Punjab University Journal of Zoology

OPEN ACCESS

38(2): 153-162 (2023)
https://dx.doi.org/10.17582/journal.pujz/2023.38.2.153.162

Check for updates

Research Article

Adequacy of Succedaneous Barley meal on Carcass Composition, Immunity and Minerals Absorption in Common Carp (*Cyprinus carpio*) Fingerlings

Muhammad Mudassar Shahzad[1*], Tehreem Shabbir[1], Syed Makhdoom Hussain[2], Fatima Yasin[1], Humayoun Huma Maqbool[1], Aasia Karim[3]

[1]*Department of Zoology, Division of Science and Technology, University of Education, Township, Lahore, Pakistan*
[2]*Departments of Zoology, Government College University, Faisalabad, Pakistan*
[3]*Department of Zoology, Sardar Bahadur Khan Women's University Quetta, Pakistan*

Article History
Received: March 14, 2023
Revised: October 05, 2023
Accepted: October 25, 2023
Published: November 03, 2023

Authors' Contributions
MMS planned, supervised and provided all materials for research. TS conducted the feeding trial and prepared manuscript. SMH co-supervised and helped in manuscript preparing. FY and HHM helped in writing, review, and editing. AK helped in preparing and reshaping the manuscript.

Keywords
BM, Partial replacement, Carcass composition, Immunity, Mineral absorption, *C. carpio*

Abstract | The global exponential growth in the human population has resulted in a decline in food availability. Thus, the production of protein-rich aquatic food is high on the agenda. Feed accounts for 60% of total expenditure in aquaculture. This study was designed to examine the optimal inclusion level of barley meal (BM), competitive plant proteins, as a fishmeal replacer in the formulation of diets, to evaluate its effects on the carcass composition, immunity, and mineral absorption in common carp. Six experimental diets using BM as an alternative protein source containing different graded levels of BM (0%, 10%, 20%, 30%, 40%, and 50%) were prepared. Three replicates were used for each treatment having fifteen fingerlings per tank. Fingerlings were fed at the rate of 4% wet weight twice a day for 70 days. The results revealed that *C. carpio* fingerlings given 20% BM, as a protein source (BM-III) had the most improved carcass composition (crude protein; 17%, crude fat; 8%, gross energy; 3kcal/g, ash; 6% and crude fiber; 2%), immunological indices (WBCs; $7.48\times10^{3}mm^{-3}$) and minerals absorption (Ca; 72.47%, Na; 67%, K; 71% and P; 73%). Results indicated that 20% replacement level of BM with the fish meal is best suited to improve carcass composition, immunity, and mineral absorption and for the production of eco-friendly feed. Moreover, 30% inclusion rate of BM was also found to be useful in the diets of carp species.

Novelty Statement | Barley meal was firstly used in fish feed specially for the common carp fingerlings. It was firstly tested for the mineral absorption and body composition. Use of the Barley was found very beneficial for these fish when was used at the level of 20-30% as replacement of fish meal.

To cite this article: Shahzad, M.M., Shabbir, T., Hussain, S.M., Yasin, F., Maqbool, H.H. and Karim, A., 2023. Adequacy of succedaneous barley meal on carcass composition, immunity and minerals absorption in common carp (*Cyprinus carpio*) fingerlings. *Punjab Univ. J. Zool.*, **38**(2): 153-162. https://dx.doi.org/10.17582/journal.pujz/2023.38.2.153.162

Introduction

The growth of the human global population is a continuous and gradual process. According to the

Corresponding author: Muhammad Mudassar Shahzad
drmudassarshahzad@gmail.com

United Nations, in 2019, the population outreached 7.7 billion, with the elevation of 2 billion humans as compared to the population of 1994. By the year 2050, the numbers will exceed 9.7 billion (United Nations, 2019). It is indicated by the studies, one-fourth of the inhabitants of the world did not have ample food and almost 1 billion humans starve every year (Godfray *et al*., 2010). The priority of several schemes is to find methods to supply sustainable food all around the world (FAO, 2020). Fisheries sector have improved food security for people from few years (Ayoola, 2010). Fish is a source of 20% of average animal protein consumption daily for almost 3.1 billion people (FAO, 2016). It is the sole source of omega-3 polyunsaturated fatty acids readily accessible for direct consumption by humans (Joordens *et al*., 2014). Fish also contains vital nutrients and its acknowledgment for nutritive and health-enhancing aspects is increasing (HLPE, 2014). Developing countries have increased their consumption of fish more than two-fold in the previous 50 years (Becker and Calado, 2021). Farmed fish do not eat natural organisms, rather they are usually provided with preserved feed. Feed formulation and manufacturing are done using raw components and ingredients (Jobling, 2001). Nutrition of farmed animals is critical because feed costs about 50% of production expenditure in fish farming (Craig *et al*., 2017). In fish feed, ingredients utilized should fulfill the nutritious requirements of fish, boost the growth and earnings of smallholder farmers while keeping the focus on sustainability (Coloso, 2015).

On account of the balanced essential amino acids (EAAs) and excessive protein content, FM has been used primarily as fish feed. It also provides farm fish with minerals, ingestible energy, vitamins, and essential fatty acids (El-Sayed, 1999). Approximately, 60-72% of crude protein is present in high-quality FM (Miles and Chapman, 2006). This elevated the requirement for FM, thus there is a notable shortage in the production of FM. Consequently, it turned out to be the highest-priced animal protein (El-Sayed, 1999). However, it is challenging to feed fish with a nourishing diet that is ethically and economically sustainable (Le Gouvello and Simard, 2017). For the production of 1 ton of FM; 4-5 tons of fish are required (Miles and Chapman, 2006). Multiple states are aware that they will not be able to bear the expenses of using FM in fish feed. Thus, many are striving to find ways for substitution of FM, totally or partially (El-Sayed, 2020).

Protein sources substitutes can decrease the price of aquafeed. These substitutes are available easily and are of low price. Like FM, plant meals have more protein and EAAs (Sullivan, 2008). Products of plants comprise of massive quantity of protein, fatty acids, and amino acids, which are unavailable in proteins of animal origin (Mondal and Payra, 2015). There is various evidence available that plant-based proteins can be used in fish feed by partial or total replacement of FM (Kumar *et al*., 2019). Recently, barley is obtaining recognition due to its proteins and therapeutic value (Narwal *et al*., 2020). Barley concentrates of protein as a substitute in fish feed has been hardly examined in the fish (Bell *et al*., 2014). There is extensive use of barley. In the majority of countries, it is fed particularly to pigs and cattle. Even though, barley use in human food is limited. It is an exceptional source of minerals and vitamins (Kerckhoffs *et al*., 2002).

The common carp, or *C. carpio*, is found all over the globe (Lowe *et al*., 2004). In 1964, common carp was brought to Pakistan for aquaculture from Thailand. One of the most prevalent cyprinid species in inland water, common carp is now a significant food supply in Pakistan. Pakistan has experienced common carp fish aquaculture since 1970. It started out slowly but is now employing more than 400,000 people, playing a significant part in the nation's economy. It is widely dispersed throughout Punjab and Sindh regions in Pakistan (Khan *et al*., 2016). This research was conducted to evaluate the optimum potential of BM as a plant protein in diet formulation of *C. carpio*, to partially replace the FM with BM, and to determine its effect on carcass composition, immunity, and mineral absorption of common carp.

Materials and Methods

C. carpio fingerlings and conditions of trial

The testing on *C. carpio* fingerlings was directed in the Fisheries Lab of University of Education in Lahore. The fingerlings were made habitual to the trial conditions for 15 days before initiating the experimentation. Fingerlings were stocked in V-shaped water tanks with 70 L water capacity. During experimentation, the fingerlings were given basal feed two times a day (Allan and Rowland, 1992). The parameters of water quality like pH (Jenway 3510) and dissolved oxygen (Jenway 970) and the temperature had been adjusted per day. The capillary apparatus with an air pump was used to supply air throughout the experimental trial. *C. carpio* fingerlings were kept in saline solution for about 1-2 min to eliminate any pathogens prior to the trial (Rowland and Ingram, 1991).

Design of experimentation

BM was used as test ingredient to prepare the test diet. This experimental diet was split into six diets. FM was partially replaced by BM with the increment level of 10% and test diets prepared were BM-I (0%), BM-II (10%), BM-III (20%), BM-IV (30%), BM-V (40%), and BM-VI (50%). The fingerlings were divided and stocked in tanks according to their weight. The diets were given to stocked fish in six tanks. The feed specified to the fingerlings in tanks was 4% of their wet body weight. The research period continued for 90-days. The different BM-based feeds were inspected and compared with the control diet to analyze different parameters like carcass composition, immunity, and minerals absorption using completely randomized design (CRD).

Feed formulation

Barley seeds were purchased from the seed market of Lahore. The oilseeds were dried through the air and then fats were removed using the press method (Salem and Makkar, 2009). The dried and defatted seeds were ground into powder.

Production of feed pellets

The ingredients of the diet were bought from commercial supplier. To pass the ingredients through the sieve of size 0.3 mm, ingredients were finely crushed (Table 1). The amount of all dry ingredients required was sieved. After weighing all the ingredients according to the formula, dry ingredients, chromic oxide, vitamin, and mineral premix were mixed. During the process of mixing, fish oil was added. All the substances were blended for approximately 5-10 min in a feed mixer. The chemical composition of all the feed ingredients was analyzed prior to the experimental feed preparation through standard methods (AOAC, 1995). To increase the moisture level in the diet, 15 ml of distilled water was further added gradually. The pellets were formed with the assistance of a hand-pelleting machine with a diameter of 2.0 mm. The feed pellets were put in the sunlight for 72 h for the purpose of drying. The diets were stored at the temperature of 4°C until use.

Table 1: Composition of ingredients (%) of experimental diet.

Ingredients	BM I	BM II	BM-III	BM IV	BM V	BM IV
BM	0	4	8	12	16	20
FM	40	36	32	28	24	20
Sunflower	19.5	21.5	23.5	25.5	27.5	29.5
Wheat flour	21	19	17	15	13	11
Fish oil	6					
Rice polish	9.5					
Vitamin premix*	1					
Mineral premix**	1					
Ascorbic acid	1					
Chromic oxide	1					

* 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. Data are three replicates' means (± show standard deviation). Mg= magnesium, P= phosphorus, Cu, Copper; Ca, Calcium; Fe, Iron; Na, Sodium; K, Potassium.

Feeding mechanism and collection of sample

Fingerlings of *C. carpio* having the average weight (7g) were fed twice 4% of their body weight a day. After 2 h feeding period, the excess diet was removed via a drain system from every tank by means of valves attached to the tanks. The water was drained out completely from the tanks and filled with purified water. The fecal samples were collected with the aid of valves (Valve I and Valve II) from feces collecting tubes after 3 h of the feeding period. To reduce the leaching of nutrients in the water fecal samples were collected carefully. These samples were dehydrated at 70 °C in the oven and then stored for additional chemical analysis as given by Shahzad *et al.* (2022).

Chemical analysis for carcass composition

When the trial was completed, blood from 3 fish was withdrawn from every tank. They were killed and dried at ambient temperature. The samples were homogenized and incubated for 12 h at 105°C. The contents of moisture in the carcass were figured out. Analysis of crude protein (N × 6.25) was carried out by Micro Kjeldahl Apparatus (InKjel M behr Labor Technik GmbH D-40599 Dusseldorf). Soxhlet system (Soxhlet Extraction Heating Mantels, 250 ml 53868601) and petroleum ether extraction (EE) process were used to determine the crude fat in carcass samples. The ash was calculated by keeping samples in an electric furnace at the temperature of 650°C for about 12 h. When the samples were digested, the ignition method was utilized to determine the contents of crude fiber. Using following formula carbohydrates were calculated i.e.

$$\textit{Total carbohydrate (\%)} = 100 - (EE\% + CP\% + Ash\% + CF\%)$$

The gross energy was calculated by means of oxygen bomb calorimeter.

Immunological analysis

Blood samples were gathered in the absence of anticoagulants to study the parameters related to immunity. The number of erythrocytes and leukocytes were counted by blood smears. The lymphocytes, monocytes, neutrophils, and eosinophils were numbered through Neubauer differential counting method. Before analyzing, serum samples were separated by centrifugation. Samples were frozen at the temperature of -20°C.

Evaluation of minerals absorption

After appropriate dilution, mineral contents were determined using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) according to AOAC (1995). The estimation of sodium and potassium was carried out through flame photometer (Jenway PFP-7, UK). Chromic oxide contents in diets and faeces were estimated after oxidation with molybdate reagent (Divakaran *et al.*, 2002) using UV-VIS 2001 Spectrophotometer at 370nm absorbance.

Table 2: Chemical composition of the carcass of *C. carpio* given control and experimental diets containing different levels of BM (BM).

Test diets	BM level (gkg^{-1})	Crude protein (%)	Crude fat (%)	Gross energy (kcal/g)	Ash (%)	Crude fiber (%)	Carbohy-drates (%)	Moisture (%)
BM-I	0	$13.62±0.68^{bc}$	$6.57±0.58^{abc}$	$2.23±0.30^{ab}$	$5.41±0.41^{ab}$	1.51±0.20	2.49±0.24	$68.16±0.77^{bc}$
BM-II	10	$15.59±0.77^{ab}$	$7.09±0.31^{ab}$	$2.34±0.41^{ab}$	$5.70±0.37^{ab}$	1.44±0.20	2.40±0.29	$65.44±1.41^{cd}$
BM-III	20	$16.70±1.00^{a}$	$7.60±0.79^{a}$	$2.63±0.35^{a}$	$6.03±0.32^{a}$	1.53±0.26	2.36±0.17	$63.15±0.92^{d}$
BM-IV	30	$15.32±0.77^{ab}$	$7.22±0.90^{ab}$	$2.50±0.30^{a}$	$5.34±0.37^{ab}$	1.48±0.15	2.44±0.13	$65.69±1.91^{cd}$
BM-V	40	$12.52±0.78^{cd}$	$5.59±0.55^{bc}$	$2.34±0.32^{ab}$	$5.09±0.34^{ab}$	1.46±0.19	2.48±0.21	$70.52±1.18^{ab}$
BM-VI	50	$11.15±0.61^{d}$	$5.33±0.47^{c}$	$1.51±0.15^{b}$	$4.87±0.35^{b}$	1.35±0.13	2.48±0.28	$73.31±1.03^{a}$

The observed values are the mean of three replicates; ± expresses SDM (Standard Deviation Mean). Superscripts ($^{a-d}$) are used to indicate the significant differences between the highest and lowest values given in the columns.

Table 3: Immunological indices of *C. carpio* given control and experimental diets containing different levels of BM (BM).

Test diets	BM levels %	WBC (10^3mm^{-3})	Lymphocyte (%)	Eosinophile (%)	Monocyte (%)	Neutrophile (%)
BM-I	0	6.93±0.45	$20.33±0.77^{bc}$	$1.42±0.24^{ab}$	$1.87±0.24^{b}$	$76.38±0.76^{a}$
BM-II	10	7.04±0.85	$19.69±0.87^{c}$	$1.75±0.13^{a}$	$2.63±0.37^{ab}$	$75.93±1.11^{ab}$
BM-III	20	7.48±0.58	$22.44±0.71^{ab}$	$1.22±0.16^{b}$	$2.74±0.27^{a}$	$73.61±0.72^{bc}$
BM-IV	30	7.22±0.28	$23.74±0.85^{a}$	$1.18±0.21^{b}$	$2.81±0.33^{a}$	$72.27±0.70^{c}$
BM-V	40	6.19±0.61	$20.72±0.86^{bc}$	$1.61±0.13^{ab}$	$2.14±0.25^{ab}$	$75.53±0.91^{ab}$
BM-VI	50	5.99±0.45	$21.65±0.84^{abc}$	$1.80±0.22^{a}$	$2.17±0.35^{ab}$	$74.38±1.30^{abc}$

The observed values are the mean of three replicates; ± expresses SDM (Standard Deviation Mean). Superscripts ($^{a-d}$) are used to indicate the significant differences between the highest and lowest values given in the columns.

Statistical analysis

ADC% (apparent digestibility coefficient) statistics of minerals (P, K, Mg, Na, Ca, and Cu) absorption, carcass composition (gross energy, crude fat, and crude protein), and immunological parameter were disclosed to Analysis of Variance ANOVA (Steel and Torriej, 1996). The differences present in treatments were then compared with Tukey's honesty significant difference test and considered significant at $p<0.05$ (Snedecor and Cochran, 1991). The CoStat Computer Package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was utilized for this statistical analysis.

Results and Discussion

The chemical composition of *C. carpio* fed with the control diets and different experimental diets are given in Table 2 and presented graphically in Figure 1. The result indicates that the maximum level of crude protein (17%), crude fat (8%), gross energy (3kcal/g), ash (6%), and crude fiber (2%) were observed in the fingerlings given BM-III (20% FM was replaced with BM) diet. The second highest values of crude protein (16%) and ash (5.7%) were seen in *C. carpio* fingerlings fed BM-II (replacement of 10% FM with BM). While the second-highest levels of crude fat (7%), gross energy (2.5%), and crude fiber (1.48%) were observed in the fish group given BM-IV (30% FM replacement with BM). The lowest crude protein (11%), crude fat (5%), gross energy (1.5kcal/g), ash (5%), and crude fiber (1.35%) were found in fingerlings that ate diet VI (50% FM replacement with BM). In all fish groups given diets with increasing levels of BM, the values of carbohydrates were significantly at par with each other. The highest level of carbohydrates (2.5%) was found in fish given BM-I (control diet with 0% BM) subsequently the second-highest values were noticed in *C. carpio* fingerlings which consumed BM-V (substitution of 40% FM with BM) and BM-VI (50% FM replacement with BM). While the lowest value (2.36%) was noticed in fingerlings fed on BM-III. The maximal moisture level (73%) was observed in the carcass of juveniles fed BM-VI in which 50% of FM was partially replaced by BM followed by (71%) in group V (FM replacing BM at 40%) whereas the minimum level of moisture (63%) was recorded in the fish group which consumed BM-III.

For the determination of the fish's health and an indication of the presence of disease, immunity indices are a good tool. The immunity parameters are presented in Table 3 and graphically in Figure 2. The immunological indices analysis reveals the contrasting results in the *C. carpio* fingerlings fed different test diets. In the fish given the BM-III (20% partial replacement of FM with BM) the highest levels of WBCs ($7.5×10^3mm^{-3}$) were noticed. While the second highest values of WBCs ($7.2 × 10^3 mm^{-3}$) were observed in the fingerlings of the fed BM-IV (30% FM substitution with BM), the minimum WBCs level was $6×10^3mm^{-3}$ observed in group VI in which 50%

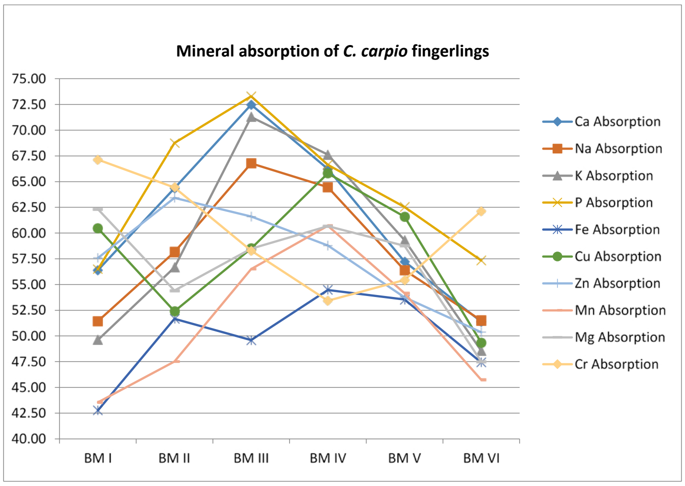

Figure 1: Cumulative carcass composition of *C. carpio* fingerlings given BM-diets.

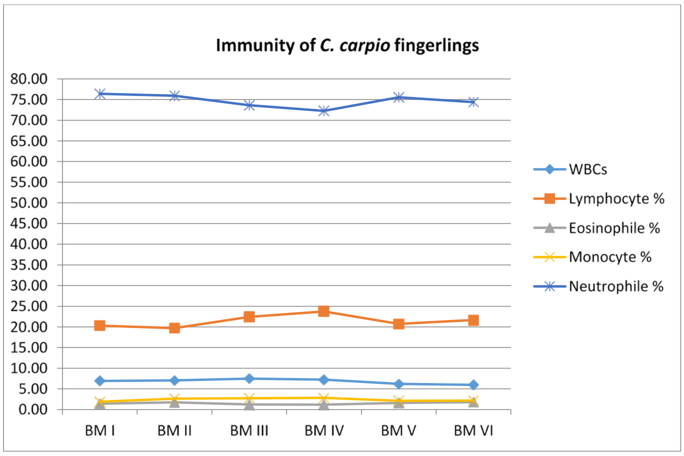

Figure 2: Cumulative immunity of *C. carpio* fingerlings given BM-diets.

FM was replaced with BM. The values of WBCs were significantly at par in six *C. carpio* fingerlings groups. The maximum levels of monocytes (3%) and lymphocytes (24%) were noticed in *C. carpio* fingerlings that fed BM-IV (30% FM substitution with BM) followed by 2.7% of monocytes and 22% of lymphocytes in the fish group given BM-III (FM replaced with BM at 20% inclusion level). Monocytes' lowest (1.87%) percentage was observed in *C. carpio* fingerlings groups fed BM-I (0% FM replacement). The lowest level of lymphocytes was 19.69% noticed in the fish-fed diet with 10% of FM replaced by BM (BM-II). The maximum percentage of eosinophil (2%) was noticed in fish given test-VI (50% inclusion of BM) afterward (1.4%) was seen in BM-I (basal diet, 0% FM replacement). The highest neutrophil level (76%) was observed in fish fed the control diet followed by group II. The least eosinophil (1.18%) and neutrophil (72%) were noticed in fish fed BM-IV with inclusion level of BM up to 30%.

Table 4 indicated that the level of minerals in all the diets including the basal diet (BM-I) was significantly at par with each other. However, differences in mineral levels in feces were observed in *C. carpio* fingerlings fed BM-based diets and a control diet according to Table 5. Ca (0.5%), Na (0.005%), K (0.79%), Mg (0.005%), Cu (0.003%), and Zn (0.02%), the level of excretion were maximum in the group fed BM-VI (50% FM substitution with BM). However, the excretion level of P (0.95%), Fe (0.03%), and Mn (0.02%) was maximal in *C. carpio* fingerlings given a control diet with 0% FM replacement. While Cr (0.018%) was found to be excreted in fish that consumed BM-V (40% FM replacing BM). Minimum excretion of minerals, Ca (0.28%), Na (0.003%), K (0.44%), and P (0.59%) were observed in BM-III (FM replacement with BM at 20%). The lowest value of Mg (0.003%) was seen in a group on the fish-fed basal diet (BM-I). In the case of Cu (0.002%) and Mn (0.01%), the lowest was witnessed in a group fed BM-IV (30% partial FM substitution with BM). While Zn (0.014%) excretion in feces was lowest in *C. carpio* fingerlings who ate a diet with 20% of BM (BM-III). The lowest level of Cr (0.012%) was seen in group I, in which 0% FM was replaced with BM. The lowest mineral discharge resulted in higher mineral digestibility.

The highest levels of absorption of Ca (72.5%), Na (67%), K (71%), and P (73%) were observed in *C. carpio* fingerlings given BM-III (20% FM replacement with BM) as shown in Table 6. The mineral absorption is shown graphically in Figure 3. The second highest values of Ca (66%), Na (64.5), and K (68%) were seen in fish fed 30% FM replacement with BM diet (BM-IV), while that of P (69%) was found in BM-II (10% FM replacement). Fe (54%), Cu (66%), and Mn (61%) were best in *C. carpio*

Table 4: Analyzed mineral absorption in the diets of *C. carpio* fingerlings containing BM

Test diets	BM level (g/kg)	Ca (%)	Na (%)	K (%)	P (%)	Fe (%)	Cu (%)	Zn (%)	Mn (%)	Mg (%)	Cr (%)
BM-I	0	0.93±0.06	0.01±0.00	1.42±0.12	2.05±0.06	0.05±0.01	0.01±0.00	0.03±0.01	0.03±0.01	0.01±0.00	0.03±0.01
BM-II	10	0.93±0.04	0.01±0.00	1.43±0.13	2.06±0.12	0.05±0.01	0.01±0.00	0.04±0.01	0.03±0.01	0.01±0.00	0.04±0.01
BM-III	20	0.93±0.05	0.01±0.00	1.42±0.14	2.05±0.10	0.05±0.01	0.01±0.00	0.03±0.00	0.03±0.01	0.01±0.00	0.03±0.01
BM-IV	30	0.92±0.04	0.01±0.00	1.44±0.10	2.06±0.12	0.05±0.00	0.01±0.00	0.04±0.01	0.03±0.01	0.01±0.00	0.04±0.01
BM-V	40	0.93±0.06	0.01±0.00	1.44±0.10	2.07±0.12	0.05±0.01	0.01±0.00	0.04±0.01	0.03±0.01	0.01±0.00	0.04±0.01
BM-VI	50	0.93±0.07	0.01±0.00	1.44±0.07	2.06±0.10	0.05±0.01	0.01±0.00	0.04±0.01	0.03±0.01	0.01±0.00	0.04±0.01

The observed values are the mean of three replicates; ± expresses SDM (Standard Deviation Mean). Ca; calcium, Na; sodium, K; potassium, P; phosphorous, Fe; iron, Cu; copper, Zn; zinc, Mn; manganese, Mg; magnesium and Cr; chromium.

Table 5: Analyzed mineral absorption in the feces of *C. carpio* fingerlings containing BM.

Test diets	BM level	Ca (%)	Na (%)	K (%)	P (%)	Fe (%)	Cu (%)	Zn (%)	Mn (%)	Mg (%)	Cr (%)
BM-I	0	0.43± 0.03^{ab}	0.00441± 0.00024^{a}	0.77± 0.04^{ab}	0.95± 0.04^{a}	0.03161± 0.00378	0.00261± 0.00040^{ab}	0.01567± 0.0026	0.01547± 0.00486	0.003493± 0.000326^{b}	0.01221± 0.00243
BM-II	10	0.36± 0.03^{bc}	0.00393± 0.00017^{ab}	0.67± 0.04^{bc}	0.69± 0.03^{de}	0.02733± 0.00335	0.00325± 0.00042^{ab}	0.01393± 0.00176	0.0142± 0.00332	0.00422± 0.000382^{ab}	0.01390± 0.00291
BM-III	20	0.28± 0.02^{d}	0.003043333± 0.00013^{c}	0.44± 0.03^{d}	0.59± 0.04^{e}	0.02867± 0.00245	0.00280± 0.00032^{ab}	0.01393± 0.00176	0.01218± 0.00244	0.003893± 0.00023^{b}	0.01507± 0.00280
BM-IV	30	0.34± 0.02^{cd}	0.003293333± 0.00027^{bc}	0.50± 0.02^{d}	0.74± 0.05^{cd}	0.02620± 0.00160	0.00229± 0.00018^{b}	0.01547± 0.0026	0.01069± 0.00252	0.0037± 0.000115^{b}	0.01507± 0.00280
BM-V	40	0.42± 0.03^{ab}	0.004013333± 0.00048^{ab}	0.62± 0.04	0.83± 0.04^{bc}	0.02680± 0.00329	0.00250± 0.00047^{ab}	0.01743± 0.00384	0.01296± 0.00427	0.003833± 0.000397^{b}	0.01813± 0.00117
BM-VI	50	0.48± 0.03^{a}	0.004503667± 0.00045^{a}	0.79± 0.04^{a}	0.93± 0.04^{ab}	0.03033± 0.00309	0.00334± 0.00033^{a}	0.01897± 0.00285	0.01499± 0.00502	0.00494± 0.000252^{a}	0.01433± 0.00223

The observed values are the mean of three replicates; ± expresses SDM (Standard Deviation Mean). Superscripts ($^{a-e}$) are used to indicate the significant differences between the highest and lowest values given in the columns. Ca, calcium; Na, sodium; K, potassium; P, phosphorous; Fe, iron; Cu, copper; Zn, zinc; Mn, manganese; Mg, magnesium; Cr, chromium.

Table 6: Analyzed Mineral Absorption of *C. carpio* fingerlings fed BM diets ± expresses SDM (Standard Deviation Mean). Superscripts ($^{a-e}$) are used to indicate the significant differences between the highest and lowest values given in the columns.

Test diets	BM level (g/kg)	Ca (%)	Na (%)	K (%)	P (%)	Fe (%)	Cu (%)	Zn (%)	Mn (%)	Mg (%)	Cr(%)
BM-I	0	56.40± 0.75^{c}	51.44± 0.72^{c}	49.62± 0.97^{e}	56.49± 0.84^{d}	42.76± 0.92^{e}	60.47± 0.81^{bc}	57.58± 0.86^{b}	43.56± 0.88^{d}	62.32± 0.92^{a}	67.14± 0.84^{a}
BM-II	10	64.36± 0.89^{b}	58.16± 0.90^{b}	56.66± 0.63^{d}	68.75± 0.94^{b}	51.68± 0.91^{bc}	52.39± 0.79^{d}	63.42± 0.96^{a}	43.56± 0.88^{c}	54.41± 0.85^{c}	64.43± 0.91^{b}
BM-III	20	72.47± 0.73^{a}	66.77± 0.99^{a}	71.29± 0.94^{a}	73.30± 0.95^{a}	49.59± 0.79^{cd}	58.51± 0.79^{c}	61.61± 0.78^{a}	56.50± 0.93^{b}	58.49± 0.56^{b}	58.29± 0.84^{d}
BM-IV	30	66.23± 0.95^{b}	64.47± 0.97^{a}	67.62± 0.94^{b}	66.63± 0.89^{b}	54.47± 0.88^{a}	65.81± 0.87^{a}	58.79± 0.99^{b}	60.71± 0.99^{a}	60.67± 0.79^{ab}	53.42± 0.57^{e}
BM-V	40	57.22± 0.97^{c}	56.39± 0.90^{b}	59.31± 0.87^{c}	66.63± 0.89^{c}	53.54± 0.73^{ab}	65.81± 0.87^{b}	53.75± 0.92^{c}	54.15± 0.99^{b}	58.76± 0.87^{b}	55.43± 0.70^{e}
BM-VI	50	51.40± 0.97^{d}	51.50± 0.95^{c}	48.58± 0.91^{e}	57.34± 0.63^{d}	47.43± 0.75^{d}	49.35± 0.76^{e}	50.37± 0.91^{d}	45.74± 0.73^{cd}	47.51± 0.88^{d}	62.13± 0.91^{c}

Ca, calcium; Na, sodium; K, potassium; P, phosphorus; Fe, iron; Cu, copper; Zn, Zinc; Mn, manganese; Mg, magnesium; Cr, chromium.

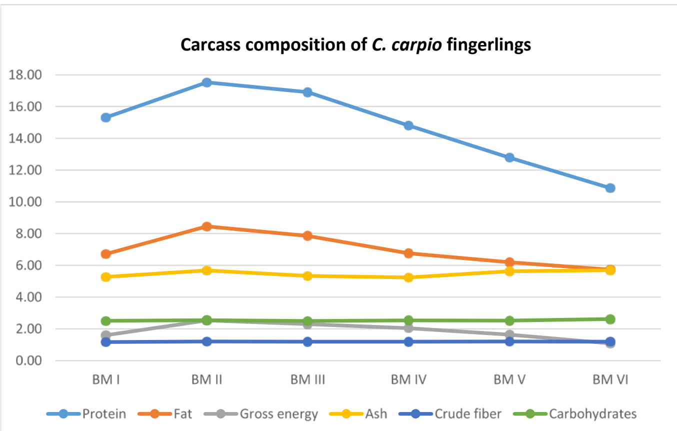

Figure 3: Cumulative mineral absorption of *C. carpio* fingerlings given BM-diets.

fingerlings that consumed BM-IV. While Mg (62%) and Cr (67%) were maximum in group I (basal diet). The highest Zn level (63%) was seen in the fish group given 10% FM replacement with BM (BM-II). The second highest levels of Fe (53.5%) and Cu (66%) were found to be in group V whereas Zn (62%) and Mn (56.5%) were noticed in BM-III. Mg (61%) secondary level was observed in BM-IV and Cr (64%) were witnessed in group II. The minimum level of Ca (51%), K (49%), Cu (49%), Zn (50%), and Mg (47.5%) were found to be in fish given BM-VI. However, Na (51%), P (56.5%), Fe (43%), and Mn (44%) were the lowest in BM-I and Cr (53%) was seen in the fish group fed BM-IV (30% FM partially substituted by BM).

In the present research, the result indicates that the maximum level of crude protein, crude fat, gross energy, crude fiber, and ash was observed in 20% partial FM replacement with BM. Similar to our results, Burr *et al.* (2013) observed in the research that inclusion of barley protein concentrate (BPC) at 22% was acceptable for *Salmo salar* (Atlantic salmon). Zaretabar *et al.* (2021) also found the alike result for *Salmo trutta caspius* (Caspian brown trout) which were given 25BPC and control diets the levels of crude protein and the crude energy were significant as compared to fish given other diets. Similarly, according to the results of the study, 35% of fish meal can be substituted by a co-blend consisting of 25% washed barley and 75% washed soybean in the diet of rainbow trout (Salh, 2020). A slightly different result from the present experiment was seen when Belal (1999) performed an experiment on *Oreochromis niloticus* (L.) in which diets were formulated in such a way that dietary corn was substituted with ground barley at 0%, 15%, 30%, and 51%. The proximate composition of fingerlings indicated that when levels of barley were elevated significant differences were not observed in the crude protein, crude fat, body moisture, and total ash. While Rossi *et al.* (2013) witnessed contradictory results in comparison to the current research trial. They concluded that Barley-PC and Soy-PC could substitute fishmeal up to 50% which can produce the chemical composition of fingerlings like that of the control diet. Whereas, in another experiment, as a source of protein, Barley Protein Concentrate (BPC) was used in feeds of *S. salar*. The outcome was in contrast to the current study as there was no significant difference in crude lipid and moisture data obtained from the whole analysis of fish. The content of ash and protein was notably lower in the fish group fed BPC50 (Bell *et al*., 2014).

The best immunity was found in *C. carpio* fingerlings fed 20% partial fishmeal replacement with BM (BM-III) in this trial. Similar results were found in another trial, in which the effects of substitution of FM with graded levels of fermented oilseed meal (0%, 25%, 50%, 75%, and 100%) on the immunity of *Pagrus major* were investigated. The findings revealed that at a medium level (25%) replacing fermented oilseed meal with fishmeal increased the immune reactions (Dossou *et al*., 2018). Reda *et al.* (2022) concluded identical to the present study that oilseed (rice) protein concentrate can be used to replace up to 25% of fish meal in the diet of *O. niloticus,* while also enhancing fish immunity and disease resistance. A bit different results in comparison with the present study were observed, where dietary inclusion of processed oilseed meal had no effect on mucous innate immunity. Five diets for Nile tilapia were created by excluding the dietary fish meal and including 0 (basal), 12.5%, 25%, 37.5%, and 50% oilseed meal (Mohammadi *et al*., 2020). A different result from the current experimentation was found in research on changing fish meal with plant sources on immunological responses of rainbow trout. There was no significant influence on white blood cells (Jalili *et al*., 2013). Contrary to the present research, Hossain *et al.* (2018b) observed that *P. major* groups with substituted fishmeal showed improved innate immunity in the fish-fed diet FM25 (fishmeal protein was substituted with oilseed protein concentrate at 75%) when compared to the control diet. Zhao *et al.* (2016) also determined that unlike the present experiment the 50% of fish meal replacement with oilseed (D-50) diet might be utilized to boost immunological responses in yellow catfish. The result was contrasting to the current experimentation.

It can be witnessed that discharge of minerals through feces was maximum in *C. carpio* fingerlings fed 50% FM partially replaced with BM (BM-VI). While mineral absorption was best in the group given BMIII, in which 20% of FM was substituted with BM. And some minerals showed the highest absorption at 30% BM partially replacing FM (BM-IV) as a source of protein. Mineral digestion in fish using plant-based alternatives has received little attention (Araújo *et al*., 2018). As the present study showed that P absorption in *C. carpio* was highest at 20% BM replacing FM (BM-III), another study done by Satoh *et al.* (2003) indicated that the retention of P was best in the type of experimental diet for rainbow trout which was formed with 20% soybean meal, 25% corn gluten meal, 5% blood meal, and 20% FM as a protein source. A slightly varying result, when compared to the current research study, was also obtained by Mbahinzireki *et al.* (2001). It was indicated that in the diet of tilapia, the substitution of FM with cottonseed meal (CSM) can be done up to 50%. The mineral composition indicated that when the plant seed meal was increased, the mineral level declined. In diet 3 (50% of CSM), the Cu and Mg were maximum and were significantly different. Ca level was maximum at 20% BM replacing FM in the current experimental trial, however, Falamarzi and Zanguee (2016) also observed a bit different result as Ca levels were significantly at par with each other in *C. carpio* fed diets formed by supplementing alfalfa at different levels. Completely opposite to the results of the present study Nwanna *et al.* (2007) observed when the plant feedstuffs were fermented with phytases, the digestibility of minerals in *C. carpio* was effectively better. The result witnessed by Dabrowski *et al.* (2009) was also contradictory to the present research as Ca was highest in *C. carpio* group fed 100% FM. The variations in findings might be attributed to differences in fish species, oilseed meals, and BM suppliers. Distinctions in feed formulation and drying technique might also provide differential results. The variations in findings might be attributed to differences in fish species, oilseed meals, and BM suppliers (Shahzad *et al*., 2022). Distinctions in feed formulation and drying technique might also provide differential results.

Conclusions and Recommendations

From the results of this experimental trial, it can be concluded that the improvement in carcass composition, immunity, and mineral absorption was seen in *C. carpio* fingerlings when fed on BM as a partial replacement of

FM. According to the analysis, 20% inclusion of BM (BM-III) enhanced carcass composition, immune system, and mineral absorption in fingerlings of common carp.

Acknowledgment

I offer my heartiest gratitude to University of Education, Lahore for providing the facilities regarding to the research work and analysis.

Conflict of interest

The authors have declared no conflict of interest.

References

Allan, G.L. and Rowland, S.J., 1992. Development of an experimental diet for silver perch (*Bidyanus bidyanus*). *Austasia Aqua*., **6:** 39-40.

AOAC, 1995. *Official methods of analysis*, 15th Ed. Association of Official Analytical Chemists, Washington D.C., USA., pp. 1094.

Araújo, J.G., Nebo, C., Pádua, D.M.C., Souto, C.N. and Guimarães, I.G., 2018. Apparent Digestibility of Minerals from Several Ingredients for Tambaqui, *Colossoma macropomum*, Juveniles. *J. World Aquacult. Soc.*, **49:** 1026–1038. https://doi.org/10.1111/jwas.12517

Ayoola, A., 2010. Replacement of fishmeal with alternative protein sources in aquaculture diets.

Becker, J. and Calado, R., 2021. Aquaculture, fish and fisheries: A new home for the Blue Revolution. *Aquacult. Fish Fisheries.*, **1:** 1-2. https://doi.org/10.1002/aff2.4

Belal, I.E.H., 1999. Replacing dietary corn with barley seeds in Nile tilapia,*Oreochromis niloticus* (L.), feed. *Aquacut. Res*., **30:** 265–269. https://doi.org/10.1046/j.1365-2109.1999.00323.x

Bell, J., Strachan, F., Roy, W., Matthew, C., Mcdonald, P., Barrows, F. and Sprague, M., 2014. Evaluation of barley protein concentrate and fish protein concentrate, made from trimmings, as sustainable ingredients in Atlantic salmon (*Salmo salar* L.) feeds. *Aquacult. Nut*., **22:** 326–334. https://doi.org/10.1111/anu.12250

Burr, G.S., Barrows, F.T. and Wolters, W.R., 2013. Growth of *Atlantic salmon, Salmo salar*, fed diets containing barley protein concentrate. *J. appl. Aquacult.,* **25:** 320-328. https://doi.org/10.1080/10454438.2013.845641

Coloso, R.M., 2015. Feed formulation for sustainable aquaculture. In: Resource enhancement and sustainable aquaculture practices in Southeast Asia: Challenges in responsible production of aquatic species: Proceedings of the International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia 2014 (RESA). Aquaculture Department, Southeast Asian Fisheries Development Center. pp. 223-230.

Craig, S., Helfrich, L.A., Kuhn, D. and Schwarz, M.H., 2017. Understanding fish nutrition, feeds, and feeding.

Dabrowski, K., Krasnicki, K. and Kozlowska, H., 2009. Rapeseed meal in the diet for common carp reared in heated waters. *Zeitschrift Für Tierphysiologie Tierernährung Und Futtermittelkunde*., **46**: 273–282. https://doi.org/10.1111/j.1439-0396.1981.tb01364.x

Divakaran, S., Obaldo, L.G. and Forster, I.P., 2002. Note on the methods for determination of chromic oxide in shrimp feeds. *J. Agric. Fd. Chem*., **50:** 464-467. https://doi.org/10.1021/jf011112s

Dossou, S., Koshio, S., Ishikawa, M., Yokoyama, S., Dawood, M.A., El Basuini, M.F., Olivier, A. and Zaineldin, A.I., 2018. Growth performance, blood health, antioxidant status and immune response in red sea bream (*Pagrus major*) fed *Aspergillus oryzae* fermented rapeseed meal (RM-Koji). *Fish Shellfish Immunol.*, **75:** 253–262. https://doi.org/10.1016/j.fsi.2018.01.032

El-Sayed, A.F.M., 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. *Aquaculture*, **179:** 149–168. https://doi.org/10.1016/S0044-8486(99)00159-3

El-Sayed, A.F.M., 2020. Chapter 7: Nutrition and feeding. *Tilapia Cult.*, pp. 135–172. https://doi.org/10.1016/B978-0-12-816509-6.00007-0

Falamarzi, Z. and Zanguee, N., 2016. Effects of different levels of meal and alcoholic extract of alfalfa (*Medicago sativa*) on growth performance, nutrition, carcass biochemical and some serum biochemical parameters in common carp (*Cyprinus carpio*). *J. Fish*., **69:** 235-251.

FAO, 2016. FAO statistical pocketbook 2015: World food and agriculture. Food and Agriculture Organization of the United Nations (FAO).

FAO, 2020. The state of world fisheries and aquaculture 2020. Sustainability in action. Rome.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. and Toulmin, C., 2010. Food security: The challenge of feeding 9 billion people. *Science*, **327:** 812-818. https://doi.org/10.1126/science.1185383

High Level Panel of Experts on Food Security and Nutrition, 2014. Sustainable fisheries and aquaculture for food security and nutrition. A report by the high level panel of experts on food security and nutrition.

Hossain, M.S., Koshio, S., Ishikawa, M., Yokoyama, S., Sony, N.M. and Islam, M.J., 2018b. Fishmeal replacement by soya protein concentrate with inosine monophosphate supplementation influences growth, digestibility, immunity, blood health, and stress resistance of red sea bream, *Pagrus major*. *Fish Physiol. Biochem.*, **45:** 613–629. https://doi.org/10.1007/s10695-018-0581-2

Jalili, R., Tukmechi, A., Agh, N., Noori, F. and Ghasemi, A., 2013. Replacement of dietary fish meal with plant sources in rainbow trout (*Oncorhynchus mykiss*); effect on growth performance, immune responses, blood indices and disease resistance.

Jobling, M., 2001. Feed composition and analysis. *Fd Intake Fish.*, **827:** 1-24. https://doi.org/10.1002/9780470999516.ch1

Joordens, J.C., Kuipers, R.S., Wanink, J.H. and Muskiet, F.A., 2014. A fish is not a fish: Patterns in fatty acid composition of aquatic food may have had implications for hominin evolution. *J. Hum. Evol.*, **77:** 107-116. https://doi.org/10.1016/j.jhevol.2014.04.004

Kerckhoffs, D.A., Brouns, F., Hornstra, G. and Mensink, R.P., 2002. Effects on the human serum lipoprotein profile of β-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. *J. Nutr.*, **132:** 2494-2505. https://doi.org/10.1093/jn/132.9.2494

Khan, M.N., Shahzad, K., Chatta, A., Sohail, M., Piria, M. and Treer, T., 2016. A review of introduction of common carp *Cyprinus carpio* in Pakistan: origin, purpose, impact and management. *CJF*, **74:** 71-80. https://doi.org/10.1515/cjf-2016-0016

Kumar, M., Gupta, G., Pathak, N., Mishra, V. and Shukla, A., 2019. A review on replacing fish meal in aqua feeds using plant and animal protein sources. Unpublished.

Le Gouvello, R. and Simard, F., 2017. Durabilité des aliments pour poisson en aquaculture.

Lowe, S., Browne, M., Boudjelas, S. and De Poorter, M., 2004. 100 of the world's worst invasive alien species: A selection from the global invasive species database. Auckland, New Zealand: The Invasive Species Specialist Group. World Conservation Union. 6.

Mbahinzireki, G.B., Dabrowski, K., Lee, K.J., El-saidy, D. and Wisner, E.R., 2001. Growth, feed utilization and body composition of tilapia (*Oreochromis* sp.) fed cottonseed meal-based diets in a recirculating system. *Aquacult. Nutr.*, **7:** 189-200. https://doi.org/10.1046/j.1365-2095.2001.00172.x

Miles, R.D. and Chapman, F.A., 2006. Benefits of fish meal in aquaculture diets. *EDIS*, **2006**. https://doi.org/10.32473/edis-fa122-2006

Mohammadi, M., Imani, A., Farhangi, M., Gharaei, A. and Hafezieh, M., 2020. Replacement of fishmeal with processed canola meal in diets for juvenile Nile tilapia (*Oreochromis niloticus*): Growth performance, mucosal innate immunity, hepatic oxidative status, liver and intestine histology. *Aquacult*, **518:** 734824. https://doi.org/10.1016/j.aquaculture.2019.734824

Mondal, K. and Payra, P., 2015. A review on use of plant protein sources in diets for fish feed formulation. *Int. Multidiscip. Res. J.*, **3:** 257- 264.

Narwal, S., Kumar, D., KharuB, A.S. and Verma, R.P.S., 2020. *Barley biofortification: present status and future prospects*. Wheat and Barley Grain Biofortification, pp. 275–294. https://doi.org/10.1016/B978-0-12-818444-8.00011-0

Nwanna, L., Eisenreich, R. and Schwarz, F., 2007. Effect of wet-incubation of dietary plant feedstuffs with phytases on growth and mineral digestibility by common carp (*Cyprinus carpio* L). *Aquaculture*, **271:** 461–468. https://doi.org/10.1016/j.aquaculture.2007.04.020

Reda, R.M., Maricchiolo, G., Quero, G.M., Basili, M., Aarestrup, F.M., Pansera, L., Mirto, S., Abd El-Fattah, A.H., Alagawany, M. and Abdel Rahman, A.N. 2022. Rice protein concentrate as a fish meal substitute in *Oreochromis niloticus*: Effects on immune response, intestinal cytokines, Aeromonas veronii resistance, and gut microbiota composition. *Fish Shellfish Immunol.*, **126:** 237–250. https://doi.org/10.1016/j.fsi.2022.05.048

Rossi, W., Moxely, D., Buentello, A., Pohlenz, C. and Gatlin, D., 2013. Replacement of fishmeal with novel plant feedstuffs in the diet of red drum *Sciaenops ocellatus*: An assessment of nutritional value. *Aquacult. Nutr.*, **19:** 72–81. https://doi.org/10.1111/anu.12073

Rowland, S.J. and Ingram, B.A., 1991. *Diseases of Australian native fishes*. In: Fisheries Bulletin 4 NSW Fisheries, Sydney, NSW, Australia.

Salem, H.B. and Makkar, H., 2009. Defatted *Moringa oleifera* seed meal as a feed additive for sheep. *Anim. Feed Sci. Technol.*, **150:** 27–33. https://doi.org/10.1016/j.anifeedsci.2008.07.007

Salh, M.J.H., 2020. Modification of plant proteins and their potential application as fish meal replacements in rainbow trout *Oncorhynchus mykiss* Feeds. South Dakota State University.

Satoh, S., Hernández, A., Tokoro, T., Morishita, Y., Kiron, V. and Watanabe, T., 2003. Comparison of phosphorus retention efficiency between rainbow trout (*Oncorhynchus mykiss*) fed a commercial diet and a low fish meal based diet. *Aquaculture*, **224:** 271–282. https://doi.org/10.1016/S0044-8486(03)00217-5

Shahzad, M.M., Hussain, S.M., Hussain, M., Ahmad, N., Tahir, L. and Akhtar, K., 2022. Effect of eco-friendly probiotics-supplemented rapeseed meal-based diet on the performance of *Catla catla* fingerlings. *Environ. Sci. Pol. Res.*, https://doi.

org/10.1007/s11356-022-22738-x

Snedecor, G.W. and Cochran, W.G., 1991. *Statistical methods*. 8th Ed. Iowa State University Press, Americans. USA 503.

Steel, R.G. and Torriej, H., 1996. *Principles and procedures of statistics*. HcGraw-Hill Book Co., Singapore.

Sullivan, K.B., 2008. *Replacement of fish meal by alternative protein sources in diets for juvenile black sea bass*. Doctoral dissertation, University of North Carolina Wilmington.

United nations, 2019. *World population prospects 2019: Highlights*. Department of Economic and Social Affairs, Population Division.

Zaretabar, A., Ouraji, H., Abedian kenari, A., Yeganeh, S., Esmaeili, M. and Keramat amirkolaee, A., 2021. One step toward aquaculture sustainability of a carnivorous species: Fish meal replacement with barley protein concentrate plus wheat gluten meal in Caspian brown trout (*Salmo trutta caspius*). *Aquacult. Rep.*, **20:** 100714. https://doi.org/10.1016/j.aqrep.2021.100714

Zhao, Z.X., Song, C.Y., Xie, J., Ge, X.P., Liu, B., Xia, S.L., Yang, S., Wang, Q. and Zhu, S.H., 2016. Effects of fish meal replacement by soybean peptide on growth performance, digestive enzyme activities, and immune responses of yellow catfish *Pelteobagrus fulvidraco*. *Fish. Sci. Res.*, **82:** 665–673. https://doi.org/10.1007/s12562-016-0996-6