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THE USE OF CORN GLUTEN MEAL AS A PARTIAL REPLACEMENT FOR FISH MEAL IN DIETS OF SEA BASS (DICENTRARCHUS LABRAX) FRY

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Key words: Sea bass, fish meal, corn gluten meal, growth performance, feed utilization.

ABSTRACT

This experiment was conducted to evaluate corn gluten meal (CGM) as an alternative protein source for fish meal in the diet of Sea bass Dicentrarchus labrax fry. Fish with an average initial body weight of 0.58±0.002g were stocked into ten glass aquaria (100 X 30 X40 cm). Each tank contains 100 liter of seawater and was stocked with ten fish. Five isonitrogenous diets (55% crude protein) containing different levels of CGM, were fed three times daily, 6 days a week for 84 days. Growth, feed and nutrient utilization of Sea bass fry Dicentrarchus labrax fed the diets replacing 40 % of FM by substitution CGM protein were not statistically different from those of fish fed the control diet (containing 75% fish meal as a sole protein source). All parameters of growth and feed utilization for fish fed the diet replacing 60% of fish meal protein by CGM protein were significantly lower than that fed the control diet ($P< o.o5$). Supplements of crystalline amino acids to the CGM diet improved the nutritive value of the diet. Since substitution up to 40% did not adversely affect whole body composition of the cultured fish. Survival rates of fish ranged from 90 to 100%, it is suggested that up to 40% of fish meal protein can be replaced with CGM in the diet of Sea bass fry.

INTRODUCTION

Fishmeal is generally recognized as the best source of dietary protein for most carnivorous fish species (Sanz *et al*, 1994). The high cost of good quality fish meal poses real problems for cost-effective feed formulations, so that a variety of protein sources have been tested with the objective of partially or completely replacing fish meal in fish diets. Most of these studies have focused on the protein portion of the raw material. Nevertheless, the different raw materials also contain non-protein ingredients that should be evaluated as well, since their use may affect the utilization of the protein (Morales *et al.*, 1994).

The carbohydrate contained in most plant protein sources is generally not very digestible. Some recent research has demonstrated the capacity of carnivorous fish to use moderate levels of carbohydrate with improved growth and protein utilization (Cho and Kaushik, 1985; and Kaushik, 1992). Several plant proteins have been examined as alternative protein sources for fish meal because of their availability and low market price. Of the plant protein feed stuffs, soybean meal is considered to be the most nutritious. It is reported that a considerable part of dietary fish meal protein can be replaced with soybean meal without serious adverse effects on their growth or feed efficiency (Gallagher 1994; Webster *et al.* 1995; Langer, 1997).

Soybean meal is an effective protein source for fish meal, even in the diet of carnivorous marine fish species such as yellowtail *Seriola quinqueradiata,* Japanese flounder *Paralichthys olivaceus,* red drum *Sciaenops ocellatus* and sea bass *Dicentrarchus labrax* (Viyakarn *et al.*, 1992; Shimeno *et al.*, 1993; Kikuchi *et al.*, 1994 Mc Googan & Gatlin 1997 and Alexis 1993)

Besides soybean meal, corn gluten meal (CGM), cottonseed meal (Robinson and Li 1994) are also available and have been evaluated as dietary replacements for fish meal with various fish species. CGM is considered to be the most promising for carnivorous fish feeds. Corn gluten meal as a substitute for fish meal has been studied with rainbow trout *Oncorhynchus mykiss* (Moyano *et al.*, 1989; Morales *et al.*, 1994), gilthead seabream *Sparus aurata* (Robaina *et al.*, 1997), and yellowtail (Shimeno *et al.*, 1993), and it is reported that 20- 40 % of dietary fish meal protein can be replaced with CGM. Use of dietary CGM in combination with other alternatives such as soybean meal has also been examined (Moyano *et al.*, 1992; Pongmaneerat and Watanabe 1992; Watanabe and Pongmaneerat 1993; Yamamoto *et al.*, 1995). The common practice in sea bass *Dicentrarchus labrax* husbandry is to partially replace fish meal proteins with less expensive protein sources to obtain least cost without lowering the quality of the feed; in other terms without adversely affecting growth and production. However, information on the potential use of CGM in the diet of Sea bass *Dicentrarchus labrax* has not been obtained. Therefore, this study was designed to determine the maximum inclusion level of CGM as a partial substitute for fish meal in the diet of Sea bass fry.

MATERIAL AND METHODS

In April 2001, Sea bass *Dicentrarchus labrax* fry of mean weight of 0.58±0.002 g were purchased from Artificial Fish Hatchery, 21 Km West of Alexandria, Egypt. Healthy fry were acclimatized and adapted for one week on a basal diet (55% protein) before being distributed randomly in the experimental aquaria. Ten-glass aquaria (100-liter capacity) at Lab.of fish nutrition (NIOF), Alex. were used for housing the fish. The fish were stocked at a rate of ten fry per aquarium. All glass aquaria were provided with aeration flow through filtered seawater with salinity of 34-35 ppt, thermostatically controlled temperature at 28±1°C and subjected to a 12 hs photoperiod using fluorescent lamps.

The ingredients and proximate composition of the experimental diets are shown in Table 1. Diet 1 (control) containing 75% of fish meal (FM) as a sole protein source. Diets 2,3 and 4 contained 15, 29 and 43 of corn gluten meal (CGM), respectively. Replacing 20,40 and 60 of the FM protein with CGM protein on an iso-nitrogenous basis produced diets 1 to 4. These diets were designed to examine the effect of dietary CGM level on the growth of Sea bass fry. Diets 2 to 4 were supplemented with the crystalline amino acids arginine, lysine, and tryptophan, based on the difference of amino acid composition between FM and CGM sea Table 2. The composition of diet 5 was the same as those of diet 3, except it did not contain the crystalline amino acids. Diets 3 and 5 were designed to examine the availability of crystalline amino acids in the diet.

The CGM used in this study contained 10% moisture, 64% crude protein, 3% crude lipid, and 2% ash, while fish meal FM contained 9% moisture, 62.6% crude protein, 4.5% crude lipid, and 23.1% ash. Mineral and vitamin mixtures were added to each diet as shown in Table (1). All feed stuffs, except cod liver oil, were mixed after grinding. Formulated diets were dried in an air drier at 20°C, after that cod liver oil was added. The level of oil added varied according to the lipid content of protein sources. The diets were packed in airtight plastic bags and stored in a deep freezer at -20°C until needed. As shown in Table1, crude lipid contents in the diet were similar in all experimental diets. The experimental diets were tested for a period of 84 days. The fry were hand fed three times daily for 6 days a week at a rate of 10% (28 days), 7% (25 days) and 5% (28 days) of the total body weight per day, respectively. At fourteen days intervals the amount of feed was adjusted according to their weight. Faces and uneaten feed remnants were siphoned out of each aquarium daily together with about one third of water volume of each aquarium and replaced with fresh seawater before the morning feeding. After that body weight of each fish was measured and samples of fish from each dietary treatment were retained for proximate chemical analysis. The chemical analyses of the experimental diets and fish carcasses were performed according to the standard methods of AOAC (1990).

Mean weight gain (g/fish), average daily gain (mg/ fish), specific growth rate (SGR, %/ day), survival rate (SR%), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV%) and energy utilization (EU%) were calculated following Zein-Eldin and Meyers (1973).

Statistical analyses of experimental results were conducted according to Snedecor and Cochran (1980). Duncen's multiple range tests (1955) were carried out to test the significance among treatments means.

RESULTS

Growth performance and survival rates of the experimental fish are shown in Table 3. All fish accepted the experimental diets and fed actively during the period of the study. Survival rates of fish ranged from 90-100%. Only one fish was died in both treatments 4 and 5 during cleaning the ponds with siphoning. Final body weight, weight gain, ADG, and SGR% were not statistically different among fish fed the diets replacing 0, 20, and 40% of fish meal protein with CGM (diets 1-3). Significant reductions (P<0.05) in all these parameters were observed in the dietary group which replacing 60% of fish meal protein with CGM (diet 4). Growth performance of fish fed the CGM diet supplemented with amino acids (diet 3) were significantly higher (P<0.05) than those in the diet without supplementation (diet 5).

Data on proximate chemical analysis of fish carcasses presented in Table 4, showed that fish had more ash and less dry matter, protein, and lipid percentage at the start of the experiment as compared with the body contents at the end. There were no significant differences (P< 0.05) in terms of DM, CP and ash contents among treatments at the end of the experiment. Lipid content increased as level of dietary CGM increased, and the lipid content of fish fed diet 4 was significantly higher (P<0.05) than that in fish fed diets1 and 2.

Feed conversion ratio FCR, and protein efficiency ratio PER are improved slightly with replacing 20 and 40% FM protein with CGM (diets 2&3), while there were no significant difference (P< 0.05) in protein productive value PPV % and energy utilization EU% between 1,2 and 3 groups which fed diets 1,2 and 3. The lowest values of feed and nutrient utilization were at the group of fish that fed diet 4 (replacing 60% of FM protein with CGM). The addition of supplemented amino acids in diet 3 increased significantly (P< 0.05) all the parameters of feed and nutrient utilization than diet 5, show Table 5.

DISCUSSION

Fish fed the diet replacing 60% of fish meal protein with CGM (diet 4) showed considerably lower growth and feed performance. However, in the dietary groups substituting 20 and 40% of fish meal protein with CGM (diets2and3), all parameters measured were comparable to those of the control diet containing fish meal as a sole protein source. Therefore, maximum replacement level of CGM for fish meal protein in the diet of Sea bass fry is considered to be 40 %, when the diet is supplemented with essential amino acids. Thies result of the present study is in agreement with the results of Moyano *et al*, 1992 and Morales *et al*, 1994 on rainbow trout; Robaina *et al*, 1997 on gilthead sea bream and Shimeno *et al*, 1993 on yellowtail, which use corn gluten meal

(CGM) as an substitute for fish meal and they reported that, 20-40 % of dietary fish meal protein can be replaced with corn gluten meal protein.

Comparing the growth and feed utilization of fish fed diet 3 to those of fish fed diet 5, revealed that the fish can utilize crystalline amino acids added to CGM diet up to 40% substitution level. It also appears that part of the problem of using CGM as a substitute for fish meal in the diet of Sea bass is that the amino acid profile is insufficient to meet the requirement of Sea bass. Fish fed the diet of 60% replacement FM (diet 4) showed poor growth and feed utilization. These results are agreement with Kikuchi (1999), in his study at Japanese flounder, *Paralichthys olivaceus* who suggest that there is an amino acid imbalance in this diet because of the limitation in availability of crystalline amino acids in the CGM diet. However, it is possible that an anti-nutritional substance exists in CGM for the Sea bass. Positive effects of crystalline amino acid supplementation on the nutritional value of diets containing plant protein, have been shown in tilapia, *Oreochromis niloticus*X *O. aureus* fed soybean meal diet supplemented with methionine (Shiau *et al.* 1987), and channel catfish fed diets having soybean meal and distiller grains as substitution protein sources with lysine and methionine (Webster *et al.* 1992), as well as in sea bream (*Sparus aurata*) fed soybean meal diet supplemented with methionine (Essa, 2000). In Jadogpanese flounder, *Paralichthys olivaceus,* crystalline amino acids additions led to better results in diets containing soybean meal and feather meal (Kikuchi *et al.* 1994).

Corn gluten meal has been utilized as a partial substitute for dietary fish meal in several species without supplementation of essential amino acids. Studies of Shimeno *et al.* (1993), on yellowtail, showed that the proper replacement level of fish meal protein with CGM was 13-26%. On the other hand, Robaina *et al.* (1997) showed that replacing 20- 40% fish meal protein with CGM in the diet of gilthead seabream had no adverse effects on the growth, feed efficiency, protein efficiency ratio, and digestibility. Similarly, a 40% substitution of fish meal protein with CGM has been reported for rainbow trout diets (Morales *et al.* 1994). In the present study at Sea bass, 40% substitution without amino acid supplementation (diet 5) resulted in reduced growth, feed efficiency ratio, PER, PPV and EU. There are several factors that may affect the high level for replacement among fish species, such as the specific amino acid requirement of fish, digestibility, and formulation of feeds.

In conclusion, 20 to 40 % of dietary fish meal protein can be replaced with corn gluten meal protein. without adversely effect on growth, feed utilization and survival performances of sea bass fry.

Table 1. The ingredients and proximate composition of five-corn gluten meal diets for Sea bass fry *Dicentrarchus labrax.*

Item	Diet No.				
	1	2	3	4	5
<u>Feed Ingredient (%):-</u>					
Fish meal	75.0	60.0	45.0	30.0	45.0
Corn gluten meal(CGM)	-	15.0	30.0	43.0	30.0
Starch	16.0	13.6	14.0	11.0	14.0
Cod liver oil	5.0	6.0	7.0	8.0	7.0
Vitamin mixture [1]	2.0	2.0	2.0	2.0	2.0
Mineral mixture [2]	2.0	2.0	2.0	2.0	2.0
Amino acid supplement [3]	-	1.4	2.8	4.0	-
<u>Nutrient (as % DM basis)</u>					
Crude protein (CP)	55.70	55.90	56.10	56.80	56.0
Ether extract (EE)	11.60	11.80	12.00	12.30	12.1
Ash	20.00	16.30	13.30	10.30	13.9
Crude fiber (CF)	4.80	4.72	4.60	4.10	4.50
Nitrogen free extract(NFE)	7.90	11.28	14.00	16.50	13.50
Gross energy kcal/100g[4]	456.12	473.03	487.22	504.28	485.55
Protein energy ratio (P/E)	122.12	118.17	115.14	112.66	115.33

[1]- Vitamin mixture / kg premix containing the following: 3300IU vitamin A, vitamin D, 410IU vitamin E, 2660mg vitamin B^{1} · 133 mg vitaminB^{2} · 580mg vitamin B^{6}, 410 mg vitamin B, ^{12} 50 mg biotin,9330mg colin chloride, 4000mg vitamin C, 2660 mg Inositol,330mg para- amino benzoic acid, 9330mg niacin, 26.60mg pantothenic acid.

[2]- Mineral mixture / k premix containing the following : 325mg Manganese, 200mg Iron, 25mg Copper, 5mg Iodine, 5 mg Cobalt.

[3]- Amino acid supplement:- Diet2 :0.4% of L-arginine-HCl(Arg), 0.9% of L- lysine-HCl (Lys), and 0.1% of L- tryptophan(Trp) were added; Diet 3: 0.8% Lys, and 0.2% of Trp were added;Diet 4: 1.2% of Arg, 2.6% of Lys, and 0.2% of Trp were added.

[4]- Gross energy(GE kcal/100g diet) calculated according to NRC(1993) using the following calorific values: 5.64, 9.44, and 4.11 kcal/g diet of protein, fat and carbohydrate, respectively.

Table 2. Average essential amino acid (EAA) composition of fish meal FM and corn gluten meal CGM (all values are expressed as % by weight).

Essential amino acid(EAA)*	FM	CGM
Arginine	4.02	1.39
Histidine	1.34	0.97
Isoleucine	2.72	2.25
Leucine	4.36	7.22
Lysine	4.53	0.80
Methionine	1.68	1.04
Cystine	0.75	0.67
Phenyl alanine	2.28	2.78
Tyrosine	1.83	1.01
Threonine	2.57	1.42
Tryptophan	0.67	0.21
Valine	3.02	2.19

* Tacon (1993).

Table 3. Growth performance and survival rate of Sea bass fry *Dicentrarchus labrax* fed at different levels of corn gluten meal diets.

Diet No.*	Initial Weight (g/fish)	Final Weight (g/fish	Growth performance			Survival Rate %
			Gain(g/fish)	ADG (mg/day/fish)[1]	SGR (%)[2]	
1	0.57	25.32 ^{a}	24.75 ^{a}	294.64 ^{a}	4.52 ^{a}	100 ^{a}
2	0.58	27.41 ^{a}	26.83 ^{a}	319.40 ^{a}	4.59 ^{a}	100 ^{a}
3	0.58	26.12 ^{a}	25.54 ^{a}	304.05 ^{a}	4.53 ^{a}	100 ^{a}
4	0.59	19.84 ^{b}	19.25 ^{b}	229.17 ^{b}	4.18 ^{b}	90 ^{b}
5	0.57	20.24 ^{b}	19.67 ^{b}	234.17 ^{b}	4.25 ^{b}	90 ^{b}
LSD (P<0.05)	-	4.36	4.36	51.85	0.22	6.81

* Diet 1 control(zero of CGM), Diet 2,3,4 replacing 20,40 and 60% FM protein with CGM protein respectively, and add supplementary amino acids. Diet 5, replacing 40% FM protein with CGM protein but without adding supplementary amino acids.

1 ADG = Average daily gain = body Gain / experimental period.

2 SGR = Specific growth rate = ln final weight – ln initial weight / time (day) X 100

a,b,c: Means with different letters within the same column differ significantly (P< 0.05).

Table 4. Carcass composition (%) on dry matter basis of Sea bass *Dicentrarchus labrax* at start and after 84 days of fed at different levels of corn gluten meal diets.

Diet No.*	Dry matter (DM %)	% On Dry Matter Basis [1]			Energy content (KCAL/100g) [2]
		Crude protein (CP %)	Ether extract (EE %)	Ash (%)	
At start:	22.36	59.28	9.08	30.64	420.05
At end:					
1	25.4 [a]	62.42 [a]	22.90 [b]	13.70 [ab]	568.22 [b]
2	25.5 [a]	62.46 [a]	23.94 [b]	13.68 [b]	578.27 [a]
3	25.2 [ab]	62.30 [ab]	24.10 [ab]	13.72 [ab]	578.09 [a]
4	25.1 [ab]	62.26 [ab]	24.86 [a]	13.76 [a]	581.22 [a]
5	25.7 [a]	62.36 [ab]	24.42 [ab]	13.74 [a]	586.39 [a]
LSD 0.05	0.31	0.11	0.92	0.04	8.76

1 Each value was the mean of three replicates.
2 Energy contents (KCAL/ 100g) calculated according to NRC (1993) using the following calorific values; 5.64 , 9.44 and 4.11 Kcal /g whole body of protein, fat and carbohydrate, respectively.

a,b,c: Means bearing different letters within the same column differ significantly ($P < 0.05$).

* Diet 1 controls (zero of CGM), Diet 2,3,4 replacing 20,40 and 60% FM protein with CGM protein respectively, and add supplementary amino acids. Diet 5, replacing 40% FM protein with CGM protein but without adding supplementary amino acids.

Table 5. Feed and nutrient utilization by Sea bass *Dicentrarchus labrax* fed at different levels of corn gluten meal diets.

Diet No.	Feed utilization		Protein utilization		EU % [5]
	FI(g) [1]	FCR [2]	PER [3]	PPV % [4]	
1	35.12 [a]	1.42 [c]	1.27 [a]	12.01 [a]	13.43 [a]
2	37.56 [a]	1.40 [bc]	1.28 [ab]	13.05 [a]	14.26 [a]
3	36.78 [a]	1.44 [bc]	1.24 [ab]	11.83 [a]	12.78 [a]
4	28.68 [b]	1.49 [ab]	1.18 [b]	8.96 [b]	9.53 [b]
5	30.10 [b]	1.53 [a]	1.17 [b]	9.61 [b]	10.45 [ab]
LSD (P< 0.05)	5.00	0.06	0.06	2.14	2.50

1 FI= Feed intake as dry matter basis.
2 FCR= Food conversion ratio = g dry feed / g live body gain.
3 PER = Protein efficiency ratio = g live body gain./ g protein intake.
4 PPV % = Protein productive value = g protein intake. /g protein intake X 100

5 EU % = Energy utilization = Energy gain in fish (kcal) / energy intake (kcal) X 100

a,b,c: Means bearing different letters within the same column differ significantly (P< 0.05).

* Diet 1 controls (zero of CGM), Diet 2,3,4 replacing 20,40 and 60% FM protein with CGM protein respectively, and add supplementary amino acids. Diet 5, replacing 40% FM protein with CGM protein but without adding supplementary amino acids.

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