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Original article

Effects of dietary replacement of fish meal by soybean meal on growth, feed utilization, and health condition of stinging catfish, *Heteropneustes fossilis*

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ABSTRACT

Soybean meal (SBM) is a cost-effective alternative protein source to replace costly fish meal in aquaculture. This present study determined to measure the effects of replacing fish meal (FM) protein with SBM on growth, feed utilization, and health condition of stinging catfish, *Heteropneustes fossilis*. Four isonitrogenous (35 %) diets were applied in four treatment groups designed as SBM_0, SBM_{25}, SBM_{50}, and SBM_{75}, where 0 %, 25 %, 50 %, and 75 % of FM protein were substituted by SBM, respectively. Significantly higher mean final weight (g), weight gain (g), percent weight gain (%), specific growth rate (% day^{-1}), and protein efficiency ratio (PER) were recorded in SBM_0, SBM_{25}, and SBM_{50} groups than SBM_{75} group. Consequently, significantly lower feed conversion ratio (FCR) was found in SBM_0, SBM_{25}, and SBM_{50} groups than SBM_{75} group. Moreover, protein content of whole-body carcass was significantly higher in SBM_{25} and lower in SBM_0 group however, lipid content was significantly higher in SBM_0 and SBM_{75} than in other groups. Hemoglobin, red blood cells, and white blood cells were significantly higher in SBM_0, SBM_{25}, and SBM_{50} groups compared to SBM_{75}. However, the higher the substitution of FM protein by SBM in diets higher the values of glucose. Morphological analysis of the intestine including villi length (μm), width (μm), and area (mm^2); crypt depth (μm); wall thickness (μm); abundance of goblet cell (GB); and muscle thickness (μm) showed an increasing trend in fish fed diet containing upto 50 % replacement of FM protein by SBM. Therefore, the results suggest that SBM could replace upto 50 % FM protein in diets of *H. fossilis* without compromising growth, feed efficiency, and health status.

1. Introduction

Heteropneustes fossilis (stinging catfish), is high-priced and widespread among the air-sac catfishes because of its suitable content of quality protein and iron (Bhatt, 1968; Anon, 1982). It can survive in less oxygen content water therefore suitable for commercial aquaculture (Haniffa and Sridhar, 2002). The production cost is low with high market demand because of its superior quality flesh having high nutritional and therapeutic value (Alam et al., 2009). As well, *H. fossilis* are highly fecund and adaptable to artificial diets at high temperatures and salinity fluctuation (Radhakrishnan and Sugumaran, 2010; Jhingran, 1991; Thakur, 1991). Due to these promising characteristics related to production, *H. fossilis* attains considerable attention for culture in Southeast Asia over the past few years.

Feed cost broadly represents around 70 % of the total operational cost because proteins are the high-priced dietary source of semi-intensive or intensive grow-out farming operations (Hossain et al., 2020a, b). One of the biggest targets of successful aquaculture is to attain highest growth by investing lowest inputs at lowest price. Fish meal (FM), a high-priced feed ingredient, is

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generally considered one of the major sources of protein for aquafeed production because of its higher protein along with stable amino acids, higher digestible energy, and micronutrients (Tacon and Jackson, 1985). The higher prices and irregular supply of FM demand the search for alternatives with lower prices and highly available plant feedstuffs such as soybean, rapeseed meal, moong, guar, sorghum, etc. (Robinson and Li, 2007; Uddin et al., 2007).

Among all plant protein sources, soybean meal (SBM) represents the high protein content, most secured amino acid profile, stable source, and realistic cost (Meng et al., 2020; Pervin et al., 2020). Several studies reported SBM to replace FM in diets for several fish species owing to the source of quality protein of soybean throughout the world (Nyirenda et al., 2000; Kalla et al., 2003). Nonetheless, the effects of replacing FM protein with SBM on the growth and physiological status of fish are species specific along with their feeding mechanisms (Zhou et al., 2018). In general, SBM is better utilized by herbivorous and omnivorous fish than carnivorous fish species. Moreover, the complete or partial substitution of FM with SBM do not affect the growth and physiology of various herbivorous and omnivorous species of fish (Liu et al., 2021). Siddique et al. (2014) suggested that 15 % replacement of FM protein with SBM did not have any significant difference in the growth of *H. fossilis* when compared with 100 % FM-containing diet. Therefore, there had been a possibility of replacing FM protein with SBM in diets of *H. fossilis* fry more than 15 %. Pervin et al. (2020) reported that 75 % substitution of FM by SBM showed no substantial changes in the growth and physiology of *Oreochromis niloticus*. Moreover, Mohammadinafchi et al. (2014) reported no considerable variations in *Mesopotamichthys sharpeyi* when the replacement level was 100 %. However, the effects of the replacement of FM protein with SBM on growth performance and physiology of stinging catfish have not been well-documented. Moreover, due to the presence of lower content of methionine and higher content of anti-nutrients, there is a limitation to using SBM as fish feed (Ollie et al., 1994). Besides, SBM may be a cost-effective and highly available alternative protein source to replace costly fish meal in aquaculture. Therefore, the present study was projected to investigate the effects of replacing FM protein with SBM at different substitution levels on growth, feed efficiency, and health condition of stinging catfish.

2. Materials and methods

2.1. Preparation of diets

Available fresh feed ingredients were used to formulate four isonitrogenous (35 % crude protein) diets where 0 %, 25 %, 50 %, and 75 % of FM protein were substituted by SBM in SBM_0, SBM_{25}, SBM_{50}, and SBM_{75} groups, respectively. The formulation and proximate composition of different diets are presented in Table 1. Dry feedstuffs were first ground with a crusher machine for diet preparation. After sieving ground ingredients, all ingredients were thoroughly mixed and added distilled water at 30 % level (El-Saidy and Gaber, 1997). Pellets were made for all experimental diets with a pellet machine. After that processed pellets (1.0 mm size) were oven dried at 55 °C and refrigerated at 4 °C until further use (Yang et al., 2004).

2.2. Feeding trials

The experimental fish (*H. fossilis*) was gathered from a renowned hatchery in the Jashore division of Bangladesh named "Maa Fatima Hatchery". Six hundred fingerlings of *H. fossilis* (average size 1.55 ± 0.00 g $fish^{-1}$) were stocked in twelve plastic tanks (150 L capacity each) under four groups with three replications (50 fingerlings in each tank) for 14 weeks in a temporary shed set up on the premises of the Faculty of Fisheries, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh. Detailed procedures of tank preparation were followed as per Billah et al. (2022). Before starting the feeding trial, all the fish were fed for one week with a basal diet to adjust to the experimental systems and conditions. During the trial, the feed was given at the rate of 3 % body weight to all fish daily (2 times at 8:00 and 18:00 h) (Yang et al., 2011). Based on the recorded data of the total weight of fish, the ration was regulated for each treatment at fortnightly sampling. Siphoning was performed to remove uneaten feeds daily. Water exchange was completed from tap water every three days intervals. To observe water quality parameters, respective monitoring systems were applied throughout the experimental periods. The observed parameters were within a suitable range for the culture of fish. The recorded values of temperature, dissolved oxygen, pH, ammonia–nitrogen, and nitrite-nitrogen were 26.6–31.6 °C, 4.5–6.1 mg l^{-1}, 6.7–8.2, 0.1–0.5 mg l^{-1}, and 0.02–0.05 mg l^{-1}, respectively.

2.3. Analysis of growth, feed utilization, and survival rate

After 14 weeks, data were recorded for the quantity and total weight of fish in each tank. Individual length (cm) and weight (g) were measured by randomly collecting fifteen (15) fishes. The growth parameters such as weight gain (g), percent weight gain (%), and specific growth rate (% day^{-1}), feed utilization parameters such as feed conversion ratio and protein efficiency ratio, and survival (%) of *H. fossilis* were measured with the noted data are as follows.

- Weight gain (g) = Mean final weight – Mean initial weight
- Percent weight gain (%) = $\frac{(\text{Mean final weight - Mean initial weight})}{\text{Mean initial weight}} \times 100$
- Specific growth rate (% day^{-1}) = $\frac{(\text{Log}_e W_2 - \text{Log}_e W_1)}{(T_2 - T_1)} \times 100$
- Feed conversion ratio (FCR) = $\frac{\text{Feed fed (dry weight)}}{\text{Live weight gain (g)}}$
- Protein efficiency ratio (PER) = $\frac{\text{Live weight gain (g)}}{\text{Crude protein fed (g)}}$
- Survival (%) = $\frac{\text{Total number of fish harvested}}{\text{Total number of fish stocked}} \times 100$

2.4. Proximate composition analysis

The proximate composition of feed ingredients, feed, and whole-body carcass from each treatment was analyzed following methods described by AOAC (2000) with little modifications. Triplicate samples were used to analyze the moisture, protein, lipid, ash, and fibre on percent basis (%). Carbohydrate was estimated by deducting the total percentages of analyzed compositions from 100 (Castell and Tiews, 1980). The values of crude protein, fat, and carbohydrate were used to calculate the calorific value of the feeds (Jauncey and Ross, 1982).

2.5. Hemato-biochemical analysis

The value of hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), and blood glucose (Glu) of stinging catfish were determined as g/dL, $\times 10^6$ /mm^3, $\times 10^3$ /mm^3, and mg/dL, respectively as per the methods described by Billah et al. (2022).

2.6. Histological examination

After 14 weeks, two randomly selected *H. fossilis* were used for each treatment to perform morphological analysis of the intestine as per the procedures followed by Billah et al. (2022). The following morphological parameters as villus length (μm), villus width (μm), villus area (mm^2), crypt depth (μm), thickness of the intesti-

Table 1
Ingredients and nutrient composition of experimental diets fed to *H. fossilis*.

Ingredients (g 100 g^{-1})	Diets			
	SBM_0	SBM_{25}	SBM_{50}	SBM_{75}
[a]Fish meal	46.23	34.68	23.11	11.57
[b]Soybean meal	0.00	17.85	35.68	53.53
Mustard oil cake	15.34	19.72	24.49	28.77
Rice bran	15.17	9.14	7.24	0.60
Wheat bran	18.29	13.63	4.52	0.58
*Premix	1.00	1.00	1.00	1.00
Molasses	4.00	4.00	4.00	4.00
Nutrient composition (%)				
Moisture	12.85	12.82	13.64	14.36
Crude protein	35.57	35.54	35.04	35.23
Crude lipid	7.87	7.34	7.56	7.33
Ash	13.38	13.04	10.42	8.54
Crude fibre	4.20	4.36	5.13	5.34
Nitrogen free extract	26.13	26.90	28.21	29.20
Energy (kcal/g)	3.81	3.80	3.84	3.87

*Premix supplied the following vitamins and minerals (mg or g or IU/2.5 kg of diet): A, 12,500 IU; B_1, 2.5 g; B_2, 5 g; B_6, 4 g; B_{12}, 12 mg; D_3, 2,500,000 IU; E, 20 g; K_3, 4 g; Nicotinic acid, 12.5 g; Folic acid, 800 mg; Biotin, 100 mg; Cobalt, 400 mg; Copper, 10 g; Iron, 60 g; Iodine, 400 mg; Manganese, 60 g; Zinc, 50 g; Selenium, 150 g.
[a] Fish meal (56.46 % crude protein), supplied by Blueline Foods (India) Pvt. ltd.
[b] Soybean meal (36.58 % crude protein), supplied by Fresh, Meghna Group of Industries (Narayanganj, Bangladesh).

nal wall (μm), abundance of goblet cell (GB), and thickness of muscle (μm) were determined for this experiment.

2.7. Statistical analysis

A statistical comparison of data obtained from the proposed study was made with SPSS software. The normality and homogeneity of variances of data were confirmed before any statistical analysis. ANOVA (one-way analysis of variance) was applied to observe significant variations ($P < 0.05$) among diets. Presentation of data was done as mean ± SE (standard error). Moreover, the Tukey test was selected for detecting variations between diets.

3. Results

3.1. Growth performance, feed utilization and survival

After 14-week feeding trials, the performance of growth, utilization of feed, and survival of *H. fossilis* were calculated (Table 2). Significantly ($P < 0.05$) higher mean final weight (g), weight gain (g), percent weight gain (%), specific growth rate (% day^{-1}), and protein efficiency ratio (PER) was recorded in SBM_0, SBM_{25}, and SBM_{50} groups than SBM_{75} group. The FCR values were significantly ($P < 0.05$) lower in SBM_0, SBM_{25}, and SBM_{50} groups compared to SBM_{75} group. Moreover, there were no significant variations ($P > 0.05$) in the survival rate of *H. fossilis* among different feeding groups (Table 2).

3.2. Proximate composition of experimental fish

The proximate composition of the whole-body carcass is presented in Table 3. The significantly higher moisture content (%) was found in SBM_{25} and SBM_{50} groups and it was significantly lower in SBM_0 and SBM_{75} groups ($P < 0.05$). However, a reverse condition was observed in case of lipid content (%). The protein content (%) was significantly higher in SBM_{25} group compared to other groups ($P < 0.05$) and significantly lower protein content ($P < 0.05$) was found in SBM_0 group (Table 3). While significantly higher content of ash (%) was found in the SBM_{50} group and lower in the SBM_{25} group ($P < 0.05$) (Table 3). No significant difference was observed in crude fibre ($P > 0.05$) content (%) of whole-body carcass among different groups (Table 3). Nitrogen-free extract was found significantly higher in SBM_0, SBM_{25}, and SBM_{75} groups and significantly lower in SBM_{50} group ($P < 0.05$).

3.3. Hemato-biochemical study

Hemato-biochemical analysis of stinging catfish for instance hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), and blood glucose (Glu) were calculated for all the fish groups (Table 4). The SBM_0, SBM_{25}, and SBM_{50} groups showed significantly higher content of Hb, RBCs, and WBCs, however, the SBM_{75} group detected significantly lower values of these blood parameters ($P < 0.05$). Moreover, glucose showed significantly higher and lower ($P < 0.05$) values in the SBM_{75} and SBM_0 groups, respectively than in other groups (Table 4).

Table 2
Growth and feed utilization of *H. fossilis* fed different experimental diets (mean ± SE).

Parameters	Diets			
	SBM_0	SBM_{25}	SBM_{50}	SBM_{75}
Initial weight (g)	1.55 ± 0.00	1.55 ± 0.00	1.55 ± 0.00	1.55 ± 0.00
Final weight (g)	8.91 ± 0.64[a]	8.33 ± 0.28[a]	8.28 ± 0.05[a]	6.10 ± 0.25[b]
Weight gain (g)	7.36 ± 0.64[a]	6.78 ± 0.28[a]	6.73 ± 0.05[a]	4.55 ± 0.25[b]
Percent weight gain (%)	475.05 ± 41.63[a]	437.38 ± 18.41[a]	433.91 ± 3.59[a]	293.55 ± 16.13[b]
Specific growth rate (% day^{-1})	1.74 ± 0.07[a]	1.67 ± 0.03[a]	1.66 ± 0.01[a]	1.36 ± 0.04[b]
Feed conversion ratio (FCR)	3.15 ± 0.7[b]	3.29 ± 0.08[b]	3.03 ± 0.07[b]	3.87 ± 0.06[a]
Protein efficiency ratio (PER)	0.77 ± 0.01[a]	0.76 ± 0.02[a]	0.76 ± 0.01[a]	0.58 ± 0.01[b]
Survival (%)	97.63 ± 0.63	94.50 ± 3.50	96.80 ± 0.58	95.61 ± 2.64

The values in the same row that are followed by the different superscript letters are significantly different ($P < 0.05$).

Table 3
Proximate composition of *H. fossilis* fed different experimental diets (mean ± SE).

Proximate composition (%)	Diets			
	SBM_0	SBM_{25}	SBM_{50}	SBM_{75}
Moisture	76.11 ± 0.12^{b}	76.60 ± 0.02^{a}	76.70 ± 0.05^{a}	76.00 ± 0.01^{b}
Crude protein	14.44 ± 0.01^{c}	14.91 ± 0.10^{a}	14.61 ± 0.01^{bc}	14.81 ± 0.02^{ab}
Crude lipid	2.13 ± 0.02^{a}	1.98 ± 0.03^{b}	1.88 ± 0.01^{b}	2.15 ± 0.02^{a}
Ash	2.69 ± 0.06^{b}	2.23 ± 0.02^{d}	3.15 ± 0.01^{a}	2.47 ± 0.03^{c}
Crude fibre	0.90 ± 0.20	1.12 ± 0.01	1.13 ± 0.01	0.96 ± 0.17
Nitrogen free extract	3.73 ± 0.06^{a}	3.16 ± 0.19^{a}	2.53 ± 0.01^{b}	3.61 ± 0.02^{a}

The values in the same row that are followed by the different superscript letters are significantly different ($P < 0.05$).

Table 4
Hemato-biochemical parameters of *H. fossilis* fed different experimental diets (mean ± SE).

Blood parameters	Diets			
	SBM_0	SBM_{25}	SBM_{50}	SBM_{75}
Hb (g/dL)	12.33 ± 0.03^{a}	12.25 ± 0.01^{a}	12.23 ± 0.03^{a}	10.38 ± 0.03^{b}
RBC ($\times 10^6$ / mm^3)	1.33 ± 0.02^{a}	1.31 ± 0.04^{a}	1.32 ± 0.03^{a}	1.09 ± 0.01^{b}
WBC ($\times 10^3$ / mm^3)	1.60 ± 0.02^{a}	1.59 ± 0.07^{a}	1.62 ± 0.01^{a}	1.25 ± 0.02^{b}
Glucose (mg/ dL)	52.22 ± 0.04^{d}	54.00 ± 0.04^{c}	57.5 ± 0.07^{b}	72.00 ± 0.03^{a}

The values in the same row that are followed by the different superscript letters are significantly different ($P < 0.05$).

3.4. Histological study

The intestinal histo-morphological measurements of *H. fossilis* such as length (μm), width (μm), and area of the villi (mm^2), crypt depth (μm), thickness of wall (μm), abundance of goblet cell (GB), and thickness of muscle (μm) are presented in Table 5 and Fig. 1. The values of these parameters varied significantly ($P < 0.05$) among the SBM_0, SBM_{25}, SBM_{50}, and SBM_{75} groups. The increasing trend of histological parameters was observed with increasing dietary soybean meal and it continued upto 50 % replacement level and then declined to 75 %. The examined values of histo-morphological parameters were significantly ($P < 0.05$) higher in the SBM_{50} group. An importantly similar tendency was detected also in the case of the number of goblet cells (immune response variable).

4. Discussion

The growth performance of stinging catfish, *H. fossilis* was significantly higher in SBM_0, SBM_{25}, and SBM_{50} groups compared to SBM_{75}. Moreover, efficient feed utilization was also found in SBM_0, SBM_{25}, and SBM_{50} groups than SBM_{75} group. This means that replacement of FM protein with SBM upto 50 % did not show any significant difference in growth and feed utilization. Liu et al. (2021) reported that SBM can replace upto 50 % of fish meal in juvenile *Liza haematocheila* based on weight gain and feed efficiency. Wang et al. (2015) reported substitution of FM protein with SBM upto 40 % in the diet of *Pseudobagras ussuriensis* had no negative effects on growth, while best feed utilization was obtained upto 50 % substitution level. Conversely, in this present study, upto 50 % replacement level showed better growth performance and this replacement level was somewhat lower than other studies for instance blue catfish (Webster et al., 1995) and rainbow trout (Yang et al., 2011), where 60 % of FM protein can be substituted with SBM without compromising growth. These differences vary from species to species, size, types of ingredients and process of formulation, inclusion and value of SBM, and different culture systems (Wang et al., 2015). Shukla et al. (2018) suggested that 100 % substitution of FM protein with SBM improved growth performance in *H. fossilis* when the protein content of the FM (23.76 %) was less than SBM (37.83 %), which means that the deteriorative quality of the fish meal. In this present study, the protein content of FM was higher (56.46 %) than SBM (36.58 %).

At large, FM can be partly substituted by SBM without hampering development in some aquatic animals. In this study, 75 % replacement of FM protein with SBM decreased the growth of stinging catfish. Liu et al. (2021) showed that SBM above 75 % in the diet of *Liza haematocheila* reduced the growth and feed efficiency. Similar reports were also suggested by Yang et al. (2011), where 80 % dietary inclusion level of SBM significantly reduced the growth of *Oncorhynchus mykiss*. Diet containing SBM protein reduce growth and feed efficiency because of the existence of anti-nutrients, bitter taste, lower digestibility of protein, scarcity of essential amino acid (Francis et al., 2001; Wang et al., 2006; Yang et al., 2011). In the present study, the reduced growth rate of stinging catfish found from

Table 5
Changes in intestinal morphology of *H. fossilis* fed different experimental diets (mean ± SE).

Parameters	Diets			
	SBM_0	SBM_{25}	SBM_{50}	SBM_{75}
Villus length (μm)	97.0 ± 6.24^{b}	101.02 ± 4.06^{b}	147.30 ± 6.40^{a}	91.55 ± 3.10^{b}
Villus width (μm)	62.67 ± 3.90^{b}	73.33 ± 5.07^{ab}	96.67 ± 4.53^{a}	64.36 ± 2.20^{b}
Villus area (mm^2)	7.75 ± 0.39^{b}	10.87 ± 1.26^{ab}	14.68 ± 1.57^{a}	8.02 ± 0.41^{b}
Crypt depth (μm)	22.67 ± 3.73^{ab}	23.33 ± 1.33^{ab}	34.67 ± 1.32^{a}	21.38 ± 0.74^{b}
Thickness of wall (μm)	9.00 ± 0.84^{b}	10.33 ± 1.13^{b}	18.00 ± 1.85^{a}	9.09 ± 0.73^{b}
Abundance of goblet cell (GB)	71.30 ± 3.25^{b}	90.00 ± 6.70^{ab}	112.00 ± 7.00^{a}	78.09 ± 1.78^{b}
Thickness of muscle (μm)	5.09 ± 0.08^{c}	8.10 ± 0.03^{b}	13.86 ± 0.85^{a}	7.00 ± 0.30^{bc}

The values in the same row that are followed by the different superscript letters are significantly different ($P < 0.05$).

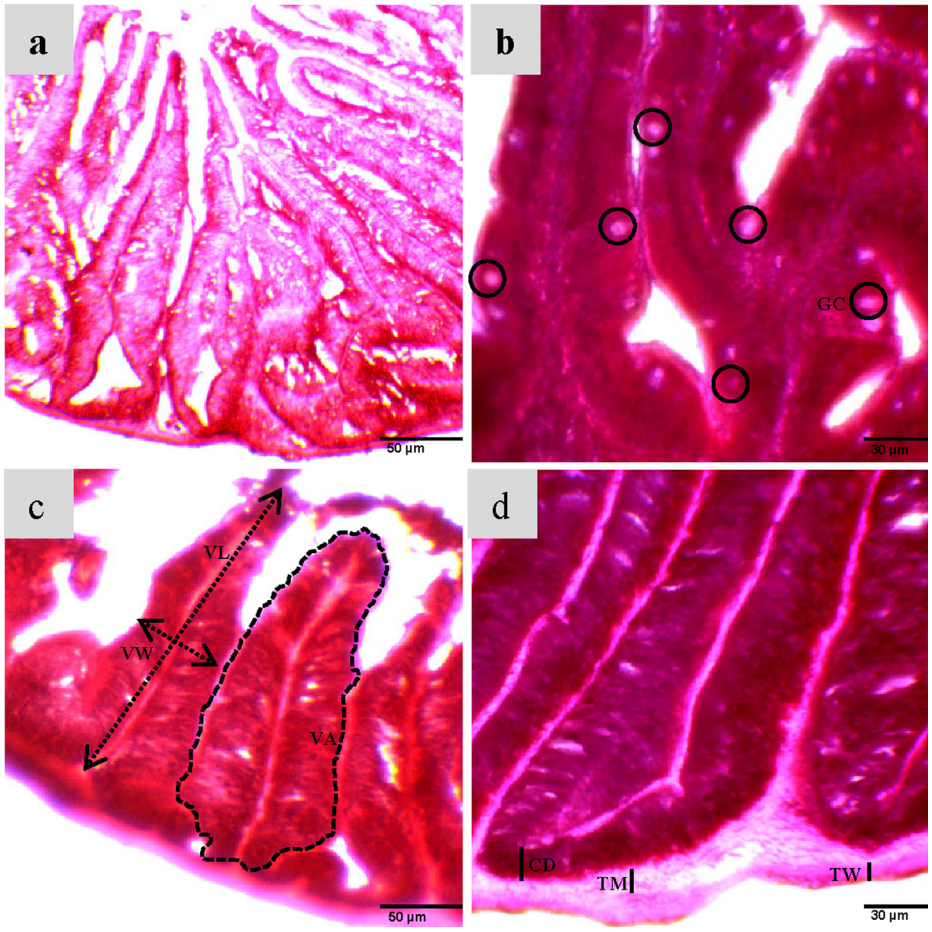

Fig. 1. Histological changes of the intestine of *H. fossilis* fed different experimental diets for 14 weeks; (a) control (SBM_0) diet, (b) GC = Goblet cell, (c) VA = Villus area, VL = Villus length, VW = Villus width, (d) CD = Crypt depth, TW = Thickness of wall, TM = Thickness of muscle.

high soybean meal-containing diet (SBM_{75}) could be due to their morphological and physiological variations for diets high in animal protein. Moreover, the FCR values of this present study were slightly higher because of the culture systems and removal of uneaten feeds. At times, leftover feeds were not possible to collect because of suspension in water, and this situation leads to excess feeding. While, in the nutritional study, the supply of feed should be smooth enough, therefore overfeeding is better than underfeeding (Tacon and Cowey, 1985). Billah et al. (2022) reported the FCR values of stinging catfish 3.55 to 4.35 when FM protein was totally replaced by SBM. In this study, the survival rate found around 94 to 98 % is similar to the results of 92 to 100 % reported by Liu et al. (2021), where FM protein was replaced with various percentages of soybean meal protein.

Substitution of FM protein with SBM in diets of *H. fossilis* affected the values of whole-body carcass composition such as moisture, crude protein, crude lipid, ash, crude fibre, and nitrogen-free extract. Comparable results were also reported in Ussuri catfish (Wang et al., 2015), Nile tilapia (Ajani et al., 2016), and European seabass (Kaushik et al., 2004). While some other studies showed that the replacement of FM protein with SBM in diets did not affect the proximate composition of rainbow trout (Yang et al., 2011) and European sea bass (Tibaldi et al., 2006). In this study, significantly higher and lower whole-body carcass protein was found in the SBM_{25} and SBM_0 groups, respectively. However, lipid content was significantly higher in SBM_0 and SBM_{75} groups than in other groups. Moreover, moisture and lipid content also showed reverse conditions.

Hemato-biochemical studies are commonly used for the evaluation of different physical conditions of fish (Pradhan et al., 2012; Sharmin et al., 2016; Jahan et al., 2019; Billah et al., 2022). In this present experiment, there was no significant variation observed in Hb, RBCs, and WBCs upto 50 % replacement of FM protein by soybean meal in diets and then there was a decreasing trend of these values at 75 % replacement level. Dawood et al. (2015) obtained Hb and Glu levels of 10.7 to 12.5 g/dL and 68 to 97.3 mg/dL, respectively in Amberjack juveniles fed 0 to 45 % soybean meal-containing diets. The increasing level of Hb in fish blood may have had better oxygen transport in tissues leading to improved growth (Esmaeili, 2021; Hossain et al., 2022). Hosseini and Khajepour (2013) reported that a higher level of replacement of FM protein by soybean meal lowers the value of Hb, RBC, and WBC in beluga. In this study, the blood glucose level was reversed with blood hemoglobin. Moreover, in this study, the higher the replacement of FM protein by soybean meal in diets higher the values of glucose in stinging catfish. Parallel reports were also stated by Liu et al. (2021) in juvenile redlip mullet and Hosseini and Khajepour (2013) in beluga. Previous reports suggested that the hematology of fish varies on fish species, size, physical and environmental conditions, feed ingredients and formulation, source of quality protein, vitamins, and probiotics (Osuigwe et al., 2005).

The morphological changes in the intestine have been observed in some fish species owing to the inclusion of SBM in the diets (Shiu et al., 2015; Garcia-Ortega et al., 2016). The present study showed that the dietary replacement of FM protein with SBM upto 50 % increased intestinal shape with increasing the histo-

morphological factors of the intestine and then decline to 75 % replacement level. Increased intestinal shape (villus length, width, area, and thickness) designates the evaginations of the intestinal mucosa, which increases the absorption of intestinal nutrients and improves fish growth performance and feed consumption (Ferguson et al., 2010; Pirarat et al., 2011; Khojasteh, 2012; Jahan et al., 2021; Islam et al., 2021). In general, plant feedstuff contains cellulose (plant fiber), which is digested by cellulase produced by the gut bacteria of many fish species such as chitinase in crustaceans. Moreover, herbivorous and some omnivorous fishes are more able to digest cellulose than carnivorous fishes. For this reason, in this present study, the shape of the intestine was increased by replacing FM protein with SBM upto 50 % in the diet. Further replacing of SBM in the diet reduced the intestinal shape of stinging catfish. Comparable effects were also described in orange-spotted grouper (Wang et al., 2017) and Japanese seabass (Zhang et al., 2018). In this study, the abundance of goblet cells increased by substituting FM protein with SBM upto 50 % level, which is responsible for the production of mucus that provides gel coating over the surface layer of epithelium and defends from bacterial invasion (Johansson et al., 2008).

Based on the results it was suggested that SBM could replace upto 50 % FM protein in diets of *H. fossilis* without compromising growth, feed efficiency, and health status. More studies should be carried out in production trials for a longer duration on *H. fossilis* by using 50 % replacement of FM protein with SBM in the diet.

Author contributions

Sumon Howlader experimented, collected, and tabulated the data; Kanij Rukshana Sumi planned the study, designed, supervised, analyzed data, and wrote the manuscript; Subroto Sarkar and Sheikh Masum Billah assisted Sumon Howlader in conducting the experiments and data collection; Mohammad Lokman Ali assisted Sumon Howlader to conduct the experiments and revised the manuscript; Md. Shahjahan facilitated the laboratory to analyze histology and hematology and edited the manuscript; Jewel Howlader helped in data analysis and revised the manuscript; All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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