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Research Article

Influence of Feeding Frequency on Feed Intake, Growth Performance and Nutrient Utilization in European Sea Bass (*Dicentrarchus labrax*) Fed Pelleted or Extruded Diets

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Abstract: In an 83-day-nutrition trial, effects of feed production technique and feeding frequency on feed intake, growth and nutrient utilization were determined in European sea bass (*Dicentrarchus labrax*). Three thousand six hundred fish (170 ± 2.6 g) were randomly allocated to experimental sea cages (approx. 5800 l) according to a 2 x 2 experimental plan [2 processing techniques: steam pelleted diet (P) vs. extruded diet (E); 2 feeding frequencies: twice (2) vs. 3 times (3)]. The P3 group consumed significantly ($P < 0.05$) more feed than the fish fed extruded diets, irrespective of feeding frequency. Growth rates of all groups did not display any significant difference ($P > 0.05$) in terms of final weight (FW) or specific growth rate (SGR), although there was a decreasing trend in final weight (E3 > E2 > P3 > P2). Feed efficiencies (FE) of E2 and E3 fish were higher than P2 and P3 treatments ($P < 0.05$). No significant difference ($P > 0.05$) was evident for the hepatosomatic index (%), mesenteric fat index (%), viscerasomatic index (%), or dress-out (%) parameters in all treatments. The carcass and muscle components (protein, lipid, and ash) did not reveal any significant difference ($P > 0.05$). According to the results, feeding sea bass twice or 3 times daily with steam pelleted or extruded diet did not influence the growth; however, extruded diet improved the feed efficiency of sea bass irrespective of feeding frequency.

Key Words: European sea bass, *Dicentrarchus labrax*, diet processing technology, feeding frequency, feed intake, growth performance

Pelet ve Ekstrude Yemlerle Beslenen Levrek Balığında (*Dicentrarchus labrax*) Yemleme Sıklığının Yem Tüketimi, Büyüme Performansı ve Nutrient Kullanımı Üzerine Etkileri

Özet: 83 günlük bir besleme denemesinde, yem üretim tekniğinin ve yemleme sıklığının Avrupa deniz levreğinde büyüme, yem tüketimi ve nutrient kullanımı üzerine olan etkileri çalışılmıştır. 3600 adet Avrupa deniz levreği (170 ± 2,6 g) 2 x 2 deney planına göre [2 yem üretim tekniği: Pelet yem (P), ekstrude yem (E); 2 yemleme sıklığı: iki kez (2), üç kez (3)] deneme kafeslerine (yaklaşık 5800 l) rasgele dağıtılmıştır. Yemleme sıklığı göz önüne alınmadığında, P3 grubu, ekstrude yemle beslenen gruplara göre daha fazla yem tüketmiştir ($P < 0,05$). Balıkların son ağırlıkları ve spesifik büyüme oranları açısından, tüm grupların büyüme oranları arasında istatistiki bir farklılık olmasa da son ağırlıklarında bir artış eğilimi belirlenmiştir (E3 > E2 > P3 > P2). E2 ve E3 gruplarının yem değerlendirmesi (YD) P2 ve P3 gruplarına göre daha yüksek bulunmuştur ($P < 0,05$). Bütün deneme gruplarında, hepatosomatik indeks, iç organ yağ oranı ve et verimi parametreleri arasında ve karkas-et bileşenleri (protein, yağ ve kül) oranları arasında önemli bir farklılık görülmemiştir ($P > 0,05$). Sonuçlara göre, levrek balıklarını pelet veya ekstrude yemle iki veya üç öğün beslemenin büyüme oranını etkilemediği, ancak, öğün sayısına bağlı kalınmaksızın ekstrude yemin, levreklerin yem değerlendirme oranını iyileştirdiği belirlenmiştir.

Anahtar Sözcükler: Avrupa deniz levreği, *Dicentrarchus labrax*, yem üretim teknolojisi, yemleme sıklığı, yem tüketimi, büyüme performansı

Introduction

Feed expense is the highest share of total cost in any intensive aquaculture operations. Therefore, feed quality and feeding strategy are of great importance in fish nutrition science (1,2). Processing technology has a direct influence on feed quality in terms of physical properties and nutrient digestibility. Extrusion technology has become more common than conventional steam pelleting since the beneficial effects of extrusion processing have been well demonstrated in fish such as the improvement

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in carbohydrate utilization, water stability, feed efficiency, and protein digestibility (3,4).

The optimal feeding strategies improve growth, survival, and food conversion ratios (FCRs), and assist in minimizing food wastage, reduce size variation, and ultimately, increase production efficiency (5-8). The optimum feeding frequency for maximum growth of fish varies depending on the fish species, fish size, culture conditions, environmental factors, dietary nutrients (protein and energy levels), and feed allowance in the previous feeding (1,8-10). The effects of feeding frequency on fish growth and food conversion efficiency have been examined for several species, including channel catfish (*Ictalurus punctatus* Rafinesque, 1818) (11,12), rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) (13), European sea bass (*Dicentrarchus labrax* Linnaeus, 1758) (14), Japanese flounder (*Paralichthys olivacues* Temminck and Schlegel, 1846) (7), and common pandora (*Pagellus erythrinus* Linnaeus, 1758) (15).

Sea bass production in Turkey has increased by almost 10-fold in the last decade from 2.229 metric tons in 1994 to 26.297 metric tons in 2002. This accounts for 28% of the total aquaculture production of Turkey (16). From a practical point of view, there are a couple of ongoing arguments among the Turkish sea bream/sea bass farmers. The first concerns whether feeding fish with extruded diets is more beneficial than steam pelleted ones or not. The second discussion is on the feed application methods; feeding fish twice or 3 times daily on a satiation basis is more efficient. Therefore, this study was designed to reveal the effects of different feeding frequencies on feed intake, growth and nutrient utilization in sea bass fed extruded or pelleted diets.

Materials and Methods

Experimental fish and rearing conditions

Adult European sea bass, *Dicentrarchus labrax*, which were obtained from a local commercial fish farm (Çanakkale, Turkey) were transported to the Net Cage Unit of the Faculty of Fisheries, Çanakkale Onsekiz Mart University. They were fed a commercial feed (47% protein; 17% fat) for 2 weeks while being acclimated. Groups of 3600 adult European sea bass (170.0 ± 2.6 g) were randomly distributed into experimental net cages (diameter: 2 m, depth: 2.5 m). Dead fish were removed and weighed daily. Average water temperature was 24.0 ± 0.2 °C, dissolved oxygen ranged between 8.6 and 9.7 mg l^{-1}, pH varied between 7.8 and 8.1, and salinity was between 23.2‰ and 24.8‰ during the study. The live body weight of each fish was measured at the beginning and at the end of the study following a 24-h starvation period.

Experimental design

A 2 (diet processing) x 2 (feeding frequency) factorial design with 2 replications was employed. Portions of the same practical sea bass diet were processed through a pellet mill (Robinson-Paladin, Holland) and through a twinscrew extruder (Sogem, France) (Table 1). Two feeding frequencies were applied in the experiment: twice (07:00 and 19:00) or 3 times daily (07:00, 13:00, and 19:00). Fish were hand fed to apparent satiation 7 days per week.

Chemical analysis

Six initial and final fish carcasses and muscles were sampled and stored at –25 °C for proximate analyses, which were performed according to AOAC (17). Dry matter was determined after drying at 105 °C until a constant weight was obtained. Ash content was measured by incineration in a muffle furnace at 525 °C for 12 h. Crude protein was analyzed by the Kjeldahl method after acid digestion using the Gerhardt system. Lipid

Table 1. Proximate composition of experimental diets.

	Pelleted	Extruded
Fish meal	55	55
Corn gluten	8	8
Full-fat soybean meal	12	12
Wheat meal	10.3	10.3
Fish oil	12.7	12.7
Vitamin-mineral premix	2	2
Chemical analyses (Dry Matter %)		
Crude protein	47.0	47.1
Crude fat	17.1	17.0
Crude ash	9.8	9.9
Crude fiber	3.1	3.0
Nitrogen-free extract	23.0	23.0
Energy (MJ/kg)	21.8	21.5

extractions were undertaken by petroleum ether extraction in a Soxhlet extraction system. Crude fiber was determined by acid alkali hydrolysis and ignition of the dried sample for 3 h. Nitrogen-free extract (NFE) was computed by taking the sum values for crude protein, lipid, ash, crude fiber and moisture and subtracting this from 100. Gross energy in the diets and fish body was calculated using the conversion factors of 23.7, 39.5 and 17.2 kJ g for protein, lipid and carbohydrate, respectively (18).

Evaluation of growth parameters

Feed intake, growth performance and nutrient utilization were calculated as follows: Feed Efficiency (FE) (%) = weight gain (g) / feed intake (g) x 100. Feed Intake (FI) (%) = daily feed intake (g) x 100 / biomass (g). Specific Growth Rate (SGR) (% day^{-1}) = 100 x [(In final fish weight) – (In initial fish weight)] / days fed. Apparent Net Protein Utilization (ANPU) (%) = final retained protein (g) – initial retained protein (g) / digestible protein intake (g) x 100. Apparent Net Energy Utilization (ANEU) (%) = final retained energy (MJ) – initial retained energy (MJ) / digestible energy intake (MJ) x 100. Protein Utilized kg^{-1} Growth (g) = protein intake (g) / weight gain (g) x 1000. (DE) Utilized kg^{-1} Growth (MJ) = digestible energy (DE) intake / weight gain (g) x 1000. Condition Factor (K) (%) = fish weight (g) / (fish length)^3 (cm) x 100. Dress-out (DO) (%) = fish weight (g) – gut weight (g) / fish weight (g) x 100. Hepatosomatic index (HSI) (%) = liver weight (g) / fish weight (g) x 100. Viscerosomatic index (VSI) (%) = viscera weight (g) / fish weight (g) x 100. Mesenteric fat index (MFI) (%) = mesenteric fat weight (g) / fish weight (g) x 100

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$) (19) using the statistical software package Statgraphics 7.0 (Manugistics Incorporated, Rockville, MD, USA). Allometric analyses of the carcasses of the experimental fish were performed as explained by Tekinay et al. (20).

Results

The moisture content of the pelleted diet was higher than that of the extruded diets, while the nutrient levels (protein, fat, energy) were similar in terms of dry weight (Table 1). Average survival rates of the experimental fish were 95.1%, 98.2%, 95.2%, and 95.7% for the E2, E3, P2, and P3 groups, respectively (Table 2). There were no significant differences in survival rate ($P > 0.05$) among the treatments. The mean feed intake of fish fed the P3 diet was significantly ($P < 0.05$) higher than that of E2 and E3 fed fish.

Table 2. Growth performance, nutrient utilization and somatic parameters of European sea bass fed the extruded or pelleted diets with different feeding frequencies.

	Initial	E2	E3	P2	P3	±SEM*
Survival (%)	-	95.1	98.2	95.2	95.7	1.32
Initial Mean Weight (g)	-	170.5	171.3	171.7	171.5	6.57
Final Mean Weight (g)	-	294.3	300.8	287.9	289.3	7.28
Feed Intake (bw %)	-	1.3^{a}	1.3^{a}	1.4^{ab}	1.5^{b}	0.05
Weight Gain (%)	-	72.6	75.6	67.7	68.7	3.41
SGR (%)	-	0.7	0.7	0.6	0.6	0.01
Feed Efficiency (%)	-	0.54^{b}	0.56^{b}	0.47^{a}	0.46^{a}	0.07
ANPU (%)	-	28.5	27.5	16.9	17.0	3.74
ANEU (%)	-	61.6	65.9	56.4	59.9	4.40
DP utilized per kg growth (g)	-	915.1	863.8	1028.1	1021.2	56.27
DE utilized per kg growth (MJ)	-	41.8	39.4	47.7	47.3	2.61
Condition factor (%)	1.1	1.3	1.2	1.3	1.3	0.03
Hepatosomatic index (%)	1.5^{a}	2.4^{b}	2.3^{b}	2.4^{b}	2.4^{b}	0.41
Mesenteric fat index (%)	0.7^{a}	4.7^{b}	4.2^{b}	4.2^{b}	4.4^{b}	0.52
Viscerosomatic index (%)	7.5^{a}	10.8^{b}	10.5^{b}	10.1^{b}	10.7^{b}	0.50
Dress-out (%)	92.5^{b}	89.2^{a}	89.5^{a}	89.9^{a}	89.3^{a}	0.50

* Values in each row allocated common superscripts or without superscripts are not significantly different from each other ($P > 0.05$).

No significant differences ($P > 0.05$) were observed in the growth performance of fish with respect to final mean weight or specific growth rate, although there was a decreasing trend in final mean weight (E3 > E2 > P3 > -P2). Feed efficiencies of the E3 and E2 groups were similar, while the FE of the E3 treatment was 21.7% and 16.7% superior compared to that of the P3 and P2 groups, respectively. The P2 and P3 groups also displayed similar feed efficiencies.

The digestible protein (DP) utilized per kg growth varied between 863.8 (E3) and 1028.1 g (P2). The digestible energy (DE) utilized per kg growth was between 39.4 (E3) and 47.7 MJ (P2). The apparent net protein utilization (ANPU) of the groups fed the extruded diet showed a similar pattern, while the ANPU of E2 was 67.6% and 68.6% better than that of P3 and P2, respectively. The apparent net energy utilization (ANEU) of E3 was the highest, but there was no clear difference among the groups.

Condition factors of the experimental groups were similar, but they were slightly higher than those of the initial fish samples. Hepatosomatic index, viscerasomatic index, mesenteric fat index, and dress-out were higher than those of the initial samples ($P < 0.05$); however, these parameters displayed similar patterns among the trial groups ($P > 0.05$) (Table 2).

The final carcass and muscle proximate compositions of European sea bass changed markedly in comparison with initial composition (Table 3). On the other hand, the carcass and muscle components (protein, lipid and ash) were not affected by dietary treatments ($P > 0.05$).

Discussion

The present feeding trial demonstrated that sea bass fed an extruded diet yielded a better feed efficiency compared to fish fed the steam pelleted diet (20-25 °C water temperature; 23‰-24‰ salinity). On the other hand, a similar growth performance was recorded in sea bass fed either extruded or steam pelleted diets twice or 3 times daily.

There were relatively low mortality rates (1.8%-4.9%) throughout the trial, which proved that environmental and dietary conditions were at optimal limits. The growth rates (SGR) determined in the present study were similar to that reported by Ballestrazzi et al. (4), who fed sea bass diets containing 48% protein and 17% fat at 23.1 °C and 21.2‰ salinity. They attained slightly higher growth because their feeding period lasted longer (195 days). The SGRs of fish fed extruded or pellet diets in this study were superior to that reported by Ballestrazzi and Lanari (21), who fed pelleted diets to sea bass at 23.7 °C and 14.2‰ for 86 days, although their diets contained more protein and fat compared to this experiment. The growth performance of the fish was also higher than that found by Ballestrazzi et al. (22). The better growth rates of sea bass in our study compared to previous ones can be explained by the progress in both ingredient processing quality and extrusion technology over time.

In this work, sea bass fed extruded or pelleted diets with the same formulation showed similar growth performance irrespective of feeding frequency. There may be 2 reasons why we were unable to see a performance difference with extruded diets. Firstly, the

Table 3. Proximate composition of European sea bass fed pelleted or extruded with various feeding frequencies.

Carcass components (%)	Initial	E2	E3	P2	P3	±SEM*
Moisture	69.1^{b}	63.2^{a}	61.8^{a}	60.7^{a}	60.6^{a}	1.37
Protein	17.3^{b}	15.8^{a}	15.2^{a}	15.2^{a}	15.6^{a}	0.64
Lipid	7.6^{a}	15.0^{b}	15.7^{b}	16.2^{b}	15.8^{b}	0.97
Ash	5.4^{b}	3.3^{a}	3.8^{a}	3.7^{a}	4.0^{a}	0.25
Muscle components (%)						
Moisture	71.7^{b}	64.1^{a}	67.1^{a}	63.9^{a}	65.4^{a}	2.06
Protein	22.2^{b}	18.6^{a}	18.2^{a}	19.1^{a}	18.5^{a}	1.12
Lipid	4.3^{a}	11.8^{b}	10.8^{b}	12.0^{b}	11.2^{b}	0.98
Ash	4.8^{b}	3.4^{a}	3.0^{a}	3.6^{a}	3.0^{a}	0.27

* Values in each row allocated common superscripts or without superscripts are not significantly different from each other ($P > 0.05$).

feeding period was not long enough to observe such difference. Secondly, fish attained the near-maximum growth potential by regulating the feed intake since they fed to satiation throughout the trial. High growth rates (SGR: 1.9%) were also reported by Peres and Oliva-Teles (23) for European sea bass fry (5.6 g) that consumed a pelleted diet having 48% protein and 12.9% fat content.

The feed intake of the P3 group was significantly higher ($P < 0.05$) than that of the E2 and E3 groups. The E2 and E3 groups apparently regulated their feed intake, although the E3 group was fed on a satiation basis. Similarly, 2 meals were sufficient for the P2 group to be satiated. The P3 group also consumed more feed than the P2 group, but this was not proven statistically. It can therefore be claimed that fish fed extruded diet can regulate their feed intake according to maximum energy requirement. This should be studied in more detail since the regulation of feed intake in fish is very complex since many factors are involved (3,24). Feed utilization rates were significantly superior ($P < 0.05$) in fish fed extruded diets irrespective of the feeding frequency. Deguara (25) also reported that extruded diets produced significantly better feed efficiency in gilthead sea bream when compared to steam pelleted diets.

The effects of feeding frequency on feed efficiency depend on fish species, fish size, dietary protein and energy levels and feeding time (7,8,10,15,26). The optimum feeding frequency may vary with species and size of fish (10). For instance, Andrews and Page (11) reported that the channel catfish *Ictalurus punctatus* (53 g) grew more slowly when fed to satiation once per day than when fed 2 or 4 times; however, no differences was detected in the food conversion ratio. In a study by Grayton and Beamish (13), who fed rainbow trout fry (16 g), 3 meals a day was better than 1 or 6. Tsevis et al. (14) reported that increasing feeding frequency resulted in an inferior feed efficiency by sea bass reared at around 20 °C. Lee et al. (7) observed that a better feed efficiency in 3.5 g flounder, *Paralichthys olivaceus*, fed to satiety was obtained at a feeding frequency of 2 or 3 times daily than once in 2 days. In this study, sea bass fed twice or 3 times daily consumed similar amounts of feed. It can therefore be stated that fish were satiated on twice-feeding regime under the production conditions. Moreover, the FE values were less than 100% for the all groups, as previously reported (14,24).

The Apparent Net Protein Utilization (ANPU) and the Apparent Net Energy Utilization (ANEU) of sea bass varied between 16.9% and 28.5%, and 56.4% and 65.9%, respectively. The ANPU determined in the present study did not differ to that reported by Lanari et al. (27), who fed sea bass diets containing different levels of fat (11%, 15%, and 19%) and nitrogen-free extract (21.5% and 28.5%) for 224 days. However, the ANEU was higher than that found by Lanari et al. (27), probably because of the variation in the nutrient profile and/or diet processing technology between the 2 studies.

The condition factor, hepatosomatic index and viscerosomatic index were used to assess the nutritional and physiological status of experimental fish. Calculated condition factors were much lower than that reported by Ballestrazzi et al. (4). This was due to the fact that they used 'standard length' for the calculation of the condition factor, whereas 'fork length' was used in this study.

Since the same formulated feeds were fed to sea bass, the hepatosomatic index did not display a significant difference among the groups ($P > 0.05$). The HSI values measured in this work were also similar to those in most previous investigations on sea bass (21,27,28).

Similar MFI data were obtained in our study since the experimental diets contained similar lipid levels. The MFI of fish increased with the dietary lipid level. However, Ballestrazzi and Lanari (21) did not find any difference in the fat index of fish ($P > 0.05$), although they used different levels of fat (14% and 21%). Dress-out percentage of European sea bass was close to 90% with similar diets (4,27).

Processing technology of diets and feeding frequency did not affect the carcass or muscle composition (protein, lipid and ash concentration) of sea bass, probably due to the similar nutrient content of the 2 experimental diets. This is supported by the allometric analysis of carcass data as explained by Shearer (29) and applied by Tekinay et al. (20) in rainbow trout, *Oncorhynchus mykiss*. Recent studies indicated that only dietary fat level may influence the carcass fat level, whereas the protein or ash level of the diet does not affect the same elements of the carcass when the fish weight is taken into account (29,30).

In terms of the commercial production of sea bass, these results revealed a continuing debate among Turkish fish farmers and feed manufacturers. Extruded diets should be preferred to pelleted diets for better specific growth rates and feed efficiency. Moreover, twice feeding daily is sufficient for sea bass to obtain energy requirements for near-maximum growth on a satiation feeding regime. Near-maximum growth can also be achieved with a steam-pelleted diet by means of consumption of more feed compared to extruded feeds.

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