



Research Article

Effects of Soybean Meal on New Muscle Generation and Enzyme Activities in Nile tilapia, *Oreochromis niloticus*

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ARTICLE INFO	ABSTRACT
Article history Received: 22 June 2026 Accepted: 12 July 2026 Published: 30 June 2026 Keywords Fishmeal, Soybean meal, Hyperplasia, Creatine kinase, Alkaline phosphate, Aspartate aminotransferase, Alanine aminotransferase, Correspondence Md. Shafaet Hossen ✉: shafaet.fbg@bau.edu.bd OPEN ACCESS	The use of fishmeal (FM) substitute ingredients in aquafeeds has lately gained attention due to the rising cost of FM associated with the rapid expansion of aquaculture. This study evaluated the effects of replacing FM with soybean meal (SBM) on new muscle generation and enzymatic activities of Nile tilapia, <i>Oreochromis niloticus</i>. Five experimental diets were formulated by replacing fishmeal with SBM at 0% (SBM0), 25% (SBM25), 50% (SBM50), 75% (SBM75), and 100% (SBM100). Nile tilapia (initial weight: 6.60 ± 0.13 g and length: 5.42 ± 0.17 cm) were randomly assigned to five treatment groups (each having three replications) and fed to apparent satiation for 180 days. Muscle histology was assessed by determining muscle fiber density (mm^{-2}) and diameter (μm). Additionally, creatine kinase (CK), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities were measured from the dorsal muscle tissues. Fish fed the SBM0 and SBM25 diets exhibited significantly higher muscle fiber density and smaller muscle fiber diameter than those fed the SBM100 diet ($p < 0.05$). Whereas muscle fiber diameter increased progressively with increasing dietary SBM inclusion. Histological observations confirmed that lower SBM inclusion maintained numerous small muscle fibers characteristic of hyperplastic growth. Higher SBM inclusion (SBM75 and SBM100) resulted in fewer but larger muscle fibers, indicating a shift towards hypertrophic growth. CK and ALP activities were not significantly affected by dietary treatments ($p > 0.05$). However, AST and ALT activities were significantly ($p > 0.05$) reduced in fish fed the SBM75 and SBM100 diets, suggesting altered amino acid metabolism at higher SBM inclusion levels. These findings indicate that replacing fishmeal with SBM up to 50% maintains normal muscle development and metabolic functions, whereas higher replacement levels ($\geq 75\%$) adversely affect muscle growth characteristics and metabolism. Therefore, partial replacement of fishmeal with soybean meal represents a suitable dietary strategy for Nile tilapia while supporting the sustainable use of alternative protein sources in aquaculture.
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Introduction

Fishmeal (FM) has long been regarded as the preferred protein source in aquafeeds due to its balanced amino acid profile, high digestibility, and palatability (Hardy, 2010; Hua et al., 2019). However, FM is one of the most expensive ingredients used in feed industry (Rana et al., 2009; Hua et al., 2019). Furthermore, the increasing demand for fishmeal from the aquaculture, livestock, and pet food industries, together with constraints on global supply has contributed to increasing prices and concerns over its long-term availability (Naylor et al., 2009; Rana et al., 2009; Hua et al., 2019; Hussain et al., 2024). Consequently, reducing the reliance on FM using sustainable and cost-effective alternative protein sources has become a major focus in aquaculture

nutrition research (Hossain et al., 2017a; 2017b; Hua et al., 2019; Hussain et al., 2024). Bangladesh has a wide variety of plant proteins that can potentially serve as an effective replacement for FM (Pervin et al., 2020a; 2020b; Jahan et al., 2021).

Several studies have been conducted to explore alternative protein sources derived from plants, with a particular focus on grains, pulses, and oilseeds (Moniruzzaman et al., 2018; 2020). Soybean meal (SBM) has drawn a lot of interest as a viable substitute protein source for FM as its affordable price, easy accessibility, and well-balanced amino acid profile (Gatlin et al., 2007). Soybean meal has high protein concentration,

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low fiber content, and superior vitamin and mineral content make it a priceworthy source of protein for monogastric animals (Sarkar et al., 2014). Different SBM products, from full-fat and defatted SBM to de-hulled SBM, extruded SBM, soy protein concentrate, and soy protein isolate are used in place of FM. There have also been differences in the substitution amounts depending on species. For instance, 20-75% of FM can be substituted with soybean products without compromising the growth performance of black sea bream, *Acanthopagrus schlegelii* (Zhou et al., 2011). Similarly, a replacement level of 25% has been noted in European seabass, *Dicentrarchus labrax* (Tibaldi et al., 2006), 30% in Japanese seabass, *Lateolabrax japonicus* (Li et al., 2012), and 33% in Atlantic salmon, *Salmo salar* (Carter and Hauler, 2000). Substantial replacement, such as 50% has been done in Japanese sea bass, *Lateolabrax japonicus* (Zhang et al., 2018), 60% in summer flounder, *Paralichthys dentatus* (Ward et al., 2016), and 75% in Senegalese sole, *Solea senegalensis* (Cabral et al., 2013). Therefore, the present study evaluated the suitability of SBM as an alternative protein source in diets for Nile tilapia, *Oreochromis niloticus*.

Nile tilapia is one of the most widely cultured freshwater fish species because of its rapid growth, tolerance to a wide range of environmental conditions, and ease of culture (Yan and Wang, 2010; El-Sayed, 2019). It is also widely used as a model species in fish nutrition research because of its ability to efficiently utilize a wide range of feed ingredients, including plant-based diets (El-Sayed, 2019). As an omnivorous species, Nile tilapia readily consumes algae and other plant-based feedstuffs, making it a suitable model for evaluating alternative plant protein source such as soybean meal (Figueredo and Giani, 2005; El-Sayed, 2019).

Muscle growth is a major determinant of fish growth and flesh yield which occurs through two complementary processes: hyperplasia, the formation of new muscle fibers, and hypertrophy, the enlargement of existing muscle fibers (Johnston et al., 2011). Unlike mammals, many fish species continue to recruit new muscle fibers throughout juvenile growth and, in some species, into adulthood (Rowlerson and Veggetti, 2001; Johnston et al., 2011). All fish species continue to recruit new skeletal muscle fibers during their larval stages, and some species that attain high adult body sizes continue to do so during their juvenile and adult stages as well (Rowlerson and Veggetti, 2001). Fish given a concentrated diet of rice protein showed a higher frequency of muscle fibers in the 20-50 μm class but a decreased frequency in the $> 50 \mu\text{m}$ class (Cai et al., 2018). Therefore, evaluating muscle fiber

generation provides valuable insight into the effects of dietary ingredients on fish growth beyond conventional growth performance measurements.

Muscle growth is supported by continuous metabolic activity and adequate cellular energy supply. Creatine kinase (CK) plays a central role in maintaining adenosine triphosphate (ATP) homeostasis by catalyzing the reversible transfer of phosphate groups between creatine and adenine nucleotides, thereby meeting the high and fluctuating energy demands of muscle tissues (Saks et al., 1996; Wallimann et al., 1998). All vertebrates have the enzyme CK that helps in mediating a reversible interaction between creatine and ATP to produce phosphocreatine and adenosine diphosphate (ADP). This vital enzyme plays a crucial role in balancing cellular energy, especially in cells with intermittent high-energy demands (Wallimann et al., 2011). Creatine kinase levels also indicate myocardial, hepatic, and skeletal muscle issues and helps in diagnosing acute myocardial infarction (McLeish and Kenyon, 2005). Although phosphocreatine has been the subject of numerous studies, CK has not received as much attention. In our previous study, we reported the beneficial effects of SBM in partial replacement of FM in terms of growth performances in Nile tilapia (Pervin et al., 2020a; 2020b). In the current study, we aim to elucidate the effects of SBM in FM replacement on fish muscle generation and assessing the CK activity.

Materials and methods

Ethical statement

The experiment was conducted at the Department of Fisheries Biology and Genetics of Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh in accordance with European Union guidelines for handling of laboratory animals (2010/63/EU). The current study was approved by the Animal Welfare and Experimental Ethics Committee of BAU under the accession number AWEEC/BAU/2020.

Experiential diets

A control diet containing 40% fishmeal, here indicated as SBM0 was formulated, and four experimental diets were prepared by replacing 25%, 50%, 75%, and 100% of the FM with SBM. The experimental diets were designated as SBM25, SBM50, SBM75, and SBM100, respectively. Cod liver oil and wheat flour were included at constant levels in all diets to maintain similar fat and starch contents. After mixing thoroughly, the diets were formed into small balls and stored at 4 °C for up to two weeks. The ingredient composition of the experimental diets is presented in Table 1. The proximate analysis of the dietary ingredients and the formulated diets was conducted using standard methods provided by the Association of Official Analytical Chemists (AOAC, 2019).

Experimental design

Nile tilapia with an initial mean length 5.42 ± 0.17 cm and mean body weight 6.60 ± 0.13 g were obtained from the Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. The fish were kept in $2.44 \times 1.23 \times 0.46$ m³ cisterns at the Mini Hatchery Cum Breeding Complex of the Faculty of Fisheries, BAU before transferring into the experimental units. A total of 15 aquaria (5 diets with 3 replications) were set for the feeding trial and each unit contained 40 fish. Fish were reared for 180 days and fed twice daily in the morning at 9:00 a.m. and in afternoon at 5:00 p.m. until satiation. Water quality parameters, such as temperature, pH, and dissolved oxygen were monitored on regular basis. Ten fish from each treatment group were randomly collected every after 30 days for observing muscle growth and biochemical parameters. MS222 as an anesthetic agent was used during the sampling process, which complied with institutional animal care principles.

Observation of new muscle generation

Muscle samples were collected directly from live fish and were fixed in 10% neutral buffered formalin. The fixed samples then went through decalcification process to remove any mineral deposits. After decalcification, the samples were dehydrated using a graded series of ethanol. The samples were then cleared in xylene to remove ethanol. The cleared samples were embedded in paraffin wax, which provides support and stability to tissue during sectioning. An automatic tissue processor

(Thermo Fisher Scientific-Shandon Citadel 1000, Waltham, MT, USA) was used during tissue processing to ensure consistency and efficiency of dehydration, clearing, and embedding processes. Once the tissue processing was completed, the paraffin blocks containing the muscle samples were sectioned using a microtome machine (Leica Jung RM2035, Wetzlar, Germany). Thin transverse slices, approximately 5 μ m were cut out of each block. To visualize the tissue structure, the sections were stained with hematoxylin-eosin, a common staining method in histology. Hematoxylin stains cell nuclei and turn to blue, while eosin stains the cytoplasm and extracellular components which turn into pink. After staining, the sections were mounted with Canada balsam, a resinous mounting medium that helps to preserve the stained tissue and facilitates microscopic examination. The morphometric analysis of muscle samples was conducted using a light microscope (BX51, Olympus, Japan) equipped with a CCD-video camera (Color View Soft Imaging System, Olympus) and an interactive image analysis system (Olympus Cell Family). These tools enable us to capture images of the tissue and to perform measurements and analysis using a specialized software (Olympus Cell Family and interactive Image J software). The total count of white muscle fibers was determined by multiplying the total area of white muscle (mm²) by the number of white muscle fibers per unit area (expressed as fibers mm⁻²). All measurements involving white muscle fibers were conducted using a 10 $\times$ magnification.

Table 1. Composition of ingredients used for the formulation of experimental diets (%).

Ingredients	Diets				
	SBM0	SBM25	SBM50	SBM75	SBM100
Wheat flour	10	10	10	10	10
Wheat bran	15	15	15	15	15
Rice bran	20	20	20	20	20
Maize meal	13.5	13.5	13.5	13.5	13.5
Fishmeal	40	30	20	10	0
Soybean meal	0	10	20	30	40
Vitamin B	0.5	0.5	0.5	0.5	0.5
Cod liver oil	1	1	1	1	1

Note: SBM0, SBM25, SBM50, SBM75, and SBM100 represent diets in which 0%, 25%, 50%, 75%, and 100% of the fishmeal component was replaced with soybean meal, respectively.

Estimation of enzymatic activities

Dorsal muscle tissues were collected from fish in each treatment group and stored at -80 °C until biochemical analysis. 100 mg of tissue sample from each group was homogenized in 0.01 M potassium phosphate buffer containing 1 mM EDTA using a chilled mortar and pestle. The homogenate was centrifuged, and the resulting supernatant was collected for biochemical analyses. The total protein quantity was calculated

using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, MT, USA). The creatine kinase (CK) assay kit (Weldon Biotech India Pvt. Ltd., New Delhi, India) was used to measure the activity of CK. In brief, 10 μ l sample was mixed with 200 μ l of reagent-1 and incubated at 37 °C for 3 minutes. 50 μ l of reagent-2 was then added and mixed into a cuvette and incubated again for 2 minutes at the above-mentioned temperature. The initial absorbance was measured at

340 nm and the timer was set simultaneously. Subsequent absorbance readings were recorded after 1, 2, and 3 minutes. The change in absorbance per minute ($\Delta A/\text{min}$) was then calculated. Creatine kinase activity was calculated by the following formula: $\text{CK (U/L)} = \Delta A/\text{min} \times k$ ($k = 4180$).

The supernatant collected from dorsal muscle tissue homogenate were subsequently used for the determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. All biochemical analyses were performed in triplicate.

Statistical analyses

All data generated in the present study were checked for normality and homogeneity of variance before analysis. Data analyses conducted using SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA). Differences among treatment groups were evaluated using one-way

analysis of variance (ANOVA). When significant differences were detected, Duncan's Multiple Range Test (DMRT) was used to compare treatment means. Data are presented as mean $\pm$ standard error (SE). Differences were considered statistically significant at $p < 0.05$.

Results

The proximate composition of the feed ingredients and experimental diets

The crude protein contents of fishmeal and soybean meal used in this study to prepare the experimental diets were 60.61% and 41.54%, respectively. The crude protein contents of the experimental diets: SBM0, SBM25, SBM50, SBM75, and SBM100 were 20.07%, 19.44%, 19.02%, 16.04%, and 15.05%, respectively. The proximate compositions of the feed ingredients and experimental diets are presented in Tables 2 and 3, respectively.

Table 2. Proximate composition of feed ingredients used in the current study to prepare experimental diets.

Feed ingredients	Percentage (%)					
	Moisture	Crude lipid	Crude protein	Ash	Crude fiber	Carbohydrates
Wheat flour	15.51	2.20	9.22	1.68	0.80	73.59
Rice bran	18.78	10.60	11.98	7.36	7.20	44.08
Wheat bran	14.96	4.80	14.51	2.89	6.90	55.94
Maize meal	14.33	3.20	16.08	7.64	6.70	52.05
Soybean meal	14.80	4.28	41.54	5.54	6.88	26.96
Fishmeal	10.17	8.46	60.61	18.03	1.80	0.93

Table 3. Proximate composition of the formulated diets.

Diets	Percentage (%)					
	Moisture	Crude lipid	Crude protein	Ash	Crude fiber	Carbohydrates
SBM0	48.36	3.20	20.07	3.77	4.20	25.42
SBM25	49.62	3.36	19.44	5.20	4.48	21.30
SBM50	46.33	3.46	19.02	6.89	4.96	19.64
SBM75	44.83	3.88	16.04	8.11	5.20	18.54
SBM100	47.43	3.96	15.05	10.63	5.86	13.55

Note: SBM indicates soybean meal.

Water quality parameters

Throughout the experimental period, the water temperature, pH, and dissolved oxygen were maintained at 26.0 ± 1.5 °C, 7.3 ± 0.41 , and 7.2 ± 0.5 mg/L, respectively.

Observation of new muscle generation in Nile tilapia

Muscle fiber characteristics were significantly altered ($p < 0.05$) by dietary SBM inclusion (Table 4; Figure 1). Fish fed the control (SBM0) and SBM25 diets exhibited the highest muscle fiber density, with 179.33 ± 6.52 and 180.00 ± 8.69 fibers mm^{-2} , respectively.

Whereas, fiber density progressively decreased with increasing SBM replacement, reaching the lowest value in the SBM100 group (134.67 ± 7.40 fibers mm^{-2}) ($p < 0.05$). In contrast, muscle fiber diameter increased with

increasing SBM inclusion, with the largest muscle fibers observed in fish fed the SBM100 diet (60.00 ± 2.82 μm) and the smallest in the SBM25 (36.00 ± 5.88 μm) and SBM0 (40.00 ± 4.63 μm) groups ($p < 0.05$). Fish fed the SBM0 (Figure 1A), SBM25 (Figure 1B), and SBM50 (Figure 1C) diets demonstrated numerous small and closely packed muscle fibers.

However, fish fed the SBM75 (Figure 1D) and SBM100 (Figure 1E) diets exhibited fewer and larger muscle fibers. These observations indicate that low levels of SBM replacement-maintained muscle growth predominantly through hyperplasia (formation of

numerous small muscle fibers), whereas increasing dietary soybean meal promoted hypertrophy (enlargement of existing muscle fibers).

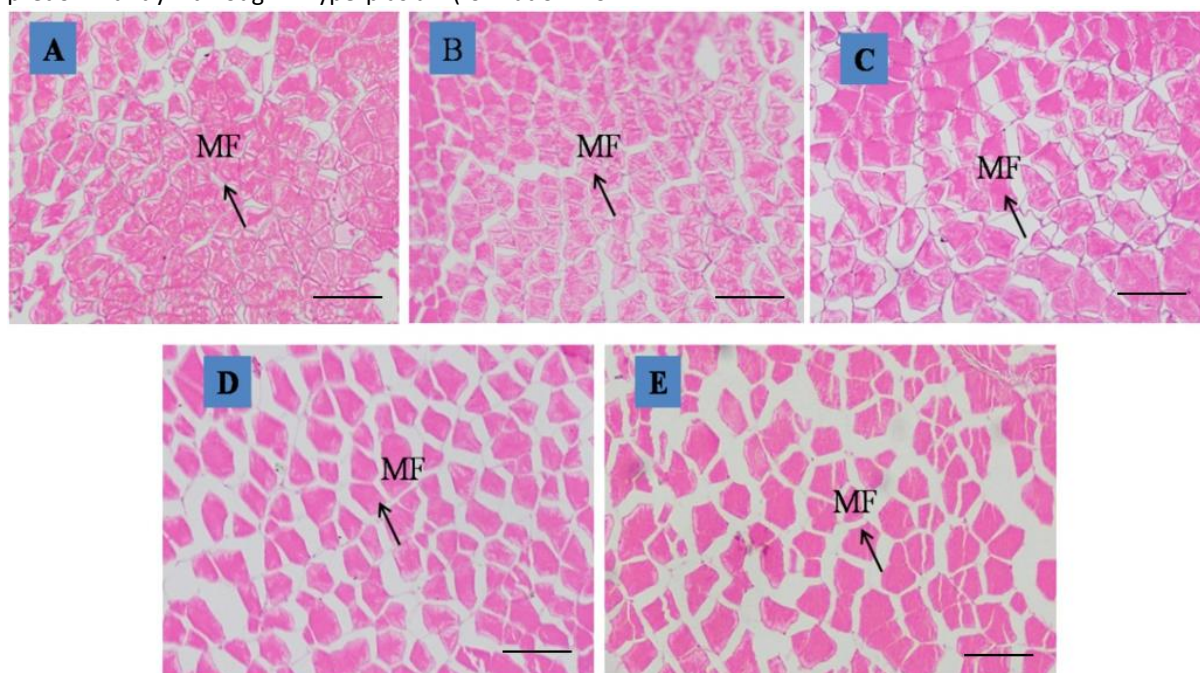


Fig. 1: Cross sectional area of dorsal muscles. A, B, C, D, E indicate cross sectional area of muscles in Nile tilapia fed with SBM0, SBM25, SBM50, SBM75, and SBM100 diets, respectively. MF denotes muscle fiber. Scale bars = 100 μ m.

Table 4. Mean number of muscle fiber/mm² in Nile tilapia fed with the formulated diets for 180 days.

Parameters	Diets				
	SBM0	SBM25	SBM50	SBM75	SBM100
Number of muscle fiber/mm ²	179.33 $\pm$ 6.52 ^a	180 $\pm$ 8.69 ^a	159.33 $\pm$ 4.29 ^b	143 $\pm$ 4.16 ^c	134.67 $\pm$ 7.4 ^c
Diameter of muscle fiber (μ m)	40 $\pm$ 4.63 ^c	36 $\pm$ 5.88 ^c	44 $\pm$ 2.42 ^{bc}	49 $\pm$ 2.09 ^b	60 $\pm$ 2.82 ^a

Note: Values are presented as mean $\pm$ SE of triplicate samples. Values with different superscript letters in the same row are significantly different (DMRT, $p < 0.05$).

Estimation of enzymatic activities

The activities of creatine kinase (CK) and alkaline phosphatase (ALP) were not significantly affected ($p > 0.05$) by increasing dietary SBM inclusion (Figure 2A and 2B). Although, a slight declining trend in CK activity was observed with increasing SBM levels. In contrast, aspartate aminotransferase (AST) activity was significantly influenced by dietary SBM level ($p < 0.05$) (Figure 2C). Fish fed the SBM0, SBM25, and SBM50 diets exhibited significantly higher AST activities than those fed the SBM75 and SBM100 diets, while no

significant differences were observed within either group.

A similar pattern was also observed for alanine aminotransferase (ALT) activity ($p < 0.05$) (Figure 2D). ALT activity was significantly higher ($p < 0.05$) in fish fed the SBM0, SBM25, and SBM50 diets compared with those fed the SBM75 and SBM100 diets. No significant differences were detected among SBM0, SBM25, and SBM50 or between SBM75 and SBM100.

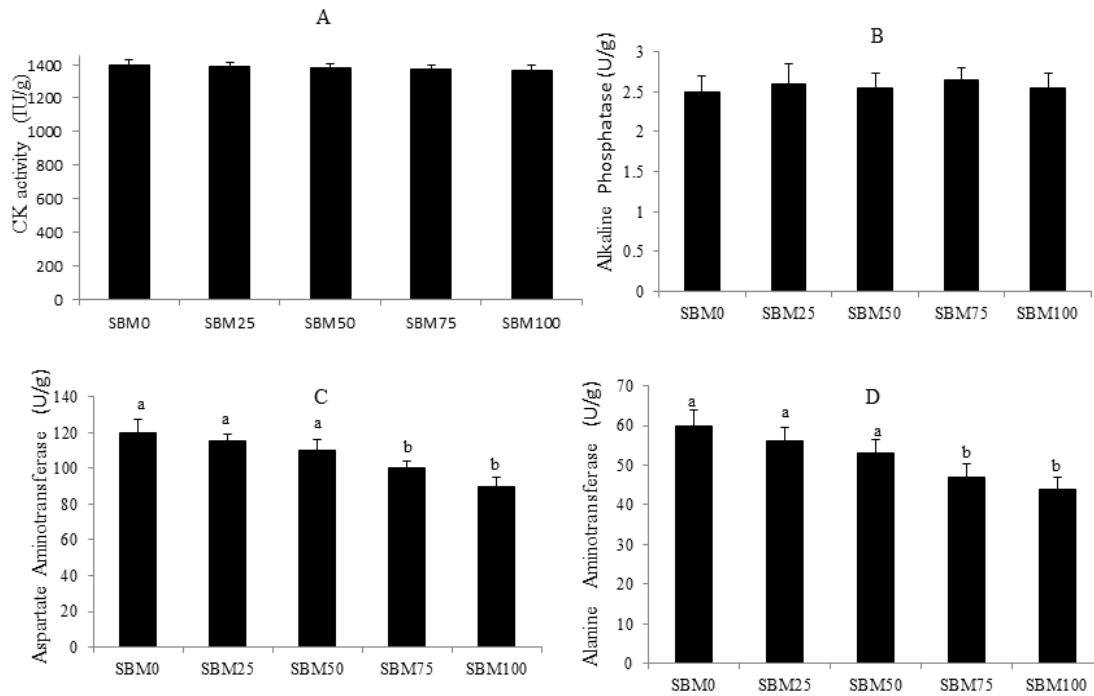


Fig. 2: CK (2A), ALP (2B), AST, (2C) and ALT (2D) activities of Nile tilapia fed SBM0, SBM25, SBM50, SBM75, and SBM100 diets, respectively. Notes: CK=Creatine kinase, ALP=alkaline phosphatase, AST=aspartate aminotransferase, and ALT=alanine aminotransferase.

Discussion

Replacing fishmeal with plant protein sources such as SBM has become an important strategy for improving the sustainability of aquaculture. However, the nutritional quality of alternative protein sources can influence muscle development and growth through differences in nutrient digestibility, amino acid availability, and the presence of antinutritional factors (Francis et al., 2000; Refstie et al., 2006; Hua et al., 2019). In teleost fish, skeletal muscle growth occurs by two complementary mechanisms: hyperplasia, involving the formation of new muscle fibers, and hypertrophy, involving the enlargement of existing fibers (Ennion et al., 1995; Johnston et al., 2011). The relative contribution of these processes influences not only growth performance but also muscle quality and flesh texture (Johnston et al., 2011). In the present study, histological observation showed increasing replacement of FM with SBM shifted muscle growth from hyperplasia towards hypertrophy, as evidenced by reduced muscle fiber density and increased muscle fiber diameter. The higher muscle fiber density and smaller muscle fiber diameter was observed in fish fed the SBM0 and SBM25 diets that indicate active hyperplastic muscle growth. While the reduced fiber density and increased fiber diameter in fish fed the SBM75 and SBM100 diets suggest a greater reliance on hypertrophic growth.

This interpretation is consistent with Cai et al. (2018), who reported that smaller muscle fibers are characteristic of active hyperplastic growth, while larger muscle fibers reflect increased hypertrophic growth during new muscle generation. In the current study, histological observations (Figure 1) further supported above-mentioned findings by showing numerous small and closely packed muscle fibers in fish fed the lower soybean meal diets (up to SBM50). In contrast, fish fed the higher soybean meal (SBM75 and SBM100) diets exhibited fewer and larger muscle fibers. Similar results have been reported by Jahan et al. (2019), who found that silver barb, *Barbonymus gonionotus* fed a diet containing 50% SBM demonstrated significantly higher muscle fiber density and smaller muscle fiber diameter than fish fed 100% SBM diet.

The shift from hyperplastic to hypertrophic muscle generation observed at higher soybean meal inclusion levels may be attributed to the reduced nutritional quality of diets containing high levels of SBM. Compared with FM, SBM contains antinutritional factors and relatively high levels of dietary fiber that can reduce protein digestibility, amino acid availability, and overall nutrient utilization (Francis et al., 2000; Richter et al., 2003; Refstie et al., 2006). As the formation of new muscle fibers through hyperplasia depends on an adequate supply of nutrients and amino acids (Johnston et al., 2011), reduced nutrient availability may restrict hyperplastic growth and increase the contribution of hypertrophy. Therefore, a

moderate replacement of FM with SBM generally maintains normal muscle development, whereas complete replacement often compromises growth performances and new muscle generation (Refstie et al., 1998; Grisdale-Helland et al., 2008; Hua et al., 2019).

Although CK activity was not significantly affected by dietary SBM inclusion in the present study, a declining trend was observed with increasing SBM levels. CK plays a central role in cellular energy homeostasis by catalyzing the reversible transfer of phosphate groups between creatine and ATP, thereby providing a rapid source of energy for tissues with high metabolic demands such as skeletal muscle (Hauser et al., 2000; Wallimann et al., 2011). The absence of significant differences in CK activity despite marked changes in muscle fiber morphology proposes that CK activity was less sensitive to dietary SBM inclusion. Similarly, the unchanged ALP activity found in the study also indicates that alkaline phosphatase activity remained relatively stable despite alterations in muscle growth. In contrast, the significant decrease in ALT and AST activities was observed in fish fed the SBM75 and SBM100 diets. ALT and AST are key enzymes involved in amino acid metabolism and are widely used as indicators of liver metabolic function in fish (Cai et al., 2015; Wang et al., 2017). The reduced activities of these enzymes observed in this study suggest decreased amino acid metabolism, likely resulting from reduced protein digestibility and amino acid availability associated with high soybean meal inclusion (Vagadia et al., 2017). Therefore, the present study indicates that fishmeal can be replaced by soybean meal at levels of up to 50% without adversely affecting muscle generation or protein metabolism in Nile tilapia. However, higher FM replacement levels (75–100%) may compromise muscle generation by altering the pattern of muscle growth and amino acid metabolism. (Jahan et al., 2019; Pervin et al., 2020a, 2020b).

Conclusion

Replacing fishmeal with soybean meal offers promising environmental and financial benefits. However, the substitution should be carefully balanced without causing any damage to the fish's overall performance. The results obtained in the present study suggest that replacing 50% of FM with SBM notably increased the muscle fiber of Nile tilapia, proposing that moderate inclusion of SBM could support muscle generation. However, the proportion increased beyond this level could abate muscle generation significantly. This is likely attributed to the high activity of protease inhibitors in raw and inadequately processed soybean meal. Overall,

our study suggests that Nile tilapia can use diets with SBM sources up to 50% of fish meal replacement, but total substitution of FM is not advisable.

Data availability

Data supporting the results of this study will be provided upon reasonable request to the corresponding author.

Competing interests

There are no commercial or financial relationships between the remaining authors that could be construed as a potential conflict of interest.

Credit authorship contribution statement

Israt Jahan Tumpa: responsible for research investigation, data collection, processing, and manuscript drafting. Mst. Arzu Pervin: responsible for research investigation, data collection, and manuscript drafting. Md. Shafaet Hossen: responsible for research co-supervision, data analysis, and manuscript editing. Maria Binte Moin and Md. Saddam Hossain: involved in research investigation and data curation. Zakir Hossain: involved in experimental design, supervision of the experiment, manuscript finalizing, and provided funding.

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