6(1): 63-73 (2012) DOI: 10.3153/jfscom.2012008

Journal of FisheriesSciences.com

E-ISSN 1307-234X

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

UTILIZATION OF CORN GLUTEN MEAL AS A PROTEIN SOURCE IN DIETS FOR GILTHEAD SEA BREAM (*Sparus aurata* L.) JUVENILES

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Abstract: The utilization of corn gluten meal (CGM) was evaluated as a partial fish meal (FM) substitute in practical diets for gilthead sea bream juveniles. Four test diets (isonitrogenous and isocaloric, 52% protein and 10% lipid, 19 kJ/g diet) containing increasing levels of CGM were formulated to replace anchovy meal at levels of 0%, 10%, 20%, and 30%. Triplicate groups of juvenile sea bream (initial body weight of 1.5 g) were reared in a Recirculating Aquaculture System (RAS) over 45 days at 18±2°C. Fish fed a diet containing 10% of CGM showed comparable growth performance similar to the control diet containing FM as the sole protein source. No mortality was observed in all treatment groups. Dietary CGM inclusion levels of 20% and 30% showed lower growth performance, feed utilization, and protein efficiency compared to the control and the 10% CGM inclusion diets. However these values were not significantly different among fish fed the CGM10 and CGM20 diets. Economical analyses also confirmed the growth related experimental results in terms of best profit obtained with the 10% CGM inclusion diet. Results in the present study showed that CGM alone without any amino acid supplements can substitute FM up to 10% with no adverse effects on growth performance, feed utilization, or economical inputs in gilthead sea bream juveniles.

Keywords: Corn gluten meal, Gilthead Sea Bream, Growth, Feed utilization

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Özet:

Çipura (*Sparus aurata* L.) Yavru Yemlerinde Mısır Gluten Ununun Protein Kaynağı olarak Kullanımı

Bu çalışmada çipura yemlerinde balık ununun bir kısmı yerine mısır glüten ununun (CGM) kullanılabilirliği incelenmiştir. Araştırmada hamsi unu, artan miktarlarda (0%, 10%, 20% ve 30%) mısır glüten unu ile ikame edilerek hazırlanan dört farklı deneysel yemlerle (izo-nitrojenik ve izo-kalorik, %52 protein ve %10 yağ, 19 kJ/g yem) yavru çipura balıkları (ortalama ağırlık 1,5 g) günde 2 kez olmak üzere beslenmişlerdir. Üç tekerrürlü olarak 45 gün süreyle gerçekleştirilen çalışma, Kapalı Devre Üretim (RAS) sisteminde ve 18±2 °C'lik su ortamında yürütülmüştür. Deneme grupları arasında %10 CGM içeren yem grubunda elde edilen büyüme performansı değerlendirildiğinde, %100 balık unu (FM) içeren kontrol yem grubu ile benzerlik gösterdiği belirlenmiştir. Deneme süresince tüm gruplarda yaşam oranı %100 olarak kaydedilmiştir. Yemdeki CGM ilavesi %20 ve %30 olan deneme gruplarında elde edilen büyüme performansı ile yem verimliliği ve protein verimliliği değerlerinin kontrol grubunda ve %10 CGM grubunda elde edilen verilere göre daha düşük olduğu belirlenmiştir. Ancak, bu farklılıklar CGM10 ve CGM20 grupları arasında istatistiksel yönden önemsiz olarak kaydedilmiştir. Büyüme performansına ilişkin ekonomik verimlilik analizlerine göre elde edilen sonuçlar da deneme grupları arasında en büyük kazancın ve karlılık oranının %10 CGM içeren yem grubunda elde edildiği anlaşılmıştır. Bu çalışmada elde edilen sonuçlara göre, herhangi bir amino asit desteği olmaksızın, sadece mısır glüten unu kullanımının, yavru çipura balıklarında büyüme performansı, yem verimliliği veya ekonomik indeksler açısından herhangi bir olumsuz etki göstermeyeceği sonucuna varılmıştır.

Anahtar Kelimeler: Mısır glüten unu, Çipura, Büyüme, Yem verimliliği

Introduction

Fish meal, the main protein source in aquaculture diets, is the most important ingredient due to its high protein quality (Yigit et al., 2006). Considering that fish meal is the most expensive ingredient in fish diets, the increasing demand and instability of supply of this product forces feed manufacturers to reduce fish meal in the diets and use less expensive animal or plant protein sources as partial or total replacements for fishmeal. It has been reported that the global aquaculture demand for fishmeal was 32% of the world supply level in 1999 (New and Wijkstöm, 2002), 37% in 2000 (Chamberlain, 2000) and it is estimated that this demand may reach nearly 70% by 2015 (New and Wijkstöm, 2002). If this trend continues to increase, soon the entire global fishmeal production might be used by the aquaculture industry alone. This trend could also potentially reduce the profitability of fish culture as feed typically accounts for 35-60% of the production costs and moreover, the protein sources, where fishmeal is the most significant dietary ingredient, account for about 50% of the total diet cost (Higgs, 1997). The sustainability of the growing aquaculture industry depends on the progressive reduction of wild fish catch as protein source for aquaculture diets (Naylor, et al., 2000). In this perspective, aquaculture nutritionists have been attracted to investigate the possible reduction of dietary fishmeal and produce cost-effective, nutritionally balanced, and environment friendly diets with alternative protein sources, supporting the sustainability of intensive aquaculture industry. However, due to the high protein requirements of carnivorous marine fishes, alternative protein sources such as animal or plant proteins are so far restricted to a few ingredients with high-protein content, high digestibility and readily acceptable by the fish (Sargent and Tacon, 1999). One of the main problems in utilization of plant protein sources are the unbalanced amino acid composition of the ingredient, and the anti-nutritional factors in most plant proteins (Krogdahl et al., 2003). For example corn gluten is known to be low in lysine or arginine, whereas soybean, the most commonly used plant protein source in aquaculture diets, is low in lysine and methionine, compared to fish meal sources and the requirement levels of fish (Halver, 1991; NRC, 1993). Hence, the replacement of fish meal with a mixture of several plant protein sources is a common approach in order to minimize the amino acid deficiencies in a formulated fish diet and meet the requirement levels of amino acid in fish species (De Francesco et al., 2007). Several studies are available on the replacement of fish meal by CGM in diets for rainbow trout (Gomes et al., 1995), yellowtail (Shimeno et al., 1993b), European sea bass

(Ballestrazzi et al., 1994); Japanese flounder (Kikuchi, 1999); turbot (Regost et al., 1999; tilapia (Pereira and Oliva-Teles, 2003; Wu et al., 1995) and gilthead sea bream (Robaina et al., 1997; Ebiary, 2005).

It is well known that the utilization of plant protein sources is species or size specific in fish. The aim of this study was to evaluate the possibility of replacing a partial portion of fish meal in the diet for gilthead sea bream at early juvenile stage, right before or after their transfer to cage farms, using practical ingredients readily available to the aquaculture feed industry and to evaluate possible effects on growth performance, feed utilization, and economical inputs in early juvenile fish.

Materials and Methods

Experimental diets

Four experimental diets, formulated from commercially available ingredients were produced in the Fish Nutrition Laboratory on the Dardanos Campus of the Faculty of Fisheries at Canakkale Onsekiz Mart University. The diets were isonitrogenous and isocaloric with 52 % crude protein and 10 % lipid (19 kJ gross energy per g diet) (Table 1). In the control diet, brown fishmeal (anchovy meal) was the sole protein source. In the other three diets, fishmeal was replaced by CGM at levels of 10%, 20%, or 30%. Amino acid profiles of the diets are given in Table 1; the proximate composition and amino acid profiles of the protein sources and gilthead sea bream are shown in Table 2; total n-3 HUFA contents of the diets, calculated according to Yigit et al. (2006) as follows are given in Table 3;

Total n-3 HUFA contents= (total fish oil in g/kg diet) x (% n-3 HUFA in fish oil)

Dry ingredients and oil were mixed in a food mixer for 15 min. Tap water was blended into the mixture to attain a consistency suitable for passing through a meat grinder with a 2-mm hole die. After pelleting, the diets were dried to a moisture content of 8-10% and stored at -20°C until use.

Growth trial and rearing conditions

Hatchery reared gilthead sea bream, *Sparus aurata*, were obtained from a commercial marine hatchery (IDA Gida Co.) in Canakkale, Turkey, and transported to the facilities of the Faculty of Fisheries of Canakkale Onsekiz Mart University in Canakkale, Turkey. After acclimation to the new environment, a total of 480 fish (1.53 ± 0.086 g initial mean weight) were randomly stocked into 12 identical 40-l rectangular polypropylene blue colored tanks (40 fish per tank, three replicate tanks per treatment). The system was an indoor Recirculation Aquaculture System (RAS) run with sea water of 30.4 ± 1.3 g/l salinity. Continuous aeration was provided by airstones. Fish were exposed to the natural light regime (40° 4'29.99" N, 26°21'35.58" E). The tanks were cleaned daily to remove uneaten feed and fecal material. Water quality was controlled periodically: pH ranged 7.5-8, total ammonia nitrogen, determined by the Nessler method using a HANNA C200 portable spectrophotometer (HANNA Instruments, Co., Italy), ranged 0.24-0.29 mg/l, ambient water temperature ranged from 14.6 to 22.6 °C (18.2 ± 2.1°C) during the course of the study. Fish were hand fed twice daily at 09:00 and 17:00 for 45 days, from August to October 2010. Feeding activity was monitored carefully to ensure an even distribution of the feed to all fish in each tank. Fish were individually weighed at the start and end of the experiment, while group weighed on days 15, and 30. Fish were deprived of feed for one day prior to weighing.

Analytical methods

Experimental diets were chemically analyzed according to AOAC (1984) guidelines as follows: dry matter, by drying in an oven at 105°C for 24 h until a constant weight was obtained; protein (N x 6.25), by the Kjeldahl method after acid digestion; lipids, by ethyl ether extraction in a Soxhlet System; ash, by incineration in a muffle furnace at 550°C for 12 h; nitrogen free extracts, as the difference between total dry matter and (crude lipid + crude ash + crude protein).

Calculations

Following calculations on growth performance and feed utilization data were performed as described by Yigit et al. (2006);

Feed conversion rate (FCR) was calculated from the amount of feed consumed (dry matter) and the total biomass gained;

FCR = feed consumed / (weight gain + weight of dead fish)

Relative growth rate was calculated as the increased biomass in percent of the initial biomass;

RGR = [(final wet weight – initial wet weight) / initial wet weight] x 100

Specific growth rate (SGR) was calculated as percent increase of body weight per day;

SGR = [(ln final wet weight - ln initial wet weight) / days] x 100

Protein efficiency ratio (PER) was calculated as weight gain for each unit weigth of protein consumed;

PER = wet weight gain / protein intake

Daily feed intake was calculated as air dry feed consumed per fish per day;

DFI = (air dry feed intake/number of fish) / days

Likewise the daily protein and energy intake values were calculated as;

DPI = (feed intake x crude protein in diet / 100) / days

DEI = (feed intake x energy in diet / 100) / days

Statistical analysis

Results were analyzed by analysis of variance (ANOVA) using the PASW Statistical Analysis Software Program for Windows, Version 18.0.0, 2009, for significant differences among treatment means. Tukey test was used to detect significant differences ($p<0.05$) in growth performance data, feed intake, feed conversion rate, protein efficiency rate, and bio-economical data.

Results and Discussion

At the end of the growth trial, survival was 100% in all experimental groups, showing that partial replacement of FM by CGM in diets did not affect survival rates of fish. No significant difference ($p>0.05$) was recorded for final body weight (FBM) and for relative growth rate (RGR) among experimental fish fed the FM100 and CGM10 diets, which had the best growth rates (Table 4). Sea bream juveniles fed the CGM20 diet demonstrated a slightly lower FBW and RGR and the values were not significantly different from fish fed on CGM10 diet. When FM was further replaced by CGM, growth performance of fish showed significant reduction ($p<0.05$) in growth performance. Specific growth rate (SGR) followed the same trend, being best for the FM100 treatment group and poorest for CGM30, with significant similarity ($p<0.05$) between the FM100 and CGM10, the CGM10 and CGM20 and the CGM20 and CGM30 groups. Best feed conversion rate (FCR) values were found for fish fed diets containing FM only, and fish fed the 10% CGM has a GCR not significantly different ($p>0.05$) from those fed the FM based diet. Values obtained for the protein efficiency rate (PER) was clearly higher for fish fed the FM100 and the CGM10 diets and showed a significant decrease ($p<0.05$) as fish meal was gradually replaced by CGM. No statistical differences ($p<0.05$) were found in daily feed intake, daily protein or energy intake per fish among experimental treatments (Table 4).

Bio-economic analysis carried out for the experimental diets showed that the Profit of the diets with no CGM inclusion (control) and the 10% CGM inclusion were significantly better than the other diets. Profit value of the 10% diet was not statistically different than the 20% CGM diet ($p<0.05$), but significantly different than the 30% CGM diet. The same relation was seen among experimental treatment groups when the feed cost values were calculated as percent of profit (Table 5). Hence, the profit from the treatment groups fed on 100% fish meal and the 10% CGM inclusion diets were significantly better than the other groups.

Growth performance obtained in the present study, were excellent for juvenile sea bream fed FM based and 10% CGM diets, however, a gradient decline was seen when dietary CGM increased up to 30% incorporation level, which is in agreement with other studies in terms of inverse relationship between fish growth and dietary level of CGM sources (Regost et al., 1999; Wu et al., 1995; Ebiary, 2005; Albrektsen et al., 2006). Daily feed intake, as well as daily protein or energy intake data were similar in all treatment groups, indicating that there were no palatability problems in the present study as fish in all groups readily accepted the experimental diets. Hence, the lower growth rates in the groups fed diets with higher levels of CGM over 10%, lead to increased FCRs, which can be attributed to the poorer utilization of the test diets with higher CGM inclusion over 10% under the conditions applied in this study.

Table 1. Ingredients and nutrient composition of diets used in the experiment.

Ingredients (g/100 g)		Replacement level (%)			
		FM100	CGM10	CGM20	CGM30
Fish meal (FM)		79.4	72.56	65.33	58.06
CGM		0.00	10.0	20.0	30.0
Fish oil		4.41	4.66	4.95	5.24
b-Corn starch		13.19	9.78	6.45	3.14
Vit.-Min. Premix[a]		2.8	2.8	2.8	2.8
Binder (Guar-Gum)		0.2	0.2	0.2	0.2
Proximate composition (g/100g air dry basis)					
Dry matter		91.12	91.19	91.23	91.11
Crude Lipid		10.52	10.52	10.52	10.52
Crude Ash		14.61	13.67	12.66	11.64
Crude Protein		52.32	52.32	52.32	52.32
Nitrogen free extracts[b]		13.34	14.07	14.83	15.43
Gross energy (kJ g^{-1} diet)[c]		18.82	18.94	19.07	19.18
P:E (mg/kJ)		27.80	27.62	27.43	27.29
PE:GE		0.66	0.65	0.65	0.65
Amino acid content[d]					
	Sea bream requirement in diet				
Arg (% DM)	2.39	3.26	3.14	2.99	2.85
Lys	2.50	4.36	4.07	3.76	3.45
His	0.83	1.40	1.39	1.37	1.35
Ile	1.35	2.68	2.71	2.71	2.72
Leu	2.24	4.31	4.78	5.22	5.66
Val	1.51	3.03	3.01	2.97	2.94
Met+Cys	1.20	2.24	2.22	2.19	2.17
Phe+Tyr	1.35	4.34	4.40	4.43	4.46
Thr	1.40	2.38	2.33	2.27	2.20
Trp	0.31	0.65	0.62	0.58	0.54

[a] Kadai, Riken Vitamin, Tokyo, Japan.

[b] Calculated by difference

[c] Calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, 17 kJ g^{-1} nitrogen free extract.

[d] Essential amino acid contents calculated from data in Table 2.

Table 2. Proximate analyses of the ingredients used and total essential amino acid composition of ingredients as compared to the requirements of sea bream

		Fish meal	Corngluten meal
Proximate analyses (%)			
Moisture		8.0	11.0
Protein		65.9	45.0
Lipid		7.7	2.7
Ash		18.4	3.2
Essential amino acid (%)*			
	Sea bream requirements		
Arg	4.60	4.11	1.54
Lys	4.80	5.49	0.88
His	1.60	1.76	1.10
Ile	2.60	3.38	2.53
Leu	4.30	5.43	8.35
Val	2.90	3.81	2.42
Met	N/A	2.16	1.10
Cys	N/A	0.66	0.66
Met+Cys	2.30	2.82	1.76
Phe	N/A	3.03	3.19
Tyr	N/A	2.44	1.10
Phe+Tyr	2.60	5.47	4.29
Thr	2.70	3.00	1.54
Trp	0.60	0.82	0.22

N/A = not available

* Data on amino acid content of fishmeal and corn gluten meal are from Halver (1991) and of sea bream from Kaushik (1998).

Table 3. Estimated n-3 HUFA content in the experimental diets.

	Replacement level (%)			
	FM100	CGM10	CGM20	CGM30
Brown fishmeal in diet (%)	79.4	72.6	65.3	58.1
Crude fat (%)	7.70	7.70	7.70	7.70
Fat from fishmeal (%)	6.11	5.59	5.03	4.47
Fish oil in diet (%)	4.41	4.66	4.95	5.24
Total fish oil in diet (%)	10.52	10.25	9.98	9.71
n-3 HUFA in fish oil (%)[a]	29.76	29.76	29.76	29.76
Total n-3 HUFA in diet (%)	3.13	3.05	2.97	2.89
n-3 HUFA requirement of Sea bream[b]	0.9[b]			

[a] According to Güner et al. (1998).
[b]According to Kalegeropoulos et al. (1992)

Furthermore, the low utilization of higher CGM levels in the diets could be also due to the quality of raw material as for example the particle size of the CGM used in this study was in some degree high. The poor growth performance of sea bream juveniles fed diets with higher levels of CGM were possibly because of the low biological value of the CGM source used here, which is far too rich in leucine and marginal in lysine, arginine, and tryptophan when compared to FM in the present study. This also might be a possible reason that caused lower feed utilization. In contrast, some studies have shown considerable success in partial replacement of FM with CGM at levels of 12-26% in diets for trout (Alexis et al., 1985; Moyano et al., 1992), 20% for sea bass (Alliot et al., 1979), 40% for Japanese flounder (Kikuchi, 1999), 20% for turbot (Regost et al., 1999), and 60% for sea bream (Pereira and Oliva-Teles, 2003). It is important to note that crude protein content of CGM used in the present study was 45%, which is much lower than those used by Regost et al. (1999) (85.4%) and Pereira and Oliva-Teles (2003) (66.4%) crude protein, respectively, in diets for sea bream. It is noted that higher incorporation levels might be achieved by using ingredients with high-protein content (Pereira and Oliva-Teles, 2003). For example, several grain legumes have also been tested as alternative protein sources for fish, but their relatively low-protein content limited the incorporation level to 20-30% of the dietary protein (Carter and Hauler, 2000; Gouveia and Davies, 2000).

The discrepancy between the findings in the present study and those of previous ones in terms of the effect of CGM on growth performance and feed utilization might be attributed to several factors, namely; differences in diet composition, protein level and quality of the alternative plant protein source used, culture conditions such as water quality, temperature and salinity fluctuations, fish size or species, or a combination of these factors. In the present study, conducted for 45 days of feeding trial, there were no significant differences between experimental groups during the first 30 days of the experiment, however, visible changes in terms of growth appeared from day 30 onwards and significant differences have been recorded on day 45. Considering the growth trend of treatment groups in the present study, it can be deducted that more effective results could have been obtained when the experiment would have continued after 45 days of trial, which also could be a one of the reasons for the lower utilization of CGM in this study compared to those in the previous ones. Overall, our results in terms of growth performance and feed utilization data of sea bream fed increasing levels of CGM are comparable to those of previous studies on sea bream nutrition (Robaina et al., 1997; Fournier et al., 2002; Pereira and Oliva-Teles, 2003; Pereira and Oliva-Teles, 2002; De Francesco et al., 2007).

In general, CGM is known to have an appropriate amino acid balance for marine fish species, however, amino acid contents of the test diets in the present study, particularly lysine and arginine were lower than those used in previous studies mentioned above. Furthermore, some of the previous studies (Davies et al., 1997; Kikuchi, 1999; Regost et al., 1999; Cheng et al., 2003; Fournier et al., 2004; Albrektsen et al., 2006; De Francesco et al., 2007) have supplemented the experimental diets with amino acids (mainly lysine and arginine), which may have contributed to an increased replacement level. In this study, however, diets were not fortified with amino acids, and also the crude protein content of CGM was lower than the previous studies, which also might have affected reduced growth performance of fish in the present study.

Even though the experimental diets in the present study were not fortified with essential amino acids, the required levels of amino acid for sea bream were provided by the experimental diets and met the requirements of sea bream reported by Kaushik (1998). However, there were still imbalances of AA contents among each test diet. Nevertheless, the imbalances in the amino acid composition between experimental groups were explicit. For example, the 20% and 30% CGM diets contained 14% and 20% less lysine, respectively than the control diet. Lysine is generally considered the first limiting amino acid in most fish species (Robaina et al. 1997), and both lysine and arginine are the two main limiting amino acids in CGM for aquaculture feeds (Amerio et al., 1998), which was reflected in the amino acid profile of the experimental diets with increasing levels of CGM. In the present study, besides lysine, arginine content in the 20% and 30% CGM diets were also less (8% and 13%, respectively) than the control diet. Therefore, the 22 to 26% lower mean final weights in the 20% or 30% CGM groups could be attributed to the lower lysine or arginine contents of the test diets compared to the control group.

All the experimental diets met the essential fatty acid requirements of sea bream. However, due to the lower amount of anchovy oil and lipids provided by the lower amount of fishmeal, the experimental diets contained less n-3 HUFA than the control. The 5% to 8% differences of n-3 HUFA content in the 20% and 30% CGM diets, respectively, compared to the reference diet could explain the differences in growth performance. Furthermore, it is well known that the quality of plant protein sources can be improved by thermal treatment and solvent-extraction (Burel et al., 2000). Based on the estimated n-3 HUFA and amino acid contents in the test diets, the poorer growth of fish fed over 10% replacement diets could be attributed to the variation of HUFA or amino acids among treatment groups, as well as to processing conditions, quality of the ingredients, poor digestibility or palatability, or to the combination of these factors.

Table 4. Growth performance and feed utilization of sea bream juveniles fed test diets during the course of the study (means ± SD for triplicate groups).

	Replacement level (%)			
	FM100	CGM10	CGM20	CGM30
Iinitial body wt.(g)	1.58 ± 0.07[a]	1.48 ± 0.16[a]	1.53 ± 0.04[a]	1.54 ± 0.04[a]
Final body wt.(g)	6.97 ± 0.44[c]	5.99 ± 0.40[b]	5.45 ± 0.23[ab]	5.15 ± 0.08[a]
Relative growth rate(%)	343.3 ± 46.4[c]	306.7 ± 15.6[bc]	257.1 ± 7.2[ab]	233.8 ± 4.9[a]
Specific growth rate(%/day)	3.30 ± 0.24[c]	3.12 ± 0.08[bc]	2.83 ± 0.04[ab]	2.68 ± 0.03[a]
Daily feed intake (g)	0.133 ± 0.00[a]	0.125 ± 0.00[a]	0.125 ± 0.01[a]	0.127 ± 0.00[a]
Daily protein intake (g)	0.069 ± 0.01[a]	0.065 ± 0.01[a]	0.066 ± 0.03[a]	0.066 ± 0.01[a]
Daily energy intake (kJ)	2.50 ± 0.03[a]	2.39 ± 0.04[a]	2.39 ± 0.10[a]	2.40 ± 0.02[a]
Feed conversion rate	1.22 ± 0.14[a]	1.37 ± 0.07[ab]	1.58 ± 0.07[bc]	1.74 ± 0.04[c]
Protein efficiency rate	1.73 ± 0.18[c]	1.54 ± 0.08[bc]	1.33 ± 0.06[ab]	1.21 ± 0.03[a]
Survival rate (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

Values (mean ± standard deviation of data for triplicate groups) with different superscripts in the same row are significantly different at 5 % level.

Table 5. Bio-economical analyses of sea bream juveniles fed different levels of dietary corn gluten meal during the course of the study (means ± SD for triplicate groups).

	Replacement level (%)			
	FM100	CGM10	CGM20	CGM30
Feed supply (kg/fish)	0.26±0.00[a]	0.25±0.00[a]	0.25±0.01[a]	0.25±0.00[a]
Mean weight gain (kg)	0.22±0.02[c]	0.18±0.01[b]	0.16±0.01[ab]	0.14±0.00[a]
Feeding cost ($/kg)	0.34±0.01[b]	0.32±0.01[a]	0.32±0.01[ab]	0.33±0.00[ab]
Gross income ($/fish)	1.12±0.11[c]	0.94±0.05[b]	0.82±0.04[ab]	0.75±0.01[a]
Total initial biomass cost ($)	0.33±0.02[a]	0.31±0.03[a]	0.32±0.01[a]	0.32±0.01[a]
Total final biomass cost ($)	1.45±0.09[c]	1.25±0.08[b]	1.13±0.05[ab]	1.07±0.02[a]
Profit ($/kg)	0.78±0.11[c]	0.62±0.05[bc]	0.50±0.03[ab]	0.43±0.01[a]
Feed cost as % of profit	44.38±7.34[a]	52.04±4.27[ab]	65.18±4.72[bc]	76.74±2.81[c]

Price of feed: 1.3 $/kg; price of fish: 5.2 $/kg; other costs than feed used are ignored and assumed to be same for all experimental groups.
Feeding cost ($/kg)= feed supply (kg/fish) x feed cost ($/kg)
Gross income ($/fish)= mean weight gain (kg) x price of fish ($/kg)
Total initial biomass cost ($)= initial fish weight (kg) x price of fish ($/kg)
Total final biomass cost ($)= final fish weight (kg) x price of fish ($/kg)
Profit ($/kg)= (total final biomass cost-total initial biomass cost) - feeding cost

Economical analyses also confirmed the growth experimental results in terms of best profit obtained with the 10% CGM inclusion diet and the worst with the highest dietary CGM of 30%. The profit from fish fed on 100% fish meal and the 10% CGM inclusion diets demonstrated better results in terms of cost-effective sea bream culture with CGM replacing fish meal in the diet.

Conclusion

As a conclusion, based on the findings in the present study, CGM can be incorporated up to 10% in diets for gilthead sea bream juveniles, with no adverse effects on growth performance, feed utilization or economical inputs, even with both low protein level and low quality raw material, and without any amino acid supplementation. It is suggested to use high quality raw material of CGM with higher protein content when replacing fish meal in aquafeeds. Furthermore, it also seems to be worth investigating the potential of dietary CGM in combination with other plant protein sources as a partial replacement for fish meal in diets for European sea bream in order to avoid any dietary imbalance of the amino acid composition and produce cost-effective and environment friendly feeds for a sustainable future of the aquaculture industry.

Acknowledgements

The financial support by the Scientific Research Commission (BAP-Bilimsel Arastirma Komisyonu, Project No. BAP-2009/43) of Canakkale Onsekiz Mart University, Turkey, is greatfully acknowledged. We would like to thank IDA Gida Co. in Canakkale, Turkey for supporting the experimental animals for this study.

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