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Efficacy of Substituted Barley Meal Based Diet on Growth Performance, Nutrients Digestibility and Hematological Indices in Common Carp (*Cyprinus carpio*)

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ABSTRACT

With continuous expansion of human population, fish is a rich protein source used as food all over the world but high cost of fish meal (FM) and its inconsistent supply has entailed researchers to find its substitute. In present experimental work, plant-based protein source was used as an alternative of fish meal as it is available at comparably low-cost and effectively accessible. The present research work was conducted to evaluate the effects of barley seed meal (BSM) by replacing FM on the growth performance, nutrients digestibility and hematological indices in *Cyprinus carpio*. Six test diets (replacing FM at 0, 10, 20, 30, 40 and 50% level) were prepared using BSM as an alternative protein source. Three replicates were used for each treatment consisting of 15 fingerlings (8.13g) per tank. Fish was fed at the rate of 4% live wet body weight two times a day for 70 days. The results revealed that fingerlings fed Barley seed meal-based diets (BSMD) II (20% FM replacement) showed best results in growth parameter (weight gain%; 249%, weight gain; 20g, SGR; 1.39 and FCR; 1.31), hematological indices (RBCs; 2.83×10^6mm^{-3}, PLT; 68.54 and Hb; 8.15g/100mL) and nutrient digestibility (CP; 72% and GE; 67%). It was noticed that further increase in BM level decreases the fish performance as compared to above mentioned diet. From results, it was concluded that we can replace FM up to 20% to improve growth performance, nutrients digestibility and hematological indices as well as making eco-friendly and cost-effective feed.

INTRODUCTION

The human population on the planet Earth is increasing day by day, predicted to cross 9.4 billion in the year 2050 (Raftery and Ševčíková, 2021). The biggest issue society is currently facing is the sustainability and availability of nutritious food (Orun *et al*., 2007; Sundram, 2023; Hall *et al*., 2017). It is feasible to meet the current fish consumption rates, regardless of the effects of environmental change on the potent fisheries and increasing human population, if resources of fish are substantially managed and the industry of feed reduces its dependency on wild fish (Mitra *et al*., 2023; Merino *et al*., 2012).

For a sustainable future, the two complementing elements are food security and food safety (Orun and Talas, 2008; Vagsholm *et al*., 2020). Food availability, assess to food, nutrients sufficiency, stability and foreseeabilty (of the mentioned conditions) are the factors that are required for sustainable food security (Alsaleh, 2023; Helland and Sorbo, 2014). It is crucial for human wellbeing to make sure that individuals are eating in a healthy environment and consuming nutritious foods (Uysal *et al*., 2023). For our future, a novel solution of food security and sustainability is needed with no compromise on safety of food (Vagsholm *et al*., 2020).

Global per capita yearly consumption of fish climbed from 9.1 kg in 1961 to 20.6 kg in 2021, with the supply growing at a higher rate than the population (Selamoglu, 2021). For utilizing fish in attaining nutritional

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dependability, it is important to acquire information on the nutritional importance of fish. Fish is a health-giving food, a rich source of animal protein (Selamoglu, 2018; Kakoolaki *et al*., 2013), micronutrients and PUFAs (polyunsaturated fatty acids) specifically presence of omega-3 DHA; docosahexaenoic acid and EPA; eicosapentaenoic acid (Talas *et al*., 2007; Mohanty *et al*., 2017).

C. carpio, commonly known as common carp, is distributed widely all over the World but mainly found in Eastern Europe and Central Asia (Selamoglu *et al*., 2015; Shahzad *et al*., 2021; Weber and Brown, 2009). It belongs to family Cyprinidae, the substantial freshwater fish family (Talas *et al*., 2012; Rahman, 2015). Common carp is viewed as a capable candidate for culturing in Asia and European countries, and is highly adaptive to food and the environment (Manjappa *et al*., 2011; Rahman, 2015).

Fish love eating FM as it is a high-quality digestive feed carrying great amount of energy; a good source of vitamins, minerals, lipids; and a rich source of protein (Hussain *et al*., 2024; Miles and Chapman, 2006). Worldwide, FM has been used all over the world as a nutritious feed for fish, however, its inconstant supply, substantial demand and ascending prices have entailed researchers to find the substitute of FM; and have stepped up their aim at perceiving alternate feed of plant and animal origin (Sarfraz *et al*., 2020; Chen *et al*., 2023; Gao *et al*., 2024). Considering the nutritional need of aquatic animals and technological advancement in aquatic feed, researchers suggest complete or partial replacement of FM with other plants (or animal) based protein sources (Soltan *et al*., 2023; Ayoola, 2010). Plant-based feed must have some nutritive values such as high protein content, low level of fibre and beneficial amino acid content (Hussain *et al*., 2018).

Because of their antioxidant qualities, which assist to combat oxidative stress, fish supplements are essential for increasing fish health, growth, and immunity (Duran and Talas, 2009; Talas *et al*., 2014). It is crucial to guarantee food safety in these supplements since it avoids contamination and promotes environmentally friendly fish farming methods (Gulhan and Selamoglu, 2016). The reason for using BSMD as feed for *C. carpio* (as well as other fish species) is its crude fibre content (i.e., 5%) which is high as compared to other cereals, for example, maize; 2.3% and wheat; 2.6% (Janković *et al*., 2011).

Current project was designed to evaluate the effects of barley meal-based diet. The goals of current research work are: to use barley-based diet to analyze nutrients digestibility; growth performance and hematological indices of *C. carpio* as well as cost effective and eco-friendly fish feed.

MATERIALS AND METHODS

The research work was based on the partial replacement of fish meal with barley meal to scrutiny its effects on some parameters (such as growth performance, nutrients digestibility and hematological indices) in *C. carpio* fingerlings. Research was performed at Fish Nutrition Laboratory, Zoology Department, University of Education Township Campus, Lahore, Pakistan.

Experimental conditions for fish

Fingerlings (*C. carpio*) were purchased from Zaman Fish Hatchery, Jhabran, Sheikhupura, 72 km from Lahore. Before the beginning of trial, fingerlings were acclimated to lab conditions for fifteen days (Örün *et al*., 2011; Fuat *et al*., 2012). V-shaped tanks were used (specially designed for experiment purpose) having capacity of 70L water. *C. carpio* fingerlings were placed in container having saline solution for 60-120 sec to exterminate the pathogens (Rowland and Ingram, 1991). During acclimatization, fish was fed on basal diet two times in 24 h (Allan and Rowland, 1992). Water quality parameters like temperature, dissolved oxygen (Jenway 970) and pH (Jenway 3510), were regulated each day. Oxygen pump was used for supplying air by capillary set-up throughout the experimentation period.

Experimental design

Fish meal was replaced with barley meal and was divided into six test diets. Each diet was compared with other diet to ascertain nutrients digestibility, growth performance and hematological indices in *C. carpio* fingerlings.

Formation of feed pellets

Barley (*Hordeum vulgare*) seeds were purchased from local shops of Lahore. Seeds were dried naturally and de-oiled by pressing method (Weiss, 1971; Salem and Makkar, 2009) with oil seed extraction machine. De-oiled barley seeds were then pulverized to make fine powder. Fish feed ingredients were purchased from commercial market and were ground exquisitely so that they could pass through the strainer of 0.3 mm size. After straining, required proportion of all the dry ingredients including chromic oxide, Ascorbic acid, mineral and vitamin premix were weighed by digital electronic weighing balance and then mixed in a feed mixer for 5-10 min. Chemical composition of all ingredients was analyzed before preparing experimental diets by standard method (AOAC, 1995). Fish oil was added gradually during the process of mixing ingredients. 15mL distilled water was added and mixed to make dough of the feed ingredients for preparing feed pellets. After this process, dough was passed through hand pelleting machine of 2.0 mm diameter. Prepared feed pellets were dried for about 72 h in sunlight and stored at 4°C until use.

Feeding and feces collection protocol

C. carpio fingerlings were fed twice a day at the rate of 4% wet body weight. Feeding time given to fish was 2 h, after which leftover was drained out by opening the valves of tank. Tanks were washed after feeding period and refilled properly. After three h of washing, feces were collected through feces collecting pipes by opening and closing the valves of tanks alternately. Feces were circumspectly collected and dried in incubator each day at 70°C for 60-90 min to avoid leeching of nutrients in water. After this, feces were stored for further inspection.

Utilization of feed and growth performance analysis

C. carpio fingerlings of average weight were stocked in each tank. To analyze the growth performance of fingerlings during the whole experimental patch, fishes were weighed after every 15 days. Parameters of growth i.e. weight gain (WG) in grams (g), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated by standard formulae (NRC, 1993).

$$WG\% = \frac{(Wf - Wi)}{Wi} \times 100$$

W_i is the initial weight whereas W_f is final weight of fingerlings.

$$FCR = \frac{DFI\ (g)}{Wet\ WG\ (g)}$$

Here DFI stands for dry feed intake.

$$SGR\% = \frac{(\ln Wf - \ln Wi)}{Trial\ Days} \times 100$$

SGR stands for specific growth rate.

Nutrients digestibility

Collected samples were subjected to 105°Cfor 12 h, after which moisture content of experimental diets and feces were evaluated. To measure of crude protein (CP) content (N × 6.25), MKS (Micro Kjeldahl System; InKjel M behr Labor Technik GmbH D-40599 Dusseldorf) was used, whereas Soxhlet Apparatus (Soxhlet Extraction Heating Mantels, 250 mL 53868601) was used for the investigation of crude fat (CF) by Petroleum Ether Extraction (EE) technique. Crude fiber content was evaluated as loss on ignition of dried lipid free residue after digesting with 1.25 NaOH and 1.25% H_2SO_4. Ash was evaluated by ignition for 12 h in an electric furnace (Naberthern B170) at 650°C to invariable weight. $C_X(H_2O)_y$ contents (N-free extract) were calculated using standard formula i.e.

Total carbohydrates % = 100 – (EE + CP + CF+ Ash)

To find the GE of samples, oxygen bomb calorimeter was used.

Hematological indices

Fish was taken from each tank to collect blood from the caudal vein of fish via heparinized syringe. Blood samples were sent to the laboratory for the purpose of analyzing hematological indices. Microhematocrit method using capillary tubes was applied for hematocrit test (Brown, 1980). Hemocytometer with Neubauer counting chamber was used for counting red blood cells; RBCs (Blaxhall and Daisley, 1973). For hemoglobin (Hb) concentration testing, description by Wedemeyer and Yastuke (1977) was used. MCH (mean corpuscular hemoglobin), MCV (mean corpuscular volume) and MCHC (mean corpuscular hemoglobin content) were calculated by using the following formula:

$$MCH = \frac{Hb}{RBC} \times 10$$

$$MCV = \frac{PCV}{RBC} \times 10$$

$$MCHC = \frac{Hb}{PCV} \times 100$$

ADC of nutrients

ADC (Apparent digestibility coefficient) of the experimental diets was calculated using standard formula (NRC, 1993).

$$ADC(\%) = 100 - 100 \times \frac{\%\ \text{maker in diet} \times \%\ \text{nutrient in feces}}{\%\ \text{maker in feces} \times \%\ \text{nutrient in diet}}$$

Statistical analysis

Statistics for growth parameters, hematological indices and nutrients digestibility parameters were directed to Analysis of Variance (one way) ANOVA (Steel and Torriji, 1996). Tukey's honesty significant difference test was performed in order to compare variations in experimental diets and were contemplated significant at $p<0.05$ (Snedecor and Cochran, 1991). CoStat Computer Package (PMB 320, Monterey, CA, Version 6.303,93940 USA) was used for statistical analysis.

RESULTS

In this research on fish nutrition, FM was replaced by BSM at different levels (0%, 10%, 20%, 30%, 40%, and 50%) to observe its effects on the nutrient's digestibility, growth performance and hematological indices of *C. carpio*.

Table I. Ingredients (%) used to prepare six BSMD to feed *C. carpio* fingerlings.

Ingredients	BSMD – 0	BSMD – I	BSMD – II	BSMD -III	BSMD –IV	BSMD –V
BSM	0	4	8	12	16	20
FM	40	36	32	28	24	20
Fish oil	6	6	6	6	6	6
Sunflower meal	19.5	21.5	23.5	25.5	27.5	29.5
Wheat flour	21	19	17	15	13	11
Rice polish	9.5					
Vit premix*	1					
Min premix**	1					
$C_6H_8O_6$	1					
Cr_2O_3	1					
Nutrients in diet						
CP	31.56±0.82	31.58±0.81	31.55±0.64	31.57±0.91	31.59±0.79	31.57±0.96
CF	6.60±0.52	6.59±0.50	6.62±0.63	6.61±0.63	6.59±0.61	6.62±0.57
GE	3.96±0.18	3.98±0.12	3.99±0.18	3.97±0.20	3.96±0.17	3.97±0.16

BSMD 0, Control diet with zero % replacement of FM; BSMD I-V, replacement of FM at different levels; Vit Premix, Vitamin premix; Min Premix, mineral premix; $C_6H_8O_6$, Ascorbic acid; Cr_2O_3, Chromic oxide. *Vit. D3, 3,000,000 IU; Vit. A, 15,000,000 IU Vit; C, 15,000 mg; Vit. B6, 4000 mg; Vit. E, 30000 IU; Vit. B2, 7000 mg Vit; B12, 40 mg; Folic acid, 1500 mg; Vit. K3, 8000 mg Ca pantothenate; 12,000 mg; Nicotinic acid, 60,000 mg. ** Mg, 55 g; Ca, 155 g; Se, 3 mg; Na, 45 g; P, 135 g; Cu, 600 mg; Mn, 2000 mg; Co, 40 mg; Fe, 1000 mg; Zn, 3000 mg; I, 40 mg. CP, crude protein; CF, crude fat; GE (kcal/g), gross energy (kcal/g).

Table II. Growth performance parameters of *C. carpio* fed on different levels of BSMD.

BSMD levels %	Test diets	Wi (g)	Wf (g)	Wg (g)	Wg (%)	Wg (fish^{-1} day^{-1}) g	FI (fish^{-1} day^{-1}) g	Feed conversion ratio	Survival growth rate	Survival rate (%)
0	BSMD-0	8.14±0.19	20.47±0.89^{cd}	12.33±0.82^{cd}	151.55±9.63^{cd}	0.18±0.01^{cd}	0.26±0.02^{c}	1.47±0.04^{ab}	1.02±0.04^{bc}	92.48±3.12
10	BSMD-I	8.12±0.23	25.53±0.66^{b}	17.41±0.47^{b}	214.53±4.07^{b}	0.25±0.01^{ab}	0.35±0.01^{ab}	1.41±0.06^{bcd}	1.27±0.01^{a}	94.44±0.00
20	BSMD-II	8.12±0.16	28.35±0.81^{a}	20.23±0.87^{a}	249.26±13.71^{a}	0.29±0.01^{a}	0.38±0.01^{a}	1.31±0.02^{d}	1.39±0.04^{a}	98.15±3.21
30	BSMD-III	8.14±0.18	26.75±0.79^{ab}	18.61±0.76^{ab}	228.60±10.48^{ab}	0.27±0.03^{ab}	0.33±0.03^{ab}	1.36±0.03^{cd}	1.32±0.04^{a}	98.15±3.21
40	BSMD-IV	8.13±0.27	22.72±0.86^{c}	14.59±1.12^{c}	179.93±19.78^{c}	0.21±0.02^{bc}	0.30±0.03^{bc}	1.43±0.02^{bc}	1.14±0.08^{b}	96.30±3.21
50	BSMD-V	8.15±0.16	19.42±0.94^{d}	11.27±0.78^{d}	138.10±7.09^{d}	0.16±0.01^{d}	0.25±0.02^{c}	1.54±0.04^{a}	0.96±0.03^{c}	90.52±3.41

Data are means of triplicates, ± shows SD; FI, feed intake; Wi, initial weight; Wf, final weight; Wg, weight gain. Superscripts represent values at $P<0.05$ (significantly different) higher to lower within column as $^{a>b>c>d}$.

Growth parameters such as weight gain %, weight gain, FCR and SGR, of *C. carpio* fingerlings fed on test diets replacing FM with BSM are shown in Table II and Figure 1A. Results indicated that initial weight was statistically similar for all six groups while other growth parameters presented variations from each other. Maximum % weight gain (249%), weight gain (20g), SGR (1.39), and survival rate (98%) values were noted in fingerlings fed BSMD-II (having 20% FM replacement) followed by (229% for weight gain%, 1.32 for SGR, 19g for weight gain and 98% for survival rate%) with BSMD - III (30% replacement of FM). In contrast, lowest weight gain (11g), SGR (0.96), weight gain % (138%) and survival rate % (91%) were observed in BSMD - V (50% replacement of FM). On the other side, best FCR (feed conversion ration; 1.31) value was noticed in fingerlings fed BSMD – II while followed (1.36) was observed at 30% replacement of FM. FCR values differ significantly in all test diets. Highest FCR (1.54) value was observed in BSMD - V. weight gain g, FCR, and weight gain % at 20% and 30% replacement were statistically at par. It was concluded that all growth parameters started increasing when fed with control diet (i.e., diet having no replacement of FM) and outreached to its maximum when fed at 20% BSM based diet while started decreasing at higher levels (i.e., 30%, 40% and 50%) of BSM based diet.

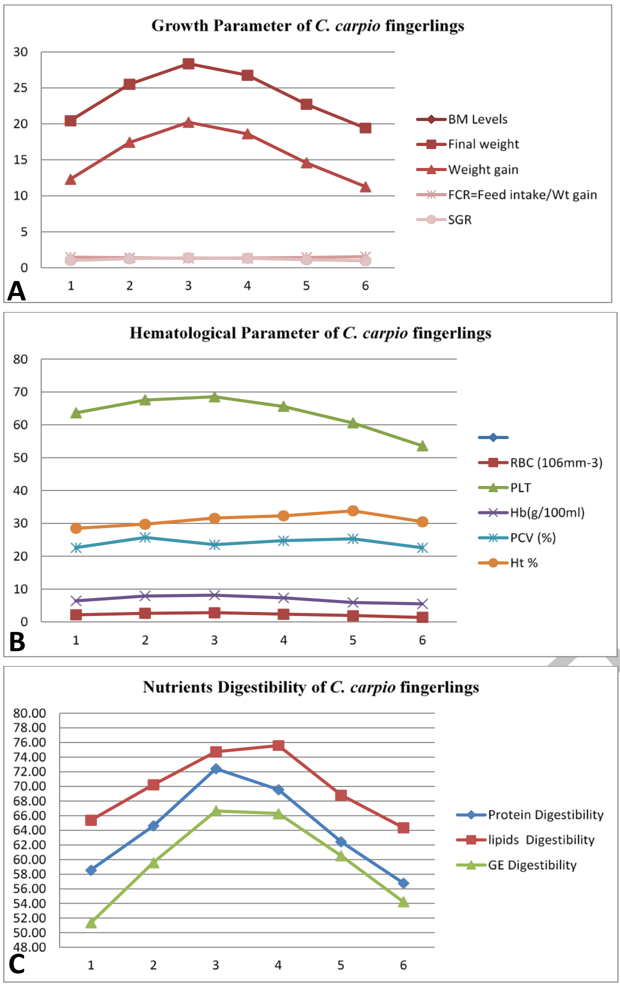

Fig. 1. Cumulative Growth parameter (A), hematological parameter (B), and cumulative nutrients digestibility (C) of *C. carpio* fingerlings fed with barley seed meal-based diets.

Hematological indices are a good tool to determine fish health. From this research work, hematological data of all fingerlings was compared with control diet and all test diets containing different levels of BSM. The hematological indices of *C. carpio* fingerlings fed with barley meal-based diets are displayed in Table III and its graphical representation in Figure 1B. From hematological indices analysis of fingerlings, it was found that there are variations in all fingerlings fed with the experimental diets (BSMD I-V) and control diet. Results showed significantly variance in all blood parameters. Maximum levels of RBCs ($2.83\times10^6 mm^{-3}$), PLT (68), and Hb (8g/100mL) were observed in the fingerlings fed BSMD–II; PCV (26%) in BSMD - I; and Ht% (34%) in the fish fed BSMD-IV. In contrast, lowest values of RBCs ($1.37\times10^6 mm^{-3}$), PLT (53.59), Hb (5.52 g/100mL) and PCV (22.59%) were observed in fingerlings fed BSMD -V; and Ht% (28.47%) at control diet. Most parameters were good at BSMD- II (having 20% replacement of FM).

Crude fat (CF), Crude Protein (CP) and gross energy (GE) were analyzed in the BSMD diets (presented in Table I) and feces of *C. carpio* fingerlings to observe nutrients digestibility in fish presented in Table IV and graphical representation in Figure 1C. Nutrients (CF, CP, and GE) were significantly similar in all experimental diets. It was found that highest amount of nutrients were passed through feces by the fingerlings fed BSMD-V (having 50% replacement of FM). Highest values of CP (14.49%) and fat (2.50%) were noticed in the feces of fingerlings fed BSMD- V followed by fingerlings (with 13.97% for CP and 2.44% for fat) fed BSMD – 0 while lowest value of CP (9.35%) was observed in feces of fingerlings fed test BSMD- II and fat (1.74%) in fish fed BSMD- III followed by second lower value of CP (10.32%) in fish fed BSMD - III and fat (1.80%) in fingerlings fed BSMD-II. Maximum value of GE 2.06% was recorded in feces fed BSMD - 0 (i.e. control diet having no replacement of FM) followed by (1.93%) in fish fed BSMD- V whereas lowest value (1.43%) in fingerlings fed BSMD- II followed by second

Table III. Haematological indices of *C. carpio* fed with different levels of BSMD.

BSMD levels %	Test diets	RBC ($10^6 mm^{-3}$)	PLT	Hb(g/100ml)	PCV (%)	Ht %
0	BSMD-0	2.14 ± 0.20^{bc}	63.65 ± 0.66^{c}	6.43 ± 0.52^{bc}	22.62 ± 0.83^{b}	28.47 ± 0.62^{d}
10	BSMD-I	2.62 ± 0.16^{ab}	67.53 ± 0.80^{ab}	7.89 ± 0.75^{a}	25.71 ± 0.71^{a}	29.73 ± 0.94^{cd}
20	BSMD-II	2.83 ± 0.18^{a}	68.54 ± 0.81^{a}	8.15 ± 0.37^{a}	23.52 ± 0.74^{ab}	31.60 ± 0.86^{abc}
30	BSMD-III	2.35 ± 0.22^{abc}	65.56 ± 0.92^{bc}	7.33 ± 0.39^{ab}	24.75 ± 0.98^{ab}	32.29 ± 0.87^{ab}
40	BSMD-IV	1.96 ± 0.23^{c}	60.56 ± 0.76^{d}	5.93 ± 0.37^{c}	25.32 ± 0.86^{a}	33.80 ± 0.92^{a}
50	BSMD-V	1.37 ± 0.16^{d}	53.59 ± 0.90^{e}	5.52 ± 0.47^{c}	22.59 ± 0.72^{b}	30.53 ± 0.89^{bcd}

Data are means of triplicates, ± shows SD; RBC, red blood cell; Hb, hemoglobin; PCV, packed cell volume; PLT, platelets; Ht, hematocrit. Superscripts represent values at P<0.05 (significantly different) higher to lower within column as $^{a>b>c>d}$.

Table IV. Analyzed composition of feces and digestibility of the nutrients by fish.

BSMD levels %	Test diets	Analyzed nutrients composition of feces			Analyzed digestibility of nutrients		
		CP in feces	Fat in feces	GE in feces	CP digestibility	Fat digestibility	GE digestibility
0	BSMD-0	13.97±0.57[a]	2.44±0.22[a]	2.06±0.10[a]	58.57±0.97[d]	65.36±0.76[c]	51.33±0.96[d]
10	BSMD-I	12.08±0.23[b]	2.12±0.20[ab]	1.74±0.12[bc]	64.60±0.83[c]	70.24±0.89[b]	59.59±0.76[b]
20	BSMD-II	9.35±0.46[c]	1.80±0.23[b]	1.43±0.11[d]	72.42±0.96[a]	74.73±0.92[a]	66.64±0.90[a]
30	BSMD-III	10.32±0.45[c]	1.74±0.24[b]	1.44±0.11[cd]	69.55±0.98[b]	75.58±0.93[a]	66.29±0.94[a]
40	BSMD-IV	12.67±0.39[b]	2.19±0.18[ab]	1.67±0.11[bcd]	62.44±0.81[c]	68.82±0.90[b]	60.53±0.92[b]
50	BSMD-V	14.49±0.50[a]	2.50±0.14[a]	1.93±0.12[ab]	56.78±0.95[d]	64.35±0.82[c]	54.21±0.89[c]

Data are means of triplicates; ± shows SD; CP, crude protein; GE, gross energy. Superscripts represent values at $P<0.05$ (significantly different) higher to lower within column as [a>b>c>d].

lower value (1.44%) in BSMD- III. On the other hand, highest value of CP digestibility (72.42%) and GE digestibility (66.64%) was observed in fish fed BSMD – II followed by fingerlings (with 69.55% for CP and 66.29% for GE) fed BSMD - III while lowest value of CP digestibility (56.78%) in fish fed BSMD-V and lowest value of GE (51.33%) was observed in fish fed BSMD- 0. For fat digestibility, maximum value (76%) was noticed in fish fed BSMD - III followed by 75% in fingerlings fed BSMD - II while the lowest value 64.35% was recorded in the fingerlings fed experimental diets replacing 50% replacement of FM followed.

DISCUSSION

Findings of this research suggested that the addition of BSM in fish diets by partially replacing FM has propitious effects on the growth performance parameter of *C. carpio* fingerlings. At 20% replacement of fish meal with BSM (i.e., in BSMD-II), maximum weight gain% (249%), SGR (1.39), survival rate% (98%) and weight gain (20g) were observed. Quite similar observations were obtained in the research of Hernandez *et al.* (2007) that showed increased weight of fish fed test diet having 20% inclusion of plant based diets (oil seeds meal) replacing FM in *Diplodus puntazzo*; sharpsnout seabream. Another experiment conducted by Lim and Lee (2009) showed that oil based seeds (plant protein source) can be used up to 20% by replacing FM in the diets of Juvenile *Oplegnathus fasciatus*; parrot fish and up to 30% in the on growing *O. fasciatus*, and concluded that oil seeds meal can be utilized better in large fish. Nearly similar observations were studied in the experiment performed by Rahmdel *et al.* (2018) to study the effects of oil seeds meal substitution (at 0, 25, 50, 75 and 100% level) by replacing FM on the growth performance of *C. carpio*. Highest fish performance (weight gain and SGR) was recorded in fingerlings fed experimental diet II (having 25% replacement of FM) while lowest growth performance was recorded in fish fed the diet replacing 100% FM. Closely similar data was obtained by Zaretabar *et al.* (2021), who performed an experiment to study the effects of barley protein concentrate (BPC) by replacing FM on the growth performance of *Salmo trutta caspius* (Caspian brown trout). This study concluded that BPC levels replacing 25% of FM showed best result followed by 50% of replacement and control diet, while BPC levels 75% and 100% showed decrease in weight gain. Quite different results were explained by Pinedo-Gil *et al.* (2016), who conducted a research to observe the enhancement of *O. mykiss* flesh by incorporating barley diets and concluded that inclusions of barley meal in test diets (ranging from 0 to 32%) did not showed significant effect on growth parameter. Another study (Tahir *et al.*, 2008) where FM was replaced with plant based diets (oil seeds meal) was carried out to evaluate its effects on the growth parameters of major carps (i.e., *Labeo rohita*, *Catla catla* and *Cirrhina mrigala*). Seven treatments were prepared (T1-T7), out of which T1 was standard diet, T2-T4 were replacing FM with sunflower meal and T5-T7 with canola seed meal. It was concluded that *C. catla* and *L. rohita* showed lessened final weight in all treatments except standard diet (i.e., T1 having no replacement of FM), while *C. mrigala* showed better results under 20% inclusion of canola seed meal (T5) replacing FM.

According to this study, the addition of BSM by replacing FM in diets has advantageous effects on the hematological parameter of *C. carpio* fingerlings. By 20% replacement of FM with BSM (i.e., in BSMD-II), highest value of RBCs ($2.83\times10^6 mm^{-3}$), PLT (68.54) and Hb (8.15g/100mL) were recorded. Similar calculations were obtained by Zhou *et al.* (2005) and concluded that hematological parameters were best in the juvenile cobia (*Rachycentron canadum*) fed test diet, replacing 20% FM with oil seeds meal while noticed poor at 60% replacement. This research suggested that FM can be replace up to 20% and results showed decrease as the inclusion level was increased. Slightly similar results were found by Sotolu and Faturoti (2011) that inclusion of higher level of seeds meal showed decrease in parameters of hematological indices of catfish (*Clarias gariepinus*). They concluded that at 50% replacement of FM and above those, hematology parameters were poor while best at 25% replacement. Nearly similar results were found in the study (Kikuchi, 1999) showing best hematology parameters in the diets for Japanese flounder (*Paralichthys olivaceous*) fed test diet that contain 15% plant based diets replacing 20% white FM. Quite different study done by Rahmdel *et al*. (2018) suggested that plant protein-based diets can be added up to 75% for the improvement of the hematological parameters in *C. carpio*. Abedalhammed *et al*. (2017) explained through his research on the comparison of effects of barley sprout powder (at level 2.5gm/kg and 5gm/kg), hydroponic planting (at level 2.5gm/kg and 5gm/kg) and control diet. They concluded that significant variance was detected among the treatments when compared with control diet in the parameters such as RBC count, Hb and PCV; and BSP (5gm/kg) showed good result than others, when observed in comparison with control diet and hydroponic planting at both levels.

Nutrients digestibility is one of the main element for assessing whether a protein source can be a suitable option for the replacement of FM. The present research showed that there was maximum absorption of nutrients i.e., crude protein and gross energy fed BSMD-II (having 20% replacement of FM) while absorption of crude fat was better in fish fed BSMD-III (having 30% replacement of FM). Quite similar calculations were obtained by the studying the research (Hernandez *et al*., 2007) that 20% inclusion of oil seeds meal in the fish diet can replace FM as the apparent digestibility coefficient was recorded maximum in sharp snout seabream (*Diplodus puntazzo*) while start decreasing at higher levels of replacement. Different results were obtained from the study of Sun *et al*. (2015) showed that nutrients (proteins and lipids) digestibility was recorded least in the sea bream (*Acanthopagrus schlegelii*) fed test diet replacing 24% FM in comparison with control diet while no significant variations were observed among other levels i.e., 0, 08 and 16%. Nearly similar findings were observed by (Zaretabar *et al*., 2021), who conducted an experiment to see the effects of barley protein concentrate (BPC) on the digestibility of nutrients in *Salmo trutta caspius* (Caspian brown trout) by replacing FM. This study showed that BPC levels replacing 25% of FM showed better result i.e., maximum digestion of protein and energy followed by 50% of replacement and control diet, while increase in BPC level showed decrease in weight gain. Different results were obtained by Xie *et al*. (2016) as their experiment showed that higher level of FM increased the digestibility of nutrients while inclusion of oil seeds meal at higher levels showed decrease in the digestion of nutrients in juvenile *Litopenaeus vannamei*; Pacific white shrimp. In another study by (Morken *et al*., 2011), explained the digestibility of nutrients in *O. mykiss*; rainbow trout, by using barley protein concentrate, FM with HCOONa and extrusion temperature cleared that BPC inclusion had a significant role in increased digestibility of nutrients while extrusion only affected the pellets durability.

Reasons behind differences in the results of all the experiment discussed in this section may be due to the difference in the fish species; diet formulation i.e., difference in the origin of ingredients, their processing, and method of collection of feces (Koprucu *et al*., 2004); water quality, culture system (Tucker *et al*., 1989), size and age of fish species (Saleh, 2020); difference in temperature (Fazio *et al*., 2018) and presence of ANFs (anti nutritional factors) in plant based diet (Silva and Anderson, 1994) used in various experiments.

CONCLUSION

From this research work, it was concluded that replacing FM with cost effective and environment friendly feed i.e., BSMD resulted in improved growth performance, hematological indices and nutrients digestibility of *C. carpio* fingerlings. It was cleared that 20% inclusion of BSM resulted in best performance of fingerlings.

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Given data is the part of thesis and will be provided on demand of journal whenever required after publication.

Statement of conflict of interest

The authors have declared no conflict of interest.

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