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Valorization Use of Amphipod Meal, *Gammarus pulex*, as a Fishmeal Substitute on Growth Performance, Feed Utilization, Histological and Histometric Indices of the Gut, and Economic Revenue of Grey Mullet

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Abstract: The future of aquaculture development is directed toward high intensification to overcome the decline in natural fishing and to provide enough protein for the increasing world population. The spread of aquaculture production and intensification requires the search for high-quality, new efficient feed ingredients with low cost and sustainable importance. Therefore, the current study aimed to detect the effects of partial or total replacement of fishmeal with amphipod meal, *Gammarus pulex*, on growth performance, survival percent, feed utilization, histological alteration of intestine and liver, and economic yield of grey mullet, *Mugil cephalus*, fry. Five diets were formulated to contain 100% fishmeal (FM), or FM replaced with 25%, 50%, 75%, or 100% amphipod meal (APM) (D_0, D_{25}, D_{50}, D_{75}, and D_{100}, respectively). A total of 300 grey mullet fry (0.097 ± 0.001 g), were divided into five groups (three replicates each) at an initial stocking density of 20 fry per aquaria (100 L). The aquarium's water is renewed at a rate of 30% daily. During a 60-day experimental period, the feeding rate was 20% of body weight, which was introduced as five meals per day. Fish fed D_{50} achieved the highest significant values of final weight (1.80 g), weight gain (1.70 g), survival (86.67%), final length (4.47 cm), and length gain (2.06 cm). In addition, the feed utilization of diets containing increasing substitution levels of FM showed that the highest protein intake (0.82 g ish^{-1}), protein efficiency ratio (0.83), protein productive value (30.65%), and the lowest significant feed conversion ratio (1.21) were recorded with D_{50}. The dose-response study revealed that the best substitution levels could range between 50% and 75%. Histological observations confirmed that the highest number of goblet cells and intestinal villi were recorded in the group fed D_{50}. No pathological effect was observed in the liver at all substitution levels. In terms of economic efficiency, the best economic conversion ratio was recorded in the group fed D_{50}. This study confirmed that 50% partial substitution of FM with APM is the ideal replacement level for grey mullet fry. In addition, the use of a new renewable alternative, such as APM to substitute FM, could relieve the pressure on the capture of wild fish and reduce the environmental impact of inland aquaculture.

Keywords: protein sources; amphipod meal; substitution; grey mullet; growth performance; histological changes

1. Introduction

Aquaculture is a promising industry that provides important animal protein at a low cost to meet the demands of an increasing global population [1]. However, aquaculture itself is also facing new challenges in terms of economic and ecological impact [2]. Aquaculture feeds are the highest recurring cost in aquaculture activities, accounting for 40–70% of the variable costs [3]. Fishmeal (FM) is the most important protein source and is considered the major ingredient component in the aqua diet. Fishmeal supply is one of the most significant difficulties facing the aquaculture industry, particularly in Egypt and developing countries, due to the low availability, high cost, cheating, and improper treatment of protein during diet manufacture [4]. According to Welcomme [5], around 70% of the world's fisheries stocks (fish used for oil production and fishmeal) are either wholly depleted or overfished, and this means that there is no chance to increase or assure the continuous supply of catches in the near future. Furthermore, the growing aquaculture industry will require alternative feed sources.

Recently, the aquaculture sector has focused on searching for alternative feedstuffs with a significant potential of success as fishmeal replacers [6–8]. For instance, plant protein sources such as high protein distillers' dried grains [9,10], fermented soybean meal [11], soy protein isolates [12], and corn gluten [13], among others. Moreover, several insects and mealworms have been used as fishmeal replacers in aquatic diets [14,15]. Meanwhile, animal protein origins could be the best alternatives to FM at higher replacement levels, such as poultry by-products [14], dairy by-products [16], crustacean meal [17], and small crustacean dried biomass (Palaemon and brine shrimp) [18,19].

Many authors have been reported that marine and freshwater zooplankton are natural live feeds for the majority of wild fish at various stages of development [20,21]. Furthermore, zooplankton meal has been suggested as a promising alternative protein source in aquatic diets [22–24]. The aquatic environment is still not fully examined and could provide feasible and sustainable solutions for FM supply shortages [25]. One possible strategy is harvesting from the lower trophic levels of the aquatic communities, and exploiting species, such as amphipods, copepods, daphnia, and total zooplankton. Each year, hundreds of millions of tons of various zooplanktons are produced, but only a small portion is harvested [26]. These aquatic organisms have recently attracted the scientific interest of aquaculture researchers because they are considered a rich source of animal protein and omega-3 fatty acids [27–29]. Our recent work revealed that the use of *Daphia magna* meal could successfully replace the FM at a level of 75% [30].

In this context, a promising alternative feedstuff could be amphipod crustaceans, *Gammarus* species. Gammarids have widespread distribution, and they have successfully adapted to both marine and freshwater ecosystems [31]. The amphipod, *Gammarus pulex* (Linnaeus, 1758) is one of the possible protein alternative sources due to its high productivity and nutritional quality [20]. Amphipoda contains up to 40% protein, with lysine, leucine, phenylalanine, and threonine amino acids dominating, as well as high levels of polyunsaturated fatty acids and phospholipids and high amounts of phosphatidylcholine, phosphatidylethanolamine, and triacylglycerols [32]. *Gammarus* has been used as a protein source in the diets of several pet animals, as well as in environmental monitoring studies [33]. The amphipod can be grown on a large scale using low-cost diets (such as fish feces or *Ulva* sp.) and offers the potential for the development of a sustainable culture [34].

The grey mullet, *Mugil cephalus* L., is one of the most traditional, economical, and important omnivorous fish in coastal aquaculture in many countries [35]. Mullet contributed 2.6% to the total production of marine fishes [5]. Practical diets used in the culture of grey mullet are still dependent on the significant containment of costly and limited marine raw materials, such as FM [35]. With the expansion of aquaculture and, consequently, aquafeed production, the use of FM in artificial nutrition must be kept to a minimum [36]. Therefore, the current work aims to investigate the effect of partial or total replacement of FM by amphipod meal (APM) on growth performance, feed and nutrient utilization, whole-body composition, histological changes of both liver and intestine, and economic yield of grey mullet fry.

2. Materials and Methods

2.1. Experimental Fish and Culture Technique

Grey mullet fry were obtained from the Edko coast, Mediterranean Sea, El Behira Governorate, Egypt. The average initial body weights and body lengths of the fish were 0.097 ± 0.001 g and 1.35 ± 0.20 cm, respectively. A total of 300 grey mullet fry were equally divided into 5 groups (three replicates per each group) and stocked into 15 glass aquariums (100 L; 66 cm $\times$ 47 cm $\times$ 33 cm) at an initial stocking density of 20 fry per aquaria. Before the start of the experiment, grey mullet fry were adapted to the experimental conditions for 2 weeks and fed a control diet, five times a day. After adaptation, the experiment lasted 8 weeks. The aquariums were renewed at a rate of 30% a day. During the experimental period, all fry were fed their respective diets at a level of 20% of the actual live bodyweight of all fish in the aquaria, which was adjusted biweekly. The daily ration was divided into five equal amounts and offered five times a day (09.00, 12.00, 15.00, 18.00, and 21.00). The uneaten feed was collected carefully and dried to accurately determine the feed intake.

During the experiment, all water quality parameters were maintained within the acceptable range for the culture of mullet fry. Water temperature ranged from 21.6 to 23.7 °C, salinity 5%, dissolved oxygen (DO) ranged from 5.40 to 6.45 mg L^{-1}, pH ranged from 7.34–7.72, the ammonia (NH_3) ranged from 0.16 to 0.30 mg L^{-1}, and photoperiods was 10 light and 14 dark. Each aquarium was supplied with air through an air pipeline using an air blower. Fish feces were removed five times every day by siphoning. The study was carried out in accordance with the Declaration of Helsinki's standards and was authorized by Alexandria University's Institutional Animal Care and Use Committee with approval No. (AU:19/21/05/27/3/18).

2.2. Preparation and Characterization of Amphipod Meal (APM)

Isolation, cultivation, nutritional composition, and preparation of APM were conducted as described previously by Abo-Taleb et al. [20]. The Amphipod has been isolated from freshwater Lake Mariout, Alexandria, Egypt. Isolated *G. pulex* were cultured (with an initial stocking density of 30 ind. L^{-1} with a sex ratio of 1:2 of male to female) for 100 days under controlled growth conditions of salinity (8%), temperature (27 ± 1 °C), pH (7.8), DO (5.5 mg L^{-1}) and photoperiod 12/12 (600 lux). Amphipods were cultured in different conditions. However, amphipoda cultured on balls of palm fibers habitat resulted in the highest yields (127 ind. L^{-1}) and wet weight (2.45 g L^{-1}). During the culture period, amphipod was fed a formulated diet consisting of 33.3% spirogyras algae, 33.3% berries leaves, 16.7% rice bran powder, and 16.7% soybean powder (1:1:0.5:0.5), respectively. After 100 days of culture under previous conditions, amphipods were harvested, dried, powdered, and preserved at −20 °C until further analysis or use as a protein meal for the investigated species. Cultured amphipod resulted in a valuable nutritional value of crude protein (40%), carbohydrates (27.4%), crude fat (5.5%), crude fiber (2.9%), ash (21.4%), β-carotene (21,602.96 IU 100 g^{-1}), folic acid (521.18 μg 100 g^{-1}), and tannic acid (223.14 mg 100 g^{-1}). The values of vitamins B_2, B_6, B_6, B_{12}, A, E, D were 338.38 mg100 g^{-1}, 635.61 mg 100 g^{-1}, 419.50 mg 100 g^{-1}, 19,623.98 IU 100 g^{-1}, 177.95 mg 100 g^{-1}, and 59.67 mg 100 g^{-1}, respectively. Moreover, the total bacterial count was less than 6500×10^2 CFU g^{-1}, while *Listeria, Salmonella*, and *Clostridium* count was 65, 25, and 25 CFU g^{-1}, respectively. No counts of *E. coli*, yeasts, or molds were obtained, as described previously [20].

2.3. Experimental Design

Isonitrogenous (35.02 0.09% crude protein) and isocaloric (4545 20.8 kcal kg^{-1}) diets were formulated. The biochemical compositions of the ingredients used in the experimental diets (% of dry matter) are presented in Table 1. In addition, the amino acid profile of fishmeal and gammarid meal (amphipod meal) were reported in Table 2 according to Sallam et al. [37] and Koprucu et al. [38].

Table 1. Proximate biochemical compositions of the ingredients used in formulating the experimental diet (% of dry matter).

Ingredients	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	NFE (%)	GE (Kcal kg^{-1})
Fishmeal (FM)	92.03	60.17	12.50	0.62	15.40	11.50	3930.14
Amphipod meal (APM)	93.15	40.02	14.21	1.50	19.82	24.51	3364.05
Soybean meal	90.23	45.32	1.10	7.31	6.34	40.32	4451.10
Yellow corn	88.16	10.10	3.62	2.32	1.30	82.80	3985.01
Rice bran	89.28	14.01	6.43	9.90	5.31	64.43	3792.04

DM: dry matter (%); CP: crude protein (%); EE: ether extract (%); CF: crude fiber (%); NFE: nitrogen free extract (%); and GE: gross energy (Kcal kg^{-1}).

Table 2. Amino acid profile of fishmeal and gammarid meal (amphipod meal).

Amino Acid Profile (% of Dry Weight) [1]		
	Fishmeal [37]	**Gammarid Meal [38]**
Methionine	1.99	1.1
Lysine	5.04	3.0
Cystine	0.86	0.5
Arginine	3.77	3.2
Glycine	4.4	2.3
Histidine	1.61	1.3
Isoleucine	3.1	1.9
Leucine	3.99	3.4
Phenylalanine	2.78	2.3
Tyrosine	2.52	1.9
Serine	3.29	1.9
Threonine	2.76	2.1
Tryptophan	0.75	-
Valine	3.5	2.3

[1] Amino acid profile was obtained as cited in the mentioned references.

In the current study, five groups (diets) were conducted. The first group is the basal control diet (D_0) that contains 100% fishmeal. Diets 2–5 are APM diets that contains 25% (D_{25}), 50% (D_{50}), 75% (D_{75}), and 100% (D_{100}) of APM with different percentages of the equivalent fishmeal replacement. Formulation (% DM), cost (Egyptian L.E. per ton), and proximate composition (%) of the control diet D_0 and four experimented APM diets D_{25}, D_{50}, D_{75} and D_{100} are presented in Table 3.

Table 3. Formulation (% DM), cost (U.S.A. \$/ton diet^{-1}), and proximate composition (%) of the experimented diets.

Diets *	D_0	D_{25}	D_{50}	D_{75}	D_{100}
Formulation (% DM)					
Fishmeal (FM) [1]	20.00	15.00	10.00	5.00	0.00
Cost	509.55	382.17	254.78	127.39	0.00
Amphipod meal (APM)	0.00	8.5.00	17.00	25.50	34.00
Cost	0.00	108.28	216.56	324.84	433.12
Soybean meal [2]	42.5.00	42.5.00	42.00	42.00	42.00
Cost	189.17	189.17	187.26	187.26	187.26
Yellow corn [2]	17.00	15.00	13.00	11.5.00	10.00
Cost	39.49	34.39	29.94	26.18	22.93
Rice bran [2]	16.50	15.00	14.00	12.00	10.00
Cost	37.58	34.39	31.85	27.39	22.93
Vitamin and mineral premix [3]	2.00	2.00	2.00	2.00	2.00
Cost	127.39	127.39	127.39	127.39	127.39
Fish Oil [4]	2.00	2.00	2.00	2.00	2.00
Cost	25.48	25.48	25.48	25.48	25.48
Total Cost (U.S.A. \$/ton diet)	928.66	901.27	873.25	845.92	819.11
Proximate composition (%)					
Crude protein	35.14	35.13	34.96	34.96	34.9
Ether extract	9.5	10.1	10.9	10.8	11.2
Ash	10.2	9.8	10.1	10.4	10.2
Crude fiber	2.4	2.6	2.5	2.7	2.5
Nitrogen free extract	37.9	37.5	36.5	36.1	36.1
Dry matter	93	95	94	93	95
Gross energy (Kcal/kg) [5]	4495	4543	4565	4548	4574

* D_0, D_{25}, D_{50}, D_{75}, and D_{100}: diets supplemented with 0%, 25%, 50%, 75%, and 100% of amphipod mail *Gammarus pulex* as alternative protein source. [1] Fish meal was purchased from Zhanjiang Haibao Feed Co., Ltd., Guangdong, China. [2] Ingredients were sourced from local feed ingredients' market, Alexandria, Egypt. [3] Vitamin and mineral premix provided the diet with (IU or mg/kg dry diet) retinol (VA), 3000 IU; cholecalciferol (VD), 1500 IU; tocopherol (VE), 40 mg; menadione (VK), 4.5 mg; thiamin (VB_1), 8 mg; riboflavin (VB_2), 8.5 mg; pyridoxine (VB_6), 6.5 mg; cyanocobalamin (VB_{12}), 0.02 mg; nicotinic acid, 45 mg; nicotinamide, 45 mg; D-Ca pantothenate, 17 mg; inositol, 40 mg; biotin, 0.15 mg; folic acid, 1.3 mg; ascorbic acid, 110 mg; copper, 6.5 mg; iron, 45 mg; selenium, 0.35 mg; zinc, 70 mg; manganese, 8.5 mg; magnesium, 100 mg; cobalt, 1 mg; iodine, 1.2 mg. [4] Fish oil was purchased from Bawa Fishmeal and Oil Co., Karnataka, India. [5] Gross energy was calculated with the following equation = total carbohydrate × 17.2 J kg^{-1} + fat × 39.5 J kg^{-1} + protein × 23.5 J kg^{-1}.

2.3.1. Growth Indices

The mean final body weight (FW) of each aquarium was determined by dividing the total fish weight by the number of fish. The following equations were used to compute weight gain (WG), feed conversion ratio (FCR), protein intake (PI), protein efficiency ratio (PER), and protein productive value (PPV):

$$\text{Weight gain (WG; g)} = \text{FW (g)} - \text{IW (g), where: FW and IW are final and initial body weight (g)} \quad (1)$$

$$\text{Length gain (cm/fish)} = \text{final total length (cm)} - \text{initial total length (cm)} \quad (2)$$

$$\text{Condition factor} = 100 \times (\text{final weight (g)}/\text{length (cm)}^3) \quad (3)$$

$$\text{Feed conversion ratio, FCR (Feed: gain)} = \text{FI (g)}/\text{WG (g), where: FI is feed intake (g)} \quad (4)$$

$$\text{Protein Intake (PI; g)} = \text{feed intake (g)} \times \text{protein (\%)} \quad (5)$$

$$\text{Protein efficiency ratio (PER)} = \text{weight gain (g)}/\text{protein intake (g)} \quad (6)$$

$$\text{Protein productive value (PPV)} = (\text{protein gain (g)}/\text{protein intake (g)}) \times 100 \quad (7)$$

2.3.2. Whole-Body Composition and Proximate Composition

The proximate composition of DM, CP, EE, ash, and GE of the dietary components, experimental diets, and whole-body composition of fish (as a pooled sample of each replicates; 10–12 fish) were determined according to the Association of Official Analysis Chemists [39]. Briefly, the dry matter was evaluated by drying the samples at 105 °C for 24 h. Crude protein (N × 6.25) was determined using the Kjeldahl system method (VELP Scientifica, UDK 149, Usmate Velate, Italy). Crude lipid content was determined by petroleum ether extraction (40–60 °C) using a Soxhlet System (VELP Scientifica, SER 148, Usmate Velate, Italy). The ash content of different samples was determined using a muffle furnace at 550 °C for 5 h (Nabertherm B150, Bremen, Germany).

2.3.3. Histological Examination

At the end of the experiment, six samples from each group were randomly selected for histological analysis of the intestine and liver. Specimens from the liver and intestine were collected and fixed in 10% neutral buffered formalin for 24 hrs, then transferred to 70% ethyl alcohol. A histological paraffin embedding technique in the traditional sense has been carried out. Then, sections (5 μm thick) were cut and stained with hematoxylin and eosin [40]. Intestine parts were subjected to muscle thickness and villus height measurements in accordance with Hamidian et al. [41].

2.3.4. Economic Evaluation

Economic evaluations of the five experimented diets were calculated, including the cost of feed and the price of fish. The cost of experimental diets was calculated in US$ according to international and local market prices as described by Goda et al. [42] using the following equations:

$$\text{Incidence cost} = \text{cost of feed to produce 1000 fry} + \text{the price of the fish} \tag{8}$$

$$\text{Value of fish} = \text{price of 1000 fry (1500 L.E.)} \times \text{survival rate (SR\%)} \tag{9}$$

$$\text{Profit index} = \text{value of fish/cost of consumed feed (1000 fish)} \tag{10}$$

$$\text{Economic conversion rate (ECR)} = \text{feed intake (g)/weight gain (g)} \tag{11}$$

The cost for 1 kg of each of the diets (D_0, D_{25}, D_{50}, D_{75} and D_{100}) were 0.93, 0.90, 0.87, 0.85 and 0.82 US$., respectively, which calculated based on the local price at 2020.

2.4. Statistical Analyses

All data were presented as mean ± standard error. Statistical analyses were conducted using SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). The effect of partial substitution of FM with DMM was investigated using a one-way ANOVA test. Duncan's multiple range test was also used to find significant variations between means [43]. In addition, to determine the fit regression model between increasing fishmeal substitution level and weight gain, and FCR, polynomial contrasts were performed [44].

3. Results

3.1. Growth Performance

The growth performance of grey mullet fry is presented in Table 1. The highest significant values ($p \leq 0.05$) of growth performance were observed for fish-fed D_{50} compared to fish-fed either replacement diets (D_{25}, D_{75}, and D_{100}) or the control diet. Grey mullet fry fed the diet contained 50% of APM as FM replacement achieved the highest significant ($p \leq 0.05$) values of FW (1.80 g), WG (1.70 g), final length (4.47 cm), and survival (86.67%) (Table 1). Whereas, the response of animals in terms of weight gain to increasing FM substitution levels with APM revealed a poly-nominal regression pattern with a strong correlation ($R^2 = 0.8524$) (Figure 1A).

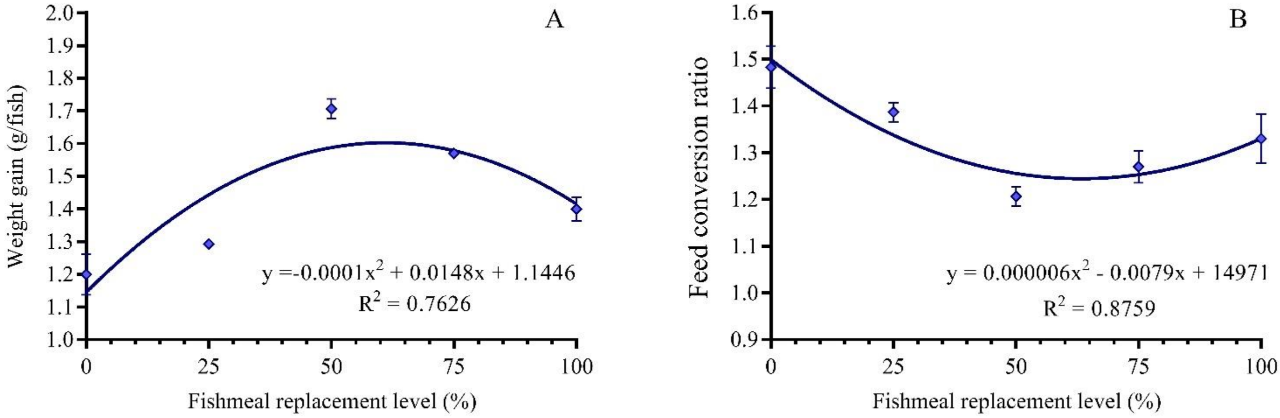

Figure 1. The fit regression model of increasing fishmeal substitution levels by amphipod meal of weight gain (**A**) and feed conversion ratio (**B**) of *Mugil cephalus*.

3.2. Feed Utilization

Feed utilization indices are presented in Table 4. Grey mullet fed on 50 and 75% APM showed higher ($p \leq 0.05$) FI than fry fed on the control diet by 16.3 and 13.5%, respectively. The FCR of grey mullet fry fed on D_{50} (1.21) was significantly lower ($p \leq 0.05$) than that of fish fed other replacing diets and the control. The FCR of the group fed D_{50} was lower than the group fed D_0 by 18.24%. The regression pattern of FCR revealed that the best value could be recorded between 60–70% replacement levels with a strong correlation (R^2 = 0.8161) (Figure 1B).

Table 4. Effect of partial or total replacement of fishmeal by amphipod meal, *Gammarus pulex*, on growth performance of *Mugil cephalus*.

Diets *	D_0	D_{25}	D_{50}	D_{75}	D_{100}
Initial weight (g/fish)	0.096 ± 0.00	0.096 ± 0.00	0.098 ± 0.00	0.097 ± 0.00	0.097 ± 0.001
Final weight (g/fish)	1.29 ± 0.03 ^{d}	1.38 ± 0.01 ^{d}	1.80 ± 0.02 ^{a}	1.68 ± 0.01 ^{b}	1.49 ± 0.03 ^{c}
Weight gain (g/fish)	1.20 ± 0.03 ^{d}	1.29 ± 0.01 ^{d}	1.70 ± 0.02 ^{a}	1.57 ± 0.01 ^{b}	1.40 ± 0.03 ^{c}
Initial length (cm/fish)	1.33 ± 0.03 ^{b}	1.37 ± 0.03 ^{ab}	1.40 ± 0.00 ^{a}	1.30 ± 0.00 ^{b}	1.40 ± 0.00 ^{a}
Final length (cm/fish)	3.83 ± 0.03 ^{c}	3.93 ± 0.03 ^{c}	4.47 ± 0.03 ^{a}	4.03 ± 0.06 ^{ab}	4.13 ± 0.03 ^{b}
Length gain (cm/fish)	2.50 ± 0.06 ^{c}	2.57 ± 0.03 ^{bc}	3.06 ± 0.03 ^{a}	3.00 ± 0.06 ^{a}	2.73 ± 0.03 ^{b}
Survival (%)	73.33 ± 3.3 ^{d}	73.33 ± 3.3 ^{d}	86.67 ± 3.3 ^{a}	83.33 ± 3.3 ^{b}	76.33 ± 3.3 ^{c}
Condition factor	2.30 ± 0.05	2.27 ± 0.04	2.02 ± 0.06	2.11 ± 0.08	2.12 ± 0.07

* Data are means ± SD (n = 3) followed by different letters (a > ab > b > bc > c > d) in the same row are significantly different (p < 0.05). D_0, D_{25}, D_{50}, D_{75}, and D_{100}: diets supplemented with 0%, 25%, 50%, 75%, and 100% of APM, respectively.

Grey mullet fry fed D_{50} and D_{75} presented significantly higher ($p \leq 0.05$) protein intake than fry fed D_0, with percentages reaching 15.4% and 12.6%, respectively. The PER of grey mullet fry fed D_{50} (0.83) was significantly higher ($p \leq 0.05$) than the PER of fish fed other replacement diets and the control diet (Table 5). The PPV of grey mullet fry fed on D_{50} (30.65%) was significantly higher ($p \leq 0.05$) than the PPV of fish fed either replacing diets D_{25}, D_{75}, and D_{100} (25.18, 28.35, and 26.50%, respectively) or control diet D_0 (23.51%). The PPV in the group fed D_{50} was 23.2% higher than in the D_0 group (Table 5).

Table 5. Effect of partial or total replacement of fishmeal by amphipod meal, *Gammarus pulex*, on feed utilization of *Mugil cephalus*.

Diets *	D_0	D_{25}	D_{50}	D_{75}	D_{100}
Feed intake (g/fish)	1.77 ± 0.03 ^{b}	1.79 ± 0.01 ^{b}	2.06 ± 0.02 ^{a}	2.01 ± 0.05 ^{a}	1.86 ± 0.01 ^{b}
Feed conversion ratio (Feed: gain)	1.48 ± 0.03 ^{a}	1.39 ± 0.01 ^{ab}	1.21 ± 0.01 ^{d}	1.27 ± 0.02 ^{cd}	1.33 ± 0.03 ^{bc}
Protein intake (g/fish)	0.71 ± 0.01 ^{b}	0.71 ± 0.01 ^{b}	0.82 ± 0.01 ^{a}	0.80 ± 0.02 ^{a}	0.74 ± 0.02 ^{b}
Protein efficiency ratio	0.68 ± 0.01 ^{d}	0.72 ± 0.03 ^{cd}	0.83 ± 0.05 ^{a}	0.79 ± 0.01 ^{ab}	0.75 ± 0.02 ^{bc}
Protein productive value (%)	23.51 ± 0.48 ^{d}	25.18 ± 0.51 ^{cd}	30.65 ± 0.54 ^{a}	28.35 ± 0.83 ^{b}	26.50 ± 0.46 ^{c}

* Data are means ± SD (n = 3) followed by different letters (a > ab > b > bc > c > cd > d) in the same row are significantly different ($p < 0.05$). D_0, D_{25}, D_{50}, D_{75}, and D_{100}: diets supplemented with 0%, 25%, 50%, 75%, and 100% of APM, respectively.

3.3. Carcass Composition

The whole-body chemical composition of the grey mullet fry is presented in Table 6. Among all diets, D_{50} achieved the highest significant ($p \leq 0.05$) protein content (67.23%) and gross energy (5424 Kcal kg^{-1}). The percent of increase reached 6.6% and 3.8%, respectively. The ash content of grey mullet fry fed on D_{50} (15.57) was significantly lower ($p \leq 0.05$) than the ash of fish fed on other replacing diets D_{25}, D_{75}, and D_{100} (18.80, 17.63, and 18.60, respectively) or the control diet D_0 (19.37). Among all diets, no significant differences ($p \leq 0.05$) were observed in dry matter content or ether extracts (Table 6).

Table 6. Effect of partial or total replacement of fishmeal by amphipod meal, *Gammarus pulex*, on carcass composition of *Mugil cephalus* (% on a wet weight basis).

Diets *	D_0	D_{25}	D_{50}	D_{75}	D_{100}
Moisture content	77.9 ± 0.88	78.07 ± 1.53	77.97 ± 1.49	77.9 ± 1.13	77.93 ± 0.71
Crude protein	13.94 ± 0.08 ^{c}	14.01 ± 0.05 ^{bc}	14.81 ± 0.09 ^{a}	14.39 ± 0.11 ^{b}	14.11 ± 0.07 ^{bc}
Ether extract	11.08 ± 0.08	11.07 ± 11.07	11.56 ± 0.04	11.22 ± 0.20	11.16 ± 0.06
Ash	3.40 ± 0.05 ^{a}	3.26 ± 3.26 ^{ab}	2.68 ± 0.06 ^{c}	3.04 ± 0.03 ^{b}	3.25 ± 0.04 ^{ab}
Gross energy (Kcal kg^{-1})	5223 ± 10.0 ^{c}	5245 ± 14.0 ^{c}	5424 ± 19.0 ^{a}	5308 ± 3.00 ^{b}	5262 ± 8.00 ^{bc}

* Data are means ± SD (n = 3) followed by different letters (a > ab > b > bc > c) in the same row are significantly different ($p < 0.05$). D_0, D_{25}, D_{50}, D_{75}, and D_{100}: diets supplemented with 0%, 25%, 50%, 75%, and 100% of APM, respectively.

3.4. Intestinal Histological Morphology Indices

Intestinal morphology is shown in Table 7. Villus length, width, and crypts depth, as well as muscle thickness of the intestine, revealed a marked improvement by APM ($p \leq 0.05$). Intestinal villi and muscle thickness reached the top in the fish group D_{50}, followed by D_{75}. The lowest values of the intestinal villi and muscle thickness were recorded in the control group. But the significant decrease in D_{25} and D_{100} when compared with D_{50} and D_{75}. When compared to the control group, the number of goblet cells in all parts of the intestine increased in the fish fed AMP-supplemented diets. (Table 7).

Table 7. Effect of partial or total replacement of fishmeal by amphipod meal, *Gammarus pulex*, on intestine morphology of *Mugil cephalus*.

Diets *	D_0	D_{25}	D_{50}	D_{75}	D_{100}
Villus length (µm)	413.7 ± 3.10 ^{d}	416.5 ± 1.10 ^{d}	475.8 ± 5.70 ^{a}	457.10 ± 3.60 ^{b}	425.70 ± 0.80 ^{c}
Villus width (µm)	90.70 ± 1.30 ^{e}	99.70 ± 1.50 ^{d}	131.80 ± 2.20 ^{a}	128.60 ± 1.10 ^{b}	108.60 ± 1.40 ^{c}
Crypts depth (µm)	44.00 ± 0.60 ^{e}	48.50 ± 1.10 ^{d}	77.5 ± 2.10 ^{a}	68.20 ± 1.10 ^{b}	55.20 ± 1.40 ^{c}
Muscle thickness (µm)	20.80 ± 0.40 ^{e}	22.00 ± 0.60 ^{d}	29.20 ± 0.70 ^{a}	27.30 ± 0.66 ^{b}	24.70 ± 0.33 ^{c}

* Data are means ± SD (n = 3) followed by different letters (a > b > c > d > e) in the same row are significantly different ($p < 0.05$). D_0, D_{25}, D_{50}, D_{75}, and D_{100}: diets supplemented with 0%, 25%, 50%, 75%, and 100% of APM, respectively.

3.5. Histological Observation of Liver

The liver of grey mullet fed D_0 showed normally organized hepatic lobules with radially arranged hepatic cords around well-formed thin-walled central veins. Elongated and branched hepatic sinusoids were seen among the hepatic cords. The hepatocytes were large, showed cytoplasmic ribosomal basophilia with centrally located, moderately enlarged nuclei. The hepato-portal area and the hepato-pancreatic structures were well-developed. Examined sections from the liver of groups (D_{25}, D_{50}, D_{75}, and D_{100}) revealed a histo-morphological structure comparable to the control group (Figure 2).

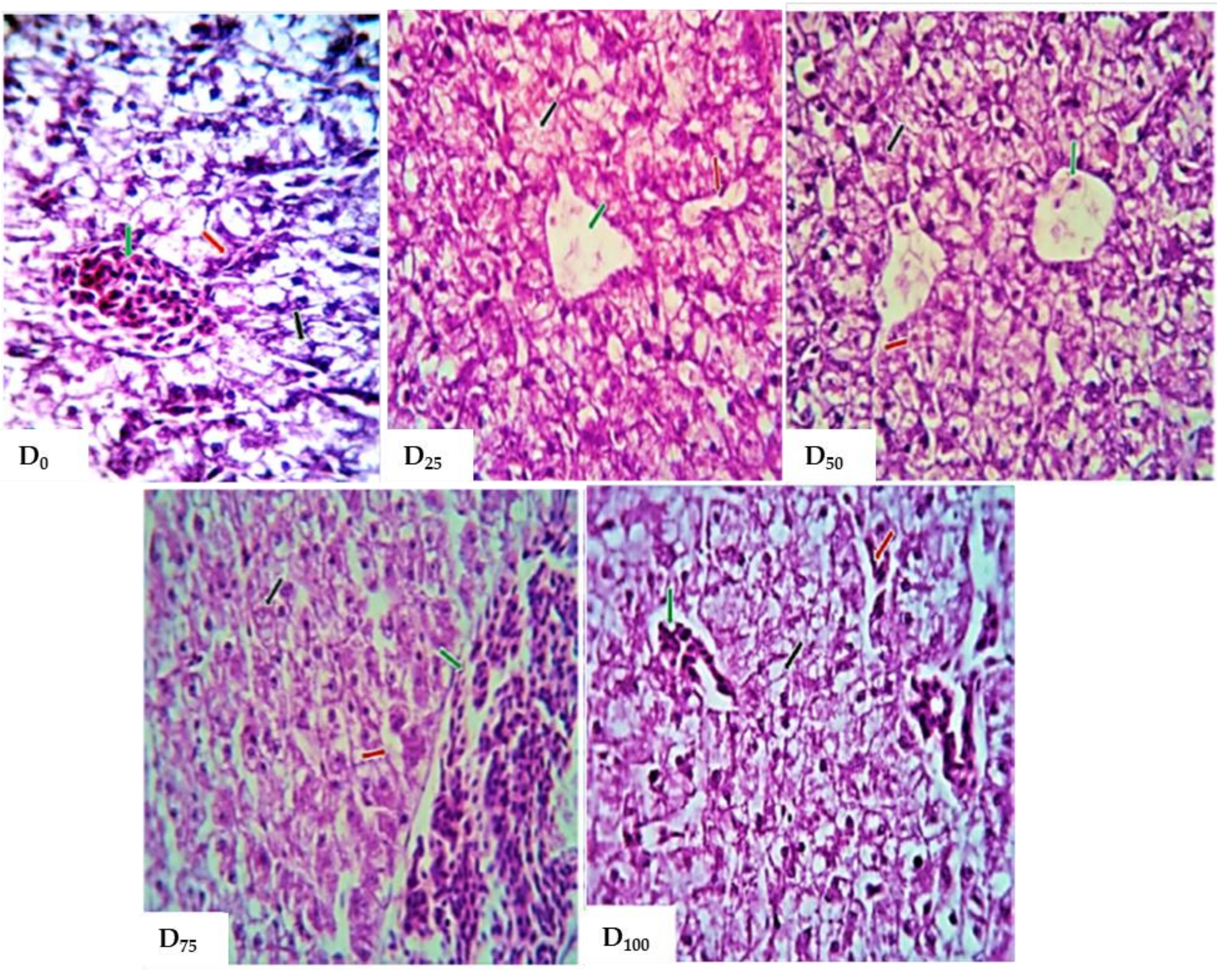

Figure 2. Photomicrograph of the effect of partial or total replacement of fishmeal by amphipod meal, *Gammarus pulex*, on the liver histology of *Mugil cephalus.* D_0 showing normally organized hepatic lobules with indistinct boundaries, radially arranged hepatic cords (black arrows) around a well-formed thin-walled central vein (green arrow). Hepatic sinusoids are seen among the hepatic cords (red arrows) for groups fed (D_{25}, D_{50}, D_{75}, and D_{100}) as in the control group. (hematoxylin and eosin staining, X 400).

3.6. Economic Evaluation

The economic evaluation of substituting FM with APM in the diets of grey mullet is presented in Table 8. Compared to fish fed the control diet, the results indicated that the lowest incidence cost (23660 L.E.), change in the incidence costs (92.22%), and the highest profit index (0.055 L.E.), and changes in the profit indices (113.50%) were obtained for fish fed D_{100}. In addition, the economic conversion rate (ECR) remarkably decreased with increasing FM replacement levels with APM than 50% without clear differences with higher levels.

Table 8. Cost-profit analysis of *Mugil cephalus* fed on experimental diets.

Diets *	Fish Price (US$ 1000 fish^{-1})	Feed Cost (US$ 1000 fish^{-1})	Value of Fish (US$)	Incidence Cost (US$)	Change in Incidence Cost (%)	Profit Index (LE)	Change in Profit Index (%)	Economic Conversion Rate (US$)	Change in Economic Conversion Rate (%)
D_0	101.91	1532.10	74.71	1606.82	100	0.049	100	20.11	100
D_{25}	101.91	1500.38	78.79	1579.17	98.06	0.053	107.68	18.29	90.95
D_{50}	101.91	1668.98	88.34	1757.32	108.38	0.053	108.50	15.39	76.52
D_{75}	101.91	1573.44	84.90	1658.34	102.53	0.054	110.65	15.61	77.60
D_{100}	101.91	1405.10	77.77	1482.87	92.22	0.055	113.50	15.77	78.42

* D_0, D_{25}, D_{50}, D_{75}, and D_{100}: diets supplemented with 0%, 25%, 50%, 75%, and 100% of APM, respectively, as an alternative protein source. The US dollar = 15.7 Egyptian pounds.

4. Discussion

The sustainable development of aquaculture could consider the environmental impact, particularly the consumption of fishmeal as a strategic feedstuff. In 2050, the demand for wild fish to produce fishmeal could reach 47.2 million tons, which increased by 133.66% than 2010 this could trigger biotic depletion [45]. The use of fishmeal in the aquafeeds is expressed as a "Fish In–Fish Out (FIFO)" ratio. The FIFO values could be decreased to maximize the production of fish against the consumed fishmeal [46,47]. The present study aimed to explore a new alternative (amphipods) to fishmeal as a partial or total replacement in the diet of grey mullet, which could alleviate the pressure of aquaculture on the environment.

The protein content of the APM used in the current study was 40%, as reported in our previous study [20]. The earlier studies showed that amphipods reared in either marine water or freshwater habitats have similar protein values to those obtained by Abo-Taleb et al. [20] with percentages ranging between 25 and 52% of DW [22,48–51]. Moreover, fatty acids have been analyzed in some gammarid species, showing high levels of polyunsaturated fatty acids [20,52–54]. In addition, Gammarus amphipods contain a high concentration of carotenoids, which may improve aquatic animal performance [55]. Accordingly, gammarid species have high protein levels, making them an excellent candidate to be used as an alternative animal protein source in complete diets of farmed fish [56]. To date, amphipods are reported to be utilized in the diets of Atlantic salmon, Atlantic halibut, Atlantic cod, and rainbow trout [51,56–58].

To the best of our knowledge, there is no feasible diet for grey mullet fry that uses APM as a partially or completely substitute for FM. The current study confirmed that APM can partially replace FM in a practical diet for grey mullet fry. In the current study, fish fed on D_{50} achieved the highest significant level of FM replacement with the highest values of FW (1.80 g), WG (1.70 g), FL (4.47 cm), SR (86.67%), FI (2.06 g), PI (0.82 g), PER (0.83 g), PPV (30.65%), and the lowest FCR value (1.21) in comparison to D_0, D_{25}, D_{75}, and D_{100}). These data confirmed that 50% partial substitution (D_{50}) of FM with APM is the ideal replacement level in the diet of grey mullet fry. Our findings are consistent with many previous studies conducted to investigate the effects of APM on different aquatic animals, such as cod, *Gadus morhua* [22], juveniles of rainbow trout, *Oncorhynchus mykiss* [59], fry of common carp, *Cyprinus carpio* [60], caspian salmon [61], juveniles of Siberian sturgeon, *Acipenser baerii* [62], fingerlings of caspian roach, *Rutilus caspicus* [52], and African jewelfish, *Hemichromis bimaculatus* [63]. In the same vein, the replacement of FM up to 50% by housefly, *Musca domestica,* maggot meal in the diet of Nile tilapia, *Oreochromis niloticus,* did not affect growth performance and ingredient utilization, and also decreased the environmental impact by reducing the release of nitrite and phosphorus in the discharge water [64]. In addition, the use of *D. magna* meal as FM substitution in the diet of grey mullet improved growth performance and FCR with increasing substitution levels up to 75% of FM [30]. The replacement of FM with poultry by-product meal, cottonseed-protein concentrate, and their combination in the diet of largemouth bass, *Micropterus salmoides,* could reduce the FM usage to 20% without compromising the fish performance and FCR without negatively affecting the environment [65].

In the current study, there was a significant decreasing trend in growth, survival, and feed utilization with increasing substitution levels of amphipod meal over 50% (D_{75} and D_{100}). Our results agree with the findings of Opstad et al. [22], who investigated the effects of replacing fishmeal with alternative sources of marine amphipod meal (25, 50, or 100%) on juvenile cod, *Gadus morhua*, growth, survival, liver index, and deformities. Their results revealed a decrease in growth and survival with an increase in the level of amphipod meal. The fish fed 100% APM replacement had the highest proportion of deformities (16% of all fish). In addition, replacing FM with APM up to 50 or 100% maintained the growth of turbot, *Psetta maxima*, without any negative effects [66]. It can also be used as a potential source of protein to replace fishmeal in shrimp feed [67]. The amino acid profile of APM showed comparable content with fishmeal in most essential amino acids (lysine, leucine, phenylalanine, threonine) [38]. Meanwhile, some key limiting amino acids (methionine and tryptophan) in APM are still lower than their values in FM. These findings could be interpreted as the feasibility of replacing FM with APM up to 50% in which case the content of the amino acids in the diets could cover the grey mullet requirements, but increasing substitution levels higher than 50% could cause an imbalance in amino acids profile of the diets. The negative effects associated with increasing FM replacement with APM could be attributed to the high content of chitin (10.4%) and fluorine in APM [22], which could have a level of effect in the diet of grey mullet. Whereas, the grey mullet intestine did not show any chitinase activity [68]. In addition, the high florid content of APM could contribute to the decrease in growth with increasing FM substitution by APM, whereas fluoride is reported to decrease fry development and increase deformities [22].

In the current study, the lowest incidence cost associated with the higher profit index was observed with fish fed D_{100}. However, there is no marked difference in the profit index between D_{50} and D_{100}. The highest incidence cost was observed for fish fed D_{50} and this was due to the high feed intake in this group. Meanwhile, the lowest ECR value was recorded with fish fed the diet containing 50% AMP as a fishmeal replacer. This result may be owing to the highest significant growth, survival, and feed utilization parameters of grey mullet fry recorded by the 50% FM replacement with AMP. In the same vein, the soybean meal replaced by a high protein distiller's dried grain up to 50% improved the ECR of sea bass [9]. Moreover, replacing FM with low-cost and available alternatives in the diet of aquatic species has a vital role in reducing the cost of the formulated diet and improving the profit margin [10]. The ECR of gilthead seabream, *Sparus aurata*, was reduced when FM was replaced with meat and bone meal, and the profit index was increased [69].

The number of goblet cells and the height of the intestinal villi are indicators of digestion and nutrient absorption capacity [70]. In addition, Pirarat et al. [71] reported that goblet cells protect the intestinal mucosal layer from dehydration, damage, and pathogens. In addition, goblet cells also protect the gut from invading pathogens [72]. In the present study, the fish fed on APM at different concentrations (D_{50} and D_{75}) showed high improvement in the villus length and muscular thickness. In the current study, the increase in villus length and width, which indicate an improvement in absorption area, revealed that the inclusion of APM in the diet of grey mullet at moderate levels did not negatively affect intestinal health. In addition, the high content of APM from β-carotene, folic acid, tannic acid, and vitamins [20], could participate in improving gut health (goblet cell number and villus length and width) compared to the control diet (FM). This improvement could explain the higher growth and feed utilization in the current study, particularly with D_{50}. The present results are consistent with those of Goda et al. [8] and Allam et al. [10], who reported that fishmeal substitution up to 50% with high protein distiller dried grains maintained gut integrity with different degrees of intestinal morphological parameter improvement. However, in the present study, increasing FM substitution levels by APM with a level over 50% had a compromising effect on gut integrity and reduced histometric parameters of grey mullet intestine, this could be attributed to the increasing level of chitin and fluoride in the diets D_{75} and D_{100} which could be considered antinutritional compounds in the fish diet [22].

5. Conclusions

Fishmeal, the most expensive component in aquatic diets, is considered one of the most important challenges in the development of the aquaculture industry. The aquatic environment is still not fully examined and could provide feasible and sustainable solutions for the supply of fishmeal. The amphipoda, *Gammarus pulex,* is one of the possible protein alternative sources, due to its high productivity and nutritional quality. The current study concluded that 50% partial replacement of fishmeal with amphipod meal has a positive effect on growth performance, feed utilization, and histological and economic status of grey mullet, *Mugil cephalus*, fry. However, increasing the total replacement of fishmeal with amphipod meal showed growth performance reduction. Accordingly, amphipoda meal could be used as a natural renewable alternative protein source for substituting 50% of FM and minimizing the environmental impact of aquaculture.

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