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RESEARCH

Growth performance, intestinal morphology, blood biomarkers, and immune response of Thinlip Grey Mullet (*Liza ramada*) fed dietary laminarin supplement

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Abstract

Laminarin is a source of immunostimulants and antioxidative biomolecules involved in supporting the performance and health of aquatic animals. Hence, this study investigated the growth performance, intestinal morphology, blood biomarkers, and immune response of Thinlip Grey Mullet (*Liza ramada*) fed dietary laminarin. For 60 days, mullets were fed diets supplemented with laminarin at 0, 200, 400, 600, and 800 mg kg^{-1}, then the growth performance was evaluated, and samples were collected. The FBW, WG, SGR, PER, and carcass lipid content were markedly increased, while the FCR was significantly lowered by dietary 600 and 800 mg kg^{-1}. Further, the lipase and protease activities were significantly higher in mullets fed laminarin at 600 mg kg^{-1} than those fed 0, 200, 400, and 800 mg kg^{-1}. The intestinal histopathological evaluation revealed that all layers of the intestinal villi and the intestinal wall appeared intact without any deteriorating changes. The intestinal mucosal lining of anterior and middle segments showed improved morphological appearance with increased goblet cells in the intestinal villi associated with increased supplemented laminarin level. The total protein, globulin, and total cholesterol were markedly higher in fish fed 400 and 600 mg kg^{-1} laminarin than those fed 0, 200, and 800 mg kg^{-1}. Furthermore, the lysozyme, catalase, and glutathione peroxidase activities were higher in mullets fed laminarin at 600 mg kg^{-1} than those fed a laminarin-free diet. The superoxide dismutase was higher in fish fed 200, 400, and 600 mg kg^{-1}than those fed 0 and 800 mg kg^{-1}. On the other hand, the malondialdehyde activity was markedly decreased by 400 and 600 mg kg^{-1} of dietary laminarin. Overall, dietary laminarin is required at 338–761 mg kg^{-1} to reveal the best growth performance, intestinal morphology, blood biomarkers, antioxidative, and immune response in mullets.

Keywords Aquafeed · Algae extracts · Antioxidative capacity · Digestion capacity · Polysaccharides

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Introduction

Aquaculture sustainability considers the necessity of diversification of finfish species to overcome the high need for valuable animal protein sources for humanity (FAO 2020). Mullet (Family Mugilidae) are suitable finfish species for farming associated with their eurythermal, euryhaline, and diversified feeding habits (Crosetti 2016). Several countries are growing mullets on a commercial scale depending on the wild fry capture (Quirós-Pozo et al. 2023). Grey mullet (*Liza ramada*) are farmed in several Mediterranean countries and are explored as a potential candidate for fish farming (Cardona 2016; Sadek 2016). Mullet are known for their high value as food and a source of income; therefore, more suitable approaches are needed for their high-quality production (Durand and Whitfield 2016). Feeding strategies are recently focused on using friendly additives to regulate feed digestion, productivity, and well-being of finfish species (Tacon et al. 2022). In this regard, a wide range of functional supplements was included in aquafeed and validated for the blue aquaculture industry (Dawood et al. 2018).

Seaweeds and their extracts have been included in aquafeed and approved as growth promotors and immunostimulants (Thépot et al. 2021). Seaweed contains necessary nutrients (e.g., vitamins and minerals), polysaccharides, and antioxidants (Liu et al. 2020; Chopin and Tacon 2021). Fucoidan, alginic acid, and laminarin are abundantly present in seaweeds and are known for their pharmacological effects (Abdel-Latif et al. 2022). More specifically, laminarin is among the polysaccharides present in brown algae (Zargarzadeh et al. 2020). Laminarin is a carbohydrate that contains β-1,3-glucans which can be formed by the conjunction between β-1,3-glucosidic and β-1,6-glucosidic bonds (Chen et al. 2021). In different studies, laminarin exhibited antibacterial (Noorjahan et al. 2022), anti-inflammatory and antioxidative roles (Rajauria et al. 2021). In addition, laminarin regulates intestinal health by modulating intestinal microorganisms' activity and metabolism (Huang et al. 2021). Markedly, laminarin regulates the adhesion of intestinal bacteria on the epithelial walls by affecting the pH and secretion of fatty acids and mucus (Vidhya Hindu et al. 2019). In aquaculture, dietary laminarin enhanced the growth performance and immune response in grouper (*Epinephelus coioides*) (Yin et al. 2014) and rainbow trout (*Oncorhynchus mykiss*) (Morales-Lange et al. 2015). Further, dietary laminarin regulated the digestive and antioxidative enzyme activities in *Ictalurus punctatus* (Jiang et al. 2021). Hence, this study aimed to evaluate the beneficial roles of laminarin on growth performance, digestive enzymes, blood biomarkers, intestinal health, antioxidative capacity, and immune response of grey mullets.

Materials and methods

Ethical approval

Experimental procedures were performed following the local Animal Care and Ethics Committee of Kafrelsheikh University, Egypt (Approval No. KFS-IACUC/111/2023).

Test diets and experimental design

The test diets were prepared as shown in Table 1. The diets were formulated using fish meal, soybean meal, and gluten as protein sources. Further, yellow corn, wheat bran, rice bran, and wheat flour were used as carbohydrates source besides their content of protein. After mixing ingredients with minerals and vitamins, fish oil was added as a lipid source then water was added to have a dough. Laminarin powder (purity of 95% sourced from *Laminaria digitata*, no. L9634, Sigma, USA) was added to the diets at 0, 200, 400, 600, and 800 mg kg^{-1}. Wheat flour was mixed with laminarin to adjust the test diets to 100%. The pelletizing machine was then used to produce pellets of 2 mm size. Subsequently, the prepared pellets were dried at room temperature and kept in plastic bags until used. The chemical composition was confirmed by following AOAC (2012).

Thinlip Mullet (*Liza ramada*) juveniles were obtained from Bughaz El-Burullus (Baltim city, Kafr El-Sheikh governorate, Egypt) and gently moved to the Fish Nutrition Laboratory, Baltim Unit, National Institute of Oceanography and Fisheries (Baltim city, Kafr El-Sheikh governorate, Egypt). To acclimatize the fish to the trial conditions, all

Table 1 Formulation and composition of the basal diet

Ingredients	g	Chemical composition	
Fish meal (65% cp)	100	Crude protein (%)	30.33
Soybean meal (44% cp)	360	Crude lipids (%)	5.65
Yellow corn	120	Ash (%)	7.55
Gluten	70	Fibers (%)	5.12
Wheat bran	120	Gross energy (kcal/kg)[2]	1822.21
Rice bran	80		
Wheat flour	89.2		
Fish oil	30		
Vitamin and mineral mix	20		
Dicalcium phosphate	10		
Vitamin C	0.8		
Total %	1000		

[1]The mixture of vitamins and minerals is detailed by Khalafalla et al. (2022)

[2]Gross energy was calculated based on the values of values for protein, lipid, and carbohydrate as 23.6, 39.5 and 17.2 kJ g^{-1}, respectively

fish were stocked in a concrete tank (3 × 2 × 1.7 m) for two weeks before the trial. The tank was provided running water in a flow-through system (1 L min^{-1}), and fish were fed the basal diet at 3% of the body weight (BW) twice daily (08:00 and 15:00). Then, five groups of 15 fish with an average weight of 6.49 ± 0.32 g $fish^{-1}$ were distributed by 15 hapas (0.5 × 0.5 × 1 m) in order to have triplicated for each treatment. Fish were offered test diets at 3% of the BW during the trial for 60 days. The photoperiod was kept at a 12:12 light: dark cycle, and the water quality indices were regularly measured throughout the trial: temperature (25.31 ± 0.28 °C), pH (7.28 ± 0.19), oxygen (5.42 ± 0.34 mg L^{-1}), salinity (11.26 ppt), and total ammonia (0.14 ± 0.01 mg L^{-1}).

Final sampling

The trial was terminated after 60 days, and all fish were starved for 24 h before the sampling day. All fish were weighed individually and counted to calculate the growth performance and survival rate. The following equations were used to calculate the weight gain (WG) and specific growth rate (SGR). Besides, the feed conversion ratio (FCR), protein efficiency ratio (PER), and feed intake were also determined.

$$WG = 100 \times (\text{final weight (FW, g)} - \text{initial weight (IW, g)})/\text{IW (g)}$$

$$SGR\ (\%/\text{day}) = 100 \times (\ln \text{FW (g)} - \ln \text{IW (g)})/\text{days}$$

$$FCR = \text{total dry feed intake (g)}/(\text{FW (g)} - \text{IW (g)})$$

$$\text{Protein efficiency ratio (PER)} = (\text{FW (g)} - \text{IW (g)})/\text{dry protein intake (g)}$$

$$\text{Survival (\%)} = 100 \times \text{final fish number}/\text{initial fish number}$$

Then fish were anesthetized with 100 mg L^{-1} tricaine methane sulfonate (Sigma-Aldrich) (Führ et al. 2012), and blood was collected from 3 fish/hapa using 5 mL gauge syringes from the caudal vein. The blood was kept in non-heparinized tubes for serum collection. After 2 h, blood samples were centrifuged at 1008 × *g* for 15 min at 4 ℃; then, serum was separated and kept at − 20 ℃ for further analysis. Then three fish per hapa were dissected and the intestines were collected and used to analyze digestive enzyme activity. Furthermore, three fish per hapa were dissected and their intestines and livers were extracted for the histological study.

Carcass composition and digestive enzyme activity

Three fish per hapa were collected, washed with fresh water and kept in the freezer for the proximate composition analysis at -20 °C. A standard method was used for the chemical composition (moisture, ash, lipids, and crude protein) of the whole fish body and the nutritional profile of test diets (AOAC 2012). The moisture content was measured using oven-drying at 110 °C to reach the constant weight while the ash samples were burnt in a muffle furnace at 550 °C for 6 h. The Kjeldahl method was used to find crude protein, while the Soxhlet extraction method was used to determine crude lipids. After being digested for 15 min in a solution of 5% sulfuric acid and 5% sodium hydroxide, the amount of crude fiber in the diets was estimated.

The collected intestines were frozen in liquid nitrogen and stored at -80 °C until used. The homogenate was prepared by rinsing the intestines in ice-cold Phosphate-Buffered Saline (PBS) (pH 7.5; 1 g per 10 mL). It was then homogenized and centrifuged at 8000 rpm for 5 min and the supernatant was collected and stored at 4 °C for further analysis. Briefly, the total amount of protein was determined using the Lowry et al. (1951) method and bovine serum albumin (BSA) as a standard. The Folin-Ciocalteu phenol reagent was then used to detect the protease activity. The amylase activity was tested using an iodine solution to identify non-hydrolyzed starch, as per Jiang (1982) and Worthington (1993). The specific activity of lipase was evaluated using olive oil as the substrate in accordance with the Borlongan (1990) and Jin (1995) procedure. Amylase and lipase activities were assessed in units per mg of protein.

Blood analysis

Serum total proteins and albumins were determined according to Doumas et al. (1981) and Dumas and Biggs (1972), while globulins content was calculated mathematically by deducting the albumins from the total proteins. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, triglycerides, creatinine, and urea were detected by RA-50 chemistry analyzer (Bayer Diagnostics, Ireland) using readymade chemicals (kits) supplied by Spinreact Co., Girona, Spain, following the manufacturer's instructions.

Serum malondialdehyde levels were determined using the thiobarbituric acid method and calorimetrically determined using a commercial kit (LPO-586 Kit, OXIS International Inc., PDX, USA) as described by Ohkawa et al. (1979). The serum samples were used to detect superoxide dismutase (SOD) (McCord and Fridovich 1969), glutathione peroxidase, and catalase (Aebi 1984) activities using colorimetric methods by commercial fish-specific kits (Biodiagnostic Co., Egypt).

Serum lysozyme activity was determined using turbidimetric assay, according to the method described by Ellis (1990) based on the lysis of *Micrococcus lysodeikticus* (Sigma, USA). Phagocytic activity was determined according to Kawahara et al. (1991). The number of phagocytized and phagocytic cells was determined to calculate the phagocytic index according to the following equations: Phagocytic activity = macrophages containing yeast/total number of macrophages × 100; phagocytic index = number of cells phagocytized/number of phagocytic cells.

Histomorphology

At the end of the experiment, samples of anterior, middle, and posterior segments in the experimental groups were dissected and fixed in 10% neutral buffered formalin. After 24 h the samples were transferred to 70% alcohol. The tissue samples were then dehydrated in ascending graded series of ethanol, cleared in xylene, and impregnated and embedded in paraffin wax. Sections of 5 µm were cut using Leica rotatory microtome and mounted on glass slides. The prepared tissue sections were subjected to conventional staining of hematoxylin and eosin (H&E), according to Gewaily and Abumandour (2021). The stained sections were examined under a light microscope.

Statistical analysis

Shapiro–Wilk and Levene tests confirmed normal distribution and homogeneity of variance. The obtained data were subjected to one-way ANOVA. Differences between means were tested at $P < 0.05$ level using the Duncan test as a post-hoc test. All the statistical analyses were done via SPSS version 22 (SPSS Inc., USA). Moreover, orthogonal polynomial contrasts were performed to determine whether the observed trends were linear or quadratic (Davis 2010). The optimum laminarin dose was evaluated by quadratic polynomial regression analysis using the results of the FW, FCR, protease activity, lysozyme activity, and SOD (Yossa and Verdegem 2015).

Results

Growth performance, digestive enzyme activity, and carcass composition

Table 2 shows the final weight (FBW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) in mullets fed dietary laminarin for 60 days. The FBW, WG, SGR, and PER were markedly increased in mullets fed laminarin at 600 and 800 mg kg^{-1} without significant differences with those fed 200 and 400 mg kg^{-1}. At the same time, the FCR was significantly lowered in fish-fed diets supplemented with 600 and 800 mg kg^{-1}compared to those fed a laminarin-free diet. The supplementation of laminarin did not significantly affect the FI ($P>0.05$). The survival showed no marked differences among dietary treatments and ranged between 93.33 ± 3.85 and $97.78 \pm 2.22\%$.

Table 3 shows the digestive enzyme activities in mullets fed dietary laminarin for 60 days. No marked effects were seen on the amylase activity after 60 days. However, the lipase and protease activities were significantly higher in mullets fed laminarin at 600 mg kg^{-1} than in those fed 0, 400 or 800 mg kg^{-1}.

No marked effects of dietary laminarin were seen on the carcass composition except for the lipid content, which was significantly higher in mullets fed 400 and 600 mg kg^{-1} than in fish fed on the other diets (Table 4).

Intestinal histology

The histopathological evaluation demonstrated normal construction of the intestine's anterior, middle, and posterior segments in the control and laminarin-supplemented fish (Fig. 1), where all layers of the intestinal villi and the intestinal wall appeared intact without any deteriorating changes. The intestinal mucosal lining displayed an improved morphological appearance in the form of an increased number of intestinal villi and augmented villous surface area (in the anterior and middle segments), as well as an abundant number of goblet cells (particularly in the middle and posterior segments) accompanying the increased level of supplemented laminarin (Fig. 1).

Table 2 Growth performance of mullets fed dietary laminarin for 60 days

Item	Laminarin (mg kg^{-1})					Quadratic (*P*-value)
	0	200	400	600	800	
IBW (g)	6.47 ± 0.04	6.49 ± 0.02	6.51 ± 0.02	6.51 ± 0.04	6.49 ± 0.02	NS
FBW (g)	28.31 ± 1.18b	29.30 ± 0.60ab	29.70 ± 0.93ab	31.27 ± 0.47a	30.43 ± 0.96a	0.001
WG (%)	337.60 ± 15.78b	351.58 ± 10.04ab	356.25 ± 15.83ab	380.37 ± 9.32a	368.85 ± 13.24a	0.001
SGR (% day^{-1})	2.46 ± 0.06b	2.51 ± 0.04ab	2.53 ± 0.06ab	2.62 ± 0.03a	2.57 ± 0.05a	0.001
FI (g feed/fish)	28.40 ± 0.41	28.96 ± 0.29	28.76 ± 0.23	29.27 ± 0.08	28.58 ± 0.92	NS
FCR	1.31 ± 0.07a	1.27 ± 0.02ab	1.25 ± 0.06ab	1.18 ± 0.03b	1.20 ± 0.04b	0.001
PER	2.54 ± 0.13b	2.60 ± 0.05ab	2.66 ± 0.12ab	2.78 ± 0.06a	2.76 ± 0.10a	0.001
Survival (%)	93.33 ± 3.85	97.78 ± 2.22	97.78 ± 2.22	97.78 ± 2.22	97.78 ± 2.22	NS

Means ± SE ($n = 3$) with different letters in the same row show significant differences ($P < 0.05$). IBW: initial body weight, FBW: final body weight, WG: weight gain, SGR: specific growth rate, FI: feed intake, FCR: feed conversion ratio, PER: protein efficiency ratio, NS: not significant ($P > 0.05$)

Table 3 Digestive enzyme activity of mullets fed dietary laminarin for 60 days

Item	Laminarin (mg kg^{-1})					Quadratic (*P*-value)
	0	200	400	600	800	
Lipase activity (unit mg^{-1} protein)	16.05 ± 0.26b	15.65 ± 0.25b	15.48 ± 0.16b	18.26 ± 0.58a	16.73 ± 0.27b	0.001
Amylase activity (unit mg^{-1} protein)	15.40 ± 0.34	15.51 ± 0.50	15.54 ± 0.18	16.25 ± 0.32	15.89 ± 0.17	NS
Protease activity (unit mg^{-1} protein)	13.98 ± 0.14b	14.14 ± 0.41ab	13.70 ± 0.28b	14.75 ± 0.26a	13.79 ± 0.29b	0.001

Means ± SE ($n = 3$) with different letters in the same row show significant differences ($P < 0.05$). NS: not significant ($P > 0.05$)

Table 4 Carcass composition of mullets fed dietary laminarin for 60 days

Item	Laminarin (mg kg^{-1})					Quadratic (*P*-value)
	0	200	400	600	800	
Moisture (%)	71.20 ± 0.06	71.01 ± 0.05	70.49 ± 0.66	70.04 ± 0.04	71.28 ± 0.16	NS
Crude protein (%)	17.82 ± 0.03	17.76 ± 0.08	17.73 ± 0.35	17.92 ± 0.19	17.86 ± 0.00	NS
Total lipids (%)	6.48 ± 0.18b	6.60 ± 0.09b	7.15 ± 0.21a	7.57 ± 0.18a	6.62 ± 0.14b	0.001
Ash (%)	4.47 ± 0.16	4.57 ± 0.03	4.44 ± 0.10	2.97 ± 1.49	4.28 ± 0.18	NS

Means ± SE ($n = 3$) with different letters in the same row show significant differences ($P < 0.05$). NS: not significant ($P > 0.05$)

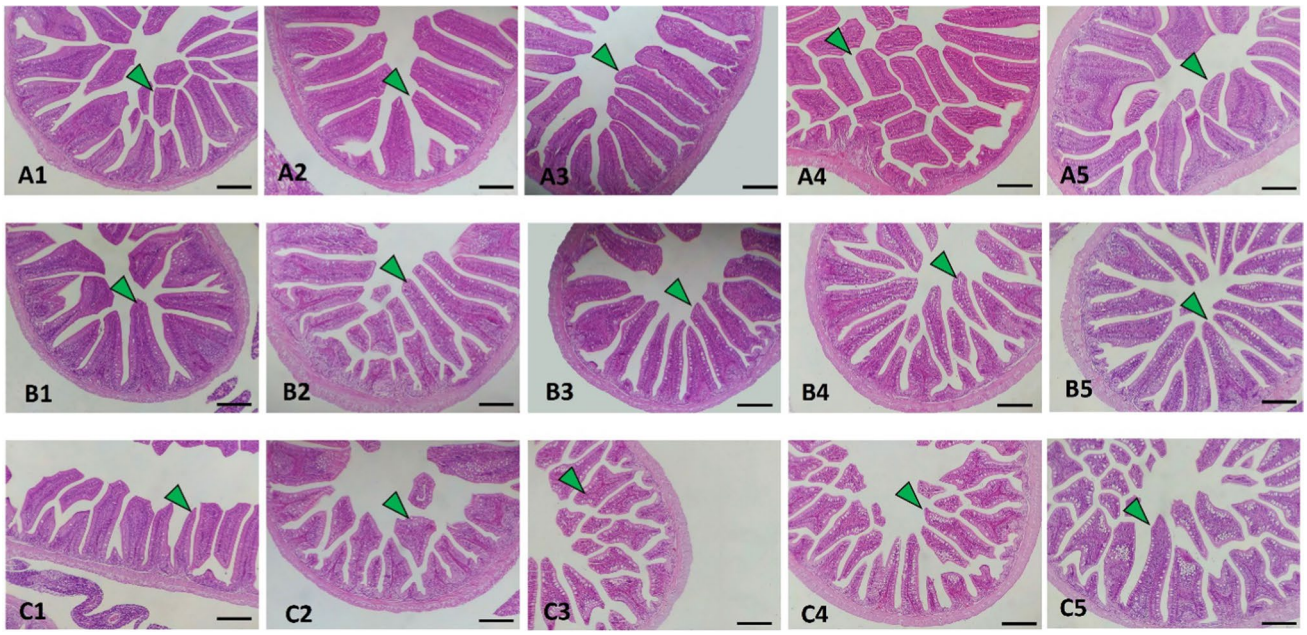

Fig. 1 Histomicrograph of anterior (upper panel), middle (middle panel), and posterior (lower panel) segments of mullet intestine in control (A1, B1, C1) and laminarin-treated groups at ascending levels; 200, 400, 600, and 800 mg kg^{-1} (A, B, C; 2–5). The intestinal morphology of groups supplemented with laminarin revealed improved, branched intestinal villi in the anterior and middle segments with increased goblet cells in the middle and posterior segments. Stain H&E. Scale bar = 100 µm

Blood biomarkers

The results of the biochemical blood indices are shown in Table 5. The results showed no marked effects on the ALT, AST, albumin, creatinine, urea, and triglycerides in mullets fed dietary laminarin for 60 days. However, the total protein, globulin, and total cholesterol were markedly higher in fish fed 400 and 600 mg kg^{-1} laminarin than those fed 0, 200, and 800 mg kg^{-1}.

Table 5 Blood biochemical indices of mullet fed dietary laminarin for 60 days

Item	Laminarin (mg kg^{-1})					Quadratic (*P*-value)
	0	200	400	600	800	
ALT (U L^{-1})	3.34 ± 0.03	3.30 ± 0.01	3.25 ± 0.03	3.19 ± 0.04	3.30 ± 0.03	NS
AST (U L^{-1})	68.44 ± 0.42	69.07 ± 0.27	68.25 ± 0.18	68.86 ± 0.17	69.38 ± 0.50	NS
Total protein (g dL^{-1})	4.24 ± 0.05b	4.39 ± 0.03b	4.50 ± 0.04a	4.61 ± 0.03a	4.25 ± 0.05b	0.001
Albumin (g dL^{-1})	2.40 ± 0.10	2.60 ± 0.19	2.34 ± 0.29	2.30 ± 0.17	2.33 ± 0.22	NS
Globulin (g dL^{-1})	1.84 ± 0.13b	1.78 ± 0.16b	2.16 ± 0.26a	2.31 ± 0.20a	1.93 ± 0.26b	0.001
Creatinine (mg dL^{-1})	0.38 ± 0.00	0.36 ± 0.01	0.36 ± 0.02	0.37 ± 0.00	0.36 ± 0.01	NS
Urea (mg dL^{-1})	5.50 ± 0.07	5.32 ± 0.05	5.39 ± 0.03	5.28 ± 0.04	5.49 ± 0.13	NS
T-CHO (mg dL^{-1})	75.62 ± 0.32b	73.54 ± 0.58b	79.37 ± 0.49a	78.55 ± 0.81a	72.47 ± 0.62b	0.001
TG (mg dL^{-1})	120.82 ± 1.75	117.78 ± 1.09	119.46 ± 1.24	118.08 ± 1.34	118.80 ± 0.91	NS

Means ± SE ($n = 3$) with different letters in the same row show significant differences ($P < 0.05$). ALT: alanine aminotransferase, AST: aspartate aminotransferase, T-CHO: total cholesterol, TG: triglycerides, NS: not significant ($P > 0.05$)

Blood immunity and antioxidative responses

The lysozyme, catalase, and glutathione peroxidase activities were higher in mullets fed laminarin at 600 mg kg^{-1} than in those fed a laminarin-free diet (Table 6). The phagocytic activity was higher in fish fed 600 mg kg^{-1} than in those fed 0, 200, 400, and 800 mg kg^{-1}, while the phagocytic index was higher in fish fed 400 and 600 mg kg^{-1} than in those fed 0, 200, and 800 mg kg^{-1} (Table 6). The superoxide dismutase was higher in fish fed 200, 400, and 600 mg kg^{-1} than in those fed 0 and 800 mg kg^{-1}. On the other hand, the malondialdehyde level was markedly decreased in fish fed either 400 or 600 mg kg^{-1} of dietary laminarin compared to those fed the laminarin-free diet (Table 6).

Quantification of optimum laminarin dose

Figure 2 shows the regression analysis of the FBW, FCR, protease activity, lysozyme activity, and SOD. The regression analysis shows that the best FBW, FCR, protease activity, lysozyme activity, and SOD in mullets require dietary laminarin 558.33, 761, 595, 645, and 338 mg kg^{-1}, respectively. Overall, dietary laminarin is required at 338–761 mg kg^{-1} to reveal the best performances in mullets.

Discussion

Seaweeds and their extracts have an effective immunostimulant activity involved in regulating local intestinal immunity (Thépot et al. 2021) and thereby, the entire body's performance (López Nadal et al. 2020). Laminarin is a functional polysaccharide with several antibacterial, antioxidation, and anti-inflammation benefits (Chen et al. 2021; Noorjahan et al. 2022). Therefore, the supplementation of laminarin at specific doses has been validated for several fish species with high potential as growth promotors, antioxidative, and immunostimulant agents (Yin et al. 2014; Morales-Lange et al. 2015; Jiang et al. 2021).

The quantitative assessment of proper doses of dietary supplementations can be determined by applying polynomial

Table 6 Blood immunity and antioxidative responses of mullets fed dietary laminarin for 60 days

Item	Laminarin (mg kg^{-1})					Quadratic (*P*-value)
	0	200	400	600	800	
Lysozyme activity (unit mL^{-1})	35.11 ± 1.16b	37.10 ± 0.29ab	37.84 ± 0.29ab	38.34 ± 0.46a	36.19 ± 0.54ab	0.001
Phagocytic activity (%)	28.99 ± 0.41b	28.78 ± 0.21b	29.37 ± 0.25b	31.24 ± 0.61a	28.95 ± 0.37b	0.001
Phagocytic index	2.03 ± 0.04b	2.08 ± 0.05b	2.17 ± 0.06a	2.20 ± 0.06a	2.00 ± 0.01b	0.001
Superoxide dismutase (IU L^{-1})	26.06 ± 0.38b	28.52 ± 0.46a	27.90 ± 0.20a	29.01 ± 0.88a	26.30 ± 0.54b	0.001
Catalase (IU L^{-1})	33.80 ± 0.16b	34.43 ± 0.45b	34.89 ± 0.45b	36.32 ± 0.64a	34.79 ± 0.16b	0.001
Glutathione peroxidase (IU L^{-1})	21.69 ± 0.23b	21.33 ± 0.39b	22.89 ± 0.12b	23.76 ± 0.31a	21.70 ± 0.27b	0.001
Malondialdehyde (nmol mL^{-1})	23.46 ± 0.38a	22.22 ± 0.46ab	21.90 ± 0.20b	21.14 ± 0.88b	22.22 ± 0.54ab	0.001

Means ± SE ($n = 3$) with different letters in the same row show significant differences ($P < 0.05$). NS: not significant ($P > 0.05$)

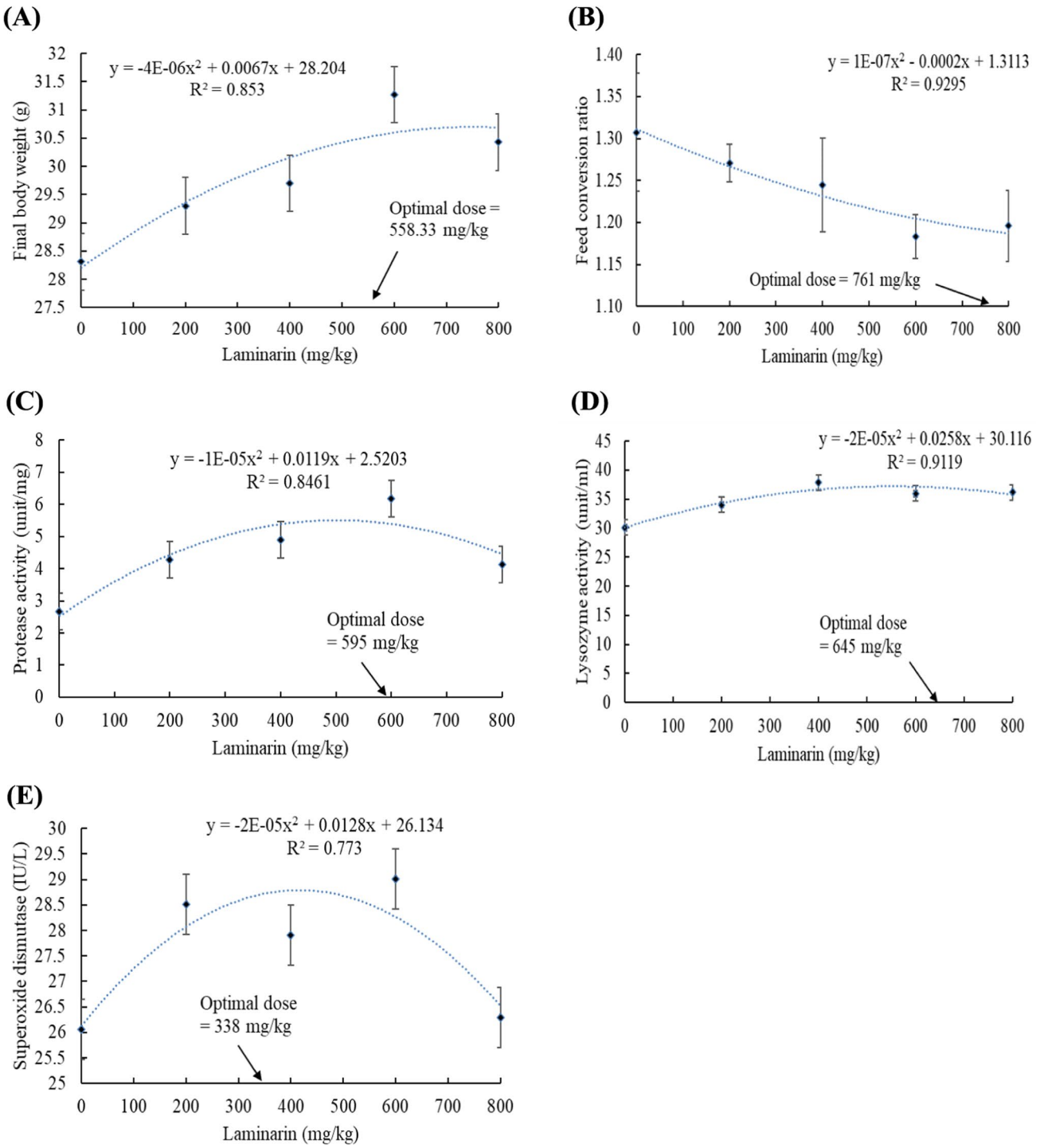

Fig. 2 Quantification of the optimum laminarin dose using regression analysis. The optimum laminarin dose was evaluated by quadratic polynomial regression analysis using the results of the final body weight (A), feed conversion ratio (B), protease activity (C), lysozyme activity (D), and superoxide dismutase (E)

regression analysis (Yossa and Verdegem 2015). In the current study, we considered representative variables for the growth performance (e.g., the final weight), feed utilization (e.g., FCR), intestinal digestion (e.g., protease activity), immune response (e.g., lysozyme activity), and antioxidative capacity (e.g., SOD) of mullets fed on dietary laminarin. Subsequently, the estimated dose for laminarin could be determined based on the overall performances of mullets.

Herein, the optimum dose of laminarin for the proper performances of mullets is 338–761 mg kg^{-1}. The results are comparable with Morales-Lange et al. (2015), who stated that laminarin is needed at 200 mg kg^{-1} to enhance the immunity of rainbow trout. Further, Jiang et al. (2021) indicated that laminarin can be added up to 8000 mg kg^{-1} in the diets of *Ictalurus punctatus* to enhance the growth performance, digestion, and immune response. Yin et al. (2014) also reported that laminarin could be added at 500–1000 mg kg^{-1} to enhance the growth performance and immunity of grouper. In light of the earlier efforts, laminarin addition differs among fish species; thus, more future studies are needed since the differences in feeding habits, fish species and sizes, and feeding duration can substantially affect the recommended doses.

In this study, mullets treated with laminarin revealed improved growth performance and digestive enzyme activity. Further, activated antioxidative and immunity were also seen in mullets treated with laminarin. The results showed marked improvements in the growth performance of *L. ramada*-fed laminarin in a dose-dependent manner. The results are in line with Yin et al. (2014) and Jiang et al. (2021), who reported enhanced growth performance in grouper and *Ictalurus punctatus*-fed dietary laminarin at 500–1000 mg kg^{-1}. The study also indicated enhanced feed utilization indices (FCR and PER), which may explain the improved growth performance in mullets by dietary laminarin. Indeed, laminarin can regulate the absorption of digested nutrients in fish intestines leading to high absorption capacity (Huang et al. 2021). Although the results showed that dietary laminarin did not significantly affect feed intake, mullets fed laminarin had improved growth performance. These results indicate enhanced feed utilization by dietary laminarin in mullets resulting in improved FCR and PER. The study indicated high digestive enzyme activity in mullets treated with laminarin to support this hypothesis. The improved digestion in the mullet's intestines can be linked to the potential antibacterial role of laminarin in eliminating harmful microorganisms (Noorjahan et al. 2022). Hence, the local intestinal digestion and immunity enhance and result in high feed utilization and the health status of the fish's entire body (Dawood 2021). Enhanced local intestinal immunity by dietary laminarin would result in regulated metabolic and physiological capacity (Li et al. 2021) and, thereby the entire body's growth performance.

The intestinal morphological features of mullets indicated the absence of undesirable effects of dietary laminarin. The intestinal and mucosal layers were intact and showed no degenerative features by dietary laminarin in mullets. In the same line, incorporating polysaccharides-rich additives enhanced the intestinal morphological features in hybrid red tilapia (Abdelrhman et al. 2022). Accordingly, fish show high digestibility and absorption capacity for lipids in the intestines, which can enhance local intestinal immunity and digestibility (Dawood 2021; Abdelrhman et al. 2022). The results also indicated enhanced lipase and protease activities in mullets by laminarin (600 mg kg^{-1}) to support the digestion capacity. The results agree with Jiang et al. (2021), who stated that improved trypsin, amylase, and lipase in *I. punctatus*-fed dietary laminarin. Improved digestion of carbohydrates, lipids, and proteins is the primary function of activated amylase, lipase, and protease activities in fish intestines (Zheng et al. 2020). Proper feed digestion results in regulated metabolic function and thereby enhanced physiological and immunological responses (Yukgehnaish et al. 2020). The current study showed a marked enhancement in feed utilization in mullets treated with laminarin through the improved PER and FCR. Concurrently, the absence of abnormal intestinal features and enhanced digestive enzyme activity may explain the improved growth performance and regulated blood biomarkers in mullets fed laminarin.

No marked effects were observed on the carcass composition except the lipid content, which showed increased levels in mullets fed dietary laminarin. Usually, the measurements of the carcass composition refer to the level of nutrient metabolism and accumulation in the entire fish body (Ahmed et al. 2022). Seaweed is involved in lipid metabolism and could reduce lipid accumulation (Ferreira et al. 2020). However, this mechanism varies among fish species and can be related to the diet composition, feeding behavior, and type of seaweed (Ragaza et al. 2021). Thus, the increased levels of lipids in mullets fed laminarin require further studies to explain the mechanism involved in lipid accumulation.

The study also tested the biochemical indices of serum samples from mullets fed with or without laminarin. Routinely, the biochemical markers can show the liver and kidney function and the metabolism of proteins and lipids in the fish's blood (Shahjahan et al. 2022). High protein biomarkers, globulin, and lipids were seen in mullets fed laminarin. The increased protein profile can be related to the enhanced feed utilization and efficient metabolic function involved in the high availability of proteins in the blood (Wang et al. 2023). Liver (ALT and AST) and kidney (urea and creatinine) function indices showed non-significant values in mullets fed with or without laminarin. The results indicate that laminarin did not harm the liver and kidney.

Oxidative stress may occur under stressful conditions and can induce health interruption, including fish's impaired physiological and immunological status (Chowdhury and Saikia 2020). Malnutritional strategies and farming stressors are the main reasons for oxidative stress in aquatic animals (Dawood 2021). Hence, incorporating suitable and functional additives in aquafeed can regulate the antioxidative responses and protect from impaired health status (Habotta

et al. 2022; Vijayaram et al. 2022). The oxidative stress resulting from the overproduction of reactive oxygen species (ROS) causes inflammation via cellular lipid peroxidation, which malondialdehyde (MDA) levels can evaluate (Birnie-Gauvin et al. 2017; Ratn et al. 2018). Consequently, antioxidative responses attenuate the ROS by activating SOD, CAT, and GPx (Martínez-Álvarez et al. 2005). Therefore, the activated antioxidative capacity of fish indicates a healthy and immunized status, thereby enhancing feed digestion and growth performance. In this regard, the current trial illustrated that laminarin enhanced the antioxidative capacity (SOD, CAT, and GPx) while decreasing the level of MDA in mullet. The CAT and GPx were higher in mullets fed laminarin at 600 mg kg^{-1} than in those fed a laminarin-free diet, while SOD was higher in fish fed 200, 400, and 600 mg kg^{-1} than in those fed 0 and 800 mg kg^{-1}. On the other hand, the MDA level was markedly decreased in fish fed either 400 or 600 mg kg^{-1} of dietary laminarin compared to those fed the laminarin-free diet. Similarly, Jiang et al. (2021) and Yin et al. (2014) reported activated antioxidative capacity in *I. punctatus* and grouper. The enhanced antioxidative capacity can be related to the role of polysaccharides, which reduce lipid peroxidation and, thereby, the possibility of forming lipid peroxides and ROS (Chen et al. 2021; Noorjahan et al. 2022). Further, the absence of stress in mullets due to laminarin supplementation explains the activation of antioxidative status.

Laminarin is a source of polysaccharides involved in fish immunomodulation and activation of antioxidative capacity (Mohan et al. 2019). Protecting host immunity is crucial since fish are suspected of biotic and abiotic stressors (Dawood et al. 2022). Instantly, phagocytosis and lysozyme activity protect fish from bacterial infection induced by immunity suppression under infection attack (Saurabh and Sahoo 2008). Through the activation of the antibacterial capacity, phagocytic and lysozyme activities defend fish by inhibiting pathogenic bacterial invasion (Magnadóttir 2006). It has been confirmed that polysaccharides extracted from seaweed can activate the immunity of fish (Chen et al. 2021). The results showed that lysozyme activity was higher in mullets fed laminarin at 600 mg kg^{-1} than in those fed a laminarin-free diet. The phagocytic activity was higher in fish fed 600 mg kg^{-1} than those fed 0, 200, 400, and 800 mg kg^{-1}. In this regard, Jiang et al. (2021), Yin et al. (2014), and Morales-Lange et al. (2015) illustrated that *I. punctatus*, grouper, and rainbow trout-fed dietary laminarin showed an increased immune response (phagocytic and lysozyme activities). Seaweed-derived polysaccharides can enhance immunity due to their high content of β-glucans which have been confirmed to pass through the walls of immune cells via specific receptors (Petit and Wiegertjes 2016; Chen et al. 2021). Further, polysaccharides can enhance local intestinal immunity and, thereby, the entire body's immunity by reducing inflammation and inhibiting harmful bacterial effects (Vidhya Hindu et al. 2019).

Conclusion

Laminarin showed a potential effect on the diets of grey mullets. In this regard, dietary laminarin enhanced growth performance, feed utilization, and digestion capacity. Further, dietary laminarin did not affect the histomorphological features of the anterior, middle, and posterior intestines of grey mullets, indicating the safe use of laminarin. Accordingly, dietary laminin in mullets regulated the blood biomarkers and immune and antioxidative responses. Comprehensively, dietary laminarin is required at 338–761 mg kg^{-1} to reveal the best growth performance, intestinal morphology, blood biomarkers, antioxidative, and immune response in mullets.

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Declarations

Competing interest The authors declare no competing interests.

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