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Effect of a New Feed *Daphnia magna* (Straus, 1820), as a Fish Meal Substitute on Growth, Feed Utilization, Histological Status, and Economic Revenue of Grey Mullet, *Mugil cephalus* (Linnaeus 1758)

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Abstract: The formulator of aquatic diets is part of a continuous search for alternative protein sources instead of depreciated fish meal. The utilization of zooplankton as a feed ingredient is an interesting trend due to their high-quality protein content and abundance of essential nutrients. The current study aims to investigate the effects of partial and total replacement of fish meal (FM) by *Dahpnia magna* meal (DMM) on growth performance, feed utilization, histological, and economic status of mullet, *M. cephalus*, larvae. In addition to the control diet, 100% FM, D_0, four diets containing DMM at different levels were used: 25% (D_{25}), 50% (D_{50}), 75% (D_{75}), and 100% (D_{100}) replacement of fish meal. A total of 300 larvae (0.097 ± 0.001 g) were equally divided into five groups (three replicate per each group) at a density of 200 larvae m^{-3}. The aquariums were renewed at a rate of 30% daily. During the 60-day experimental period, all larvae were fed their respective diets at a level of 20% of live body weight, five times a day (9.00 a.m., 12.00, 15.00, 18.00, and 21.00 p.m.). The results indicated that compared to D_0, fish fed D_{75} recorded the highest significant value of growth, and feed utilization parameters, while fish fed D_{100} achieved the lowest feed cost and incidence cost, and the highest profit index and economic efficiency ratio. A strong correlation was reported among weight gain, feed conversion ratio and fish meal replacement with DMM; R^2 = 0.94 and 0.91, respectively. The fit regression model representing mullet response to FM replacement with DMM is a poly-nominal regression model with maximum response at 75–100%. The histological investigation of the intestine revealed an improvement of histomorphometric indices and goblet cell number with increasing DMM inclusion levels. These findings confirmed that 75% to 100% partial substitution of FM with DMM is the ideal replacement for mullet, *M. cephalus*, larvae for improving fish growth performance and feed utilization.

Keywords: *Daphnia*; substitution; flathead grey mullet; growth performance; histomorphometry; economic revenue

1. Introduction

In aquatic food webs, natural zooplankton is a secondary producer. Furthermore, the natural productivity of zooplankton species is vital for assessing the environmental potential of aquaculture. [1]. Zooplankton is the main source of fish feed in their early life stages [2,3]. With the increase in the number of fish farms, the need for zooplankton has increased and the trend of cultivating zooplankton, as live food, has increased significantly in the last decades [4]. Several zooplankton species were successfully cultured and utilized in aquaculture, include rotifer, *Brachionus plicatilis* [5], *Artemia franciscana* [6], amphipod, *Gammarus pulex* [3], copepods, *Oithona nana* [4], and *Daphnia magna* [7]. For many nutritional aspects [8,9], algal cells are the basic and the recommended food item for zooplankton [5,6], while, several food items have been utilized in feeding for different zooplankton species, include agriculture, industrial waste products, and animal manures [10–12].

Aquaculture feeds are the major recurring cost in aquaculture operation, accounting for 40% to 70% of the variable costs, therefore, it is urgently necessary to find new aqua-feed sources [13]. Aquaculture development faces many problems, especially in developing countries. One of the most important problems is the availability and sustainability of fishmeal (FM), which is considered the main protein source and the most important ingredient in the aquaculture diet [14]. Until now there are only a few potential protein sources that could be investigated as an FM replacer [15].

The utilization of zooplankton dried biomass as a feed ingredient showed promising results to replace fish meal in aquatic diets [16]. It has been used to replace FM in the diet of sea bass up to 100% [17], crustacean (Callianassa) meal succeeded in substituting 25% of FM in the diet of Nile tilapia, *Oreochromis niloticus* [18], and Gammarus meal with partial or total replacement of FM in the diet of Siberian sturgeon, *Acipenser baerii* [19], among others.

Among zooplankton species, *D. magna* has excellent nutritional content that is recommended for use in feeding of fish larvae [7]. Cultivation of this species is not difficult as it can feed wide varieties of unused food residues. Herawati et al. [20] mentioned that rice bran has high nutritional value and enhances the growth of *D. magna*. Additionally, it has widespread overall freshwater bodies. Recently, it was used as a promising feedstuff for feeding fish fry in aquaculture [21]. It is characterized as a suitable size for the mouth opening of many fish larvae and fry, and it has a high nutritional composition [22]. Its nutrient content varies according to the culture medium and the degree of availability of phytoplankton [20,23]. Daphnia meal is successfully used as a fish meal replacer in the diet of *Pelteobagrus fulvidraco* [21]. Moreover, *D. magna* can be used as a bioencapsulation of probiotics during fish larvae feeding, as indicated by [24] who reported that Persian sturgeon (*Acipenser persicus*) larvae fed with bioencapsulated *D. magna* with *Saccharomyces cerevisiae* had higher growth performance and better feed utilization. The bioencapsulation of *D. magna* with commercial probiotic (*Bacillus* bacteria; Protexin Aquatic®) enhanced the resistance of *Acipencer percicus* larvae against different stresses [25].

Mullet, *Mugil cephalus* L, is a commercially important euryhaline and eurythermal marine teleost that contributes to large estuarine and coastal fisheries in many countries, especially in the Mediterranean. [26]. According to FAO [27], the overall production of mullet in aquaculture was 2.6% in the total marine aquaculture production and flathead grey mullet, *M. cephalus*, contributed substantially as the most dominant species. Due to its lower trophic level grazing on plant detritus and microflora, the flathead grey mullet, *M. cephalus*, is an excellent candidate species for both monoculture and polyculture [28]. Mullet, *M. cephalus,* is commonly cultured in intensive and semi-intensive culture in marine, brackish, and freshwater [29], and can eat both supplementary feed and natural food [30].

The present study is the first work that aims to investigate the effect of total or partial replacement of fish meal (FM) by daphnia, *D. magna*, meal (DMM), on growth, feed utilization, chemical composition, histological changes, and economic evaluation of mullet, *M. cephalus,* larvae, as one of the most common fish species in coastal regions in many countries, especially the Mediterranean area.

2. Materials and Methods

2.1. Experimental Fish and Culture Technique

The current experiment was conducted at a private freshwater fish farm (Sahary Masr Co. Fish Farm, 31°12′30.07″ N and 29°58′41.66″ E) located in Alexandria Governorate, Egypt, (Figure 1) which supplied fresh water. Mullet, *M. cephalus*, larvae were obtained from Edko, El Behira Governorate, Egypt. The initial body weight and length of the fish were 0.097 ± 0.001 g fish^{-1} and 1.35 ± 0.01 cm fish^{-1}, respectively. A total of 300 *M. cephalus* larvae was equally divided into 5 groups (three replicate per each group) and stored in 15 aquariums (66 cm × 47 cm × 32 cm) at a density of 200 larvae m^{-3}. Before the start of the experiment, *M. cephalus*, larvae were adapted to experimental conditions for two weeks and fed the control diet (D_0), five times a day. The aquariums were renewed with a rate of 30% daily. During the 60-day experimental period, the larvae were fed their respective diets at a level of 20% of body weight. The daily ration was divided into five equal amounts and offered five times a day (9.00 a.m., 12.00, 15.00, 18.00, and 21.00 p.m.). Random samples from each replicate were weighed biweekly and the amount of daily allowance was adjusted accordingly. During the experiment, the water quality parameters of water temperature (21.6 to 23.7 °C), dissolved oxygen (5.40 to 6.45 mg L^{-1}), pH (7.34–7.72), and the ammonia (NH_3, 0.16 to 0.30 mg L^{-1}) were maintained within the acceptable range of the culture of mullet, *M. cephalus.* Each aquarium was supplied with air through an air pipeline using an air blower.

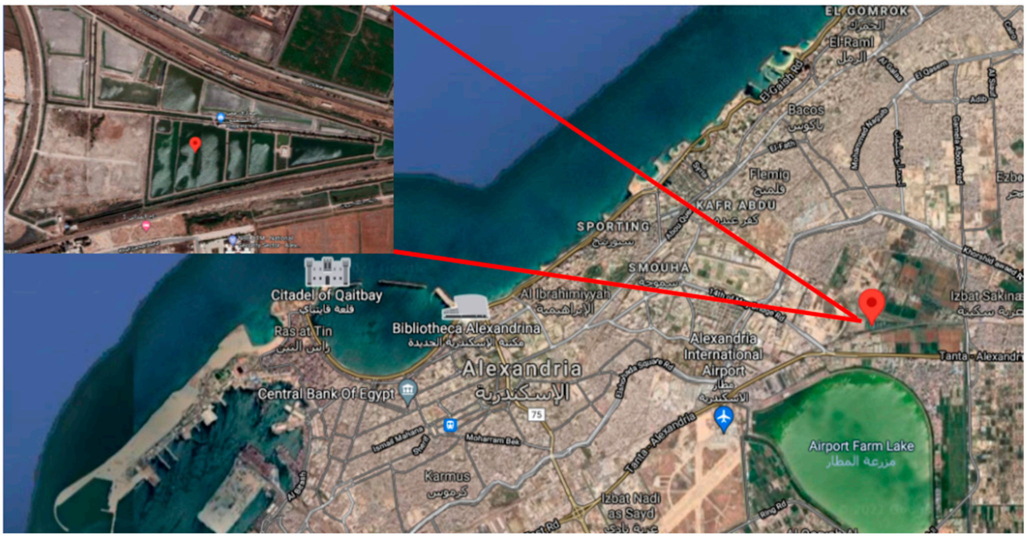

Figure 1. Location map of the farm (Sahary Masr Co. Fish Farm, 31°12′30.07″ N and 29°58′41.66″ E) according to Google Maps (20 June 2021).

2.2. Preparation of Daphnia Meal (DMM)

Isolation, cultivation, nutritional compositions, and preparation of Daphnia meal, *D. magna*, (DMM) were conducted as described previously by El-feky and Abo-taleb [7]. At the end of the culture of *D. magna* as described previously [7], *D. magna* were harvested, dried, powdered, and preserved at −20 °C until further analysis or utilization as DM. Dry matter content (DM%), crude protein (CP%), ether extract (EE%), crude fiber (CF%), nitrogen-free extract (NFE%), and gross energy (GE Kcal kg^{-1}) of DMM are presented in Table 1. As described previously by El-Feky and Abo-Taleb [7], DMM resulted with strong nutritional values of dry matter content (92%), crude protein (47.7%), ether extract (10.2%), crude fiber (2.1%), ash (17.8%), nitrogen-free extract (22.2%), caloric value (3337 K cal kg^{-1}), β-carotene (6510 IU/100 g), folic acid (110 µg 100 g^{-1}), and tannic acid (56 mg 100 g^{-1}). Values of vitamins B_2, B_6, B_6, B_{12}, A, E, and D were 203 mg 100 g^{-1}, 176 mg 100 g^{-1}, 191 mg 100 g^{-1}, 318 IU 100 g^{-1}, 17 mg 100 g^{-1}, and 19.2 mg 100 g^{-1}, respectively. Moreover, the total bacterial count was less than 6500 × 10^2 CFU g^{-1}, while *Listeria* count was 10 CFU g^{-1}. No counts of *E. coli*, *Salmonella*, and *Clostridium* yeasts or molds were obtained.

Table 1. Biochemical compositions of ingredients used in the experimental diet (% of DM).

Ingredients	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	NFE (%)	GE (K cal kg^{-1})	DE (Kcal kg^{-1})
Fish meal (FM)	92	60	12.5	0.6	15.4	11.5	5055	3791
Daphnia meal (DMM) *	92	47.7	10.2	2.1	17.8	22.2	4360	3472
Soybean meal	90	45	1.1	7.3	6.3	40.3	4550	3413
Yellow corn	88	10	3.6	2.3	1.3	82.8	4309	3232
Rice bran	89	14	6.4	9.9	5.3	64.4	4368	3276

DM: dry matter content; CP crude protein; EE: ether extract; CF: crude fiber; NFE: nitrogen free extract = 100 − (CP + CF + EE + Ash%); GE: gross energy (Kcal kg^{-1}); DE digestible energy, estimated according to Jobling [31] using digestible energy = gross energy × 0.75. * Biochemical compositions of DM% according to El-Feky and Abo-Taleb [7].

2.3. Experimental Design

Diets were formulated to be isonitrogenous (35.08 ± 0.75% CP) and isocaloric (4706 ± 6.4 kcal k g^{-1}). Biochemical compositions of the ingredients used in the experimental diets (% of DM) are presented in Table 1.

In the current study, five diets were formulated. The first diet is a basal control diet (D_0) that contained 100% of FM. The other diets contained 25% (D_{25}), 50% (D_{50}), 75% (D_{75}), and 100% (D_{100}) of DMM with different percentages of the equivalent fish meal replacement, respectively. Proximate analysis (%), formulation (based on the % of DM) and preparation cost (Egyptian L.E. per ton) of five experimental diets (D_0, D_{25}, D_{50}, D_{75}, and D_{100}, respectively) are presented in Table 2.

Table 2. Formulation, cost, and the proximate composition of the formulated diets.

Diets *	D_0	D_{25}	D_{50}	D_{75}	D_{100}
Formulation (% DM)					
Fish meal (FM)	18	13.5	9	4.5	0
Cost	720	540	360	180	0
Daphnia meal (DMM)	0	6.5	13	19.5	26
Cost	0	130	260	390	520
Soybean meal	45.5	45	44	44	43.5
Cost	318.5	315	308	308	304.5
Yellow corn	16.5	16	15	14	13.5
Cost	60.2	58.4	54.7	51.1	49.2
Rice bran	16	15	15	14	13
Cost	57.6	54	54	50.4	46.8
Vitamin and mineral premix *	1	1	1	1	1
Cost	50	50	50	50	50
Fish Oil	3	3	3	3	3
Cost	60	60	60	60	60
Total (% DM)	100	100	100	100	100
Total Cost (Egyptian L.E. 100 kg $diet^{-1}$)	1266.3	1207.4	1146.7	1089.5	1030.5
Proximate composition (%)					
Dry matter (%)	92	92.1	92.3	92	92
Crude protein (%)	35.1	35.1	35	35.1	35.1
Ether extract (%)	13.5	13.1	13.9	14.2	14.9
Fiber (%)	4.2	4.3	4.4	4.8	5.2
Ash (%)	11.6	12.3	10.1	10.9	11.7
NFE (%)	35.6	35.2	36.6	35	33.1
GE (Kcal kg^{-1})	4859	4801	4931	4917	4923
DE (Kcal kg^{-1})	3638	3601	3698	3688	3692

* D_0, D_{25}, D_{50}, D_{75}, and D_{100}: diets supplemented with 0%, 25%, 50%, 75%, and 100% of *D. magna* meal (DMM); DM%: dry matter content; CP% crude protein; EE% ether extract; CF%: crude fiber; NFE%: NFE%: nitrogen free extract = 100 − (CP + CF + EE + Ash%); GE: gross energy (Kcal kg^{-1}); and DE: digestible energy, estimated according to Jobling [31] using digestible energy = gross energy × 0.75.

2.4. Parameters Calculations

2.4.1. Growth Indices

The fish length was measured, weighed, and counted to determine the zootechnical performance and survival at the end of the experiment. Weight gain (WG), final body weight (FW), feed conversion ratio (FCR), protein intake (PI), protein efficiency ratio (PER), and protein productive value (PPV) were calculated using the following equations:

$$\text{Weight gain, WG (g)} = \text{FW} - \text{IW} \tag{1}$$

where: FW and IW are final and initial body weight (g)

$$\text{Feed conversion ratio, FCR (Feed: gain)} = \text{FI (g)/WG (g)} \tag{2}$$

where: FI is feed intake (g)

$$\text{Protein intake, PI (g fry}^{-1}\text{)} = \text{feed intake (g)/protein\%.} \tag{3}$$

$$\text{Protein efficiency ratio, PER (g)} = \text{weight gain (g)/protein intake (g)} \tag{4}$$

$$\text{Protein productive value, PPV (\%)} = \text{(protein gain (g)/protein intake (g))} \times 100 \tag{5}$$

2.4.2. Whole-Body Proximate Composition

Biochemical composition of dry matter content, crude protein, ether extract, gross energy, and ash of the experimented diets and fish carcass composition were determined according to AOAC [32].

2.4.3. Histological Observations of Fish Intestine

At the end of the experiment, five samples from each group were sacrificed with an overdose of aesthetic for conducting the histological examination of the intestine and liver. The samples were fixed in 10% formalin for 24 h before being transferred into 70% ethanol for dehydration in a graded series of ethanol. Classical histological processing was undergone. Then, sections (5 μm thick) were cut and stained with hematoxylin and eosin [33]. Intestine sections were submitted to the measurement of muscular thickness and villus height according to Hamidian et al. [34].

2.4.4. Economic Evaluation

A simple economic evaluation of the formulated diets was calculated based on the price of fish and the cost of feed. According to national and the international market prices, the cost of supplementation diets was calculated in Egyptian Pound (L.E.) then converted into US Dollars ($), using the following equations, as described by Goda et al. [35]:

$$\text{Incidence cost} = \text{the price of the fish (102.11 US\$)} + \text{cost of feed to produce 1000 fish} \tag{6}$$

$$\text{Value of fish} = \text{the price of 1000 fish (102.11 US\$)} \times \text{survival rate (SR\%)} \tag{7}$$

$$\text{Profit index} = \text{value of fish/cost of consumed feed (1000 fish).} \tag{8}$$

$$\text{Economic conversion rate (ECR)} = \text{feed intake (g)/weight gain (g).} \tag{9}$$

The cost is for 1 kg of each of D_0, D_{25}, D_{50}, D_{75}, and D_{100} which were 0.81, 0.77, 0.73, 0.70, and 0.66 US$, respectively.

2.5. Statistical Analyses

Statistical analyses were carried out using SPSS program (SPSS for Windows, Version 16.0., SPSS Inc., Chicago, IL, USA). One-way ANOVA test was used in order to test the effect of partial replacement FM with DMM. In addition, significant differences among means were determined by Duncan [36].

3. Results

3.1. Growth Performance and Feed Utilization

During the 60 days of the experimental period, no mortality was reported for mullet, *M. cephalus,* fed the studied diets with or without DMM inclusion. Growth performance and feed utilization of fish fed the control diet (D_0) or diets containing DMM as fish meal replacer (D_{25}, D_{50}, D_{75}, and D_{100}) are presented in Table 3. Significantly ($p \leq 0.05$) higher growth indices were reported for fish fed either D_{75} or D_{100} diets compared to the control and other diets. Compared to the control diet (D_0), fish fed diet D_{75} reported an increase of 38.57%, 40.77%, 43.48%, 18.09%, and 29.25% of FW, WG, FL, and LG, respectively. The relation among WG, LG, and increasing FM replacement levels with DMM showed polynomial regression trends with strong correlation R^2 = 0.94 and 0.78, respectively, which noted that the best replacement level of FM with DMM could be between 75% and 100% replacement level (Figure 2A,B).

Table 3. Effect of fish meal replacement with *Daphnia magna* meal on growth performance and feed utilization indices of mullet, *Mugil cephalus,* larvae.

Diets	D_0	D_{25}	D_{50}	D_{75}	D_{100}
Growth performance indices					
Initial weight (g fish^{-1})	0.096 ± 0.001	0.096 ± 0.001	0.098 ± 0.00	0.098 ± 0.00	0.097 ± 0.001
Final weight (g fish^{-1})	1.40 ± 0.03 ^{d}	1.55 ± 0.03 ^{c}	1.69 ± 0.03 ^{b}	1.94 ± 0.01 ^{a}	1.87 ± 0.01 ^{a}
Weight gain (g fish^{-1})	1.30 ± 0.03 ^{d}	1.45 ± 0.03 ^{c}	1.59 ± 0.03 ^{b}	1.83 ± 0.01 ^{a}	1.77 ± 0.01 ^{a}
Initial length (cm fish^{-1})	1.33 ± 0.03 ^{c}	1.37 ± 0.03 ^{b}	1.40 ± 0.00 ^{a}	1.30 ± 0.00 ^{c}	1.40 ± 0.00 ^{a}
Final length (cm fish^{-1})	3.87 ± 0.03 ^{d}	4.07 ± 0.03 ^{c}	4.23 ± 0.03 ^{bc}	4.57 ± 0.03 ^{a}	4.40 ± 0.06 ^{ab}
Length gain (cm fish^{-1})	2.53 ± 0.07 ^{d}	2.70 ± 0.00 ^{cd}	2.83 ± 0.03 ^{bc}	3.27 ± 0.03 ^{a}	3.00 ± 0.06 ^{b}
Condition factor	2.43 ± 0.11	2.30 ± 0.10	2.22 ± 0.08	2.03 ± 0.03	2.20 ± 0.08
Feed utilization indices					
Feed intake (g)	1.76 ± 0.012 ^{c}	1.82 ± 0.014 ^{c}	1.95 ± 0.023 ^{b}	2.08 ± 0.021 ^{a}	2.08 ± 0.023 ^{a}
Feed conversion ratio (feed: gain)	1.35 ± 0.030 ^{a}	1.26 ± 0.030 ^{ab}	1.23 ± 0.010 ^{bc}	1.13 ± 0.010^{c}	1.18 ± 0.010 ^{bc}
Protein intake (g)	0.84 ± 0.006 ^{c}	0.87 ± 0.006 ^{c}	0.93 ± 0.011 ^{b}	0.99 ± 0.009 ^{a}	0.99 ± 0.011 ^{a}
Protein efficiency ratio (g)	0.74 ± 0.18 ^{c}	0.80 ± 0.19 ^{bc}	0.82 ± 0.007 ^{b}	0.88 ± 0.006 ^{a}	0.85 ± 0.007 ^{ab}
Protein productive value (g)	21.47 ± 0.78 ^{d}	23.47 ± 0.87 ^{c}	24.30 ± 0.09 ^{bc}	27.73 ± 0.33 ^{a}	26.19 ± 0.62 ^{ab}

Values are means ± SE. Means (n = 3) in the same row with different superscript are significantly ($p \leq 0.05$) different. D_0, D_{25}, D_{50}, D_{75}, and D_{100} were diets with 0%, 25%, 50%, 75%, and 100% replacement of fish meal with *Daphnia magna* meal (DMM) as alternative protein source, respectively.

Fish fed diets D_{75} and D_{100} showed a significantly ($p \leq 0.05$) higher FI, FCR, PI, PER, and PPV. Compared to the control diet (D_0), fish fed diet D_{75} was showed an increase by 18.18%, 6.30%, 17.86%, 18.92%, and 29.16% of FI, FCR, PI, PER, and PPV, respectively (Table 3). In addition, the polynomial regression is the fit regression model to present the relation among FCR, PPV, and FM replacement levels with strong correlation coefficient R^2 = 0.91 and 0.81, respectively (Figure 2C,D).

3.2. Whole-Body Proximate Composition

The whole-body proximate chemical composition of fish fed the control diet and DMM as FM replacer is presented in Table 4. Among all diets, fish fed the diet D_{75} achieved high significant ($p \leq 0.05$) whole-body proximate chemical compositions of DM (22.35%), CP (68.00%), and GE (5325 kcal kg^{-1}), while they achieved the lowest significant ($p \leq 0.05$) value of ash (16.30%). In conclusion, compared to the control diet (D_0), fish fed diet D_{75} experienced an increase of DM (76.2%), CP (25.6%), EE (19.0%), and GE (4.49%) and decrease of ash (−19.82%) (Table 4). No significant differences were recorded in EE among fish fed either D_0 or DMM supplementation diets (Table 4).

3.3. Histological Status

The histological investigation of the proximal intestine of mullet, *M. cephalus*, showed normal intestinal histological structure of fish fed the control (D_0) and low FM replacement level (D_{25}) (Figure 3 and Table 5). The histo-morphometric parameters of the proximal intestine, including villus length, width, muscular thickness, crypts depth, and goblet cells number were markedly increased ($p \leq 0.05$) with increasing DMM inclusion levels. The villus length and width, crypts depth, and muscular thickness of fish fed diet D_{75} and D_{100} were the highest among DMM groups (D_{25}, D_{50}) (Table 5). In addition, no different pathological effects occurred on the hepatopancreas of fish fed the control group or FM replaced with DMM diets (Figure 4).

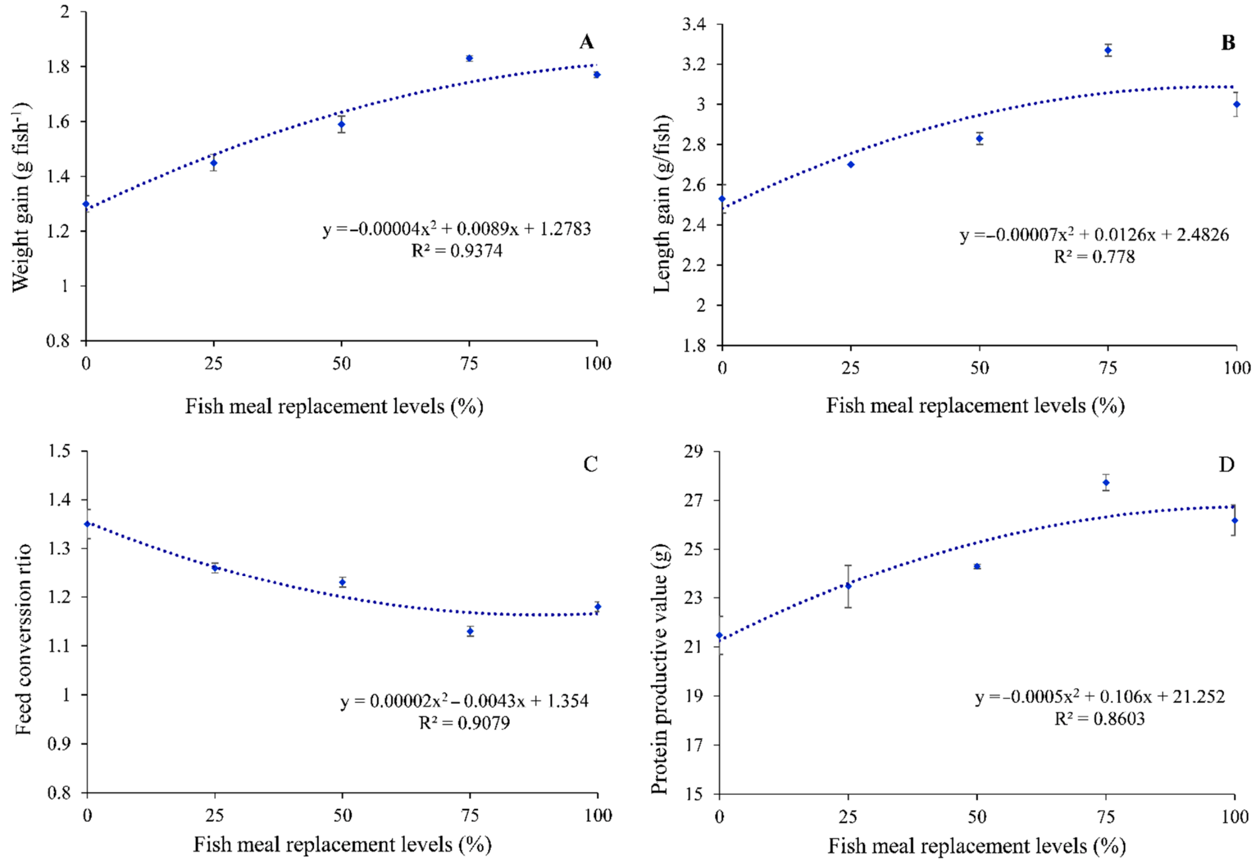

Figure 2. (**A**–**D**) Poly-nominal regression of different growth and feed utilization parameters and fish meal replacement levels with *Daphnia magna* meal in the diets of mullet, *Mugil cephalus* larvae.

Table 4. Effect of fish meal replacement with *Daphnia magna* meal on whole-body proximate composition of mullet, *Mugil cephalus*, larvae.

Diets	D_0	D_{25}	D_{50}	D_{75}	D_{100}
Dry matter (%)	21.70 ± 0.31 [b]	21.80 ± 0.19 [b]	21.91 ± 0.33 [b]	22.35 ± 0.22 [a]	22.07 ± 0.24 [a]
Crude protein (%)	64.00 ± 0.15 [c]	64.50 ± 0.47 [bc]	64.83 ± 0.24 [bc]	68.00 ± 0.36 [a]	65.93 ± 0.28 [b]
Ether extract (%)	15.67 ± 0.88	15.67 ± 0.27	15.57 ± 0.33	15.70 ± 0.31	15.40 ± 0.15
Ash (%)	20.33 ± 0.88 [a]	19.83 ± 0.2 [a]	19.60 ± 0.21 [a]	16.30 ± 0.15 [c]	18.67 ± 0.13 [b]
Gross energy (Kcal kg^{-1})	5096 ± 40.0 [d]	5124 ± 3.00 [cd]	5134 ± 10.00 [c]	5325 ± 13.00 [a]	5180 ± 2.00 [b]

Values are means ± SE. Means ($n = 3$) in the same row with different superscript are significantly ($p \leq 0.05$) different. D_0, D_{25}, D_{50}, D_{75}, and D_{100} were diets with 0%, 25%, 50%, 75%, and 100% replacement of fish meal with *Daphnia magna* meal (DMM) as alternative protein source, respectively.

3.4. Economic Evaluation

Cost–profit analysis of *M. cephalus* fed different diets with FM substitution by DMM is presented in Table 6. Compared to fish fed the control (D_0), the results indicated that the lowest feed cost (1.368 US\$ 1000 $fish^{-1}$), incidence cost (103.47 US\$ 1000 $fish^{-1}$), and the highest profit index (74.64 US\$) were obtained with fish fed D_{100} (Table 6). No marked changes were noticed in the calculated economical parameters among the control diet and other FM replacement diets. However, the ECR improved by increasing the DMM replacing FM up to 75 and 100 by 14.88% and 15.91%, respectively.

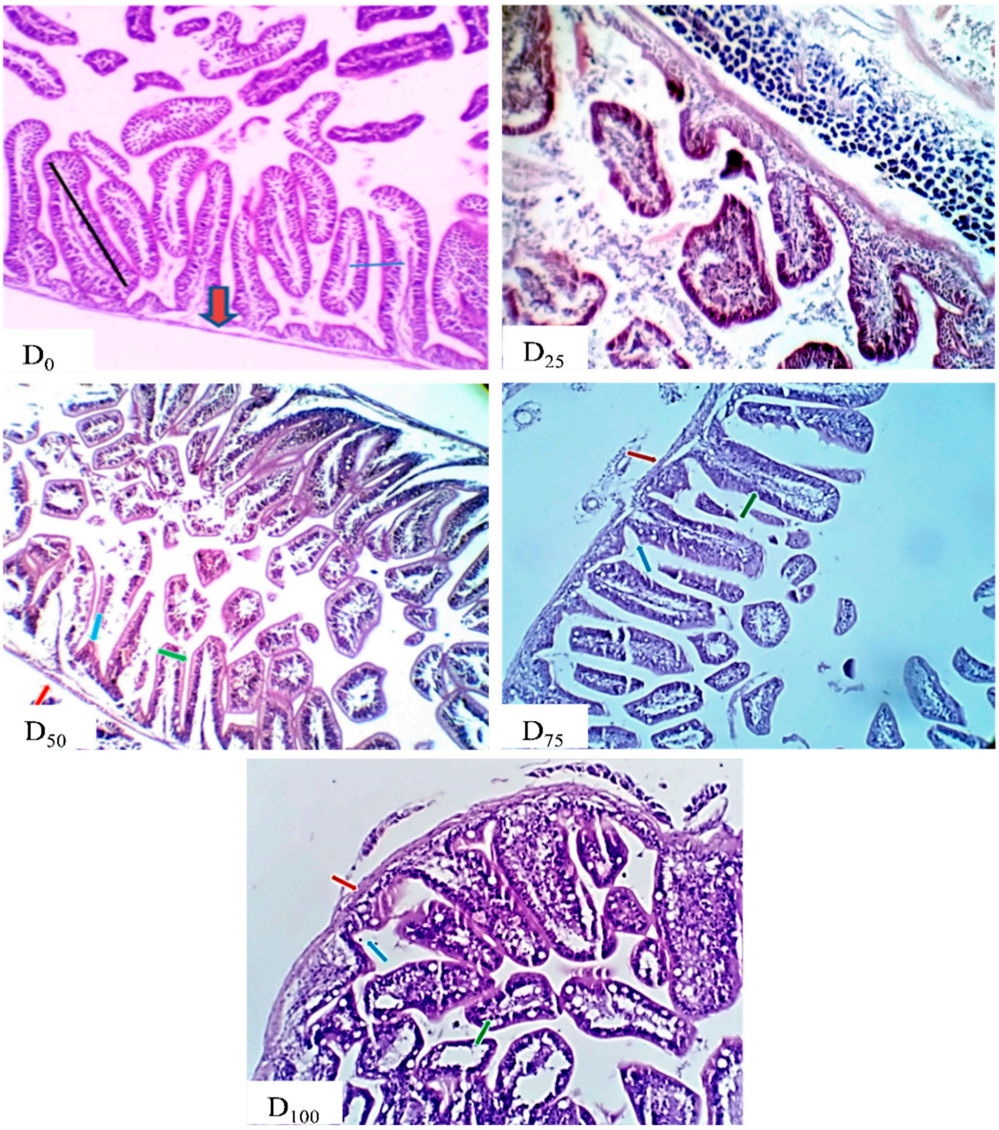

Figure 3. Photomicrograph of the proximal intestine of mullet, *Mugil cephalus,* larvae fed diets with different fish meal substitution levels with *Daphnia magna* meal. D_0, D_{25}, D_{50}, D_{75}, and D_{100} were diets with 0%, 25%, 50%, 75%, and 100% replacement of fish meal with *D. magna* meal (DMM) as alternative protein source, respectively. The control group (D_0) and D_{25} showed normal histological structure with few goblet cells numbers and the increasing of DMM showed an increase of different intestinal morphometric parameters. Villi (black arrow), crypts (blue arrow), submucosa (red star), and muscle layer (red arrows). A few goblet cells were detected (yellow arrow) (H&E; ×400).

Table 5. Effect of fish meal replacement with *Daphnia magna* meal on proximal intestinal morphometric parameters of mullet, *Mugil cephalus,* larvae.

Diets	D_0	D_{25}	D_{50}	D_{75}	D_{100}
Villus length (µm)	415.80 ±0.90 ^{d}	419.5 ± 2.60 ^{d}	429.2 ± 1.40 ^{c}	459.70 ± 3.20 ^{b}	446.0 ± 3.00 ^{a}
Villus width (µm)	91.10 ± 1.20 ^{e}	101.0 ± 0.90 ^{d}	106.2 ± 1.10 ^{c}	120.5 ± 1.10 ^{b}	111.0 ± 1.40 ^{a}
Crypts depth (µm)	45.30 ± 0.40 ^{d}	47.80 ± 0.50 ^{d}	56.80 ± 0.90 ^{c}	73.80 ± 0.80 ^{b}	65.2 ± 0.70 ^{a}
Muscle thickness (µm)	20.50 ± 0.20	21.20 ± 0.60	20.30 ± 0.80	20.80 ± 0.30	21.00 ± 0.40
Goblet cells number (cells/high power field)	3.50 ± 0.50 ^{b}	3.50 ± 0.50 ^{b}	5.00 ± 1.0 ^{b}	11.00 ± 1.0 ^{a}	13.00 ± 1.0 ^{a}

Values are means ± SE. Means (n = 3) in the same row with different superscript are significantly ($p \leq 0.05$) different. D_0, D_{25}, D_{50}, D_{75}, and D_{100} were diets with 0%, 25%, 50%, 75%, and 100% replacement of fish meal with *Daphnia magna* meal (DMM) as alternative protein source, respectively.

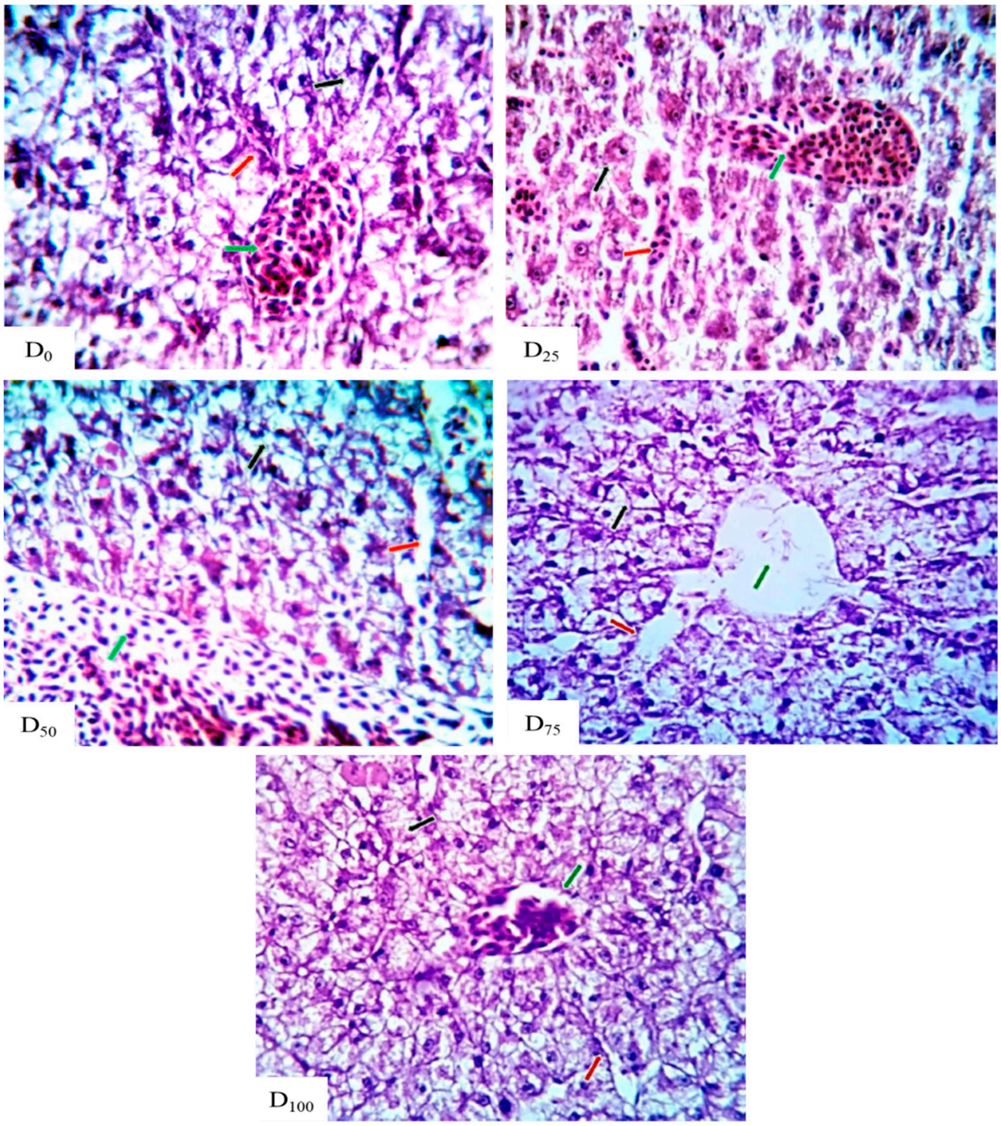

Figure 4. Photomicrograph of the hepatopancreas of mullet, *Mugil cephalus,* larvae fed diets with different fish meal substitution levels with *Daphnia magna* meal. D_0, D_{25}, D_{50}, D_{75}, and D_{100} were diets with 0%, 25%, 50%, 75%, and 100% replacement of fish meal with *D. magna* meal (DMM) as alternative protein source, respectively. Control group (D_0) showing normally hepatic lobules with clear boundaries, radially arranged hepatic cords (black arrows) around a well formed thin walled central veins engorged with nucleated erythrocytes (green arrow). Compared to the control group (D_0), the hepatic sinusoids are seen among the hepatic cords (red arrows), and did not show any pathological effect with using *D. magna* meal as a protein source (D_{25}, D_{50}, D_{75}, and D_{100}) (H&E; ×400).

Table 6. Cost–profit analysis of mullet, *Mugil cephalus,* larvae fed fish meal replacement diets with *Daphnia magna* meal.

Diets	Fish Price (US$ 1000 Fish^{-1})	Feed Cost (US$ 1000 Fish^{-1})	Incidence Cost (US$)	Change in Incidence Cost (%)	Profit Index (US$)	Change in Profit Index (%)	Economic Conversion Rate (U$)	Change in Economic Conversion Rate (%)
D_0	102.11	1.422	103.53	100.00	71.791	100	1.92	100.00
D_{25}	102.11	1.402	103.51	99.98	72.811	101.42	1.77	92.02
D_{50}	102.11	1.427	103.53	100.02	71.554	98.27	1.76	91.43
D_{75}	102.11	1.446	103.55	100.02	70.604	98.67	1.63	85.12
D_{100}	102.11	1.368	103.47	99.92	74.646	105.73	1.64	84.09

D_0, D_{25}, D_{50}, D_{75}, and D_{100} were diets with 0%, 25%, 50%, 75%, and 100% replacement of fish meal with *Daphnia magna* meal (DMM) as alternative protein source, respectively.

4. Discussion

Daphnia magna is considered one of the most important zooplankton species utilized as feed for fish, due to its high content of animal protein that ranges from 30.8% to 61% [22]. In addition, this organism contains a considerable amount of chitosan, vitamins, antioxidants, and non-saturated fatty acids [7,22,37]. Previous studies reported that zooplankton meal has high protein values, which qualifies it as a good ingredient source that could be utilized as an alternative animal protein source in the practical diet of farmed fish [38–40]. Several studies have focused on alternative fishmeal protein sources in aquatic diets, including zooplankton or crustacean protein-based meals [7,27,41,42], fermented soyabean meal [43], lipid-extracted microalgal (Sarker et al., 2018), poultry by-product [44], insect meal [45], and silages of fishery by-products [46], among other alternatives. *D. magna* could easily be produced in laboratories using microalgae [47]. In addition, the natural collected *D. magna* has a biochemical composition and carotenoids content comparable to FM to be used in aquaculture [48].

According to our knowledge, until now, there is no practical diet for mullet, *M. cephalus*, larvae containing DMM as a total or partial replacement of FM. The current study revealed that DMM could replace FM in a practical diet of mullet, *M. cephalus*, larvae up to 75% and 100%. The increasing levels of DMM in the diet presented a tendency towards better growth performance, feed utilization, carcass composition as well as lower diet preparation cost when compared to fish fed the control diet (D_0). The improvement of different zootechnical performance parameters in the group fed D_{75} was higher than the control group (D_0) by 38.57%, 40.77%, 29.25%, 16.30%, 18.92%, and 29.16% with FW, WG, LG, FCR, PER, and PPV, respectively.

The current results strongly confirm that DMM is a promising protein source to replace FM in the mullet, *M. cephalus*, diet. In the same vein, replacing fish meal with DMM up to 60% significantly improved growth performance, PER, and FCR of *Pelteobagrus fulvidraco* [21]. The use of *D. magna* and spirulina, *Arthrospira platensis* as complementary protein and lipid sources improved the growth performance and the total biomass of common carp [49]. In addition, the barramundi, *Lates calcarifer*, fed diets containing 5–10% *D. similis* meal have high immune surveillance and disease resistance compared to the control group [37]. The supplementation of common carp, *Cyprinus carpio,* diet with 5% DMM improved growth, feed utilization, and immuno-biochemical parameters [50]. The utilization of *D. magna* as a nutritional bag for *S. cerevisiae* significantly improved growth performance and feed utilization of Persian sturgeon, *A. persicus* [24].

The ability to use DMM in the diet of mullet, *M. cephalaus*, with high replacement levels could be due to its favorable content of essential amino acids, which is an amount adequate for omnivorous fishes, with also 5.0% lipids consisting of 18.7% and 66.2% saturated and unsaturated fatty acids, respectively [22]. Furthermore, the apparent digestibility of DMM was determined as 80.6% and 82.6% for energy and protein, respectively [51].

In addition, the improvement of growth performance and feed utilization in the present study could also be attributed to the considerable contents of antioxidants (tannic acid and β-carotene) as well as water-soluble (B2, B6, B12, and folic acid) and fat-soluble vitamins (A, E, and D) in DMM [3,7]. Hongxia et al. [21] reported that the use of DMM at a level of 60% replacing FM significantly improved superoxide dismutase and catalase activities, and significantly decreased the free radical level. Moreover, *D. magna* contains 3–7% chitin [52], which mainly consists of chitosan (77%), considered a growth promoter and immunostimulant for fish [37].

The histological observations of the fish intestine confirmed that compared to the control diet, fish fed diets D_{75} and D_{100} resulted in the highest villus length, width, muscular thickness, and crypts depth. The improvement of histomorphometric parameters in the present study could reveal an increase of absorption area and digestive system functions [53]. These results could explain the growth and feed utilization improvement obtained in the present study. In addition, the replacement of soybean with high protein distiller dried grains up to 50% improved the area of absorption and goblet cell number in the proximal intestine of sea bass, *Dicentrarchus labrax* [54]. In accordance, using zooplankton feeding (*Artemia*) against dry feed revealed an improvement of mucosal fold length and development of the intestinal tract [55] (Adamek-Urbańska et al., 2021). The intestine of fish fed increasing levels of DMM significantly showed an improvement in the number of goblet cells in the present study. Goblet cells are a mucus secreting cell that protects the intestinal wall from pathogens and toxic materials and increasing goblet cells abundance improves the absorption of nutrients, especially polypeptide [55,56].

Meanwhile, no histological changes were reported on the hepatopancreas of fish fed the control diet or DMM-based diets. These results indicated that DMM did not have any noxious or toxic effects on fish. In accordance, the mullet fed diets with different plant protein sources replacing FM revealed healthy liver [57]. Hongxia et al. [21] reported that using DMM as a protein replacer decreased the activity of aminopeptidase enzymes. In addition, the somatic indices of common carp, including hepatic, gills, viscera, kidney, and spleen somatic index did not significantly change with DMM supplementation [50].

In the current study, the cost–profit analysis showed that the lowest feed cost, ECR, and the highest profit index were obtained for fish fed D_{100} compared to the control diet (D_0). Although D_{75} achieved the highest growth rate, feed utilization, and chemical composition, it came in the second-order after D_{100} in terms of economic evaluation, but with a small margin. These findings confirmed that 75% to 100% substitution (D_{75} and D_{100}) of FM with DMM is the ideal replacement of FM for mullet, *M. cephalus,* larvae. In agreement with the current findings, the replacement of fish meal with different protein source in the diet of mullet up to 50% and 75% levels reduced the price of the formulated diet by 15.5% and 23.6%, respectively, while maintaining normal growth performance [57]. Moreover, one of the main aims of replacement of FM is maintaining the cost-efficiency of the formulated diet by keeping the biological and the environmental aspects as a favorable approach [53,54,58].

Some previous studies on different fish species revealed that FM substitution could be conducted with bone and meat meal up to 50% [59], black soldier fly pre-pupae meal up to 22.5% [60], meat and blood meal up to 80% [61], and hydrolyzed feather meal up to 25% [62] without compromising fish performance, feed utilization, and improving economic revenue. In addition, increasing gambusia meal reduced the cost of Nil tilapia diet, however, the cost–benefit analysis revealed that gambusia could only replace 50% of FM in terms of lower cost per unit of weight-gain compared to the control or other higher replacement levels [63]. The maximum profit was recorded by replacing 31.6% of dietary FM with poultry by-product meal in the diet of dourado, Salminus brasiliensis [64]. Interestingly, the present findings revealed that the use of DMM as a FM replacer reached 75–100% with a positive follow in the mullet performance and economic benefit. Consequently, these results could direct the industrial application of using this alternative protein source. Much consideration should therefore be given to ensure that even zooplankton meal or DMM are not harvested for meal production causing deprivation of natural food from their predators [16].

5. Conclusions

Fish meal is considered one of the most important challenges facing the aquaculture industry. According to our knowledge, until now there is no practical diet for mullet, *Mugil cephalus*, larvae prepared from *Dahpnia magna* meal (DMM) as a total or partial replacing fish meal. Based on the results obtained in the current study, DMM may be regarded as a valuable protein source for mullet, *M. cephalus*, larvae. It could replace up to 75% to 100% of dietary fishmeal with significant improvements in growth performance, feed utilization, histological, and economic status of mullet, *M. cephalus,* larvae.

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