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Nutritional Evaluation of Fishmeal Free Diets in European Seabass, Dicentrarchus labrax, Feeds Reared in Fresh Water

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ABSTRACT

Decreases of aquaculture feed costs is one of the main targets of aqua culturists, because it represents more than 80% of the running costs. Until recently, fishmeal used in fish feeds represented a significant percentage in the composition of the diets. However, with the recent shortage and significant increases in fishmeal prices, it becomes necessary to search for alternatives that perform the same function as fishmeal and help in the process of reducing feed prices. Use of fishmeal free diets (FMFD) may be one the solutions to achieve this goal. The present study aims to evaluate FMFD for sea bass, *Dicentrarchus labrax*, juvenile reared in freshwater from nutritional view and study their effects on growth performance, body composition, feed utilization efficiencies and cost-benefit analysis. Five experimental FMFD were formulated to contain meat meal (MM), poultry by-product meal (PBM), mixed of MM+PBM instead of fishmeal (control) and plant protein ingredients. Methionine and lysine were supplied) when it is needed)to adjust amino acid profile in FMFDs. All the tested diets were offered to fish in triplicate tanks, two times daily, satiated for 90 days feeding period.

Growth and survival rate values of fish fed FMFD containing mixture of plant protein were significantly ($P<0.05$) lower than those fed the control diet. However, nutritional parameters improved in case of diets had MM or PBP. Although, no significant differences ($P>0.05$) were observed among fish group fed diet of MM+PPM and those fed the basal diet in specific growth rate, feed conversion, protein efficiency ratio, productive protein value and energy retention. The best-feed conversion ratio was achieved with the fish group fed MM+PBP diet and it was not significantly ($P>0.05$) among the basal diet. Histological observations of individual villi indicated several symptoms of non-infectious sub – acute gastrointestinal enteritis in the group feeding plant protein, many of which were not present in the group feeding fishmeal. In the economic analysis, the best Profitability Index was achieved with group feeding mixed of meat meal and poultry by product meal diet, meat meal diet, poultry by product diet and fishmeal diet respectively. Therefore, the findings of this study will encourage feed manufacturers to utilize alternative proteins more efficiently in generating low-cost and sustainable aqua feeds.

Fish fed diet MM+PBP gave not significant differences with the basal diet showing the possibility to unused of FM in diets sea bass juveniles reared in fresh water.

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INTRODUCTION

Sea bass has now been introduced as a farmed fish species to the w orldwide including Egypt. Its production becomes increasingly important due to its high commercial value **(FAO, 2020)**, high growth rate and possibility to rear in fresh water **(Elaraby *et al*., 2018; Shalaby *et al*., 2023)**.

As a typical many marine species, sea bass requires high level of dietary crude protein may reached to about 50% of whole pellet-diets **(Hassanen *et al*., 1998; Shalaby *et al*., 2001: Shalaby *et al*., 2002; Shalaby *et al*., 2010; El-Dakar *et al*., 2011; Chowdhury *et al*., 2013)**. Sea bass like carnivorous fish relay mainly on fishmeal in their diets **(Cashion *et al*., 2017; Shalaby *et al*., 2023)**. The increasing consumption of fishmeal for feeding poses direct pressure on wild aquatic capture and gave a negatively impact on marine ecosystems. Moreover, the global fishmeal production industry faces major challenges e.g. the limited productivity of marine resources and increase their demand to use in poultry diets and aquafeeds. Which had an effective impact on increasing its competition and increasing its price **(Tacon and Metian, 2009)**.

With greater attention to blue economy, nutritionists had reduced the inclusion of fishmeal level in aquafeeds, by using alternative protein sources from land crops such as soybean, sunflower meal, corn gluten or DDGs **(Davis *et al*., 2021; Shalaby *et al*., 2023)**. More recent study by **Shalaby *et al*. (2023)** showed the success in reduction of fishmeal inclusion level in sea bass feeds from 20% down to 14% by using plant protein ingredients without side effect on growth rate, feed conversion ratio and efficiency utilization of protein and energy. However, the increase replacement percent up to 7% resulted negative response in growth performance and other nutritional parameters. Depression of growth rate and efficiency utilization of feed, protein and energy may be attributed to deficiency in energy and essential amino acids in plant protein ingredients. In this respect, use of animal protein sources e.g. Slaughterhouses by products either poultry or farmed animals may be giving a positive effect on growth performance, feed efficiency and feeding costs as replacers of fishmeal in carnivores fish diets. Hence, the present work aimed to study the of total replacement fish meal by poultry byproducts, meat meal and plant protein and their effects on growth performance, body composition, feed utilization survivor rate and cost- benefit analysis of sea bass juvenile reared in freshwater conditions.

MATERIALS AND METHODS

A feeding trial was carried out at the Wet Lab. of Fish Nutrition in Faculty of Fish Resources, Suez University, Suez, Egypt. The experimental facilities used in the present work were 15 round plastic-flat bottom tanks with capacity of 100-L, field with fresh water, that aerated overnight before use for de-chlorinating. Natural illumination was the source of light through four windows (170 x 150 cm) in the north direction. An air blower of 37 KW (Vortex MODEL: HG-1500SB) was used to supply each experimental tank with air through one air stone. The experimental fish of sea bass juveniles were obtained from K-21 Marine Fish Hatchery, Alexandria, Egypt, (General Authority for Fish Resources Development, GAFRD). Fishes were packed in transparent polythene bags filled to one third of its volume water and the rest volume of bags was injected with oxygen. All polythene bags were placed in Styrofoam boxes supplied with about 0.5 kg ice and loaded in trunks. The experimental fish were acclimatized to laboratory conditions for two weeks before the experiment start. Twenty juvenile's fish with initial body weight of 3.51±0.3 g/fish were randomly allocated in the experimental units. Removing of feces and excreta were removed by siphoning with water exchanged of one third of the water volume daily. Fish were fed two times daily until satiation. Water quality parameters values were 7.1±0.5, 5 ±0.5mg/L, 25.1±1.7°C and 12-12 D/L cycle for pH, dissolved oxygen, temperature and photoperiod, respectively.

1. Experimental diets

Five experimental diets were formulated from local ingredients feedstuff including fishmeal, meat meal, poultry by product meal, soybean meal, soy protein concentrate, corn gluten, wheat gluten and rice bran. All the dietary components were obtained from commercial factory SPAA FEED, Al manzala, Egypt.

Fishmeal was the main source of dietary protein in the basal diet (control diet) with a level 20%. Meat meal (MM), poultry by-product meal (PPM) and mixture of MM+PPM and mixture of plant protein feedstuffs were used in tested four fishmeal free diets. All the fishmeal free diets were provided with agar-coated methionine and lysine to complete amino acid profile of the tested diets according to **NRC (1993)**. Amino acid methionine was coated with agar to delay absorption time **Peres and Oliva-Teles (2006)**.

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

Ingredients (%)	Experimental diet*				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	FM	MM	PBM	PBM+MM	PPM
Fish meal	20	-	-	-	-
Meat meal	-	25	-	15	-
Poultry by product meal	-	-	25	15	-
Soybean meal	32	24	24	20	35
Soy protein concentrate	-	7	8	6	21
Corn gluten	10	10	10	10	10
Wheat gluten	10	10	10	10	10
Rice bran	11.5	5.80	4.82	5.85	5.23
Oil mixture[1]	12	12	12	12	12
Methionine	-	0.49	0.45	0.46	0.67
Lysine	-	1.21	1.23	1.19	1.60
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	1	1	1	1
Dibasic calcium phosphate	2	2	2	2	2
Chemical composition					
Dry matter %	91.1	91.37	92.59	91.33	92.11
% on DM basis: -					
Crude protein	40.07	40.09	40.13	40.23	39.97
Crude lipid	15.05	16.15	15.65	16.46	13.15
Crude Fiber	3.21	2.97	3.41	3.12	3.53
Ash	5.55	6.68	5.06	6.12	4.48
NFE[4]	36.12	34.11	35.75	34.07	38.87
Calculated values					
Gross energy [5] kcal/kg	5170	5190	5210	5230	5090
Digestible energy [6] kcal/kg	4400	4420	4440	4450	4340
Protein/energy ratio [7] (mg/kcal)	7.750	7.724	7.673	7.692	7.853

* FM: fishmeal diets, MM: meat meal diets, PBM: poultry by product meal, PBM+MM: poultry by product meal+ meat meal diets and PPM: plant protein meal.

[1]Oil mixture has fishoil : linseed: soybean oil with ratio of 1:1:1. Respectively.

[2]Vitamins (mg/kg diet): calciferol, 2,000 (IU/kg diet);; thiamine, 15; riboflavin(VB2), 25; pantothenate acid(VB4), 50; alpha-tocopherol, 35; nicotinic acid, 100; pyridoxine, 5; folic acid, 5; biotin, 1.5; ascorbyl monophosphate, 50; inositol,200 retinol, 18,000 (IU/kg diet); cyanocobalamin, 0.02; menadione sodium., 10.

[3] Minerals (mg/kg diet): zinc oxide, 37.5; manganese oxide, 26; cobalt sulphate, 1.91; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; copper sulphate, 19.6; sodium selenite, 0.66; dicalcium phosphate, 8.02 (g/kg diet); potassium chloride, 1.15 (g/kg diet); sodium chloride, 0.4 (g/kg diet).

[4] Nitrogen free extract =1-(%lipid+%moisture+%protein+%fiber+%ash)

[5] 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)**.

[6] Digestible energy calculated from standard physiological values of protein (4 kcal/g), carbohydrate (4 kcal/g) and lipid (9 kcal/g) **(Garlling and Wilson, 1976)**.

[7] (protein / gross energy) * 10 **(El-Dakar *et al*., 2015)**.

Fish oil, linseed oil and soybean oil were supplied equally as lipid sources. The diet composition is presented in Table (1). All the dietary ingredients were first ground into small particle size in a Grinder (SH-C70; SONAI, China). The diets were prepared by thoroughly mixing all the dry ingredients and amino acid in a food mixer (Mienta HM13529, Stand Mixer, French) for five min. then added oil to the dry ingredients and mixed for another five min. The required amount of water (25–30% of the dry ingredients) was then added to the premixed ingredients and mixed for another 10 min and then-passed through a meat grinder (Moulinex, ME605131 meat grinder, French) with an appropriate diameter (1.5 mm) to prepare pellets, which were then dried in a dry-air oven (CMKO model ESM-4420, Guangdong, China) at 105°C for 6 hours. The test diets were kept in refrigerator until use.

At the start of the experimental period, a pool of 20 fish were randomly selected from the remaining fish and stored at −20°C until use for chemical analysis of body composition. Every two weeks fish were anaesthetized in 60 mg/L clove oil according to **El-Dakar *et al*. (2021)** for fish counting and weighting to record tank biomass and calculate the average individual body weight per tank until the end of the experimental period. Any dead fish were recorded over the course of the experiment. At the end of the trial, ten fish per tank were anaesthetized and sampled to determine protein, lipid and ash contents. In addition, another five fish were sampled for distal intestine sections for histological analysis.

2. Chemical analyses

All ingredients, diets and the experimental fish bodies were chemical analyzed by method described by **AOAC (2007)**. The amino acid profile of the feed ingredients was analyzed by the Amino acid analyzer of the Reginal Center of Foods and Feeds - Agricultural Research Center, Ministry of Agriculture, Doki, El Giza, Egypt.

3. Cost-benefit analysis

Incidence cost (IC) and profit index (PI) of the experimental diets were calculated according to El-**Dakar *et al*. (2011)** to evaluate the cost-benefit analysis.

4. Gut histological examination

The intestine was excised, flushed with 10% formalin solution, freed of visceral fat. After that, a 1 to 2 cm section of intestine was taken, immediately fixed in 10% formalin and stored at room temperature. Later, samples were dehydrated through a series of ascending concentrations of ethanol, embedded and blocked-in paraffin wax. 5µ transverse sections were cut and stained with hematoxylin and eosin protocol **Feldman and Wolfe (2014)**. Distal intestine segments were three replications x3. Examined fields in each slide for viewed under light microscopy at 50-fold eyepiece ×10-fold objective lens and were photographed by a fluorescence microscope Leica DM2500, Germany.

5. Statistical analysis

Analysis of variance (ANOVA) was carried out 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 AND DISCUSSION

Growth performance, FCR, nutrient utilization and survival rate of fish fed FMFDs Survival rate (percentage) was not significantly (P>0.05) different at the end of the feeding trial among all treatments.and the basal diet are presented in Table (3).

(4) containing mixed of meat and poultry by-products meal were not significantly different (P>0.05) with that of the fishmeal diet (basal diet).

The results indicated that body weight, weight gain, SGR %/day of sea bass juvenile fed diet However, feeding on diet containing either meat

concentrations of lysine, isoleucine, and phenylalanine as fishmeal. Whereas, the meat meal was almost identical to the fishmeal in the concentrations of methionine, leucine and threonine. As for the soy concentrates, they contain concentrations close to fishmeal in histidine, leucine and phenylalanine, and are superior to it in the concentration of arginine. Therefore, the use of mixtures of fishmeal, poultry by-products and soy concentrates can compensate for the absence of fish meal in sea bass diets (diet 4). On the contrary, the fish group received only plant protein sources in their feeds (diet 5) had more poor response to growth and feed conversion efficiency than their counterparts that received an animal protein

meal (diet 2) or poultry by-products (diet 3) as a sole animal protein source gave lower growth rate, feed conversion and utilization of protein and energy than that fed the basal diet (FM diet). While fish fed diet had plant protein ingredients (diet 5) were the lowest in weight gain, SGR, FCR, PER, PPV% and energy retention%. This means that the possibility of successfully using a diet free of fishmeal in the sea bass feeds, consisting of a mixture of non-fishmeal animal proteins, soybean meal and gluten of wheat and corn, if provided with 0.5% of the amino acid methionine

and lysine. The main reason of improvement in the performance of sea bass fish in the case of feeding on diets free of fishmeal may be due to the integration of essential amino acids from the non-fishmeal proteins and other plant ingredients, soybean meal, soy protein concentrates, wheat gluten and corn gluten.

Fig (1) shows that both MM and PBM are rich in lysine and methionine when compared with soybean meal. Comparison circles show the amino acid content between different protein sources and fishmeal PBM contains the same were poor content in essential amino acids specially, methionine and lysine than fishmeal **(Fig. 1)**.

sources e.g. soybean meal, soybean concentrates. This is because the essential amino acids are not available in these plant protein sources. It is clear all tested PPSc (soybean meal, corn gluten and wheat gluten)

Table 2. Amino acid profile of the experimental diets.

Proximate composition	Experimental diets*				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	FM	MM	PBM	PBM+MM	PPM
Essential amino acids (AAs)					
Arginine	2.92	3.08	2.7	2.95	3.3
Histidine	0.98	0.99	0.95	0.93	1.23
Lysine	2.64	2.57	2.55	2.71	2.64
Methionine	0.75	0.75	0.71	0.76	0.73
Leucine	3.15	3.67	3.6	3.58	3.08
Isoleucine	1.97	1.77	1.81	1.78	1.96
Threonine	1.98	1.77	1.7	1.85	1.71
Valine	2.71	2.06	1.87	2.94	2.17
Phenylalanine	2.09	2.2	1.89	1.99	1.45
Non-essential amino acids (AAs)					
Glutamic acid	5.67	4.05	4.1	5.01	4.5
Tyrosine	1.88	1.51	1.48	1.89	1.68
Aspartic acid	5	6.15	4.14	5.18	5.4
Cystine	0.84	0.68	0.61	0.74	0.71
Serine	2.73	2.17	1.98	2.61	2.54
Proline	3.04	3.48	3.36	3.61	2.57
Glycine	2.05	2.51	2.73	2.79	1.9
Alanine	2.11	2.75	2.63	2.8	2.21

** FM: fishmeal diets, MM: meat meal diets, PBM: poultry by-product meal, PBM+MM: poultry by product meal+ meat meal diets and PPM: plant protein meal.

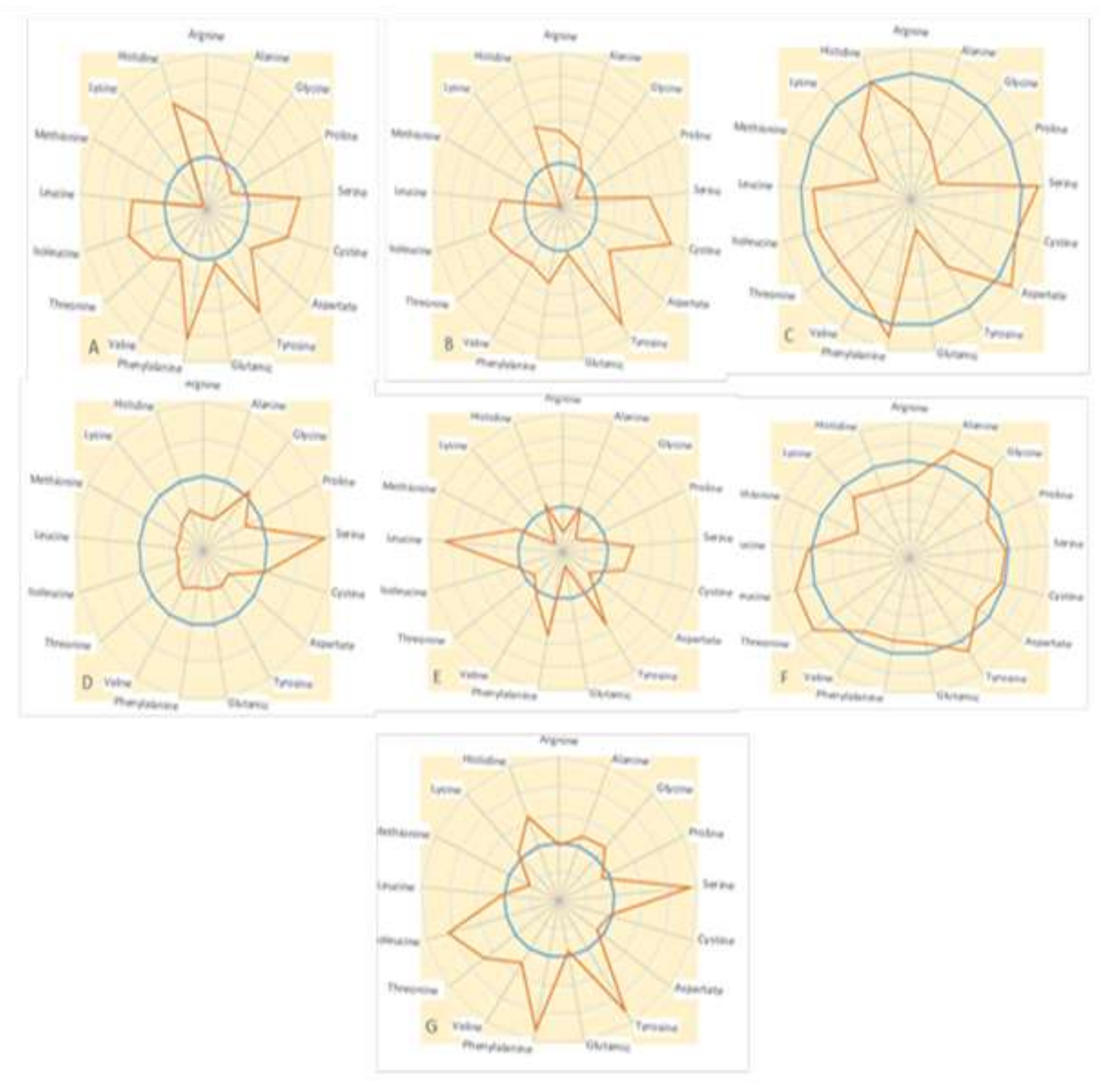

Fig.1. Comparison circles show the amino acid content between different protein sources e.g. soy protein concentrate, rice gluten, soybean meal, wheat gluten, corn gluten, meat meal, poultry by product meal and fishmeal represent A;B;C;D;E;F;G respectively.

Therefore, diet (5) gave the lowest performance (final body weight. weight gain, SGR, FCR, PER, PPV and ER% than the other tested diets. At the same time, these plant protein sources are rich in leucine, serine, aspartate, phenylalanine acids. These results are in agreement with many researchers with different fish species, e.g. Carp **(Zhan *et al*., 2020)**; Nile tilapia **(Ismail *et al*., 2020)** and catfish **(Han *et al*., 2022)**. The decrease in the efficiency of fish performance with the complete replacement of fishmeal with plant protein sources is due to the lack of essential amino acids and minerals and the high content of cellulose and nutrient inhibitor factors **(Han *et al*., 2022)**. The use of an animal protein source such as meat meal (diet 2) or poultry by-products meal (diet 3) of sea bass cultured under the present conditions led to an improvement in growth, but it remained less efficient than that of fish fed on the basal diet (diet 1).

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

Parameters	Experimental diets*					SE
	Diet 1 FM	Diet 2 MM	Diet 3 PBM	Diet 4 PBM+MM	Diet 5 PPM	
Initial weight (g)	3.3	3.3	3.42	3.39	3.27	0.021
Final weight (g)	22.26[a]	19.20[b]	16.70[c]	21.59[a]	11.66[d]	0.039
Weight gain[1] (g)	18.96[a]	15.90[b]	13.28[c]	18.2[a]	8.39[d]	0.035
Specific growth rate (%/day)	2.12[a]	1.96[b]	1.76[c]	2.06[a]	1.41[d]	0.069
Feed intake (g/fish)	32.28[a]	31.30[a]	28.26[b]	30.05[a]	22.00[c]	1.151
Protein intake (g/fish)	11.78[a]	11.46[a]	10.50[a]	11.04[a]	8.10[b]	0.417
Feed conversion ratio[2] (g/g)	1.70[a]	1.97[ab]	2.13[b]	1.65[a]	2.62[c]	0.102
Protein efficiency ratio[3] (g/g)	1.61[a]	1.38[b]	1.26[b]	1.65[a]	1.04[c]	0.058
Protein productive value[4] (%)	22.2[a]	17.43[ab]	14.26[b]	18.2[a]	12.77[c]	0.688
Energy retention[5] (%)	21.68	18.62	21.32	24.38	17.59	1.065
Survival rate[6] (%)	96.7	95	96.7	98.3	93.3	1
Condition factor[7] (%)	1.892[ab]	2.298[b]	1.371[a]	1.855[ab]	2.064[ab]	0.122

1. Weight gain = (final body weight − initial body weight)
2. Specific growth rate = 100 x [Ln (final weight) − Ln (initial weight) / da]
3. Feed conversion ratio = feed intake (g) / weight gain (g)
4. Protein efficiency ratio = weight gain (g)/ protein intake (g)
5. Protein productive value = [(final crude protein × final body weight) − (initial crude protein, × initial body weight.)] × 100 / total protein intake.
6. Survival =100 × (final no. of fish / initial no. of fish)
7. Condition factor = 100 × (fish weight / fish length^3)

The differences in body weight, weight gain and SGR were significantly (P<0.05) higher among fish fed the basal diet and those fed either meat meal (diet 2) or PBM (diet 3) as animal protein source supplemented with methionine and lysine instead of fishmeal (Table 3). While the use of these two animal protein sources (diet 4) mixed with soybean meal, soy protein concentrate, corn gluten and wheat gluten and supplemented with the essential amino acids of methionine and lysine improved growth rates of sea bass reared in fresh water conditions. The results indicated that the differences in body weight, weight gain and specific growth rate of fish that received the basic diet (20% fishmeal) were not significant (P>0.05) with that of the fish that were fed on a diet completely free of fishmeal. These results confirm the success of using fishmeal-free diets using a mixture of plant and animal proteins supplied with both methionine and lysine essential amino acids in fish feeds. They agree with the findings of the researchers **(Webster *et al*., 1999; Jiang *et al*., 2016; Shalaby *et al*., 2023)**. **Webster *et al*. (1999)** reported that use mixture of plant and animal protein source with complementary amino acid profiles may help to decrease negative effects of fish performance that cased by amino acid deficiencies. Similar results are found by **Furuya *et al*. (2004); El-Sayed (2014); Chi *et al*. (2017)** reported that MM is rich in protein and lipid which make it suitable as a fish meal replacer in carnivorous fish such as Cobia, *Rachycentron canadum*; olive flounder **(Cho, 2011)**; barramundi, *Lates calcarifer* **(Williams *et al*., 2008)**.

Supplementation of lysine and methionine are beneficial in recovering amino acid balance and palatability in different diets. Likewise, essential amino acid (EAA) deficiency is one of the most important issues regarding FM substitution with alternative ingredients **(Kaushik and Seiliez, 2010)** and unbalanced EAA levels in the diets have been reported as one of the main causes for growth depression

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

Item	Experimental diets *						SE
	Initial **	Diet 1 FM	Diet 2 MM	Diet 3 PBM	Diet 4 PBM+MM	Diet 5 PPM	
Dry matter %	20.47	26.13[ab]	24.49[abc]	22.98[bc]	27.92[a]	22.37[c]	0.683
% on dry matter basis:-							
Crude protein %	50.8	58.23[a]	57.44[a]	55.5[a]	56.4[a]	49.58[b]	0.909
Total lipid %	18.69	20.67[b]	18.81[bc]	24.2[a]	21.5[ab]	17.26[c]	0.743
Crude ash %	26.51	18.1[a]	19.75[a]	17.3[a]	18.1[a]	28.16[b]	1.360

.* FM: fishmeal diets, MM: meat meal diets, PBM: poultry by product meal, PBM+MM: poultry by product meal+ meat meal diets and PPM: plant protein meal.

** Initial values are not included in the statistical analysis.

In fish fed animal by-products-based diets **(Millamena, 2002; Xavier *et al*., 2014)**. **Shalaby *et al*. (2023)** found that FMFDs supplemented with essential amino acids led to compensate the deficiency of amino acids in plant protein sources that can be partially or completely replace fishmeal in sea bass reared in freshwater environment. The above studies confirmed the present results. All the test diets are similar EAA profile by different protein sources and pure Meth and Ly supplementation (Table 2). In this respect, it was already reported that single EAA deficiency led to a reduction of feed intake in gilthead sea bream **(Peres *et al*., 2011)**. Moreover, dietary supplementation with crystalline amino acids showed only limited improvement on the growth performance of Japanese flounder and rainbow trout **(Moutinho *et al*., 2017)**. The good growth performances obtained in the present study are probably a result of both the dietary supplementation of crystalline amino acids, ensuring the required essential amino acids for this species were fulfilled, and the good palatability of the experimental diets. However, PBM-diet in this research has decreased the feed intake and increased the feed conversion ratio. This suggests that PBM-diet is difficult for seabass to utilize with apparent digestibility 68% **(NRC, 1993)**, which was also supported by intestinal tissue sections with shorter plica and thinner intestinal wall. In addition, high proportion PBM-di*et al*so affected the body composition of seabass. The content of body fat in seabass increased significantly when the dietary content of PBM was added, which indicates that poultry by product meal have affected the fat metabolism of seabass this agrees with **Dong *et al*. (2016).**

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

Items	Experimental diets*					
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SE
	FM	MM	PBM	PBM+MM	PPM	
Feed price (LE/kg)	16.63	10.25	12.22	11.17	14	
Fish price (LE/kg)	100	100	100	100	100	
Incidence cost (IC)	28.32[b]	20.19[a]	26.07[b]	18.39[a]	52.33[c]	0.315
Incidence cost change	100[b]	71[a]	92[b]	65[a]	185[c]	0.831
Profit index (PI)	3.54[b]	4.95[a]	3.89[b]	5.46[a]	1.92[c]	0.337
Profit index change %	100[b]	140[a]	110[b]	154[a]	54[c]	0.239

* FM: fishmeal diets, MM: meat meal diets, PBM: poultry by product meal, PBM+MM: poultry by product meal+ meat meal diets and PPM: plant protein meal

Previous studies showed that MM could be replaced up to 40–60% of FM in diets of other fish species without negatively affecting fish performance **(Hunter *et al*., 2000; Stone *et al*., 2000; Ai *et al*., 2006)**. The replacement level might be increased up to 67 and 75% in diets of Sutchi catfish (*Pangasius hypophthalmus*) and African catfish (Clarias gariepinus), respectively **(Goda *et al*., 2007; Kader *et al*., 2011)**. However, **Millamena (2002)** illustrated use higher levels of MM, up to 80% in diets for grouper (*Epinephelus coioides*) by blend of meat and bone meal. These findings are confirmed by the present study. The group of sea bass that were fed MM (diet 2) that contained meat powder as a sole source of animal protein with a mixture of plant protein sources supplemented with methionine and lysine gave a clear improvement in the growth rate compared to the group of fish fed on a diet containing a mixture of protein plants only. The same result was obtained in the case of using PBM (diet 3). This improvement in the growth rate was due to the ability of sea bass fish to obtain some of its needs of EAA that are not available in plant proteins from animal protein sources, whether it is MM-diet (diet 2) or PBM-diet (diet 3) but not do for fish group fed the 20% FM (basal diet).

Decrease performance of sea bass fed ether MM or PBM instead of FM may be attributed to higher ash content of MM that can also limit its use in fish feeds. High levels of indigestible inorganic matter (i.e., bones) may increase intestinal transit, leading to a higher feed intake but decreased feed efficiency and growth performance (**Goda *et al*., 2007; Xavier *et al*., 2014)**. In addition, the nutritive value of MM is highly dependent of the freshness and quality of the raw materials and of the processing technologies used **(Kureshy *et al*., 2000)**. Similar findings were obtained with PBM **Moutinho *et al*. (2017)**. Mixed diet PBM+MM led to the highest voluntary feed intake, which suggests that palatability was not compromised by the inclusion of animal protein and may reflect an attempt of fish to adjust digestible energy intake. Indeed, it is accepted that, up to a certain level, animals adjust feed intake to meet digestible energy needs **(Yamamoto *et al*., 2000; Moutinho *et al*., 2017)**. Although fish increased feed intake when fed the high mixed diet PBM+MM, they were able to maintain the same growth of the fishmeal diet group.

The present work showed no significant differences in body composition (protein, lipid, ash and moisture) among fish fed FM diet, mixed diet and meat meal diet (Table 4). The relatively steady protein and essential amino acid contents in body suggested that fish could keep protein and essential amino acids to maintain normal physiological function when facing different diet. Previous studies showed

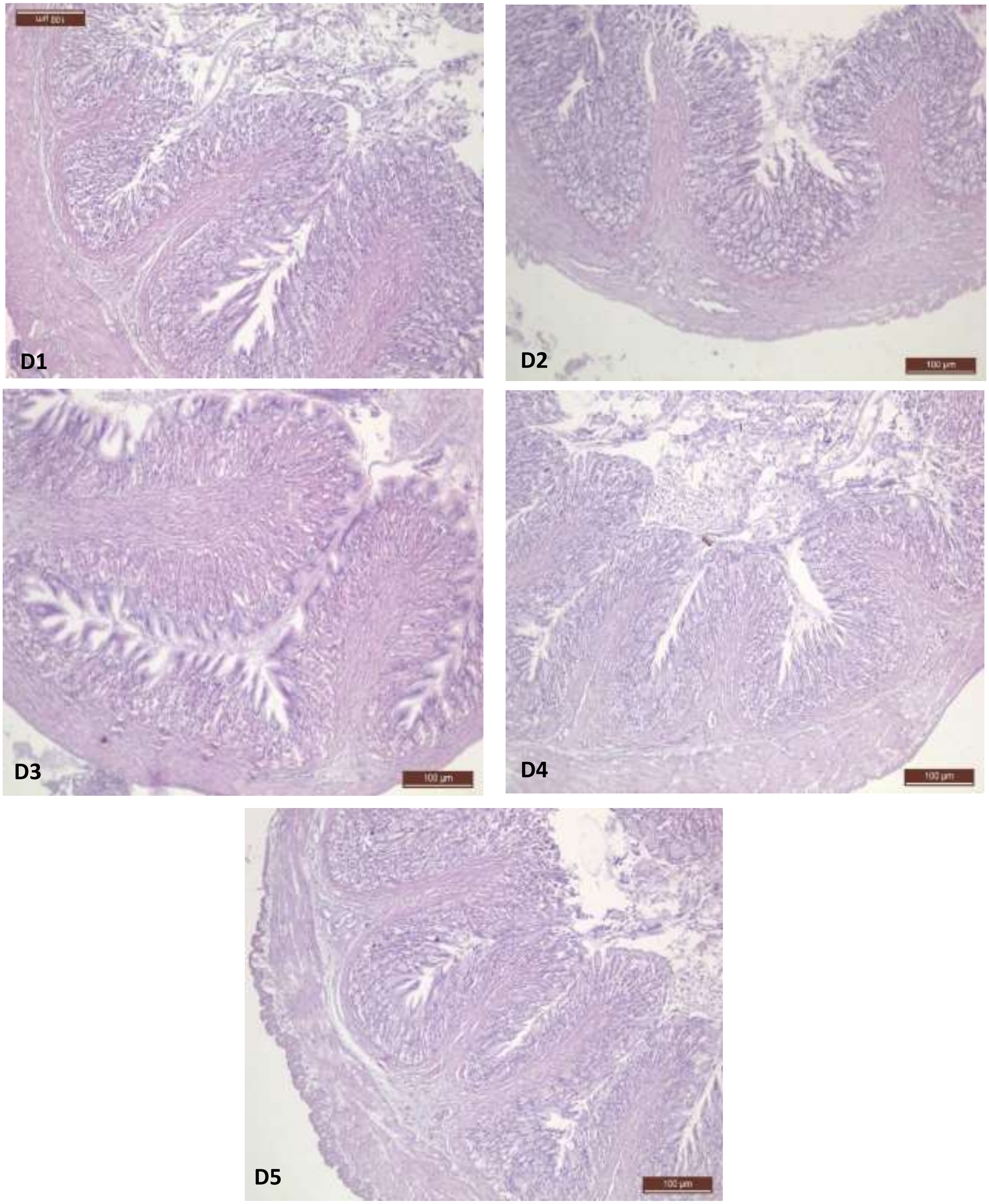

Fig.2. Transverse section of seabass juveniles (*Dicentrarchus labrax*) villus in distal intestine fed different diets for 90 days, D1, D2, D3, D4, D5 represent fish feed diet 1,2,3,4 and 5 respectively. (bar length = 100.0 μm).

that there were no significant differences in body content of protein, lipid, ash and moisture among fish fed the diets with graded levels of MM **(Robaina *et al*., 1997; Bureau *et al*., 2000)**. These agree with **Campos *et al*. (2017)** who found the hydrolyzed feather inclusion did not significantly affect final whole-body composition of the fish. **Hao *et al*. (2020)** found that high proportion poultry by-products di*et al*so affected the body composition of turbot. The content of body fat in turbot decreased significantly when the dietary content of EHPB increased.

The present work illustrated that use of MM as a sole animal protein source led to decrease of incidence cost by 29% and increase profit index by 40%. While PBM resulted in decrease 8% of incidence cost and increase of 10% (Table 5). It is clear the MM is an economically cheaper source of protein than PBM and FM. **Jayathilakan *et al*. (2012)** stated in the last few decades, huge amounts of by-products, such as skin, viscera, heads, feet, and blood have become available as a result of the modern methods of animal meat processing. Recycling wastes from meat processing plants and animal slaughterhouses has economical, biological, and environmental benefits **(Zaman *et al*., 2022)**.

As showed in figure 2 normal in vacuolar size and thickening of the lamina propria and villus size in diet 1; Villus in distal intestine of Sea bass fed diet 2 showing increase in the villi, minor lamina propria and slightly reduced microvilli; Villus in distal intestine of Sea bass fed diet 3 showing slightly reduced microvilli and intestinal tissue sections with shorter plica and thinner intestinal wall; Villus in distal intestine of Sea bass fed diet 4 showing normal size and thickening of the lamina propria; Villus in distal intestine of Sea bass fed diet 5 showing slightly reduced microvilli size. Histological analysis indicated a strong effect on plant ingredients on height and width of distal intestine simple folds and enterocyte vacuolization, and these effects corresponded directly with reduced weight gain and growth rate in the group of plant protein diet. However, the noted physiological changes to the distal intestine did not include enhanced inflammation of the lamina propria or sub-mucosal tissues.

Replacement of FM with mixed animal protein appears to be economically feasible. The cost of formulating present diets for sea bass was reduced. As resulting in a lower cost index and high profit index. As apparent in the present study the economic parameters evaluated improved with the dietary inclusion of mixed animal protein diet and meat meal diet MM, resulting in lower CI (i.e. the feed cost to produce 1 kg of fish) with a higher PI for this diets. On the contrary in the group feeding plant protein diet with high incidence cost and low profit index. Since PI is a more suitable parameter to evaluate economic profitability, as it considers production, feed costs, and selling price, our results suggest that there is a greater economic return when replacing FM protein with mixed animal protein, at least during the on-growing phase of sea bass. Therefore, the findings of this study will encourage feed manufacturers to utilize animal proteins more efficiently in generating low-cost and sustainable aqua feed. Furthermore, since these ingredients were obtained by rendering locally produced by-products, its use could help reduce the need for imported feedstuffs, thus diminishing the fishmeal of the aqua feed sector.

In conclusion, mixed animal protein with amino acid supplementation can replace fishmeal protein in feeds for sea bass juveniles raised in fresh water without compromising growth and feed efficiency, histological examine and with a positive outcome in economic efficiency.

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