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Research Article

Effect of Partial or Total Replacement of Fish Meal by Plant Protein Sources on Productive Performance of Gilthead Sea Bream (*Sparus aurata* L.) Fry under the Water Environment of Qaroun Lake

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ABSTRACT

This experiment was conducted to investigate the effect of partial or total replacement of fish meal by plant protein (PP) sources (0%, 50% and 100% PP) with not amino acids were added in diets on growth performance, survival rate, feed utilization and fish body chemical composition of gilthead Sea bream (*Sparus aurata* L.) fry under the water environment of Qaroun Lake. Survival rate was within the range 65–83.75%. The results cleared that the partial or total replacement of fish meal protein by plant protein had significant effects (P≤0.05) on the growth performance parameters such as final weight, total weight gain, daily gain and specific growth rate. The highest growth performance parameters were obtained with the diet contained 0% of plant protein (100% fish meal protein (FM)) compared with the other diets (50 and 100% PP). Feed intake values were highest with diet containing (100% FM). Also, the best feed conversion ratio (FCR) was recorded with diet containing (100% FM). While, the worst FCR was recorded with diet containing (100% PP). The lowest crude protein and ether extract body content were with diet contained (100% PP). But, the highest body content of crude protein and ether extract were with diet (100% FM), however moisture body content value was highest with diet (100% PP). Accordingly, the improvement of all growth performance parameters in diets containing higher levels of fish meal in Sea bream. And growth performance parameters were decreased with increasing plant protein in diets without not amino acids were added under the experimental conditions.

Keywords: Gilthead Sea bream; Fish meal; Plant protein sources; Growth performance; Feed utilization; Body chemical composition; Survival rate

INTRODUCTION

Gilthead Sea bream (*Sparus aurata*) is one of the most important marine fish species reared in the Mediterranean region. Gilthead Sea bream is a carnivorous species with high protein requirement [1]. As a carnivorous fish, it requires a high level of fish meal in its diets to provide an ideal amino acid profile and reach high digestibility and growth. Despite this, fish meal substitution by plant protein sources in sea bream diets is necessary to maintain the profitability of the farms. Therefore, in recent years a large research effort has been made in this field to reduce fish meal and/or fish oil in aqua feeds by plant sources [2,3]. However, plant protein sources contain certain undigestible components (non-starch polysaccharides) and anti-nutritional factors (protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins and allergens) [4,5]. These compounds can affect nutrient digestibility and absorption, as well as gut integrity, promoting bacteria ingress and, therefore, change the gut microbiota in terms of microbial abundance and species richness [6-9].

Despite these problems associated to vegetable proteins, a successful replacement of total fish meal by a vegetable protein concentrate mixture has been reported [10]. However, alterations in the gut histology of sea bream have been observed with fish meal replacement above a 60% level, as well as immuno suppression above 75% of fish meal substitution [9,11]. An imbalanced microbiota may provoke an alteration of the immune regulatory functions of the gut and contribute to the development of diseases [12].

Marine fish farming in Egypt began in 1976, with the culture of gilthead Sea bream (*Sparus aurata*) as this fish was notably adaptable to brackish and marine pond conditions [13].

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Fish meal (FM) is the dominant ingredient in commercially prepared diets for many fish species. As a consequence of rapid growth in fish and shell-fish farming, FM prices have increased significantly in the past few years and are likely to increase further with continued growth in demand [14]. As with general aquaculture nutrition, a priority area of major research is the reduction and possible elimination of FM and fish oil [15]. The uncertain future of FM availability and its potential high cost has forced to investigate alternative protein sources of good nutritional quality, which are ideally readily available and more cost effective than FM. This will reduce production costs and create a good quality product suitable for any small or large-scale fish production system [16].

Previous studies in gilthead Sea bream have shown that partial replacement of FM by plant protein is possible [17-20]. In a short-term trial (3 months), we also explored the possibility of total and graded replacement of FM by a mixture of plant protein supplemented with indispensable amino acids [21]. On the other hand Santigosa et al., found that final body weight and feed intake decreased progressively and significantly with the increase of dietary plant protein content.

The aim of the present study was, to evaluate the overall effects of partial or total replacement of fish meal protein by plant protein sources (a mixture of corn gluten, soybean meal and yellow corn) without amino acids were added in diets on growth performance, survival rate, feed utilization and fish body composition of gilthead Sea bream fry.

MATERIALS AND METHODS

Fish- rearing conditions

Gilthead Sea bream (*Sparus aurata* L.) fry (0.21 ± 0.02 g, initial body weight) were obtained from a private hatchery in Ismaelia Governorate. Fish were acclimated to laboratory conditions for 14 days before being randomly distributed into concrete ponds of 1 m^3 water capacity, in Shakshok Fish Research Station, El-Fayoum Governorate, National Institute of Oceanography and Fisheries (NIOF), Egypt. All ponds were provided with continuous aeration. The water used in the trial was obtained from Lake Qaroun (Table 1) [22]. About 65% of water ponds were changed twice every day. Water temperature ranged from 28°C to 33°C and water salinity ranged from 30% to 33%. Oxygen concentration ranged from 5.3 to 6.8 mg/l and pH ranged from 7.07 to 7.55. Fish were held under natural photo period condition throughout the experimental period (90 days experimental period).

Running water system

The system contained on water pump, sand filter unit and two large tanks (1000liter/tank) used to storage the water at a point between the water source (Lake Qaroun water) and experimental ponds. The water pump was drowning the water from water source to the sand filter unit, hence to the large tanks and hence to experimental ponds.

Aeration system

The system contained on air pump or blower connected to a network of plastic pipes this pipes transport the air to each pond, the air was controlled by tap of each pond and the air diffusers was used to distribute of air in all experimental ponds trends.

Table 1: Water quality of Lake Qaroun (Saleh, 2016).

Parameters	Value	Parameters	Value
Temperature, °C	18.2	Sulfate (SO_4), mg/l	912.57
pH	8.25	Potassium (K), mg/l	297.4
EC*, mS/cm**	35.9	Phosphorus (P), mg/l	0.1423
Salinity, mg/l	33300	Boron (B), mg/l	2.655
Total suspended solids, mg/l	223	Iron (Fe), mg/l	0.0965
Chloride (Cl), mg/l	11879.1	Lead (Pb), mg/l	0.0025
Calcium (Ca), mg/l	440.88	Nickel (Ni), mg/l	0.0063
Sodium (Na), mg/l	7034.55	Cadmium (Cd), mg/l	0.0041
Magnesium (Mg), mg/l	301	Zinc (Zn), mg/l	0.0517
Carbonate (CO_3), mg/l	24	Manganese (Mn), mg/l	0.0078
Bicarbonate (HCO_3), mg/l	256.24	Copper (Cu), mg/l	0.0087
Microbial count			
Total coliforms, per 100 ml	190	Fecal coliforms, per 100 ml	140
Fecal streptococci, per 100 ml	260		

* EC, Electrical Conductivity
** mS/cm, milli-siemens/centimeter

Experimental design

This experiment was conducted to investigate the effect of partial or total replacement of fish meal by plant protein sources (PP). Fish were fed on three diets where the animal protein was substituted by 0%, 50% and 100% PP with stocking density (60 fry/concrete pond) by using 6 experimental concrete ponds of 1 m^3 water capacity each. During the growth trial each diet was randomly allocated to duplicate concrete ponds of fish.

Diets and feeding

Diets were formulated based on Fish meal (FM) as the only animal protein source and a mixture of corn gluten, yellow corn and soybean meal as plant protein (PP) sources. Linseed oil was added as the major dietary lipid source to the all experimental diets. The diets formulated to be almost containing 45% crude protein (Table 2). No amino acids added to the diets. Diets were hand made. Feed was offered by hand at three meals/day (8:00, 12:00 and 15:00h) at 10% of body weight daily and the amount of diets were readjusted after each weighing (every 15 days). Feed consumption was recorded daily. Initial and final data for growth performance was obtained by sampling all the experimental fish. Prior to weighing, 70 fish were sacrificed for determination of body chemical analysis. At the end of experiment, 20 fish from ponds were randomly taken for the determination of body chemical analysis.

Parameters measurements

At the end of the experiment, growth performance, survival rate and feed utilization were calculated as follows:

- Weight gain (g) = final weight, g - initial weight, g.
- Average daily gain (g) = average weight gain, g/ experimental period, day.
- Specific growth rate (SGR, %) = [(ln final weight -ln initial weight)/ period in days] × 100, where ln is the natural log.
- Feed conversion ratio (FCR) = feed intake, g/ weight gain, g.

- Protein efficiency ratio (PER) = weight gain, g/ protein intake, g.

- Protein productive value (PPV, %) = (retained protein, g/ protein intake, g) × 100.

- Energy efficiency ratio (EER) = weight gain, g/ energy intake, Kcal.

- Energy productive value (EPV, %) = (retained energy, Kcal/ energy intake, Kcal) × 100.

- Survival rate, % = (number of fish at end/ number of fish at start) × 100.

Chemical analysis

Diets used and body composition were analyzed for their proximate composition in triplicates following the methods described by AOAC [23]. Gross energy was calculated according to NRC [24] for formulated diets the factors 5.5, 9.08 and 4.1Kcal/g for protein, fat and carbohydrates, respectively, for fish 5.5 and 9.5Kcal/g for protein and fat, respectively [25].

Statistical analysis

The data were analyzed by one-way ANOVA and significant differences were determined by Duncan Waller Multiple Range Test at 5% level using SPSS Statistical Package Program (SPSS, 2008) 17, released version [26].

RESULTS

Growth performance and survival rate of gilthead Sea bream

Results of growth performance parameters and survival rate of fish fed with the three different diets are shown in Table 3. Acceptance of the diet (0% PP (100% fish meal protein (FM)) was very good. but, acceptance of the diets (50% and 100% PP) was weak.

Table 2: Composition of the diets used in this experiment.

Items	Diets		
Ingredients %	**0% PP**	**50% PP**	**100% PP**
Fish meal, (CP 71%)	64	32	-
Soybean, (CP 44%)	-	20	35
Yellow corn, (CP 10%)	-	14	-
Corn glutin, (CP 60%)	-	21	50
Starch	24	-	-
Calcium phosphat	1	1	1
Vit & Min. *	2	2	2
Linseed oil	9	10	12
Chemical composition		**(as fed, %)**	
Dry matter, DM	88.08	89.85	89.73
Crude protein, CP	45.44	45.52	45.40
Ether extract, EE	15.40	15.61	15.42
Crude fiber, CF	-	4.21	4.97
Ash	7.20	7.44	8.40
Nitrogen free extract. NFE**	20.04	17.07	15.54
GE, kcal/g***	4.719	4.891	4.738

*Vitamins and minerals mixture each 3 kg of mixture contains: 12000 000 IU Vit. A, 2000 000 IU Vit. D_3, 10000 mg Vit. E, 2000 mg Vit. K_3, 1000 mg Vit. B_1, 5000 mg Vit. B_2, 1500 mg Vit. B_6, 10 mg Vit. B_{12}, 50 mg Biotin, 10000 mg Pantothenic acid, 30000 mg Nicotinic acid, 1000 mg Folic acid, 60000 mg Manganese, 50000 mg Zinc, 30000 mg Iron, 10000 mg Copper, 1000 mg Iodine,100 mg Selenium, 100 mg Cobalt, add to 3 kg carrier ($CaCO_3$).
**Calculated by differences.
*** Calculated according to NRC, 1993.

Table 3: Effect of partial or total replacement of fish meal protein by plant protein sources on growth performance and survival rate of gilthead Sea bream.

Items	Diets			SED*
	0% PP	50% PP	100% PP	
Initial weight, g/fish	0.21	0.21	0.21	-
Final weight, g/fish	12.46[a]	1.79[b]	1.45[b]	0.954
Total gain, g/fish	12.25[a]	1.58[b]	1.24[b]	0.954
Daily gain, mg/fish/day	136.11[a]	17.50[b]	13.78[b]	10.609
SGR, %/day	4.54[a]	2.31[b]	2.15[b]	0.358
Survival rate, %	82.00[a]	83.75[a]	65.00[b]	4.514

(a and b) Average in the same row having different superscripts are differ significantly ($P \leq 0.05$).
*SED is the standard error of difference

There was no significant difference in the initial body weight of the fish among treatments. Survival rate was within the range 65-83.75%, with significant differences were observed. The results cleared that the partial or total replacement of fish meal protein by plant protein had significant effects (P ≤0.05) on the growth performance parameters such as final weight, total weight gain, daily gain and SGR. While the highest values were obtained with the diet contained 0% of plant protein (100% FM) compared with the other diets (50% and 100% PP).

Feed utilization of gilthead Sea bream.

As shown in Table 4 The results showed that significant differences (P ≤0.05) were obtained in all feed utilization parameters between treatments except the FCR. Feed intake values were highest with diet containing (100% FM). The best FCR was recorded with diet containing (100% FM). The worst FCR was recorded with diet containing (100% PP). Similar results were obtained for PER and EER, although values obtained for fish fed 0% PP diet were relatively highest than those for 50 and 100% PP diets. PPV and EPV values were highest with diet containing (100% FM).

Fish body composition of gilthead Sea bream

Body chemical composition and energy content of gilthead Sea bream at the beginning and at the end of the experiment are shown in Table 5. The results showed that significant differences (P ≤0.05) were obtained in moisture, crude protein (CP), ether extract (EE) and gross energy (GE) of body composition at the end of the experimental period. However, ash had insignificant differences. The lowest CP and EE content were with diet contained (100% PP). The highest content of CP and EE were with diet (100% FM). However, moisture value was highest with diet (100% PP).

DISCUSSION

Fish meal (FM) supplies the largest portion of dietary protein of carnivorous fish in aquaculture. Alternative protein sources have been studied intensively during the last few decades because of the declining availability and high cost of fish meal [27]. Other protein sources are required to replace FM in finfish carnivorous diets. In this regard, high percentages of FM can be replaced by plant protein (PP) meals without compromising fish growth as long as diets are balanced to match amino acid requirements of the different fish species [28]. However, plant protein (PP) sources are also possibly rich in anti-nutritional factors [29]. Soybean meal (SBM) is one of the most interesting alternatives to fish meal for diets of carnivorous fish, as it has a high protein content, high availability and a competitive price [30]. The nutritive value of SBM to fish is compromised by the presence of anti-nutritional factors such as saponins, phytoestrogens, trypsin inhibitors, phytic acid, and allergens, which may have detrimental effects on growth and nutrient utilization of fish [4]. In addition, SBM is limiting in sulfur-amino acids [31]. Digestibility of corn gluten meal (CGM) is generally high, being 95% for carp [32] and also it has a high protein content.

In the present study, three different diets were animal protein

Table 4: Effect of partial or total replacement of fish meal protein by plant protein sources on feed utilization of gilthead Sea bream.

Items	Diets			SED*
	0% PP	50% PP	100% PP	
Feed intake, g/fish	38.01a	5.99b	4.98b	2.864
FCR	3.11	3.96	4.04	0.33
Protein utilization				
PER	0.72a	0.57b	0.55b	0.047
PPV, %	11.26a	8.39b	6.69b	0.736
Energy utilization				
EER	0.068a	0.052ab	0.043b	0.008
EPV, %	13.63a	7.82b	6.55b	1.138

- (a and b) Average in the same row having different superscripts are differ significantly (P£0.05).

* SED is the standard error of difference

Table 5: Effect of partial or total replacement of fish meal protein by plant protein sources on fish body chemical composition and energy content (% wet weight) of gilt head Sea bream.

Items	Start	Diets			SED*
		0% PP	50% PP	100% PP	
Moisture, %	71.94	67.17^{c}	72.82^{b}	73.76^{a}	0.261
Crude protein (CP), %	12.83	15.66^{a}	14.60^{b}	12.55^{c}	0.285
Ether extract (EE), %	7.06	11.92^{a}	7.13^{c}	8.94^{b}	0.100
Ash, %	6.17	5.19	5.17	4.72	0.261
gross energy (GE), kcal/g	1.376	1.994^{a}	1.481^{b}	1.539^{b}	0.032

-(a, b and c) Average in the same row having different superscripts are differ significantly (P ≤ 0.05).

* SED is the standard error of difference

was substituted by 0%, 50% and 100% plant protein sources (No amino acids added to the diets) were tested for gilthead Sea bream (*Sparus aurata* L.) fry. The highest growth performance parameters such as final weight, total weight gain, daily gain and SGR were obtained with the diet contained 0% of plant protein (100% FM) compared with the other diets (50% and 100% PP). These results led to believe that the fish meal presented in the diet contain a good smell which attract the fish to consume the diet and resulted in improving growth rate as it has better essential amino acid profile and a good source of essential minerals and vitamins [33].

In the present study, growth performance parameters were decreased with increasing plant protein in diets with no amino acids were added. Therefore, amino acids must be added to diets containing plant protein to compensate for essential amino acids in plant protein. In this regard, high percentages of fish meal can be replaced by plant protein meals without compromising fish growth as long as diets are balanced to match amino acid requirements of the different fish species [28]. On the other hand, although amino acid are added to diets, growth decreased gradually with increasing dietary plant protein in diets of Sea bream [9,21].

Estruch et al. mentions that the total fish meal replacement in diets for gilthead sea bream was nutritionally satisfactory and introduced no change in the total microbial diversity or richness in gastrointestinal tract, but altered the gastrointestinal tract microbiota profile at hindgut level, being a gastrointestinal tract section rich in immune cells. There was also an increase in the mortality rate. Further studies will determine if the adverse effect observed, possibly at immune level, was due to vegetable components of the diet or if it was the consequence of the microbial imbalance that they caused or both. Development of new diets with new sources of ingredients, and possibly probiotics, will help in these investigations that constitute the keystone to the development of more efficient, economic and sustainable feeds in aquaculture.

The results of the present study agreed with the results of Abou-Zied et al. who reported that final body weight was progressively decreased with increasing plant protein. The improvement of all growth performance parameters in diets containing higher levels of fish meal in Sea bream. Also, Gomez-Requeni et al; Vega-Rubin et al. and Sitja-Bobadilla et al. [34] who obtained lower significant growth performance parameters in Sea bream fed on plant protein sources diets compared with those fed fish meal diet. This condition was not found in fish fed the 100% PP diet and their reduced growth performance was even exacerbated over the course of time, such trend was mentioned by Sitja-Bobadilla et al. Fish fed diets high in dietary level of plant protein (Soybean meal (SBM)) generally exhibit progressive impairment of growth and increased feed conversion ratios, such trend was mentioned by Krogdahl et al. and Rumsey et al. [35,36]. Sensitivity of gilthead Sea bream to dietary level of plant protein (SBM) inclusion was higher at lower fish weights, such trend was mentioned by Martinez-Llorens et al. [37].

Growth decreased gradually with increasing dietary plant protein. This observation was in line with that of Kissil et al. who reported that there was an inverse relationship between growth and dietary level of plant protein, and growth was reduced in the diet for gilthead sea bream with only 30% soy bean replacement level.

On the other hand, these results disagree with Robaina et al. [38], who showed that there was no observed significant differences in growth where fish fed on plant protein sources diet (Corn gluten meal) compared with those fed fish meal diet. Also, most studies have shown considerable success in partial (40-75%) or total replacement of FM with soybean, such as Atlantic halibut, Atlantic salmon, common carp, rainbow trout and Senegalese sole [39-45].

In the present study; feed intake, PER, PPV, EER and EPV values were highest with diet containing (100% FM (0% PP)). The best FCR was recorded with diet containing (100% FM (0% PP)). The worst FCR was recorded with diet containing (100% PP). This may due to that Sea bream could digest and assimilate the FM protein in their bodies better than plant protein. The higher feed consumption observed for those fish fed on fish meal diet.

These results agreed with the results of Abou-Zied et al. who reported that the improvements in FCR for Sea bream fed higher levels of fish meal. Also, Gomez-Requeni et al., Vega-Rubin et al. and Sitja-Bobadilla et al. who obtained lower significant feed utilization parameters in Sea bream fed on plant protein sources diet compared with those fed fish meal diet.

A reduction in diet acceptability was observed in this study when fish were fed diets with high FM replacement level by plant protein. The reduction in diet palatability usually results in a decrease in feed intake, which could in turn cause reduced growth [17,40]. Diet palatability differences could have been resulted from the removal of more palatable factors (e.g. FM) replaced by plant protein [17].

The higher feed consumption observed for those fish fed higher levels of FM, Such trend was showed by Gomez-Requeni et al., Sitja-Bobadilla et al. and Vega-Rubin et al. On the other hand, the result disagreed with data obtained by Robaina et al. who reported that the higher feed consumption observed for those fish fed higher levels of Corn gluten meal compared with those fed fish meal diet and Kokou et al. who showed feed consumption increased with increasing bio processed soy product inclusion [46].

The higher feed intake observed in Sea bream fed diet higher levels of FM with respect to those fed diet based on levels of plant protein sources, differs from data obtained by Robaina et al, who did not find any difference in feed intake of Sea bream fed diets with different levels of plant protein sources through 2 months. The lower feed intake with highest diet PP could be due to lower palatability of this feed as also suggested by the data of Pereira and Oliva-Teles [18]. The Similar obtained results for PER agreed with the results of Robaina et al. who showed similar results for PER at feeding Sea bream fed on plant protein sources diets.

In the present study, the lowest CP and EE body content were with diet contained (100% PP). The highest body content of CP and EE were with diet (100% FM (0% PP)), however moisture value was highest with diet (100% PP). These results agreed with the results of Gomez-Requeni et al. who obtained significant differences with body chemical composition in Sea bream fed on plant protein sources diets compared with those fed fish meal diet, differs from data obtained by Kokou et al., De Francesco et al., Venou et al.; Robaina et al. and Nengas et al. who did not find any difference in body chemical composition in Sea bream fed diets with different levels of plant protein sources [47,48].

CONCLUSION

From the results of the present study, growth performance parameters were decreased with increasing plant protein in diets without not amino acids were added. The higher feed consumption observed for those fish fed higher levels of fish meal. The best feed conversion ratio was recorded with diet containing (100% fish meal). The worst feed conversion ratio was recorded with diet containing (100% plant protein). The improvement of all growth performance parameters in diets containing higher levels of fish meal in Sea bream under the experimental conditions. Therefore, amino acids must be added to diets containing plant protein to compensate for essential amino acids in plant protein.

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