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Reduction of Dietary Fishmeal Inclusion in Practical Diets of the Seabass, *Dicentrarchus labrax*, Juveniles by Plant Protein Feedstuff

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ABSTRACT

Plant protein feedstuffs (PPF) may play an essential role in decreasing feed costs in aquaculture by reducing the use of fishmeal to minimal inclusion levels. Further supplementation may be needed to fulfill the fish's nutritional requirements. A feeding experiment was conducted to reduce fishmeal in practical diets of seabass juveniles reared in fresh water. The first diet was formulated to contain fishmeal as the only animal protein source. Four mixtures of rice gluten (R) soy protein concentrates (S) and corn gluten (C) in different formulations expressed as 10-0-21, 17-0-21, 0-10-21, and 0-17-21 RSC and named diets 2, 3, 4 and 5, respectively, were used to reduce fishmeal in the experimental diets from 20% to 14% (diets 2 and 4) or 7% (diets 3 and 5) of whole ingredients percentage. A reduction of fishmeal inclusion level in sea bass diets from 20% down to 14% had a positive effect by using PPF mixture of 10-0-21 RSC. However, reducing dietary fishmeal to 7% gave a negative response in growth parameters, specific growth rate, protein efficiency and energy retention of juvenile seabass. Final body weight, weight gain, feed conversion ratio, protein and energy utilization efficiency were not significant (P>0.05) among fish fed the basal diet that had 20% and those fed 14% fishmeal diet, with a PPF mixture of 10-7-21 RSC. Feed intake of sea bass juveniles was significantly (P<0.05) low upon reducing fishmeal in the experimental diets. The Feed conversion ratio of fish-fed diets containing a high percentage of rice gluten was higher, compared to the control diet. Histological examination of individual villus showed several symptoms of sub-acute intestinal enteritis of fish fed rice gluten, several of which appeared fewer in fish fed soy protein concentrates. The present study concluded that dietary soy protein concentrate seems to be more suitable to reduce fishmeal in seabass diets than rice gluten at level 33% replacement. In addition, incidence cost and profit index were improved as fishmeal was replaced by PPF in the diets of sea bass.

INTRODUCTION

Fish demand for human consumption increases as the world population increase. The annual per capita of fish reached 20.2 kg/year in 2020, compared to 9.9 kg/year in the 1960s **(FAO, 2020)**. Fish production from wild captures is nowadays declining from 1990 tile due to overfishing and un-rational fishing techniques. Simultaneously, aquaculture sector is growing with a dramatically increase than the other sectors. World fish production recorded 214 million ton in 2020, including 178 million ton of cultured animals, largely due to the growth of aquaculture sector **(FAO, 2020)**. Thus, the need to increase aquaculture production while keeping their costs lower is highly required, especially the feeding cost which represents about 60-70% of total culture operations. Fishmeal is one of the fewest major aspects in aquaculture industry existing successfully today. However, fishmeal has a globally shortage production and higher prices because it is widely used in feeds of aquatic and terrestrial animals and poultry diets. Fishmeal is served as an animal protein source in poultry, ruminant and fish feeds **(Ružić-Muslić *et al*., 2014 Živković *et al*., 2021)**. Remarkably, the inclusion level of fishmeal in poultry and ruminant feeds is smaller than that used in aquafeed **(Nasopoulou & Zabetakis, 2012)**. Yet, the reduction in relying on fishmeal amount in the diets of farmed fish will allow the aquaculture industry to keep growing into the next century. This trend is detected worldwide, especially in Egypt, where fishmeal is imported, raising feeding costs, and subsequently lowering profitability **(Soliman & Yacout, 2016)**.

Compared to the terrestrial animal's feeds, higher demand on the protein and energy has been observed in the composition of aqua-feeds, which are certainly required in huge amounts in the form of the fishmeal consumed in aquaculture due to its superior nutritive value **(Daniel, 2018)**. In general, 30% of fishmeal world production is consumed in marine fish diets such as seabass "*Dicentrarchus labrax*", gilthead sea bream "*Sparus aurata*", meagre "*Argyrosomus regius*" and Japanese sea bass "*Lateolabrax japonicas*" **(El-Dakar *et al*., 2015; Torrecillas *et al*., 2017; Estévez *et al*., 2022**; **Yao *et al*., 2022)**.

For a sustainable culture of marine fish, the aqua-feed industry should exert efforts to develop diets with little or zero level of fishmeal and fish oil, without negative effects on growth performance, feed utilization, welfare or the health of fish. It is economically necessary to find other protein sources for the aqua-feed industry to minimize the use of fishmeal to sustainable levels. Many plant protein ingredients, including soybean meal, corn gluten and wheat gluten can be further processed, serving as altarnatives for fishmeal in aqua-feeds. These sources possess higher protein content and low indigestible carbohydrate fractions, and consequently can be afforded at a lower price than fishmeal. In addition, concentrates and isolated soybean are more suitable ingredients to replace fishmeal in high protein aqua-feeds than other plant meals **(Gaylord & Barrows, 2009)**. It may be possible to use different mixtures of plant protein sources that differ in their content of essential amino acids to match the amino acids profile of the fishmeal.

Therefore, the present research aimed to evaluate some practical diets containing different plant protein sources to reduce the used fishmeal percentage on the growth performance, feed utilization, survival rate and gut health for the European seabass juveniles reared in fresh water.

MATERIALS AND METHODS

A feeding experiment was performed at the laboratory of Fish Nutrition in the Faculty of Fish Resources, Suez University, Suez Governorate, Egypt. Round plastic tanks (each holds 100L) were used in the experiment. Each tank was filled with aerated fresh water one day before use for water de-chlorination. Air blower "a .37 KW, Vortex MODEL: HG-1500SB" was used to supply each experimental tank with aeration through one air stone. Natural illumination was the source of light through four windows (170 x 150 cm) in the north direction. Sea bass juveniles were obtained from the General Authority for Fish Resources Development (GAFRD); (K-21 Marine Fish Hatchery, Alexandria, Egypt). Fish were packed in transparent polythene bags, filled to the 1/3 of its volume with water and 2/3 parts for oxygen. The experimental fish were adapted to laboratory condition for fifteen days prior the experiment, and they were fed two times daily till satiation. The average initial weight was 3.51±0.3 g/fish. Fish were randomly allocated in 15 round plastic tanks with a flat bottom. The experimental fish were stocked at a carrying capacity of 20 fish/ tank with continuous aeration until the end of the experiment. A daily routine of feces and excreta siphoning were applied along with replacing one third of the water volume. Water quality parameters values were adjusted at dissolved oxygen 5mg/ L, temperature 25.1±1.7°C, pH 7.1±0.6 and normal photoperiod as time regime.

At the beginning of the trial, twenty fishes were randomly taken for body composition and put in freezer at −10°C until analysis. Every two weeks, fish were anaesthetized in 60mg/ L clove oil **(Abou Shabana & Absawey, 2018)** to calculate the number of fish per tank, then samples were weighed to record tank biomass until the end of the experimental period **(El-Dakar *et al*., 2015)**. Dead fish were recorded over the trial period. By the end of the trial, ten fish per tank were anaesthetized and taken to determine protein, lipid and ash contents. In addition, another five fish were taken to address the intestine sections bisected for histological examination.

Experimental diets

The five experimental diets were formulated from local ingredients feedstuff including fishmeal, rice gluten, wheat gluten, soybean concentrates and wheat milling by-products. All the dietary ingredients were obtained from the commercial feed plant Al-Husseini Factory, Manzala, Egypt, except for the rice gluten which was obtained from Tiba Starch & Glucose Manufacturing Company, Egypt. Based on the chemical analysis and amino acid profiles of components, five experimental diets were formulated nearly isonitrogenous (41 % crude protein) and isocaloric (4900 kcal/kg diet as gross energy). A 20% fishmeal was used as a sole animal protein source of a basal diet (Diet 1). Fishmeal was gradually reduced at 14 and 7%, by adding 33 and 66% of plant protein ingredients including rice gluten, corn gluten and soy protein concentrates in different practical diets expressed as 10-0-21, 17-0-21, 0-10-21, and 0-17-21 RSC and were named as Diet 2, Diet 3, Diet 4 and Diet 5. All the experimental diets were formulated by spreadsheet excel of El-Dakar- Nutritive calculation. The ingredients composition of the experimental diets and chemical composition are found in Table (1). All the diets were supplemented with agar coated methionine and lysine according to **Peres and Oliva-Teles (2006)**. All the experimental diets were supplied with corn gluten and wheat gluten. Free amino acid was added to complete amino acid profile of the test diets according to **NRC (1993)**. Fish oil, linseed oil and soy oil were equally supplied as lipid sources in 1:1:1 ratio and wheat flour as the carbohydrate source. All the components were ground into small size in a grinder (SH-C70; SONAI, China). The diets were prepared by mixing all the dry components and amino acid in a food mixer (Mienta HM13529, a stand mixer, French) for 5min. The oil was added to the components and mixed for five minutes to ensure the distribution of the oil. Then, a necessary amount of water was added (about 25–30 % of components) and the mixture was passed through a meat grinder (Moulinex, ME605131, French) with small diameters (1.2 mm) for producing pellets with suitable diameters for fish feeding. Afterwards, the pellets were dried in oven (CMKO model ESM-4420, Guangdong, China) at 105°C for 6 hours. The experimental diets were kept at 4°C in a refrigerator until use.

Chemical analysis

Chemical analyses of components, diets and fish body were conducted according to **AOAC (2000)**. Gross energy (GE) value was calculated using factors of 5.65, 4.22 and 9.45 kcal per gram of protein, carbohydrate and lipid, respectively, according to **NRC (1993)**. Digestible energy was calculated from standard physiological values of protein (4 kcal/g), carbohydrate (4 kcal/g) and lipid (9 kcal/g) **(Garlling & Wilson, 1976)**. The amino acid profile of ingredients and diets analyses were done in the Regional Center of Food and Feed, Agricultural Research Center, Ministry of Agriculture, Doky, Giza, Egypt. The amino acid profiles of the experimental diets are displayed in Table (2).

Cost-benefit analysis

To evaluate the cost-benefit analysis of the experimental diets, incidence cost (IC) and profit index (PI) were calculated according to **El-Dakar *et al*. (2004)**.

Gut histological examination

The intestine was removed then washed with a solution of 10% formalin to excise visceral fat. After that, a 2cm section of intestine was taken, fixed in 10% formalin and stored at room temperature. Later, samples were dehydrated through a sequence of ascending concentrations of ethanol, embedded and blocked-in paraffin wax. 5µ transverse sections were cut and stained with hematoxylin and eosin protocol Intestine segments were three replications x3 examined fields in each slide for viewed under light microscopy examination at 50-fold eyepiece ×10-fold objective lens and were photographed by a fluorescence microscope Leica DM2500, Germany **(Feldman & Wolfe, 2014)**.

Table 1. Composition of the experimental diet (percentage dry matter basis)

Ingredient (%)	Diets no.				
	1	2 10-0-21	3 17-0-21	4 0-10-22	5 0-17-22
Fish meal (72.38 %)	20	14	7	14	7
Rice gluten (70.32 %)	-	10	17	-	-
Soy protein concentrate (65.88 %)	-	-	-	10	17
Corn gluten (58.04 %)	25	21	21	22	22
Wheat gluten (54.2 %)	25	25	25	25	25
Wheat milling by-products	12.5	11.22	10.71	10.22	9.71
Oil mixture[1]	12	12	12	12	12
Methionine	-	0.16	0.22	0.16	0.22
Lysine	-	0.33	0.58	0.33	0.58
Taurine	-	0.79	0.99	0.79	0.99
Vitamin premix[2]	1.0	1.0	1.0	1.0	1.0
Choline chloride	0.2	0.2	0.2	0.2	0.2
Stay-C (35 %ascorbate)	0.3	0.3	0.3	0.3	0.3
Mineral premix[3]	1.0	1.0	1.0	1.0	1.0
Dibasic calcium phosphate	3.0	3.0	3.0	3.0	3.0
Chemical composition % on DM basis					
Crude protein	41.39	41.65	41.46	41.69	41.28
Crude lipid	15.25	15.06	14.72	14.65	14.04
Crude Fiber	4.73	4.80	4.91	4.84	5.00
Nitrogen free extract	29.26	28.44	29.08	28.29	30.04
Ash	3.87	3.27	2.55	3.74	3.34
Calculated values					
Gross energy kcal/kg	5010	4980	4960	4930	4930
Digestible energy kcal/kg	4200	4160	4150	4120	4120
Protein/energy ratio (mg/kcal)	8.26	8.36	8.36	8.46	8.37

[1]: A mixture of fish oil, linseed oil and soybean oil in a 1:1:1 ratio.

[2]: Water soluble vitamins: vitamin B1, 15(mg/kg diet); vitamin B2, 25(mg/kg diet); pantothenic acid, 55(mg/kg diet); Niacin, 100(mg/kg diet); vitamin B6, 5(mg/kg diet); vitamin B9, 20(mg/kg diet); vitamin B12, 0.02(mg/kg diet); vitamin B7, 1.5(mg/kg diet); ascorbic, 50(mg/kg diet); inositol, 400(mg/kg diet). Fat soluble vitamins: retinoid,15,000 (IU/kg diet); vitamin D, 1,000 (IU/kg diet); vitamin H, 35 (mg/kg diet) ; menadione, 10 (mg/kg diet).

[3]:Minerals: cobalt, 2.11(mg/kg diet); copper, 20(mg/kg diet); iron, 190(mg/kg diet); fluoride, 1.99(mg/kg diet); iodide, 0.8(mg/kg diet); magnesium, 799(mg/kg diet); manganese, 25(mg/kg diet); selenite, 0.7(mg/kg diet); zinc, 35.5; calcium, 7.92 (g/kg diet); potassium, 2. (g/kg diet); sodium, 0.5 (g/kg diet).

Table 2. Amino acid profile of the experimental diets

Proximate composition	Diets no.				
	1	**2**	**3**	**4**	**5**
Essential amino acids (AAs)					
Arginine	2.1	2.54	2.78	2.54	2.77
Histidine	0.82	0.92	1	0.96	1.07
Lysine	1.61	1.63	1.56	1.97	2.15
Methionine	0.95	1.07	1.16	0.93	0.92
Leucine	3.86	4.04	4.34	4.08	4.36
Isoleucine	1.37	1.57	1.73	1.57	1.72
Threonine	1.22	1.39	1.5	1.39	1.48
Valine	1.66	1.98	2.21	1.86	2
Phenylalanine	1.71	1.99	2.24	3.49	4.78
Non-essential amino acids (AAs)					
Glutamic acid	3.11	4.37	5.11	4.62	5.53
Tyrosine	1.43	1.74	2.00	1.63	1.80
Aspartic acid	2.55	3.01	3.29	3.35	3.86
Cystine	0.60	0.73	0.84	0.66	0.71
Serine	1.59	1.84	2.04	1.92	2.18
Proline	3.24	2.91	2.59	3.12	2.91
Glycine	2.03	2.09	2.05	2.07	2.00
Alanine	2.41	2.56	2.68	2.44	2.45

Statistical analysis

Analysis of variance (ANOVA) was calculated according to **Snedecor and Cochran (1982)**, using a completely randomized design (CRD). Differences were subjected to Duncan′s multiple range-test **(Duncan, 1955)** at a significance level of 0.05. All statistical analyses were performed using SPSS 21.0 software package for Windows.

RESULTS

Growth performance and feed utilization

Sea bass juveniles displayed a linear increase in growth througout the feeding period. The differences in body weight were observed during the feeding period continued as shown in Fig. (1). Survival, growth performance, nutrient utilization and energy efficiency values of fish are presented in Table (3). No significant (P>0.05) differences were detected between fish fed 20% fishmeal (basal diet) and those fed reduced fishmeal diet to 14%, with a PPF mixture of (0-10-22, RSC) (diet 4). Fish fed diet (2) showed a lower growth rate than the control and fish fed diet (4), although fishmeal was reduced to 14% with the same PPF, except for the use of rice gluten instead of soy protein concentrates (10-0-21 of RSC).

Table 3. Growth performance and feed, nutrients and energy utilization parameters of sea bass juveniles feeding the experimental diets

Item	Diets no.					SE
	1	2 10-0-21	3 17-0-21	4 0-10-22	5 0-17-22	
Survival rate (%)	98.0[a]	97.0[a]	95.0[a]	97.0[a]	97.0[a]	0.35
Initial weight (g/fish)	3.29[a]	3.27[a]	3.27[a]	3.32[a]	3.23[a]	0.01
Final weight (g/fish)	17.58[a]	13.78[b]	11.23[c]	15.89[a]	12.22[bc]	0.26
Weight gain (g/fish)	14.29[a]	10.51[b]	7.96[c]	12.57[a]	8.99[bc]	0.36
Specific growth rate (%/day)	1.76[a]	1.51[b]	1.30[c]	1.65[a]	1.40[bc]	0.05
Feed intake (g/fish)	24.84[a]	20.95[b]	18.89[c]	23.67[a]	20.08[bc]	0.23
Feed conversion ratio (g/g)	1.74[a]	1.99[abc]	2.37[c]	1.88[ab]	2.23[bc]	0.08
Protein efficiency ratio (g/g)	1.43[a]	1.22[abc]	1.03[c]	1.31[ab]	1.10[bc]	0.05
Protein productive value (%)	23.81[a]	20.25[b]	16.04[c]	21.74[ab]	16.71[c]	0.49
Energy retention (%)	25.56[a]	27.95[a]	23.16 [a]	26.25[a]	22.45[a]	0.26

Specific growth rate (SGR %, day^{-1}) = Ln (final weight) − Ln (initial weight) / Time ×100
Feed conversion ratio (FCR) = feed intake (g) / weight gain (g)
Protein efficiency ratio (PER) = weight gain (g) / protein intake (g)
Protein productive value (PPV %) = [(final crude protein × final body weight) − (initial crude protein, × initial body weight.)] × 100 / total protein intake.

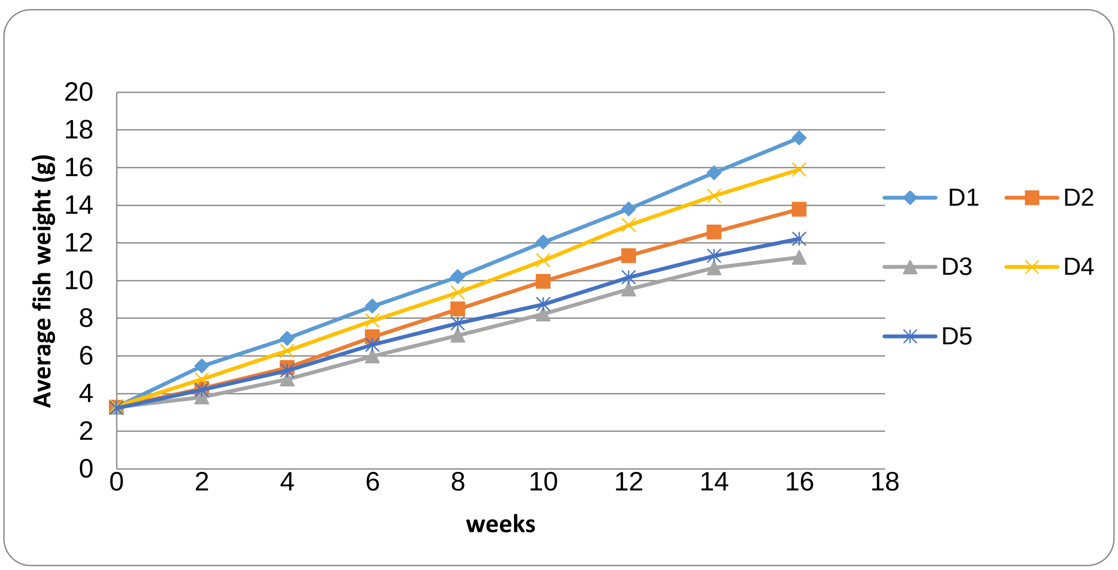

Fig. 1. Growth rate curves of sea bass juveniles (initial average weight=3.1± 0.1g/fish) fed the experimental diets

The reduction in fishmeal level to 7% for the sea bass juveniles (diet 3 and 5) resulted in a decline in their growth performance, feed conversion and efficiency of utilizing protein and energy. The lowest body weight, weight gain, SGR, FCR, PER, PPV and ER values were obtained in case of fish fed diet (3), containing PPF mixture of 17-0-21, RSC and 7% of fishmeal. Weight gain of sea bass juveniles fed diet (4) with 14% FM were statistically similar to weight gain of fish fed the diet (1), but for the final weight, weight gain and SGR of fish fed the diet (5) and diet (3) were significantly (P<0.05) lower. Among the treatments, the lowest growth performance parameters were statistically found in fish fed the diet with a 66% fishmeal replacement of plant protein feedstuff (diet 3). However, the feed intake was significantly (P<0.05) increased in fish fed diet (1) and diet (4) while decreased in diets (2), (3) and (5). Similarly, PER and PPV% showed also the lowest in fish fed the diet (3), while these parameters were not significantly (P>0.05) different among the (1) and (4) diets. Survival rate (%) was not significantly (P>0.05) different between the treatments using all the experimental diets.

Chemical analysis

In regard to the chemical composition of the fish body at the beginning of the experiment and the rest of the treatments at the end of the experiment, significant differences were recorded between them, as shown in Table (4). However, there was no significant difference (P>0.05) in the dry matter among the treatments of fish fed on the different experimental diets; however, fish fed a high rate of fishmeal showed high rate of protein (1, 2 and 4 diets) in body composition. While, fish fed diets with low percentage of fishmeal showed low percentage of lipid (3 and 5 diets) in their body composition, compared to fish fed other diets. Nevertheless, the ash was significantly higher in fish fed diets 3 and 5, then successively reduced in fish fed 1, 2 and 4 diets group.

Table 4. Proximate analysis of sea bass juvenile reared in fresh water feeding the experimental diets

Item	Diets no.						SE
	Initial	1	2	3	4	5	
Dry matter %	16.47	27.04^{a}	26.75^{a}	25.83^{a}	26.71^{a}	25.48^{a}	0.29
% on dry matter basis:-							
Crude protein	50.8	57.56^{a}	55.11^{ab}	52.7^{b}	56.3^{ab}	53.12^{b}	0.46
Total lipid	18.69	24.93^{a}	23.36^{a}	20.14^{b}	24.63^{a}	22.21^{ab}	0.29
Crude ash	26.51	15.41^{a}	18.53^{a}	23.16^{b}	17.07^{a}	22.67^{b}	0.39

Histological investigation

Images of distal intestine villi representing fish fed (1-2-3-4-5) diets are displayed (A-B-C-D-E), respectively, in Fig. (2). The villi in the distal intestine of the fish fed the control diet (Fig. A) show normal arrangement of microvilli (M), lamina propria (LP) thickness, appeared of acid goblet cells (AGC) and reduction in super-nuclear vacuolization (SNV). Although not quantified, abnormal villi structure, notable reduction in the number of enterocyte absorptive vacuoles, increased villus infiltration of super-nuclear vacuolization (SNV), reduction in villus length and thickness of mucosal layer (ML) was seen in distal intestine samples of fish fed increasing levels of rice gluten (Fig. 1B, C). However, fish feed high level of soy protein concentration showed normal arrangement of microvilli (M), thickness of mucosal layer (ML), as well as maintained lamina propria thickness with an observable slight reduction in villus length and increased villus infiltration of super-nuclear vacuolization (SNV) (Fig.D, E), compared to fish fed control diet.

Economic analysis

Upon increasing the substitution of fishmeal percentage with plant protein feedstuffs, a reduction was recorded in diets' costs. Economically, the highest incidence cost was recorded with fish group feeding diets 2 and 3, while the lowest one was noticed with the group feeding with PPF mixture of (0-10-22, RSC) diet 4. At the same time, the best profit index was achieved in-group feeding with PPF mixture of (0-10-22, RSC) (diet 4). The lowest profit index was achieved in the fish group feeding diets 3 and 5, as show in Table (5).

Table 5. Incidence costs and profit index of the experimental diets

Item	Diets no.					SE
	1	2	3	4	5	
Feed price (LE/kg)	16.30	14.89	13.80	13.87	12.56	-
Fish yield (LE/kg)	100.0	97.98	95.96	93.95	86.52	-
Incidence cost (IC)	28.53^{b}	29.98^{ab}	31.94^{b}	26.08^{a}	28.25^{b}	1.04
Incidence cost change %	100.0^{a}	105.1^{ab}	111.9^{b}	91.42^{a}	99.01^{a}	1.03
Profit index (PI)	3.53^{a}	3.31^{ab}	3.03^{b}	3.61^{a}	3.09^{ab}	0.11
Profit index change %	100.0^{a}	89.0^{ab}	76.0^{b}	98.0^{a}	90.0^{ab}	0.11

**ingredient prices in January 2020.

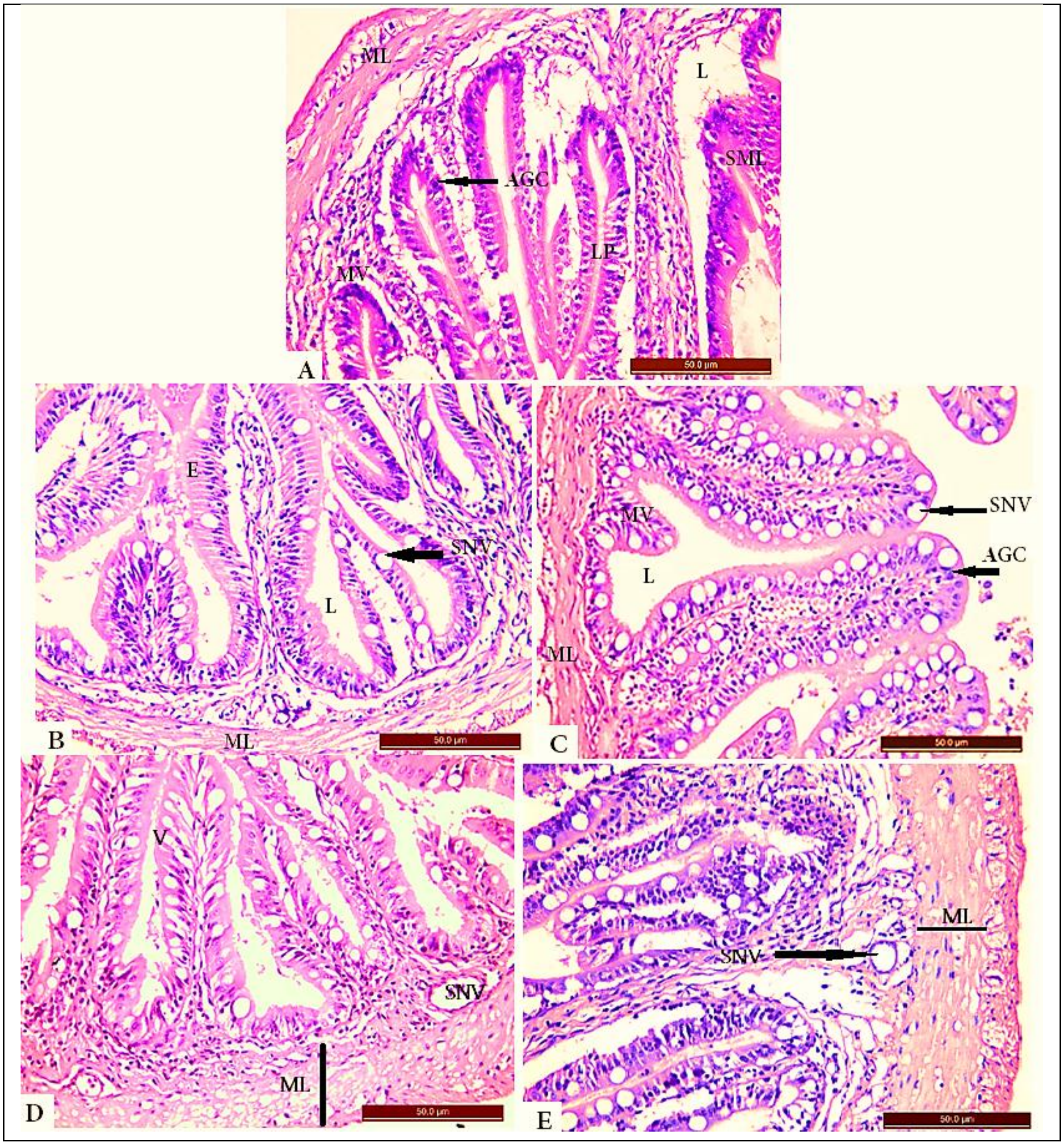

Fig. 2. Transverse section of seabass juveniles (*Dicentrarchus labrax*) villus in distal intestine fed different diets for 90 days showing, normal primary arrangement of enterocyte nuclei, lumen (L), lamina propria (LP), acid goblet cells (AGC), micro villi (MV) and mucosal layer (MS) and submucosal layer (SML) in diet 1 treatment; loss of enterocyte vacuolization, Epithelium (E), lumen (L), semi-large Mucosal layer (ML) and numerous of super-nuclear vacuoles (SNV) in diet 2 treatment; abundance of super-nuclear vacuoles (SNV), acid goblet cells (AGC), organizational loss of enterocyte nuclei, and thickened lamina propria and submucosa in diet 3 treatment; increase in supranuclear vacuole size (SNV), as well as maintained lamina propria thickness, nuclei organization in diet 4 treatment; normal villi structure, thickening in Mucosal layer (ML), increase in super-nuclear vacuoles size (SNV)in diet 5 treatment.(50.0 μm).

DISCUSSION

Fishmeal is an essential protein source in fish diets, especially for marine fish species. Fishmeal is one of the major components in aquaculture industry existing today that still relies on fishing from wild resources, which negatively affects fish stock. However, fishmeal are sold at high prices besides the global shortage in its production. Therefore, it is better to reduce the percentage of fishmeal in the diet.

The linear growth curves of seabass juveniles fed different practical diets containing low fishmeal percentage path were detected in this trial; this finding was generally found in several fish and is assumed possible for different growth verses in sea bass {for example grey mullet **(Hassanen *et al*., 1998)**, marbled spinefoot rabbit fish **(Shalaby *et al*., 2001: Shalaby *et al*., 2002; El-Dakar *et al*., 2011a, 2011b)**, white seabream **(Shalaby *et al*., 2010)**, red tilapia **(El-Dakar *et al*., 2011a)** and tilapia **(Chowdhury *et al*., 2013)**. Difference in growth rate among fish feeding experimental diets showed between week 4 and 16 of the trial because fish were biweekly weighed, and the fish began to adapt to the feeding after the 2nd week, so that the difference in weight appeared from the 4th week. Then, a lower growth rate was recorded in fish fed diets 3 and 5, compared to a formulated sea bass diet containing a high level of fishmeal (Diet 1).

In this study, results showed a decrease level of fishmeal in sea bass diets from 20% (control diet) to 7%, representing 33% from FM inclusion (diets 5), which resulted in a decline in the growth performance parameters. However, the diet with 14% fishmeal (represented 66% from FM inclusion) gave similar values of weight gain, SGR and FCR with diet 1 (control diet). No differences were observed among the diet with 20% fishmeal and the diet containing a lower fishmeal percentage (14%). Reduction in fishmeal percentages for the seabass diets may be restricted in case of diets containing soybean concentrations, corn gluten, wheat gluten and a mixture of amino acid from methionine and lysine. In contrast, an increasing inclusion level of fishmeal up to 20% resulted in a decrease in growth rate and feed convention. These results correspond with those obtained by different plant protein sources when substituting fishmeal with soybean meal for marine species such as rabbit fish **(Shalaby *et al*., 2001)** and sea bream **(Kaushek *et al*., 2004)** and freshwater species including the tilapia **(Sharda *et al*., 2017)** and carp **(Xu *et al*., 2022)**.

The present results showed that reducing FM inclusion percentage to 66% in sea bass diets reared in freshwater conditions gave a lower SGR, FCR, PER, PPV % and energy retention. These results may be due to lack of energy in alternative plant protein sources such as soybean meal and soybean concentrates that deficient in gross energy and Sulphur amino acids **(Shalaby *et al*., 2001** and **Xuquan *et al*., 2022)**. As increase of the utilization of plant products in aquafeed continues, a supplementation of limiting amino acids is required. In order to reach the level of amino acid composition similar to fishmeal required for normal growth of fish. To increase utilization of plant protein sources, supplementation of crystalline amino acids and the coating methods were tested **(Lund *et al.*, 2011)**. These results may be attributed to probability the plant protein mixture of rice gluten, corn gluten and soy protein concentrates gave a complete qualitative similar amino acid profile that in fishmeal. The lower palatability led to lower feed intake and thus depressing FCR, PPV and energy retention. These results are in agreement with **Kaushek *et al*. (2004)** who found that marine fish show a higher acceptability on feeding highly processed or value-added plant ingredients such as protein concentrates or protein isolates as an alternative to using plant meal. This increased on acceptance is due to an increase in the concentration of plant nutrient, protein availability, energy retention and improvement in digestibility thus an improvement in the overall feeding efficiency of the fish. This reduction in growth of the experimental fish feeding diet 2, 3 was attributed to differences in level of feed intake and not for differences in dietary nutrient (amino acid) content. There was an effect of the type of plant protein used, whether soybean concentrates or rice gluten, as well as the levels of addition on feed intake and compositional analysis of the fish body. Although the decrease in growth with the increase in the addition of plant protein, soybean concentrates gave a better growth rate than rice gluten to the replacement 33% of fishmeal.

Another reason for decreasing growth rate of fish fed lower fishmeal diets (diets 2 and 3) than the control diet is low palatability of replacer plant protein ingredients e.g., rice gluten and corn gluten fishmeal. **Reda *et al*. (2022)** who found that impaired growth performance of carnivorous fish fed plant protein-based diets contain rice protein concentrate or fermented rice protein **(He *et al*., 2021)** is caused by reduced feed palatability and reluctant feed intake because of unfavorable diets taste. **Gary *et al*. (2012)** who observed the linear depressing effect of soy protein concentrates on weight gain with graded inclusion levels for Atlantic salmon and rainbow trout. Moreover, higher fishmeal replacements (75–94 and 100 %) with soybean protein concentrate were also reported for other carnivores fish for example rabbit fish **(Shalaby *et al*., 2001 & 2002)**: rainbow trout **(Kaushek *et al*., 2004)**, sole **(Aragão *et al*., 2003)** and cobia **(Salze *et al*., 2010)**.

In this study, the group of fish feeding on diets (3 and 5) showed a significant decrease in body content of protein and fat compared to fish in other treatments, in addition to an increase in ash content. These results are consistent with **Zhang *et al*. (2018)** who found a decrease in the body fat content of Japanese sea bass with a decrease in fishmeal ratio in the diet and an increase in plant protein content. **Li *et al*. (2012)** recorded that there is no significant change in the body composition of Japanese sea bass when replacing fishmeal up to 60% with plant protein. From these results, it is clear that increasing in replacement of fishmeal with plant protein in diet leads to a decrease in the ability to utilize nutrients with plant protein, which leads to a decrease in growth performance, feed nutrients and energy utilization parameters, and this is consistent with growth measures, nutrition analysis, and energy utilization criteria.

Replacing fishmeal with PPF showed shortening of villus height, reduced villus and muscular thickness in intestine of sea bass juvenile. Increasing of plant protein level led to aggravate the negative effects of increase in supranuclear vacuole size and number. This result is similar to **Zhang *et al*. (2018)** who reported that reduction of microvillus height, muscular thickness and incidence of enteritis of sea bass when 75% of fishmeal was replaced with soybean meal in diet.

CONCLUSION

It may be possible to use different mixtures of plant protein sources to replace fishmeal in diet of sea bass juveniles rearing in fresh water. It showed no significant (P>0.05) effect on growth performance, nutrient utilization efficiencies, body composition and incidence cost in case of replacing of 33-66% fishmeal by plant protein feedstuff.

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