly lower compared to the fish fed to 100% and 90% levels of satiation (P<0.05). Fish fed to 60% level of satiation had the lowest growth performance. There was no increase in size heterogeneity with the decreasing ration levels in both trials I and III, since the coefficients of variation for fish body weight remained unvarying throughout the growth trials.

Table 2. Effect of feeding ration levels on growth performance and feed utilization of turbot (initial body weight: 28.90±0.07g) in Trial I (Mean ± SE).

	Feeding ration levels					
	100%	*90%*	*80%*	*70%*	*60%*	*50%*
Final body weight (g)	$51.5±1.3^{a}$	$48.9±1.2^{ab}$	$48.9±1.2^{ab}$	$47.5±1.2^{bc}$	$44.9±0.4^{cd}$	$42.6±0.2^{d}$
Specific growth rate(%/d)[1]	$1.28±0.06^{a}$	$1.16±0.05^{ab}$	$1.16±0.06^{ab}$	$1.10±0.06^{bc}$	$0.97±0.02^{cd}$	$0.86±0.01^{d}$
Feeding rate(%/d)[2]	$1.09±0.02^{a}$	$0.97±0.01^{b}$	$0.91±0.01^{c}$	$0.83±0.01^{d}$	$0.73±0.00^{e}$	$0.64±0.00^{f}$
Feed efficiency (%)[3]	$113.9±3.1^{a}$	$117.1±4.0^{ab}$	$124.5±6.7^{ab}$	$127.8±5.9^{ab}$	$131.3±3.1^{b}$	$129.2±1.6^{b}$
ΔCV_w (%)[4]	1.05±0.21	1.02±0.09	1.02±0.14	1.01±0.22	1.03±0.13	1.06±0.25

Values with different superscripts in the same row are significantly different (P < 0.05).

[1]Specific growth rate (SGR, %/d) = 100× [ln W_f - ln W_i] /feeding days, where W_f= final wet body weight, W_i= initial wet body weight.

[2]Feeding rate (%body weight/day) =100×total feed intake/[feeding days×(W_i + W_f)/2]

[3]Feed efficiency (%) =100×wet weight gain/dry feed intake

[4]Interindividual variations in body weight were assessed by calculation of the coefficient of variation: CV (%) = (S.D.× (mean weight)$^{-1}$) ×100. Changes in the coefficient of variation during the growth period were calculated as: ΔCV_w (%) = $CV_{t=45}/CV_{t=0}$.

Table 3. Effect of feeding ration levels on growth performance and feed utilization of turbot (initial body weight: 181.75±1.05g) in Trial III (Mean ± SE).

	Feeding ration levels				
	100%	*90%*	*80%*	*70%*	*60%*
Final body weight (g)	266.49±3.25[a]	266.15±1.99[a]	260.46±1.73[b]	254.71±0.57[b]	244.31±3.17[c]
Specific growth rate(% d^{-1})[1]	0.84±0.03[a]	0.86±0.02[a]	0.79±0.02[b]	0.74±0.01[b]	0.65±0.02[c]
Feeding rate(%/d)[2]	0.71±0.03[a]	0.63±0.00[b]	0.56±0.00[c]	0.50±0.00[d]	0.44±0.00[e]
Feed efficiency (%)[3]	110.72±3.52[a]	128.47±1.99[b]	139.06±1.73[c]	145.16±0.57[c]	141.96±3.17[c]
ΔCV_w (%)[4]	1.01±0.01	1.01±0.05	1.01±0.02	1.01±0.02	1.02±0.06

[1]Specific growth rate (SGR, %/d) = 100× ［ln W_f - ln W_i］/feeding days, where W_f= final wet body weight, W_i= initial wet body weight.
[2]Feeding rate (%body weight/day) =100×total feed intake/[feeding days×(W_i + W_f)/2]
[3]Feed efficiency (%) =100×wet weight gain/dry feed intake
[4]Interindividual variations in body weight were assessed by calculation of the coefficient of variation: CV (%) = (S.D.× (mean weight)$^{-1}$) ×100. Changes in the coefficient of variation during the growth period were calculated as: ΔCV_w (%) = $CV_{t=45}/CV_{t=0}$.

Feeding rate ranged from 1.09 to 0.64% of the fish body weight/day in trial I and from 0.71 to 0.44% in trial III. In both trials I and III, the FE first showed a linear increase with decreasing ration levels and then reached a plateau. Small turbot fed to satiation had a significant lower FE compared to fish fed to 50%, 60% satiation. Large turbot showed a significant increase of FE with decreasing ration levels from 100% to 80% satiation ($P<0.05$).

Water quality. Ration levels significantly affected water quality in trial I (table 4). The concentration of ammonia nitrogen, nitrite, and phosphate decreased with decreasing ration levels ($P<0.05$). Fish fed to satiation had higher nitrite concentrations compared to fish fed 80%-50% satiation levels. Fish fed to 100%, 90% of satiation had higher ammonia compared to fish fed 70%-50% of satiation. The concentration of phosphate was higher with fish fed 100%-70% satiation levels compared to others.

Table 4. Effect of feeding ration levels on rearing water quality in Trial I (Mean ± SE).

	Feeding ration levels					
	100%	*90%*	*80%*	*70%*	*60%*	*50%*
NO_2-N (mg/L)	0.019±0.001[a]	0.018±0.000[ab]	0.014±0.002[bc]	0.012±0.002[c]	0.010±0.001[c]	0.011±0.001[c]
NH_4-N (mg/L)	0.083±0.003[a]	0.082±0.004[a]	0.073±0.001[ab]	0.070±0.006[b]	0.067±0.003[b]	0.053±0.002[c]
Phosphate (mg/L)	0.084±0.008[a]	0.073±0.005[a]	0.077±0.001[a]	0.071±0.001[a]	0.055±0.003[b]	0.059±0.001[b]

Values with different superscripts in the same row are significantly different ($P < 0.05$).

Growth and feed utilization of turbot at different feeding frequencies. There were no significant differences in final body weight, SGR, feeding rate, and feed efficiency among turbot fed at different feeding frequencies both in trial II and trial IV (tables 5 & 6) ($P>0.05$). Feeding rate was 1.03-1.08% body weight/day in trial II and 0.63-0.70% body weight/day in trial IV.

Table 5. Effect of feeding frequency on growth performance and feed utilization of turbot (initial body weight: 28.90±0.07g) in Trial II (Mean ± SE).

	Feeding frequency		
	1meal/day	*2 meals/day*	*3 meals/day*
Final body weight (g)	49.8±0.3	50.7±2.1	52.0±2.6
Specific growth rate(%/d)[1]	1.21±0.01	1.24±0.09	1.30±0.11
Feeding rate(%/d)[2]	1.03±0.02	1.05±0.03	1.08±0.06
Feed efficiency (%)[3]	115.2±1.0	114.9±5.1	117.4±3.7

[1]Specific growth rate (SGR, %/d) = 100× ［ln W_f - ln W_i］/feeding days, where W_f= final wet body weight, W_i= initial wet body weight.
[2]Feeding rate (%body weight/day) =100×total feed intake/[feeding days×(W_i + W_f)/2]
[3]Feed efficiency (%) =100×wet weight gain/dry feed intake

Table 6. Effect of feeding frequency on growth performance and feed utilization of turbot (initial body weight: 181.75±1.05g) in Trial IV (Mean ± SE).

	Feeding frequency		
	2 meals/day	*1meal/day*	*1meal/2 days*
Final body weight (g)	257.82±4.54	256.28±3.29	254.74±6.32
Specific growth rate(%/d)[1]	0.81±0.04	0.79±0.04	0.85±0.05
Feeding rate(%/d)[2]	0.70±0.03	0.65±0.03	0.63±0.01
Feed efficiency (%)[3]	115.23±7.10	117.19±6.41	119.70±8.47

[1]Specific growth rate (SGR, %/d) = 100× [ln W_f - ln W_i] /feeding days, where W_f= final wet body weight,
W_i= initial wet body weight.
[2]Feeding rate (%body weight/day) =100×total feed intake/[feeding days×(W_i + W_f)/2]
[3]Feed efficiency (%) =100×wet weight gain/dry feed intake

Discussion

Curvilinear regressions between ration levels and growth performance have been well demonstrated in fish (Wang et al., 2007; Zheng et al., 2015). Turbot showed a similar pattern in the current study. Small sized turbot fed with restricted ration levels of 80% satiation and large sized turbot fed with 90% of satiation showed no significant reduction in growth performance compared to fish fed to satiation. More importantly, turbot showed improved feed efficiency with restricted ration levels. In small sized turbot greatest FE was exhibited in fish fed 60% satiation level, and in large turbot greatest FE was in fish fed 70% satiation level. This is in agreement with another study on turbot fed 35%, 65%, and 100% of satiation at different temperatures (Van Ham et al. 2003). The best feed conversion efficiency was found at 65% ration level. Many other studies have shown that the maximum FE was at feeding levels below that required for maximum growth (Storebakken and Austreng 1987; Wang *et al.* 2007; Blanquet and Oliva-Teles 2010). Conversely, there are studies showing that moderate feeding restrictions had no significant effect on feed efficiency ratio (Alanärä 1994; Azevedo et al., 1998). These studies with rainbow trout (*Oncorhynchus mykiss*) showed that feed efficiency was not significantly influenced by moderate feed restriction (85%, 70%, and as low as 50% of near satiation).

Feeding hierarchy formation is a major concern with restricted feeding in carnivorous fish farming, and leads to increased individual variation in fish body size and weight (Sæther and Jobling 1999). The restricted rations applied in the current study on turbot did not cause an increase in size heterogeneity, since there was little change in the coefficients of variation of weight (CV) during the trials and they were similar in all groups. This absence of a change in CV is in agreement with observations on turbot fed restricted rations (Sæther and Jobling, 1999; Van Ham *et al.,* 2003). Therefore, it should be no impediment to apply restricted feeding rations in turbot farming for better feed utilization.

Overfeeding of turbot increases fish production costs and causes deterioration of water quality, which can eventually affect the growth and health of fish. Juvenile turbot growth may be depressed by usual ambient ammonia concentrations in intensive farming (Person-Le Ruyet, et al. 1997). It appears from the water quality results we monitored in trial I, that reducing ration levels to 70-80% of satiation in turbot could significantly reduce waste output. Concerns regarding the environmental effects of marine fish farms have arisen because fish farms release solid waste and discharge effluent directly into the water. Regulating feeding strategies reduces waste output and maintains better water quality. Further details about feeding strategies and the impact on environmental factors from on nitrogen and phosphorus waste output (g/kg fish produced) should be investigated. It was found that feeding levels did not influence total solid wastes, solid and dissolved nitrogen waste, and phosphorus waste in rainbow trout at 6, 9, or 15°C, but higher total solid waste and nitrogen waste were observed at the satiation level at 12°C (Alanärä 1994).

Hand feeding is the most common method in turbot farming in China. Costs are higher when cultured fish are fed more frequently. Optimum feeding frequencies may change according to fish species and growth stages, also in different rearing conditions, including feed quality, feeding rate, and water temperature (Bin et al., 2014, Lee et al., 2000; Kestemont and Baras 2001). In low temperatures (5-7°C), juvenile turbot between 15-25g can be fed once every other day with without affecting growth (Türker, 2006). In the current study, we found that feeding small sized turbot (initial body weight=28.9g) once a day and feeding large sized turbot (initial body weight=181.7g) once every two days to satiation was enough to attain maximal growth. Similarly, feeding juvenile Black Sea turbot (initial body weight=23.8g & 38.6g) once a day to satiation was sufficient to attain maximal growth (Aydın *et al.* 2011). Since food intake is related to stomach capacity, the time interval between feeding is also important for gastric evacuation and digestion rate (Brett, 1971). After satiation, the appetite in juvenile turbot was found to be closely related to the degree of stomach emptiness (Grove et al. 1985). When food is regularly available, juvenile turbot take feed steadily at a rate which equals 85% maximum fullness of their stomachs. Another study found that when fed continuously every three hours to apparent satiation under similar rearing conditions, juvenile turbots with an average body weight of 29g showed a rhythm with a single peak once every 18 hours (Zheng *et al.* 2010). Protein nitrogen and energy assimilation efficiency in juvenile turbot remained unchanged irrespective of feeding rates and frequency when fed with energy-rich diets (Grove et al., 1985). Our study is in agreement with this finding.

In conclusion, our results provide useful information in feed management for commercial turbot farmers. For better feed utilization and less waste output, in rearing temperatures of 15±0.5°C, small sized turbot can be fed to 80% of satiation. Feeding small turbot (initial body weight=28.9g) once a day, and large turbot (initial body weight=181.7g) once every two days seems to be sufficient for effective growth.

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