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Effects of Dietary Enzyme Supplement on Growth of Gilthead Sea Bream (*Sparus aurata* L., 1758)

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Özet: ***Soya küspesi ağırlıklı (%40) rasyona bakteriyel enzim karışımı ilavesinin çipura balıklarının büyüme performansı, et kompozisyonu üzerine etkileri.*** Çalışmada yaklaşık 86,94±1,77 g 220 adet çipura 2 tekrarlı olacak şekilde 2 gruba rasgele dağıtılmıştır. Çalışma 14 hafta sürdürülmüştür. Deneme süresince 2 haftada bir boy ve ağırlık ölçümleri yapılmıştır. Deneme ve kontrol grupları için başlangıç ağırlıkları sırasıyla 86,5±2,54 g, 87,5±2,44 g ve sonuç ağırlıkları ise sırasıyla 126,8±3,15 g, 124,1±2,33 g olarak ölçülmüştür. Ancak, grupların ortalama ağırlıkları arasında önemli bir farklılık saptanmamıştır (P > 0,05).

Anahtar Kelimeler: *Sparus aurata*, enzim ilavesi, Allzyme Vegpro.

Abstract: This study was carried out to find out the effects of supplements, mixed bacterial enzyme, added to feed intensified with soy bean meal (40%), on the growth rate of sea bream and the composition of its flesh. In the study, 220 sea bream, approximately 86.94±1.77g, were randomly divided into two groups which have two replicate. The study lasted 14 weeks. Throughout the course of the experiment, the length and weight measurements were fulfilled every two weeks. For the experimental and control groups the initial masses were 86.5±2.54 g and 87.5±2.44 g, respectively, and the final masses were 126.8±3.15 g and 124.1±2.33 g, respectively. Nevertheless, no difference was found out between the groups (P > 0.05).

Key Words: *Sparus aurata*, enzyme treatment, Allzyme Vegpro.

Introduction

Aquaculture production of gilthead sea bream has increased over the last years (Sitja-Bobadilla et al. 2003). Besides, this fish is a major aquaculture species in the Mediterranean (Gomez-Requeni et al. 2003) and Europe (Sarropoulou et al. 2005). Studies being done an over-cultured species are significant matters. This is the reason why gilthead sea bream was chosen as a material.

The major ingredient used in gilthead sea bream feed is fish meal. Total fish meal production is 6548000 tons of which 32% used by aquaculture sector in 1992 in the world. Considering the growth in the aquaculture in the last decade, the consumption of fish meal seems to be increased (Hardy 2000). Nevertheless, unlike the fast increase observed in aquaculture worldwide, there has been no increase in the production of fish meal. In addition, the availability of fish mail for aqua-feeds has turned in to a constraint factor (Sitja-Bobadilla et al. 2003). Unfortunately, growth of aquaculture can not be supported by fish-meal-based diets (Gomez-Requeni et al. 2003).

To support aquaculture production, the alternative feed ingredients are to be used (Sanchez-Muros et al. 2003, Nengas et al. 1999, Sitja-Bobadilla et al. 2005, Lanari et al. 1998, FAO 2002). Research in aquaculture nutrition is being directed towards the improvement of feeding schedule, promoting as well the partial replacement of fish meal by plant protein and oils (Sitja-Bobadilla et al. 2003, Fournier et al. 2003). The suitable one, among feed ingredients, is soybean meal (Akiyama 1991). In spite of high nutritional value, antinutritional factors contained in soybean meal restrict of use of this raw material (Deguara et al. 1999).

What needs to be done is that the existing materials are to be used as much as possible and loss is to be minimized. Important way to reduce nutrient wastage is the use of optimally balanced diets and using improved feeding practices at the farm level (Lanari et al. 1998). The supplementation of various enzyme mixtures in feed (FAO 2002) and fermentation (Refstie et al. 2005) are potential ways of increasing to facilitate nutritional substance.

This study was carried out for the purpose of finding out the effects of supplements, bacterial enzyme mixture (Allzyme Vegpro), added to feed intensified with soy bean meal, on the performance of growth rate of sea bream and the composition of its flesh.

Materials and Methods

The gilthead sea bream, each of which is average 86.94±1.77g, was provided from Mediterranean Aquaculture Research, Production and Education Institute.

Experimental Diets

Feed used in this study was prepared in Mediterranean Aquaculture Research, Production and Education Institute. Bacterial enzyme mixture (Allzyme Vegpro), containing α-Galaktosidase, amylase, cellulase, protease and xylanase,

was used as a feed supplement. The formulations of mixtures used in the experiment were prepared according to Oliva-Teles (2000). The contents of nutritional values and formulations of feeds prepared were given table 1.

In preparation of two mixtures (control and experimental) which were containing approximate 47% crude protein with the same formulations, one (experimental) was added 2‰ enzyme mixture, and then each mixture was pelleted without any heating process was carried out. These pelleted diets with 4 mm diameter were dried in the open air for 24 hours. Diets were stored in the dark in a dry cool room at a temperature of +5˚C (Kiesseling et al. 2005).

Whole experiments were carried out in Mediterranean Aquaculture Research, Production and Education Institute.
Four tanks each of which had 2.5m^3 capacities were used. Every tank was given equal amount of water, 35 l/min, during the study.

Fish were randomly divided into two groups which have two replicate. Each replicate had 55 fish.

Through the study natural water conditions and sun light were maintained in the tanks. More over, water parameters were recorded at every other week. Besides, ammonia, nitrite and pH were found out as 0.041±0.027 mg/l, 0.001±0.0003 mg/l, and 7.5±0.17, respectively. Daily measured salinity was 30.93±0.83 ‰ with respect to variations of water temperature 20.61±2.92˚C. The level of dissolved oxygen was supplied 7.98±0.23 mg/l throughout the study.

Table 1. Formulations and Composition of Feeds Prepared.

Ingredients	Experimental Diet (With enzyme mix.) (g/kg)	Control Diet (Without enzyme mix.) (g/kg)
Fish Meal (68% CP)	250	250
Soybean Meal (45% CP)	400	400
F.F. Soybean Meal (35% CP)	92.5	92.5
Blood Meal (93% CP)	50	50
Bonkalit (12.5% CP)	92.5	92.5
Fish Oil (Sardine oil)	75	75
Vitamin mixture [a]	20	20
Mineral mixture [b]	10	10
Vitamin C	3	3
Binder (Cellulose)	4	4
Antioxidant	3	3
Enzyme mixture	2	0
Composition		
Dry Matter (DM) (%)	88.426[a*]±0.142	88.975[a]±0.705
Crude Protein (% DM)	47.085[a]±0.145	46.909[a]±0.133
Ether Extract (% DM)	12.928[a]±0.189	13.019[a]±0.191
Ash (% DM)	7.997[a]±0.088	7.971[a]±0.016
Crude Fiber(% DM)	7.345[a]±0.129	7.465[a]±0.094

Means followed by the same super script are not significantly different based on a t-test (P < 0.05) in the same line [a] Vitamin mixture was the Abernathy vitamin premix no. 2 [b] Mineral mixture was Rangen trace mineral mix F1.

The study lasted 14 weeks, two weeks of which were the adaptation intervals before the research. Initially, 10 fish were chosen randomly for flesh analysis. 15 fish of every group were measured for body mass (0.1g) and total length (1mm) by their being anaesthetized by immersion in seawater containing 2-phenoxy ethanol (0.3 ml/l) (Fostier et al. 2000) supported with O_2. Besides, in every measurement, each population was being weighed and individuals were counted (Morris et al 2005).

The mortality was recorded daily and body mass was monitored at 14 day intervals. At the end of the study 5 fish were randomly taken from each group for the flesh analysis. There was no feeding on the days when periodical measurements were carried out. Fish was fed *ad libitum* in three times (08:00; 13:00; 18:00) a day.

Growth parameters and feed efficiency were calculated as follows:

Specific Growth Rate (SGR) (%/day) = [(ln W_t-ln W_i)/T]x100 (Thompson et al. 2005),

Where W_t and W_i are the final and initial individual weights of the gilthead sea bream, respectively and T is the length of the culture period in days;

Weight gain (%) = 100[(W_t-W_i)/W_i] (Thompson et al. 2005),

Where W_t and W_i are the final and initial individual weights;

Feed conversion ratio = total diet fed (kg)/total wet weight gain (kg) (Thompson et al. 2005);

Condition factor (CF) was calculated as 100W/L^3 (Imsland et al. 2001);

Where W is the weight of the fish and L the corresponding total length;

Protein efficiency ratio (PER) was calculated as biomass per unit protein consumed (Imsland et al. 2001).

Experimental diets and flesh samples were analyzed by standard method for crude protein (CP), ether extract (EE), dry matter (DM), ash (A) and crude fiber (CFiber) (Lovell 1981, AOAC 1990).

Each group of the study was used for statistical analysis. Data were calculated for percent weight gain, SGR, FCR, PER, CF and flesh composition parameters.

Data were analyzed according to a 2 x 2 factorial design. For this purpose t-test was used to compare differences among individual means at the P = 0.05 level of significance. Averages and standard deviations were calculated by SAS (Software version 8.2; SAS, 1999).

Results

There was no statistical difference between experimental and control groups in initial weights meaning the conditions of the groups were the quite similar.

The growth pattern was prepared according to 2 weeks periodical measurements (Figure 1). Average initial weights were 86.5±2.54 g for experimental groups and 87.5±2.44 g for control groups. Final average weights were 126.80±3.15 g for experimental groups and 124.10±2.33 g for control groups with 46.70% and 41.80% weight gain, respectively. What's more, no significant difference was found out between the average weights of groups (P > 0.05). Throughout the course of the study 5939 g feed was consumed in experimental groups, 5889 g feed in control groups.

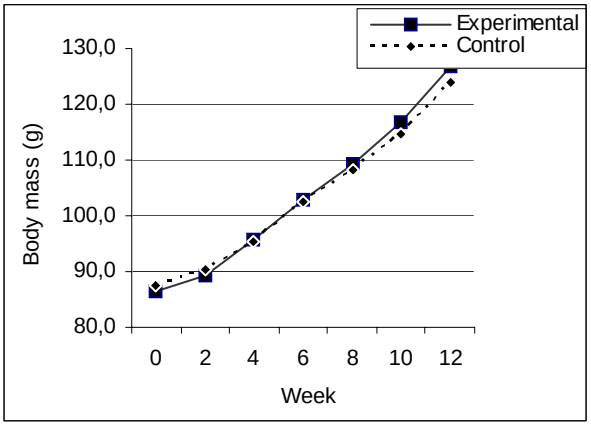

Figure 1. Weight Gain (The chart was prepared by using 2 week periodic measurements)

So, average SGRs of experimental and control groups were 0.46±0.001 and 0.42±0.018, respectively. Nevertheless, there was no statistical significant difference between these groups (P > 0.05). In addition to this, no significant difference about average FCRs (P > 0.05) was found between experimental and control groups with 2.68±0.048 and 2.94±0.307, respectively. PERs were 0.90±0.016 in experimental groups and 0.83±0.085 in control groups, but no statistical significant difference was calculated (P > 0.05). Initial and final CF's; 1.96±0.024, 1.97±0.025 in experimental groups and 1.91±0.014, 2.08±0.022 in control groups, respectively, were found out. Thus, no differences were observed (P > 0.05). Throughout the course of the study, totally 5 fish of which 3 in experimental, 2 in control groups were died. No statistical significant difference was found (P > 0.05). Besides, it was found out that there was no difference in statistical respect (P > 0.05) between the results (Table 2) obtained by the analysis carried out with the flesh samples taken from fish at the beginning and at the end of the study.

Table 2. Results of Flesh Analysis.

Parameters (%)	Initial Flesh	Experimental Flesh	Control Flesh
DM	24.491 ^{a*} ±0.180	27.840 ^{a} ±0.313	26.594 ^{a} ±0.362
CP	20.387 ^{a} ±0.264	21.183 ^{a} ±0.047	20.896 ^{a} ±0.120
EE	0.752 ^{a} ±0.002	2.851 ^{a} ±0.008	2.540 ^{a} ±0.012
A	1.106 ^{a} ±0.021	1.179 ^{a} ±0.007	1.225 ^{a} ±0.076

** Means followed by the same super script are not significantly different based on a t-test (P < 0.05) in the same line*

Discussion

In present study no positive results were gained for gilt head sea bream. This result is in agreement with Divakaran and Velasco (1999). Similar results were reached for sea bass, *D. labrax*, which were fed with feed supplemented digestive enzyme, pancreatin, (Kolkovski et al. 1997). No differences were seen in increases of body masses in a study carried out with gilthead sea bream whose feed was added virginamycine (Zünbülcan 1996) and low pH protease + α-galaktosidase (including 440 g/kg soybean meal) but positive results were gained from low pH protease + α-galaktosidase (including 320 g/kg soybean meal) (Deguara et al. 1999). Positive results were observed for tilapia, *Oreochromis sp*. (added Allzyme-Vegpro) (Ng et al. 2002) and shrimp, *Panaeus monodon* (added Enzyme Mixture-Porzyme) (Buchanan et al. 1997) as well.

In this study SGR was not found out different between control and experimental groups. This result is parallelism with the research which was on feed with low pH active protease + α-galaktosidase with 440 g/kg soybean meal; however, the results achieved from fish fed with high pH active protease + α-galaktosidase (Deguara et al. 1999) do not show similarity with the former one. Even though, the results of our study were in agreement with the results of fish fed with food containing 20% palm kernel meal and enzyme mixture, there is no parallelism between ours and than that of the fish fed with feed containing enzyme mixture and 40% palm kernel meal (Ng et al. 2002). In this study, PER showed no difference between all groups. This result is in harmony with the research of gilthead sea bream feed added low pH active protease + α-galaktosidase (feed with 440 g/kg soybean meal) but it is not in harmony with added high pH active protease + α-galaktosidase (Deguara et al. 1999). Statistically no difference was seen in this study for the average FCRs of the groups. Though, the present results are similar to the ones achieved from feeds with low and high pH active proteases but they are not parallel with the results of high pH active protease + α-galaktosidase supplemented feed which contain 440 g/kg soybean meal (Deguara et al. 1999). It is harmony with Ng et al. (2002). However, in the study on which Buchanan et al. (1997) added enzyme into shrimp feed, they found out adding enzyme positively affects FCR. In the study, averages CFs were not different between groups. Nevertheless, at the end of the study CF of the control group more increased than experimental group. This difference seemed meaningful (P < 0.05). Unlike this result, Deguara et al. (Deguara et al. 1999) found out no difference between the groups in respect of CFs. Besides, considering the dead, there were no significant differences in the study which is parallel with Deguara's (Deguara et al. 1999).

At the end of the study, it was observed that there were no significant differences between average EE, CP, A and DM results of fish flesh (P > 0.05). In a similar way, Zünbülcan (1996) stated that adding virginiamycin into gilthead sea bream feed brought about no changes.

We determinated no considerable differences on weight gain and flesh composition of gilthead sea bream between the groups which were fed with diets with and without enzyme mixture (2‰). Inefficiency of the enzyme addition could be thought that the enzyme mixture did not show the expected affect in examining temperature (20.61±2.92˚C). Because of no differences between examining groups, It is clear that the feed with enzyme mixture is more expensive than the other. According to the results, it seems that there are need to be done more new researches on different enzyme rates and temperatures for this species in the same body mass.

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