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Original Article

Effects of silverside and sorghum meals on growth performance, proximate composition and histological features of Thinlip Grey Mullet (*Liza ramada*, Risso, 1826) fingerlings

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ABSTRACT

Future aquaculture growing may be constrained by fishmeal availability, which is influenced by environmental factors (such as climate changes). As a result, finding cheap sources of carbohydrates or alternative animal protein has become essential for aquaculture production to be sustainable. A total of 1200 fingerlings of Mullet (*L. ramada*) were obtained from the private farm at Fayoum Governorate and transported to the National Institute of Oceanography and Fisheries (NIOF) to use the local ingredients from silverside and sorghum to replace imported fish meal and yellow corn in mullet diets. The experiment was applied using a completely randomized design. Fingerlings were distributed in twelve fiberglass tanks with a volume of 2 m3 with a stocking density of 100 fingerlings/ tank with an initial average weight of (3.8 ± 0.1g). Four experimental diets were used: Control (D0), which contains fish meal and yellow corn, the second, third, and fourth diets incorporated each silverside and sorghum meals to replace, 50, 75, and 100% (D50, D75 and D100) of fish meal and yellow corn. The results showed that D100 gave the best gain and growth performance, followed by D75 without a significant difference between them. However, less performance was obtained with D0 and D50. The proximate carcass composition and muscle fatty acids contents of fish were not significantly affected by the incorporation of silverside and sorghum meals in mullet diets. In the same trend, the intestinal villi from the experimental groups did not show any changes in the mucosal layer, immune cells, and lymphatic vessels between tested groups. The present results confirmed that the incorporation of 100% silverside and sorghum meals can be used in mullet diets to spare the expensive imported meals from fish meals and yellow corn .

Keyword: Mullet, Liza ramada, Fingerlings, Growth performance, Histological features.

1. INTRODUCTION

By 2050, there will be 10 billion people on the earth, indicating that 70–100% more food and meat must be produced to meet demand (FAO, 2020).

The growing greenhouse gas emissions and the scarcity of agricultural land and water provide many obstacles for the world's food production system in satisfying this demand. Due to the scarcity of water in the primary production regions, competition from plant production, the high cost of conventional feed materials, and the disruptions brought on by climate change, which have a detrimental impact on the supply of certain feed ingredients like fishmeal, new technologies are thought to be required in the production of fish protein. In light of this, new sustainable protein sources will allay these worries (Godfray *et al*., 2010; Nyachoti *et al*., 1997).

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Sorghum is a drought-resistant cereal grain typically cultivated in semi-arid environments. In terms of production and planted area, this grain is rated fifth globally after wheat, corn, rice, and barley (Stamenkovic *et al*., 2000; Aderolu *et al.,* 2009; Valin *et al.*, 2014).

The production of sorghum reached 59.71 million MT in 2023/2024 (USDA-NASS, 2024), and accounted for 2.2% of global grain production in 2013 (Bean *et al.,* 2016). The United States is considered the main producer of sorghum, with 8.7 million metric tons while Egypt produces to 750 thousand MT. According to Jiddere and, Filli (2015) sorghum is used more than other cereal grains because of its unique qualities, which are useful in poor agricultural conditions.

Additionally, the fact that it is mostly farmed by subsistence farmers, its resilience to wild plants, weather and soil conditions. Sorghum has long been a principal food crop and a major source of protein, energy, and minerals for millions of people in Asia and Africa (Obizoba and Atii *et al.*, 1991; Mohammed et al., 2011). The elaboration of fish diets has included increasing quantities of plant-based ingredients, which are in some cases its exclusive components, justified by their low cost, wide availability, and easiness of acquisition (Guimarães *et al.,* 2008). As a consequence, the food industry has been testing processes to promote higher availability of nutrients in plant-based foods, which due to the presence of phytic acid, tannin, gossypol, and others, inhibit the action of several enzymes, making several minerals unavailable and compromising protein absorption of diets (Bergamin *et al.,* 2013). Though sorghum is a potentially adequate ingredient for diets, it contains anti-nutritional factors like phytic acid, which form stable *in vitro* complexes with many minerals, thus inhibiting their absorption (Duarte *et al.,* 2011). Low-tannin sorghum has nutrients similar to corn, with higher levels of crude protein and starch, as well as a higher fraction of amylose and a high antioxidant effect (Ratnavathi *et al.,* 2016; Rostagno *et al.,* 2017). However, numerous energy ingredients that may be used in animal diets. Aquaculture's diversification has been considered for the last decades as one of the major tools for a greater and more sustainable expansion of the sector. Among the different fish species considered for aquaculture diversification, mullets (Family Mugilidae) are great candidates due to their euryhaline, eurythermal, and opportunistic feeding nature, which have propitiated its extensive culture for centuries in different regions worldwide (Quiros-Pozo *et al.,* 2023).

Mullets are—very significant marine species, occupied both fresh water and brackish water and expressed as omnivorous, particularly opportunistic feeders, successful on all accessible foods (El-Dahhar, 2007; El-Dahhar *et al.,* 2013). Egypt is the first country in the world to produce the mullet. However, this fish has not yet been commercially applied hatched. The fry of the grey mullet (*L. ramada*) is collected from natural sources and incubated in nursery ponds for a period of 6 to 12 months (FAO, 2022).

Furthermore, Mullets have been very considerably high fish species due to their high productivity for various types of culturing methods like pond, cage, and pen culture in various parts of the world such as China, Egypt, Italy, and Japan (El-Dahhar *et al.,* 2023).

Grey Mullet (*L. ramada*) can be farmed in Egypt mainly in polyculture systems because of their high growth rate, tolerance of a wide range of environmental conditions, resistance to disease and stresses, and high ability to benefit from the natural food cycles in the pond and the ability to utilize the formulated pellets, regardless of their protein content (Toutou *et al.,* 2023).The present study was detected to evaluate the effect of incorporation silverside and sorghum meals to replace fish meal and yellow corn on growth performance, proximate composition and intestine histology of Mullet (*L. ramada*) fingerings.

2.Material and Methods

2.1. Ethics approval

All experimental procedures including, rearing, handling, sampling, and euthanasia, followed the guidelines of the Committee for Ethical Care and

Use of Animals/Aquatic Animals of the National Institute of Oceanography and Fisheries (NIOF, Egypt) which approved this study with a code (NIOF-IACUC, Code: NIOF-AQ4-F-24-R-021). The authors confirm that all methods were performed in accordance with the relevant guidelines and regulations and that the study is reported in accordance with the ARRIVE guidelines.

2.2.. Fish and experimental design

Thinlip Mullet (*L. ramada*) fingerlings were purchased from the private farm located in Fayoum Governorate, Egypt. Fish were transported to Aquatic Research Station Lab. National Institute of Oceanography and Fisheries, Fayoum, Egypt. Fish were acclimated to laboratory conditions and fed with a commercial feed for 2 weeks before starting the trial. After 24 hours of starvation, 1200 fish (initial body weight (3.8 ± 0.1g) were randomly selected from the acclimatized fish and allocated into 12 rectangular tanks (size of each pond was 2 m^3) in equal number (stocking rate of 50 fish/m^3).

Each experimental diet is presented in three-replicates. During the experiment, fish were fed the experimental diets to apparent satiation twice daily (9:00 a.m. and 5:00 p.m.). The water system includes two pumps and upstream sandy filter units at a point between the water source (Earthen pond) and tanks. The pumps were drowning the water to the storage tanks and forced it through polyvinyl chloride (PVC) tubes in to the rearing in open system. The experimental period lasted 120 days after start. Physicochemical properties of water tanks were examined every week according to (APHA, 2005).

2.3. Experimental diets

Four isonitrogenous diets (27% crude protein) were formulated. The control diet (D0) consisted of fish meal and yellow corn as protein and carbohydrate sources. Three experimental diets (D50, D75, D100) were formulated with silverside and sorghum meals were used to substitute 50, 75 and 100% of the fish meal and yellow corn in the control formulation, respectively.

Table 1 gives the ingredient composition of each of the diets. All diet ingredients were ground to a fine powder by a 150-µm mesh sieve. A suitable amount of water was then added to the powder to create a stiff dough, which was then pelleted using a 2-mm diameter die using a California type pellet mill. The pellets were air-dried and then kept at -20 o C until use.

Table (1). Ingredients and proximate composition of the experimental diets (% DM basis).

Ingredients with % crude protein	D0	D50	D75	D100
Fish meal (69.9 % CP)	6.00	3.00	2.00	0.00
Silverside meal (69 % CP)	0.00	3.00	4.00	6.00
Yellow corn (8.1% CP)	32.00	16.00	8.00	0.00
Sorghum meal (9.2% CP)	0.00	16.00	24.00	32.00
Soybean meal (46% CP)	23.00	23.00	23.00	23.00
Gluten meal (62% CP)	10.00	10.00	10.00	10.00
Wheat bran (14.8% CP)	23.80	23.80	23.80	23.80
Fish oil	3.00	3.00	3.00	3.00
Vit. Min. Mix.[1]	2.00	2.00	2.00	2.00
Methionine	0.20	0.20	0.20	0.20
Proximate composition				
Crude protein	27.20	27.40	27.60	27.50
Crude lipid	6.26	6.35	6.36	6.45
Total carbohydrate	61.56	61.20	60.96	60.94
Ash	4.96	5.03	5.06	5.09
Tannin[2]	0.06	0.12	0.18	0.24
Gross energy (MJ g^{-1} diet)[3]	19.83	19.85	19.87	19.87

1-Vitamin, mineral premix (g/kg of mixture): L-ascorbic acid monophosphate-120.0;
L-α-tocopherylacetate-20.0; thiamin hydrochloride-4.0; riboflavin-9.0; pyridoxine hydrochloride-4.0; niacin-36.0; D-pantothenic acid hemicalcium salt-14.5; myoinositol-40.0; D-biotin-0.3; folic acid-0.8; menadione-0.2; retinyl acetate-1.0; cholecalciferol-0.05; cyanocobalamin-0.01; $MgSO_4{\cdot}7H_2O$-80.0; Na H $2PO_4{\cdot}2H_2O$-370.0; KCl-130.0; $FeSO_4{\cdot}7H_2O$-40.0; $ZnSO_4{\cdot}7H_2O$-20.0; Calactate-356.5; $CuSO_4$-0.2; $AlCl_3{\cdot}6H_2O$-0.15; $Na_2Se_2O_3$-0.01; $MnSO_4{\cdot}H_2O$-2.0; $CoCl_2{\cdot}6H_2O$-1.0.

2- Tannin=percent tannin on a catechin equivalent basis.

3- Gross energy (MJ Kg-1 diet) was calculated by using the following calorific values: 23.9, 39.8 and 17.6 KJ g-1 diet for protein, ether extract and nitrogen free extract, respectively (NRC, 1993)

2.4. Chemical analysis

Twenty fish from the same replicate were chosen at random prior to the experiment in order to determine the initial whole-body proximate composition. Fish were starved for a day before sampling at the end of the feeding period. Fish in each tank were weighed and counted for growth performance, feed efficiency and survival evaluation. Twenty fish from each tank were randomly selected and anesthetized with tricaine methane sulfonate (MS-222, 50 mg/L) for individual weight measurements. Then, the fish were quickly dissected for organ and tissue sampling. Finally, twenty fish per tank were randomly collected for determination of final whole-body proximate composition. Dry matter was determined by drying samples in an oven at 105°C until constant weight, crude protein was determined by measuring nitrogen (N ×6.25) after acid digestion using the Kjeldahl method, crude lipid was determined by petroleum ether extraction using the Soxhlet method, and ash was determined by incineration in a muffle furnace at 550°C for 16h (Bhatnagar et al., 2013). Tannin content of sorghum starch was determined using a modified version of Price's vanillin-HCl assay (AOAC, 2010). One gram of sorghum starch was placed in a 50 ml conical flask and 50 ml of analytical grade methanol was added. The flask was covered with a cork stopper, shaken thoroughly every few minutes for 2 hours and then left to stand at room temperature for an additional 20 h. Two ml of 2% vanillin, 4% HCl were added to one of the test tube and 5 ml of 4% HCl to other. The differences in two optical densities (the 4% HCl acting as the blank) were read on a Beckman spectrophotometer at 500 nm, and then compared to catch in standard curve.

2.5. Lipid profile analysis

Total lipid in samples was extracted after homogenisation, using an ultra turrax tissue disrupter (Fisher Scientific, Loughborough, UK), in ten volumes of chloroform–methanol (2:1, v/v) containing 0·01% butylated hydroxytoluene as antioxidant, basically according to (Price et al., 1978) and essentially as described by (Christie,1982).

Fatty acid methyl esters were prepared from aliquots of total lipids by acid-catalysed transmethylation for 16 h at 508ºC, using tricosanoic acid (23:0) as internal standard (Folch *et al*., 1957).

Fatty acid methyl esters were extracted and purified as described previously (Turchini et al., 2007) and were separated using a Hewlett-Packard 5890A series II gaschromatograph (Hewlett-Packard, Barcelona, Spain) equipped with a chemically bonded (PEG) supelcowax-10 fused silica wall coated capillary column (30m £ 0·32mm i.d.; SupelcoInc., Bellefonte, PA, USA), using an 'on column' injection system and flame ionization detection. Hydrogen was used as the carrier gas with an oven thermal gradient from an initial 508ºC to 1808ºC at 258ºC/min and then to a final temperature of 23 to 58ºC at 38ºC/ min, with the final temperature maintained for 10 min. Individual fatty acid methyl esters were identified by comparison with known standards and quantified by means of a direct-linked PC and Hewlett-Packard Chem. Station Software.

2.6. Growth evaluation

Growth performance and diets efficiency were assessed by using these equations:

Body gain= [Final body mass-initial body mass].
Specific growth rate (SGR, %/day) =100×(ln final weight-ln initial weight)/time.

Feed conversion ratio (FCR) = (feed given per fish)/ (weight gain per fish).
Protein efficiency ratio (PER)= (weight gain per fish)/ (protein intake per fish).
Net protein Utilization (NPU%) = 100 (Final body protein - initial body protein)/protein intake).
Hepatosomatic index (HSI%) = (liver weight)/ (fish weight) ×100.

2.7. Histological indices

Fish were killed by immersion in anesthetic baths (same procedure as aforementioned) and the cross-section of the intestine was collected. The samples were fixed in Bouin solution at 10% for 24h, after which were transferred to70% ethanol for the preparation of the histological slides. For the preparation of the slides all gonads were cut into 0.5-cm segments, dehydrated in increasing concentrations of alcohol, diaphanized in xylol, and embedded in paraffin, to be sectioned to the

5μ thickness and stained by hematoxylin-eosin (HE) (Genten et al., 2009). The measurements were performed under light microscopy, AX10 Zeiss, Axio Cam MRC camera, with the aid of the ZEN 2012 software.

2.8. Statistical analyses

Statistical analyses were carrying out using SPSS version 23, SPSS Institute, Cary, NC, USA). Fish performance data were tested for treatment effect using one –way analysis of variance (ANOVA). Significant differences (*$P \leq 0.05$*) between means were revealed using Duncan test. The results are expressed as means ± standard error (SE).

3.RESULTS

Water temperature, pH, dissolved oxygen, and salinity were recorded daily, and water ammonia was measured weekly. Water temperature ranged from 26 to 28°C, dissolved oxygen ranged from 6.53 to 7.86 mg/L, salinity ranged from 5.2 to 5.6, water pH ranged from 7.4 to 8 mg/L, total ammonia ranged from 0.523 to 0.741 mg/L, nitrite from 0.041 to 0.056 mg/L and nitrate 0.112 to 0.222 mg/L. Water quality parameters were maintained within the acceptable ranges as recorded by (Bhatnagar et al., 2009).

The results presented in (Tables, 1,2) showed that the all tested diet contain the recommended values from crude protein and essential fatty acids, which cover the requirements of this specie (Hua et al., 2019; NRC, 2011).

Table (2). Fatty acid composition (% total fatty acid) of experimental diets (Mean±S.E. *n=3*).

Fatty acids	**Diets**			
	D0	**D50**	**D75**	**D100**
14:0	4.2±0.2	3.8±0.1	3.4±0.2	3.5±0.3
16:0	10.2±0.1	10.8±0.1	9.6±0.2	10.1±0.1
18:0	2.6±0.3	2.5±0.2	2.1±0.1	2.1±0.2
Σ- saturated	17.0±0.2	17.1±0.1	15.1±0.2	15.7±0.2
16:1n-7	5.2±0.2	5.4±0.1	5.6±0.2	5.0±0.3
18:1n-9	11.6±0.3	11.2±0.1	11.6±0.1	11.1±0.2
Σ-mono-unsaturated	16.8±0.2	16.6±0.1	17.2±0.1	16.1±0.2
18:2n-6	2.4±0.1	2.4±0.4	2.4±0.1	2.5±0.1
20:2n-6	0.5±0.1	0.4±0.1	0.5±0.1	0.5±0.1
20:4n-6	1.4±0.1	1.3±0.1	1.2±0.1	1.4±0.1
Σ-n-6fatty acids	4.3±0.1	4.1±0.1	4.1±0.1	4.4±0.1
18-3-n3	1.6±0.1	1.5±0.1	1.6±0.1	1.6±0.1
18-4n-3	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1
20-5n-3	6.6±0.2	6.5±0.2	6.4±0.2	6.5±0.2
22-5n-3	1.2 ±0.2	1.2 ±0.2	1.2 ±0.2	1.1 ±0.2
22-6n-3	8.5±0.4	8.5±0.4	8.6±0.4	8.5±0.4
Σ-n-3fatty acids	18.4±0.2	18.2±0.2	18.3±0.2	18.2±0.2
n-3:n-6 ratio	4.27±0.1	4.44±0.1	4.46±0.1	4.14±0.1

As can be seen in (Table, 3) final weight and weight gain did not significantly differ among all the groups D100, D75, D25 and D0. In the same line, no significance differences in the other parameters of SGR, FCR, CF, PER and NPU as presented in the same table.

Photomicrographs of the intestinal villus from the experimental groups not showed any changes in mucosal layer, immune cells and lymphatic vessel between tested groups as illustrated in (Plate, 1).

The carcass analysis of *L. ramada* fed different experimental diets not significantly different between them as presented in (Table, 4). There was no significant variation (*P<0.05*) in the muscle content of fish from varied fatty acid diets (Table, 5). While Σ-mono-unsaturated, Σ-n-6 fatty acids, and the n-3: n-6 ratio in muscles fish fed D100 were completely similar with D0, Σ-n-3 fatty acids recorded the greatest average with D100. In the same concern, statistical analysis didn't reveal any significant differences among the tested diets.

Table (3). Growth performance mean values (Mean± S.E *n=3*) of *L. ramada* fed on different experimental diets for 120 days.

Parameters	Diets			
	D0	D50%	D75%	D100%
Initial weight (g /fish)	3.8±0.12	3.9±0.10	3.9±0.10	3.8±0.08
Final Weight (g /fish)	38.2 ±1.4	38.6±1.2	40.4±1.5	41.3±1.6
Weight gain (g /fish)	34.4±1.2	34.7±1.2	36.5±1.4	37.5±1.2
SGR %/day	1.54±0.2	1.56±0.1	1.58 ±0.2	1.62±0.2
FCR	1.65±0.1	1.66 ±0.2	1.62 ±0.2	1.65±0.1
CF (g/cm-3)	1.55±0.2	1.58±0.1	1.61±0.2	1.63±0.2
PER	1.43 ±0.1	1.54 ±0.1	1.56 ±0.2	1.61±0.1
NPU (%)	24.5±1.2	24.8±1.1	25.1±1.2	25.4±1.1
HSI (%)	1.56±0.2	1.54±0.1	1.64±0.2	1.67±0.1

Table (4). Carcass analysis (%w/w basis) of *L. ramada* fed on the experimental diets, (Mean± S.E, *n=3*)

Items		Diets			
	Initial	D0	D50	D75	D100
Dry matter	26.30±0.20	28.90±0.30	27.80±0.2	28.90±0.1	28.10±0.2
Protein	17.60±0.20	17.60±0.10	17.80±0.1	17.50±0.2	17.50±0.3
Lipid	5.40±0.10	7.80±0.20	7.60±0.3	7.40±0.3	7.60±0.1
Ash	3.20±0.20	3.70±0.10	3.60±0.1	3.80±0.1	3.60±0.2

Table (5). Muscle fatty acid composition (% total fatty acid) of *L. ramada* fed different experimental diets (Mean± S.E., *n=3*).

Fatty acids	Diets			
	D0	D50	D75	D100
14:0	7.5±0.1	7.4±0.1	7.6±0.2	7.8±0.3
16:0	3.6±0.2	3.8±0.1	3.6±0.2	3.4±0.1
18:0	3.4±0.2	3.5±0.2	3.1±0.1	3.3±0.2
Σ- saturated	14.5±0.2	14.7±0.1	14.3±0.2	14.5±0.2
16:1n-7	9.6±0.1	9.4±0.1	9.3±0.2	9.5±0.3
18:1n-9	11.2±0.3	11.1±0.1	11.2±0.1	11.2±0.2
Σ-mono-unsaturated	20.8±0.2	20.5±0.1	20.5±0.1	20.7±0.2
18:2n-6	2.5±0.2	2.4±0.4	2.5±0.1	2.6±0.1
20:4n-6	3.4±0.1	3.3±0.1	3.4±0.1	3.5±0.1
Σ-n-6fatty acids	5.9±0.1	5.7±0.1	5.9±0.1	6.1±0.1
18-3-n3	1.4±0.1	1.5±0.1	1.4±0.1	1.6±0.1
18-4n-3	2.4±0.2	2.5±0.1	2.7±0.1	2.4±0.1
20-5n-3	7.1±0.3	7.3±0.2	7.1±0.2	7.4±0.2
22-5n-3	3.0 ±0.2	3.2 ±0.2	3.2 ±0.2	3.1 ±0.2
22-6n-3	4.2±0.2	4.4±0.4	4.3±0.4	4.1±0.4
Σ-n-3fatty acids	18.1±0.1	18.9±0.2	18.7±0.2	18.6±0.2
n-3:n-6 ratio	3.06±0.2	3.31±0.1	3.17±0.1	3.05±0.1

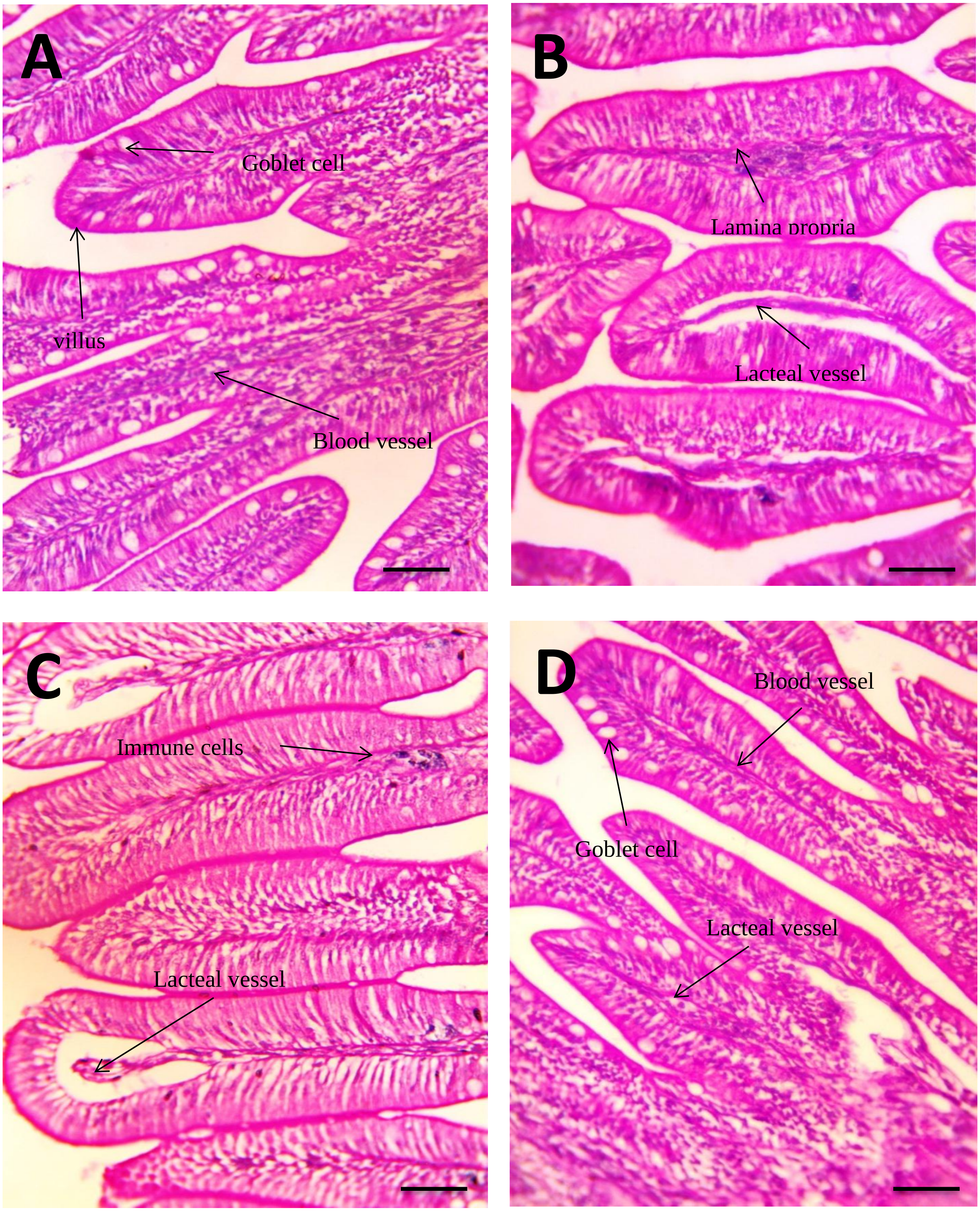
A
Goblet cell
villus
Blood vessel
B
Lamina propria
Lacteal vessel
C
Immune cells
Lacteal vessel
D
Blood vessel
Goblet cell
Lacteal vessel

Plate 1: Photomicrographs of the intestinal villus from the experimental groups stained with hematoxylin and eosin (H&E, X 400). (A: D0) control showing: normal organization of mucosal layer, also Goblet cells with their clear mucous droplets are interspersed between these enterocytes. The lamina propria supports the epithelial cells and makes up the core of the villus. Present in this layer are blood vessels, immune cells, and a lymphatic vessel, or lacteal were observed; (B: D50) showing: normal organization of mucosal layer, also atrophy and shrinkage the lamina propria, and disappearance of goblet cells were recorded, present in this layer are blood vessels, immune cells, and a lymphatic vessel, or lacteal were observed ;(C: D75) showing: normal organization of mucosal layer, also atrophy and shrinkage the lamina propria, and disappearance of goblet cells were recorded, present in this layer are blood vessels, immune cells, and a lymphatic vessel, or lacteal were observed; (D: D100) showing: normal organization of mucosal layer, also atrophy and shrinkage the lamina propria, and disappearance of goblet cells were recorded, present in this layer are blood vessels, immune cells, and a lymphatic vessel, or lacteal were observed. Scale bar = 500µm.

4. DISCUSSION

In aquaculture, feed accounts for about 60% of overall expenses. Fish meal is a major protein component in the diet of fish. It has non-anti-nutritional components, high protein content, well-balanced essential amino acid profile, and good nutritional digestibility. However, it is the primary cause of feed price increases brought on by high market prices and strong demand (Hua *et al.,* 2019). Efforts by fish nutritionists and feed producers to prepare feeds at a lower cost have had a direct impact on the economics of fish farms. By using less expensive plant protein sources to partially or completely replace fishmeal, many studies have been conducted in the past decades (Yones *et al.,* 2016a; Mambrini *et al.,* 1999).

At present, the challenge is not limited to facing the high prices and unavailability of fish meal. Another challenge facing aquafeeds is providing traditional raw materials for plant sources of carbohydrates and proteins, such as raspberries and soybeans. Hence, scientific research in recent years has tended to find alternatives such as soy bean (Abdel-Aziz *et al.,* 2019), azolla meal (Ahmed *et al.,* 2023), and seaweed (Abdel-Aziz *et al.,* 2017).

Our study investigated the potential use of silverside as a substitute for fish meal and sorghum as a substitute for yellow corn. One of these promising ingredients is sorghum grain, which is produced widely throughout the world and has appropriate nutritional value (Lemlioglu-Austin *et al.,* 2014). But when utilized as an aquafeed, sorghum has benefits and drawbacks just like any other food. According to several studies, cooked sorghum protein is less digestible than that of other grains like corn (Duodu *et al.,* 2002). Where ~~in~~ reduced digestibility is primarily associated with the formation of disulfide bonds (Damodaran, 2008). Protein digestion may be hampered by this interaction between proteins and non-starch polysaccharides, which may result in indigestible complexes or decreased accessibility to enzymes (Damodaran, 2008). The size, shape, and range of sorghum starch granules are comparable to those of corn (Wall and Blessin, 1969). Besides, the rate at which sorghum's starch and sugar are released is slower than that of other cereals (Lodge *et al.,* 1997).

Interestingly, the growth performance in our study revealed that the use of 100% ~~from~~ sorghum and silverside meal in *L. ramada* recorded the high performance. Despite limited studies are available regarding the application of sorghum as an aquafeed ingredient, there are several possibilities to justify this finding, firstly, the capacity of the digestive tract for protein or carbohydrate digestion and absorption appeared to be utilized to a greater extent when fed the mixed proteins or starch sources or than when fed only a single source. Hence, starch digestibility also varies depending on the combination of starch sources in the diet (Hamaker *et al.,* 1986).

Secondly, Sorghum varieties are further classified into α-, β-, γ-, and δ-kafirin. α-Kafirin is the main protein store in sorghum (80–84%) (Watterson *et al.,* 1970). Increasing the α-kafirin ratio can improve the protein digestibility (Dowling *et al.,* 2002). This protein is rich in nonpolar amino acids and is found primarily as monomers and oligomers. Sorghum contains high levels of minerals, ranging from less than 1% for some forms of iron to greater than 90% for sodium and potassium. In silver Catfish (*Rhamdia quelen*), the inclusion of sorghum in diet led to increased FCR, likely linked to its adverse effects on nutrient absorption in the fish. The predominant protein in sorghum is kafrin, which exhibits a rigid structural configuration within the starch protein matrix, low solubility, and an imbalanced amino acid profile, contributing to reduced food digestibility (Rodrigues *et al.,* 2020).

Furthermore, sorghum is a rich source of B-complex vitamins, contains fat-soluble vitamins, namely D, E, and K, and is not a rich source of vitamin C. The concentrations of thiamin, riboflavin, and niacin in sorghum are high (Bean *et al.,* 2016).

Thirdly, the chemical composition of sorghum diets showed low concentrations of tannins and then there are agreeing with (Aderolu *et al.*, 2009) who reported that low-tannin sorghum can fully replace corn in diets for silver catfish *C. gariepinus* without affecting the animal's development. Fourthly, fatty acids profile in the D50, D75, and D100 compared to D0 did not significantly vary especially in Σ-n-6fatty acids, Σ-n-3fatty acids, and n-3: n-6 ratio. In the same context, sorghum lipids are valuable nutrients that influence the taste and storage time of sorghum meals; they consist primarily of unsaturated fatty acids, with polyunsaturated fatty acids being the most abundant (Xiong *et al.,* 2019). The major lipid class in sorghum seeds is triacylglycerols (accounting for approximately 90% of total lipids), with linoleic acid being the predominant fatty acid. Generally, sorghum oil is similar to corn oil, and due to its higher content of essential fatty acids, sorghum has a high potential to be used as another grain in human and animal nutrition (Osman *et al.,* 2000).

Fifthly, feeding behavior of the fish species and morphological digestive system tract are very important factors in benefiting from feedstuff that contain a high level of non-polysaccharides. Wherein omnivorous fish such as carp and tilapia can digest and metabolize sorghum-based diets. In silver catfish, the sorghum dry matter and protein digestibility are higher than corn (Signor *et al.,* 2016), suggesting that sorghum is a favorable ingredient in catfish feed. Other work confirmed herbivorous and omnivorous species can consume sorghum as the main feed ingredient without negative impacts on growth and digestibility. Beneficial microorganisms in guts of herbivorous and omnivorous species showed a vital role for utilizing the contents of sorghum from protein and carbohydrate through the disruption in intestinal microbiota induced by feeding habit via diet usually affects digestive host functions through disturbance in bacterial digestive enzyme production (Ghanbari *et al.,* 2015).

The higher amylase activity in the herbivorous fish studied is related to carbohydrate utilization of *Ctenopharyngodon idella* in the diet. Similarly, omnivorous fish are capable of using higher carbohydrate levels than carnivorous fish (Li *et al.*, 2015).

Mullet primarily forage on detritus and microalgae that are undergoing microbial decomposition (Galvão *et al.,* 1997). However, it is unknown if the mullet is able to use any fibrous fraction of the detritus or if ingesting this material is a strategy for consuming the microbial matter that decomposes it.

Histological analysis of the mullet stomach demonstrated that the pyloric region – with deep folds, highly developed muscles, and no digestive glands – has the primary function of grinding food, comparable to the gizzard in birds. The absence of secretory glands for both enzymes and hydrochloric acid in the pyloric region creates a proper environment for microbial colonization, which could explain the high bacterial density in the stomachs of these animals. Thus, it is very likely that *L. ramada* utilizes the bacterial biomass produced after the decomposition of consumed food. If this is the case, mullet fish should have a ruminant-like feeding behavior, with the incorporation of bacteria into its biomass (Galvão *et al.,* 1997). Overall, this finding agrees with the previous results of Toutou *et al.* (2020) with using different carbohydrate sources in *L. ramada* diets. In the same manner Yones *et al.* (2016b) recorded enhancement in growth performance in red tilapia *O. mossambicus* × *O. niloticus* fed sorghum meal. Also, the growth performance in pangasius is not affected when cassava and corn are replaced with sorghum (US *et al.*, 2017).

On the other side, the incorporation of silverside fish improves diets palatability, as previously suggested with sole (Yones *et al.*, 2014) and other fish species (Cabral *et al.*, 2011; Espe *et al.*, 2006; Zhang *et al.*, 2012) fed on plant protein-based diet supplemented with palatable material such as squid meal and protein fish hydrolyses meal. Similarity, a study of (Gumus, 2011) showed that no significant in growth performance between the use of sand smelt meal (SSM) at a rate of 100% and the control this is due to sand smelt in Egyptian water containing essential amino acid proportions with convergent Herring fish meal where he excels in the proportions of certain acids such as (Arginine, Leucine, Tyrosine, Valine and Tryptophan) and SSM has a favorable effect on the lipid composition in the muscle of fish. Increase in n-6 levels, the other is the constant n-3: n-6 ratio. Both are thought as important factors that affected the nutritional quality of fish muscle. Further research should be conducted on usage of SSM as a substitute of FM to determine acceptable fatty acid composition for large pond fish. Moreover, Gumus (2011) stated that sand smelt meal (SSM), among other sources of animal origin, is a rich protein source. He also reported adequate growth increase in the Nile tilapia fry, fed with diets replaced by SSM for FM. Similarly, when used as replacement of FM as protein source has supplied for mirror carp fry. It is unknown how replacement rates of SSM would affect the fatty acid composition of fish muscle, and consequently their fat quality.

Additionally, progressively higher SSM protein percentages also resulted in higher PUFA levels in the diet and the fish. This was due to an increasingly higher total content of n-6 fatty acids of D50, D75 and D100 that included different proportion of SSM compared D0. Hence, these observations were in agreement with findings that were showed in Table (4). As it's known that, marine fish generally cannot transform 18C fatty acids into HUFA so they must obtain these nutrients from the diet as it covered in the diets and correct n3/n6 ratios may be taken under consideration (Table, 4). However, it has been described how high availabilities of HUFA precursors can increase elongation and desaturation processes in some fish species (Ling *et al*., 2006; Jaya-Ram *et al*., 2008).

In addition, it has been defined a certain capacity of HUFA biosynthesis for mullets (Garrido *et al.*, 2019; Galindo *et al.*, 2021), including fatty acyl desaturase 2 with n-6 activity in *L. aurata* (Quiros-Pozo *et al*., 2023). Also, as described for *L. aurata* juveniles, this finding was agreed with *Liza ramada* in the present study. The histopathological evaluation demonstrated normal construction of the intestine's segments in the control and the groups fed different diets as shown in (Fig. A, B, C and D). Sorghum inclusion levels not affected 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 as well as an abundant number of goblet cells. The present results were in agreement with the previous results in *Liza ramada* fed laminarin supplement (Abdel Mawla *et al.,* 2023). In the same line, incorporation of sorghum in silver catfish didn't effect on histomorphometry indices (Rodrigues *et al.,* 2020) but polysaccharides-rich additives enhanced the intestinal morphological features in hybrid red tilapia (Abdelrahman *et al*., 2022).

On the other hand, the inclusion of 50 % VMO (Veramaris®algal oil) in Atlantic salmon had improved intestinal structures.

Regarding, carcass chemical composition, total proximate composition data are important for nutritionists and food scientists to help them in dietary formulations, nutrient labeling, processing and fish quality. Usually, the measurements of the carcass composition refer to the level of nutrient metabolism and accumulation in the entire fish body. No marked effects were observed on the carcass composition contents of fish fed different diets. Similar results were recorded in mullet species (Abdel Mawla *et al.*, 2023; Abdelrahman *et al.*, 2022) in same trend, Yones *et al.*, (2016b) the fish fed on the sorghum-based diet was not statistically significant differences ($P > 0.05$) comparing to the other fish fed on *Zea maize*. This observation agrees with the reported results of (De *et al.*, 2012). In the same trend, the muscle body contents from fatty acids refer to will utilization by 100% inclusion levels from sorghum and silverside meal. The present results were agreeing with the results of (Mourente and Tocher, 1993). Additionally, as previously observed by Yones *et al.* (2014) the dietary on silverside meal (SM) in the present study didn't significantly effect on the proximate body composition. However, this mechanism varies among fish species and can be related to the diet composition and feeding behavior (De Carvalho *et al.*, 2010).

CONCLUSION

The present study confirmed that the inclusion 100% of silverside and sorghum meals in mullet diets led to increased growth performance of *Liza ramada*. Also, the carcass composition and muscle fatty acids contents of fish not affected with the inclusion level of these meals. The histopathological evaluation demonstrated normal construction of the intestine's segments in the control and the groups fed different diets. From the economic point of view, it can be recommended that dietary silverside and sorghum meals as local and less expensive ingredients can spare in diets costs of mullet fish.

Data availability

Data available on request from the author

Competing interests

Authors declare no competing interests.

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