Incorporation of corn gluten meal as a replacement for fish meal in the diets of two banded seabream (*Diplodus vulgaris*) juveniles

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ABSTRACT
This study aimed the evaluation of corn gluten meal (CGM) as a fish meal (FM) replacer in diets of two banded seabream, *Diplodus vulgaris,* juveniles. Five isonitrogenous and isocaloric diets were formulated containing %0, %15, %30, %45 and %60 CGM, and two banded seabream juveniles with an average weight of 5.92±0.004 g. were fed. Experimental fish stocked in to 84 L glass aquariums with a stocking density of 20 fish.aquarium^{-1} were hand fed twice a day for a 60 days period. Fish fed on the diet containing %15 and %30 CGM showed the best growth performance compared to the other experimental groups. During the course of the study, no mortality was recorded in any treatment groups. The results of the study showed that CGM, up to %30, can replace FM with no negative effects on growth performance and feed utilization of two banded seabream.

***Keywords*:** *Diplodus vulgaris, Corn gluten, Growth performance, Feed efficiency*

INTRODUCTION
In recent years, the rapid growth trend of the aquaculture industry in Turkey is based on the production of a few fish species, such as gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*). Farmers need to concentrate on alternative species for profitability and sustainability of the aquaculture industry. Two banded seabream (*Diplodus vulgaris*), an important candidate species for aquaculture industry, is an omnivorous sparid fish that easily adapts to aquaculture conditions (Jug-Dujakovic and Glamuzina 1988). There is limited information about the nutrient requirements of this species. It is well known that there is a limitation in marine based protein sources in the world and that reducing fish meal in fish diets may increase the profitability of fish farms and improve the sustainability of aquaculture operations. Considering that around 50% of the annual fish meal production is being used in the aquaculture industry for carnivorous species such as salmon, trout, seabream and sea bass (Hardy 2010), the incorporation of alternative protein sources in the diet by reducing

dietary FM is an important issue for the rational utilization of marine resources and the sustainability of the aquaculture industry.

As the two banded seabream is an omnivorous species, it is likely that it needs less animal based protein in the diet compared to other sparid. Excess dietary level of animal proteins may cause to increased nitrogenous excretion. Due, the use of dietary animal based protein and lipid sources at optimum level will support the aquaculture operations in terms of economic and environmental perspective (Ozório et al. 2009).

From this point of view, researchers in fish nutrition have focused their studies on plant protein sources, as an alternative protein source for fish meal. Corn gluten and soybean meal are considered as the most preferred plant based proteins in the fish diets, due to many advantages (Carter and Havler 2000, Cheng et al. 2013). The fact that plant protein sources contain low amino acid levels and that their digestibility is low in most cases are the main problems for replacing FM in aquafeeds (Krogdahl et al. 2003). However, two banded seabream is an omnivorous species and thus, it does not seem to be a significant problem for two banded seabream.

It is well known that corn gluten meal contains less methionine and lysine compared to fish meal. This deficiency might be overcome by using fish meal as the main protein source and incorporate corn gluten meal at a certain level in the diet. Similar approach has been done in previous studies on European seabass (Ballestrazzi et al. 1994), rainbow trout (Gomez et al. 1995), gilthead seabream (Yigit et al. 2012), and the yellow croaker *Pseudosciaena crocea* (Cheng et al. 2013).

The aim of this study was to investigate the adaptability of two banded seabream juveniles to the aquaculture conditions and the replacement of the fish meal by corn gluten meal in the diets.

MATERIAL AND METHODS

Three-hundred juvenile two-banded seabream (*Diplodus vulgaris*) with a mean weight of 5.92±0.004 g were captured in Dardanos (Çanakkale, Turkey) and kept at the Marine Aquaculture Research Facilities of the Aquaculture Department of the Faculty of Marine Science and Technology at Canakkale Onsekiz Mart University in Turkey. Fish were held in two circular tanks of 750 L. for one month to adapt to the experimental conditions. During the adaptation period, fish were fed to satiety two times a day, 7 days a week with commercial gilthead seabream diets (%49 crude protein; Bioaqua, Turkey). After the adaptation period, 20 fish were individually weighed and distributed into 20 glass aquariums of 84 L each (35 cm x 45 cm x 60 cm) in a recirculation seawater system equipped with aeration and filtration systems. Fish were kept under a constant photoperiod (12 h light-12 h dark). Three replicates per treatment were performed during the course of the study.

Experimental diets and feeding

Five experimental diets were formulated using commercial ingredients (Table 1). The diets were isonitrogenous and isocaloric (%39 protein and %15 lipid) according to (Ozório et al. 2009). The control diet included fishmeal as a main protein source. In the other four diets, fish meal was replaced by corn gluten meal at increasing levels of %15, %30, %45 and %60, respectively. All ingredients were dry weighed and carefully mixed using a laboratory food mixer. Water was added to the mixture to obtain suitable dough of feed. Wet diets were made into 2 mm pellet size and dried at 40 °C in a drying chamber, and finalized diets were stored at -20°C until the start of experiment. Experimental fish were

hand fed twice daily (10:00 h and 17:00 h) until satiation for a 60 days period.

Table 1. Feed ingredients (g.100g^{-1}) of experimental diets

	Control	CGM15	CGM30	CGM45	CGM60
Fish Meal	59.3	47.5	35.8	24	12.2
Corn Gluten Meal	-	12.3	24.5	36.7	49.1
Starch	28.7	27.2	25.5	23.8	22.1
Fish Oil	9	10	11.2	12.5	13.6
Vitamins[1]	1	1	1	1	1
Minerals[2]	0,5	0.5	0.5	0.5	0.5
Binder (Guar-Gum)	1.50	1.5	1.5	1.5	1.5
Proximate composition (g.100 g^{-1} air dry basis)					
Crude Protein	39.9	39.8	39.7	39.5	39.4
Crude Lipid	14.9	15.1	15.4	15.8	16.1
Crude Ash	11.8	9.79	7.75	5.7	3.64
Nitrogen free extracts[3]	30.3	32.4	34.2	36	37.9
GE (kJ.g^{-1} diet)[4]	20.45	20.87	21.27	21.68	22.10
P:E (mg.kJ^{-1})[5]	1.95	1.91	1.87	1.82	1.78

[1]Vitamin Mix: Vit. A, 18000 IU; Vit. D3, 2500 IU ; Vit. E, 250 mg.kg^{-1}; Vit. K3, 12 mg.kg^{-1}; Vit. B1, 25 mg; Vit. B2, 50 mg; Vit. B3, 270 mg; Vit. B6, 20 mg; Vit. B12, 0.06 mg; Vit. C, 200 mg; Folic acid, 10 mg; Calcium d–pantothenate, 50 mg; Biotin, 1 mg; Inositol, 120 mg; Choline chloride, 2000 mg.
[2]Mineral Mix: Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.350 mg; Co, 1 mg.
[3]Nitrogen free extracts = dry matter- (crude lipid + crude ash + crude protein)
[4]Gross Energy calculated using energy values of 23.6 kJ/g for protein, 39.5 kJ.g^{-1} for lipid and 17 kJ.g^{-1} for NFE.
[5]Protein-to-energy ratio in mg.kJ^{-1}.

Calculations

Following calculations on growth performance and feed utilization data were performed):

Feed conversion rate was calculated on the amount of feed consumed (in dry state) and the total biomass gained;

FCR = feed consumed x (weight gain + weight of dead fish) $^{-1}$

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

RGR = [(final wet weight – initial wet weight) x initial wet weight^{-1}] x 100

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

SGR = [(ln final wet weight - ln initial wet weight) . days^{-1}] x 100

RESULTS

During the growth trial, survival was % 100 in all experimental groups. At the end of the study, significant differences ($p<0.05$) were recorded for RGR, SGR and FCR. Table 2.

Two banded seabream juveniles fed on CGM15 and CGM30 diets showed the best growth performance, SGRs and FCRs compared to the other experimental groups. A significant reduction ($p<0.05$) in growth performance was recorded when dietary CGM was increased and FM was decreased. Specific growth rate and feed conversion rate followed the same trend. The second–order polynomial regression between dietary CGM levels and weight gain in each group demonstrated that the most suitable CGM meal level for maximum growth was determined to be % 21.1 replacement level (Figure 1).

DISCUSSION

The two-banded sea bream has a potential as being an alternative commercial marine fish species for the aquaculture industry. Nevertheless, information on the nutritional requirements of this species is scare. This is the first attempt to study on the determination of optimum dietary corn gluten meal levels in two banded sea bream

juveniles. The level of acceptance or rejection by fish is a common problem when plant-based protein sources are used as an alternative feedstuff in fish diets (Rodriguez et al. 1996). In the present study, however, all diets were accepted by fish. Therefore, dietary CGM did not affect the palatability of the test diets as described for other fish species (Moyano et al. 1992, Ergün et al. 2008a, Ergün et al.2008b, Yigit et al. 2010). Growth performance was found very successful for juvenile two banded sea bream fed with %15 and %30 CGM diets, and these results are in agreement with Alexis et al. 1985, Moyano et al. 1992, Wu et al. 1995, Albrektsen et al. 2006 and Yiğit et al. 2012. Earlier studies on different fish species reported successful utilization of dietary CGM levels around %20 for sea bass (Alliot et al. 1979), 12-%26 for trout (Alexis et al. 1985, Moyano et al. 1992), %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 likely that the low lysine and methionine levels in the diets with increasing CGM has led to poor growth performance when compared to those fed on FM based diet. However, Pereira and Oliva-Teles (2003) used corn gluten in sea bream diets at %60 replacement level with a %67 fish meal protein content.

Table 2. Growth performance of *Diplodus vulgaris*

Groups	RGR (%)	SGR (%.day^{-1})	FCR
Control	98.07±4.5^{b}	1.14±0.04^{b}	2.03±0.14^{b}
CGM15	151.7±5.7^{d}	1.54±0.04^{c}	1.36±0.04^{a}
CGM30	126.9±19,2^{c}	1.36±0.14^{c}	1.43±0.04^{a}
CGM40	93.22±10,3^{b}	1.10±0.09^{b}	1.49±0.10^{a}
CGM60	42.66±14,1^{a}	0.59±0.01^{a}	2.96±0.08^{c}

Mean with common superscripts in the same column are not significantly different(p>0.05).

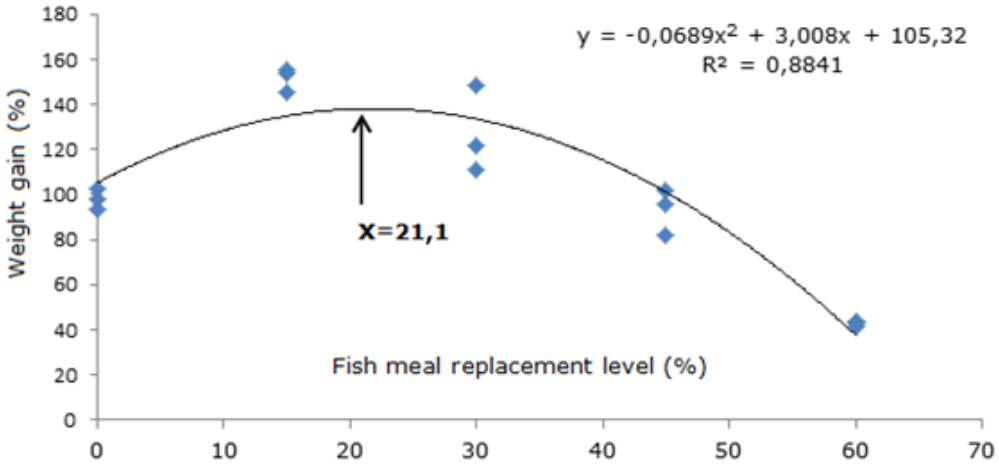

Figure 1. Relationship between weight gain of two banded seabream and dietary fishmeal replacement by corn gluten meal.

According to the results obtained in the present study, it can be concluded that the diets containing CGM, up to %30 level, might be used without any adverse effect on

fish growth performance and feed utilization while higher inclusion levels of CGM has caused negative effects on these parameters. Reduction of the growth rate due to high dietary plant protein levels, in terms of anti-nutritional factors or poor palatability, can be alleviated by heat treatment or addition of enzymes (Francis et al. 2001, Yigit and Koca, 2011). Therefore, further investigations on diets with higher CGM incorporation levels and addition of enzymes are encouraged.

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