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Research Article

Dietary Energy Requirement in Bullseye Snakehead (*Channa marulius*)

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Abstract

The bullseye snakehead (*Channa marulius*) is commercially important fish species and depends largely on live feed because of its cannibalistic nature. Therefore, to ensure its sustainable production development of a cost effective optimized energy based nutrients diets is essential. This study was carried out to evaluate the impact of four isonitrogenous diets with varying dietary lipid levels (0, 4, 8 and 12 % fish oil) in triplicate groups of bullseye snakehead fish fingerlings. The formulated diet containing the optimal energy level of fish oil (F12 %) effectively enhanced the growth performance of bullseye snakehead. After the 90-day feeding trial, the highest net weight gain (63.45 ±5.78 g) was observed in F12 upon increasing lipid content. The fish fed with F8, F12 diets that contained 8, 12% fish oil showed significantly lower FCR than others. The activity of protease, lipase and amylase enzymes was found to be significantly higher ($P<0.05$) in F12 group for both liver and intestine, while significantly lower values were observed in control (F0) diet. The proximate composition and hematological parameters of fish showed significant differences among treatments. In conclusion, fish oil supplementation at 12 % (F12) can be recommended for enhanced growth, increased physiological and enzymatic activity in bullseye snakehead fingerlings, providing valuable practical insights for sustainable culture of bullseye snakehead.

Keywords: Dietary protein, dietary lipid level, growth performance, enzymatic activity, snakehead fish

1. Introduction

Worldwide aquaculture is a rapidly developing food producing sector that fulfils the protein needs of the increasing population. To overcome the increasing demand for protein and malnutrition issues, the intensive system of aquaculture has been established to boost the production of quality protein [1]. *Channa marulius* is an important commercial snakehead fish, commonly known as sole belongs to order Perciformes and family Channidae. It is native to Indo-Pak subcontinent and can survive in low oxygen environments. The Channa species are distributed all over South Asia and are in high demand for their market value and superior flesh quality [2]. They have considerable value as food fish due to their delicious taste and have many characteristics of culturable fish such as rapid growth, tolerance, ability to live in low water quality and resistant to many diseases. In 2020, the production of all species of snakehead reached 536,000 tonnes globally [3].

The problems concerned with culturing of snakehead fishes are its cannibalistic and predatory nature starting from larval stage to the adult phase, as they prey on fishes about half to 1/3rd of their body length. The cannibalistic behaviour is the main obstacle in the earthen ponds for culturing *Channa marulius*.

Different studies showed that cannibalistic behaviour is difficult to be stopped in fishes but can be controlled or reduced by providing natural feed or through weaning on formulated feed. Therefore, the culturing of snakehead fish depends mainly on the dietary requirements during the growth and development to raise the fingerlings in captivity [4].

High growth and survival rate can be observed in young fish fed on live feed as compared to compound feed. Fresh by products feed have disadvantages as compared to artificial feed because it could transmit various disease agents to fish. Moreover, it is difficult to find the nutritional quality of live feed and it is expensive as well [5]. That is why researchers are trying to find proper diet in place of live feed in primary stages of marine fish larvae [6]. The formulation of feed is utmost important because it can greatly affect the utilization of feed in the gastrointestinal tract, the enzymes activity during feed digestion, morphological changes and the diversity of microbes in the intestine of the aquatic animals thereby influencing their health and rate of growth [7].

Growth of fish can only be obtained by feeds that provide enough energy for maintaining the body functions. The major source of energy for fish is protein that is why optimal protein level in the diet is necessary to maximize growth of fish [8]. If good quality protein is not provided to fish, its growth will be affected which may result in weight loss or termination of growth [9]. The most expensive nutrient in fish feed is protein that is why it should be balanced in optimum range with all important amino acids needed for better growth [10].

The bullseye snakehead being a carnivorous fish requires high protein feed as compared to other herbivorous fishes. The crude protein level for the carnivorous fish ranges between 45% and 55% [11]. The requirement of lipid differs among different species of fish which is an important component of the diet after protein. It enhances tolerance to low temperatures by improving the performance [12]. The maintenance of optimum levels of energy in the formulated feed is necessary because low energy level will result in the consumption of proteins for energy instead of protein synthesis.

Lipids remain rich energy source and affect the growth rate as well as feed efficiency including palatability and dustiness of feed. Mostly they are made up of triglycerides, which are important for animals due to their high energy value [13]. The chief lipid source in the diet of fish is fish oil for its higher level of unsaturated omega-3 fatty acids crucial for better health and growth of cultivated fish [14]. Fish is not capable to synthesize the n-3 fatty acids therefore it is added in their diet during feed formulation [15]. The present study is designed to determine the optimal dietary lipid level required for enhancing growth and physiological health and improving enzymatic efficiency in *Channa marulius* fingerlings. Thus, this study will assess the effect of varying energy level diets (0%, 4%, 8%, and 12%) on the growth, hematology, digestive enzymes activity and nutrients profile of *Channa marulius* during fingerling stage.

2. Material and Methods

2.1. Fish and research facilities

Bullseye snakehead fish *Channa marulius* (Sole) was sourced from within the Department of Fisheries and Aquaculture farm facility at the University of Veterinary and Animal Sciences, Punjab, Pakistan. The trial was undertaken over twelve replicate earthen ponds with a dimension of (4.5 × 3.0 × 1.5 m) and each dietary treatment allocated into three ponds (n-3). The experimental fishes of 25-30 g were stocked in ponds, with stocking density of 10 fish per pond. Feed was given twice daily (08:00 and 16:00hours) at a body ration level of 3 % of the fish wet body weight for 90 days. The procedures and methods used in the study followed the research ethical and animal welfare guidelines of the University of Veterinary and Animal Sciences, Lahore, Pakistan.

2.2. Test diets formulation and production

Four test diets with increasing levels of fish oil (0 % (F0), 4% (F4), 8 % (F8), and 12% (F12)) were formulated whilst maintaining the same dietary protein level. Increasing amount of fish oil was combined to provide graded lipid levels (Table 1). The feed ingredients were grounded into powdered form and mixed in domestic food processor. The dough for further pelletisation was prepared by adding tap water. The dough was passed through laboratory pelleting machine of 2 mm diameter.

Table 1. Ingredients composition of experimental diets (% dry weight).

	Dietary treatments			
Ingredients	F0%	F4%	F8%	F12%
Fish meal[a]	25.00	25.00	25.00	25.00
Poultry / Offal meal[a]	10.00	10.00	10.00	10.00
Corn gluten[a]	14.00	14.00	14.00	14.00
Soybean meal[a]	20.00	20.00	20.00	20.00
Meat & bone meal[a]	11.00	11.00	11.00	11.00
Wheat flour	16.00	12.00	8.00	4.00
Fish oil	0.00	4.00	8.00	12.00
Vitamin premix[b]	2.00	2.00	2.00	2.00
Mineral premix[c]	2.00	2.00	2.00	2.00
Proximate composition (%)				
Protein	42.16	41.46	41.22	40.76
Lipid	7.48	13.36	15.32	19.24
Fibre	2.68	2.53	2.48	2.38
Ash	11.04	10.94	10.91	10.84
Crude energy; MJ/kg	12.09	13.35	14.03	15.00

[a]Fish meal (50 % CP), Offal meal, Soybean meal, Meat and bone meal and Corn gluten 30 % purchased from Aqua Feeds Pvt ltd.
[b]Vitamin mixture: Vitamin A 3500,000 IU/kg, vitamin B1 3500 mg/kg, Vitamin D3 1,750,000 IU/ kg, Ca gluconate 40 g/kg, vitamin E 3.500 mg/kg, vitamin PP (nicotinamide) 30 g/kg, sorbitol 20 g/kg (Fivevet, Central Veterinary Medicine JSC No. 5, Ha Noi, Vietnam).
[c]Mineral mixture: Ferrous sulphate 25 g/kg, calcium phosphate 397 g/kg, calcium lactate 328 g/kg, magnesium sulphate 137.5 g/kg, sodium chloride 60 g/kg, potassium chloride 50 g/kg, potassium iodide 150 mg/kg, manganese oxide 800 mg/kg, copper sulphate 780 mg/kg, cobalt carbonate 100 mg/kg, manganese oxide 820 mg/kg.

The pellets were dried for 24 hrs at 60 °C and finished diets were stored at 4 °C until use.

2.3. Sampling collection

The initial weight of fish body was measured at the time of stocking for chemical analysis. After every 15-day interval random samples of fish from all replicates were netted out by using drag net to record the morphometric measurements through digital weight balance.

2.4. Proximate whole body composition

The analysis of proximate composition of *Channa marulius* was determined in terms of crude protein, crude fat, ash content, moisture contents to assess the nutritional profile by following the protocol of Association of Official Analytical Chemist [16]. Six fish samples were collected randomly from each treatment and overdosed with buffered tricane methanesulphate (MS-222, Syndel's Canada, 150 mg L^{-1}). Then samples were oven dried at 70 °C. The dried fish were subjected to homogenization by mortar and pestle to prepare a fine powder. The Crude protein was determined by using Kjeldhal apparatus (Digestion block 60,079,776 and Kjeltec distillation 8100, FOSS, Hilleroed, Denmark), moisture by oven dry (Universal Oven UN260, Memmert GmbH, Schwabach, Germany), crude fat by using Soxhlet apparatus (R106S, Behr Labor-Technik, Düsseldorf, Germany). The proximal portion of the gut was used to study the activity of protease enzyme which was assayed through method of Azocasein [17], and activities of amylase [18] and lipase [19] were determined by following specific procedures.

Blood was collected from three fish per pond at the caudal vein using EDTA coated tubes. The hematological parameters like WBC (white blood cells), PLT (platelet), RBC (red blood cells), HCT (haematocrit), HGB (hemoglobin), MCV (mean corpuscular volume), MCHC (mean corpuscular haemoglobin concentration), MCH (mean corpuscular hemoglobin), were determined by auto-hematological analyser (Celltac NIHON KOHDEN 6550, Raiffeisenstrasse, Germany) in general laboratory of the Department of Fisheries and Aquaculture, UVAS Ravi Campus, Pattoki. The physico-chemical parameters of pond water, such as temperature, total dissolved solids (TDS), dissolved oxygen, salinity, and pH were monitored regularly by using Hanna HI 9828/4–01 meter (Chelmsford, UK).

2.5. Growth performance indices

The growth parameters like feed conversion ratio (FCR), net weight gain (NWG) and specific growth rate (SGR) were calculated by using following equations

$$\text{Weight gain (g)} = \text{average final weight} - \text{average initial weight} \quad \text{Equation (1)}$$

$$\text{SGR (\%/day)} = \frac{\ln(\text{final wet body weight} - \text{initial wet body weight})}{\text{Number of days}} \times 100 \quad \text{Equation (2)}$$

$$\text{FCR} = \frac{\text{Feed consumed (g)}}{\text{weight gained (g)}} \quad \text{Equation (3)}$$

$$\text{Survival rate (\%)} = \frac{\text{total number of harvested}}{\text{total number of stocked}} \times 100 \quad \text{Equation (4)}$$

2.6. Statistical analysis

Data was subjected for statistical analysis to SPSS statistical software (version 20.0) for one-way ANOVA for growth and body composition and correlation matrix for physico-chemical parameters. Means of significant treatments were compared through Duncan's Multiple Range Test [20].

3. Results

The inclusion of fish oil in the experimental diet significantly and positively affected the growth of *Channa marulius* in the study by increasing varying energy levels (Table 2) (Figure 1A and B). The results of this study showed a significant increase in weight gain, specific growth rate and final weight from the beginning of the experimental trial in F0 (0% fish oil), F4 (4% fish oil), F8 (8% fish oil) and F12 (12% fish oil) to the end of trial respectively. The fish fed with F8, F12 diets that contained 6 and 12% fish oil, respectively showed significantly best FCR than all others that were significantly less efficient. The results were found non-significant (P<0.05) for all groups regarding feed intake of *Channa marulius*. The outcomes showed that increasing lipid level did not significantly affect the survival of fish. However above 90% survival was observed among all the groups (Table 2).

Table 2. Impact of varying energy levels on the growth of bullseye snakehead (*Channa marulius*).

Parameters	Dietary treatments				
	F0	F4	F8	F12	*P value*
Initial weight; g/fish	28.34±0.44[a]	29.13±2.10[a]	28.04±0.51[a]	27.92±0.73[a]	0.600
Final weight; g/fish	55.60±2.63[a]	66.98±1.05[b]	78.30±2.72[c]	91.37±6.21[d]	0.000
Net weight gain; g/fish	27.26±2.80[a]	37.84±2.75[b]	50.26±2.39[c]	63.45±5.78[d]	0.000
Weight gain; %	96.27±10.62[a]	130.77±18.46[b]	179.24±7.21[c]	227.17±12.35[d]	0.000
SGR; % /day	0.56±0.04[a]	0.69±0.06[b]	0.85±0.02[c]	0.98±0.04[d]	0.000
FCR	1.65±0.18[c]	1.15±0.09[b]	0.83±0.08[a]	0.68±0.06[a]	0.000
Feed intake; g/fish	44.66±0.57[a]	43.66±2.08[a]	41.66±2.08[a]	43.00±1.00[a]	0.208
Survival rate %	98.33±0.57[a]	97.33±1.15[a]	97.66±1.15[a]	98.00±1.73[a]	0.777

Mean±SD, n=3. Dietary treatments: 0 % (F0); 4 % (F4); 8 % (F8); 12 % (F12) fish oil inclusion level. *Mean values in the same row with different superscripts were significant (P<0.05).

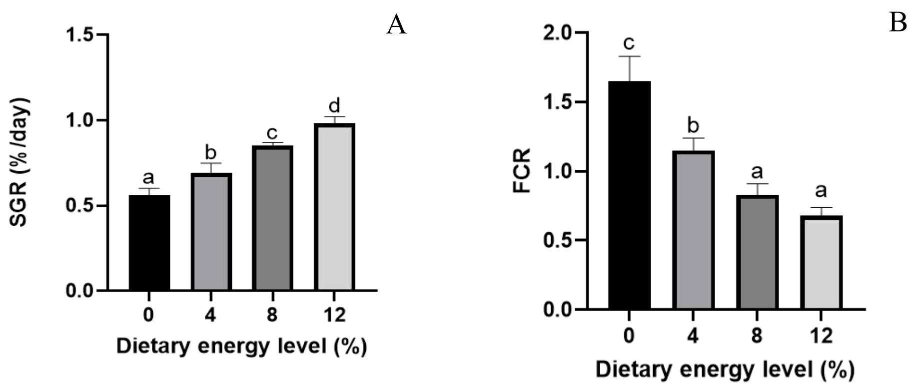

Figure 1. (A) Specific growth rate (SGR) and (B) Feed conversion ratio (FCR) of *C. marulius* in response to varying energy levels.

Table 3. Proximate composition of *Channa marulius* after experimental trial (%, wet weight)

Parameters	Dietary treatments				
	F0	F4	F8	F12	***P value***
Moisture	76.40 ±0.43^{a}	75.10 ±0.03^{b}	74.18 ±0.04^{c}	72.64 ±0.48^{d}	0.000
Crude Protein	16.5 1±0.34^{a}	17.87 ±0.15^{b}	18.64 ±0.10^{c}	19.84 ±0.22^{d}	0.000
Crude Lipids	2.60 ±0.08^{a}	2.91 ±0.41^{ab}	3.95 ±0.66^{bc}	4.30 ±0.11^{c}	0.033
Ash	1.88 ±0.05^{a}	1.98 ±0.5^{b}	2.31 ±0.08^{c}	3.09 ±0.06^{d}	0.000

Mean±SD, n=3. Dietary treatments: 0 % (F0); 4 % (F4); 8 % (F8); 12 % (F12) fish oil inclusion level. *Means with in a row with dissimilar superscripts showed significantly different values.

Table 4. The effect of increasing dietary energy on haematological status of bullseye snakehead (*Channa marulius*).

Parameters	Dietary treatments				
	F0	F4	F8	F12	*P value*
White blood cells (WBCs; 10^3/µL)	37.12±2.45^{a}	56.06±1.62^{b}	88.43±1.94^{c}	94.06±1.60^{d}	0.000
Red blood cells (RBCs; 10^3/µL)	0.90±0.05^{a}	0.74±0.03^{a}	2.68±0.12^{b}	4.55±0.12^{c}	0.000
Hemoglobin HGBs; g/dL	12.36±0.25^{a}	13.06±0.15^{b}	13.80±0.10^{c}	15.46±0.20^{d}	0.000
Haematocrit HCT;%	6.23±0.15^{a}	4.66±0.15^{b}	7.50±0.20^{c}	9.50±0.10^{d}	0.000
Mean corpuscular volume (MCV; fL)	87.57±0.92^{a}	95.67±1.31^{b}	123.06±1.52^{c}	138.43±1.13^{d}	0.000
mean corpuscular haemoglobin concentration MCHC; g/dL	31.40±0.90^{a}	35.73±1.09^{b}	38.54±0.94^{c}	43.54±0.94^{d}	0.000
platelet (PLT)	45.44±0.81^{a}	49.40±0.79^{b}	53.59±1.06^{c}	57.45±0.91^{d}	0.000
Red blood cell distribution width (RDW; %)	6.31±0.84^{a}	7.47±0.35^{a}	12.07±0.66^{b}	15.89±0.50^{c}	0.000
Red blood cell distribution (RDW; %)	17.36±0.61^{a}	18.54±0.26^{b}	16.41±0.41^{c}	19.76±0.16^{d}	0.000

Mean±SD, n=3. Dietary treatments: 0 % (F0); 4 % (F4); 8 % (F8); 12 % (F12) fish oil inclusion level. *Means with different superscripts in a row show statistically significant ($P<0.05$) difference.

Table 5. The effect of dietary fish oil variation on endogenous enzymatic activity in bullseye snakehead (*Channa marulius*).

Parameters	Dietary treatments				
	F0	F4	F8	F12	*P value*
Amylase for liver	0.55±0.01^{a}	1.15±0.07^{b}	1.59±0.02^{c}	1.94±0.01^{d}	0.000
Amylase for intestine	0.81±0.00^{a}	1.15±0.03^{b}	1.51±0.03^{c}	1.87±0.02^{d}	0.000
Lipase for liver	2.64±0.11^{a}	3.72±0.25^{b}	4.75±0.11^{c}	7.10±0.15^{d}	0.000
Lipase for Intestine	2.77±0.10^{a}	3.88±0.12^{b}	5.0±0.02^{c}	7.14±0.03^{d}	0.000
Protease for liver	2.23±0.15^{a}	5.39±0.38^{b}	6.68±0.77^{c}	8.16±0.06^{d}	0.001
Protease for intestine	3.22±0.63^{a}	4.38±0.37^{b}	7.98±0.12^{c}	8.37±0.07^{c}	0.000

± SD, n=3. Dietary treatments: 0 % (F0); 4 % (F4); 8 % (F8); 12 % (F12) fish oil inclusion level. *Means in a row with different superscripts show significant ($P<0.05$) difference among treatments.

The impact of varying energy level diets on the whole-body proximate composition of *Channa marulius* is presented in table 3. The statistical analysis revealed a decline in moisture content as fish oil increased in the feed. In experimental groups, the crude lipid and protein levels increased with an increase in dietary lipid content. The proximate composition of fish showed significant (P<0.05) differences among treatments. This study suggested that fat content increased significantly by increasing lipid levels. The F12 showed the highest levels of dry matter, crude protein and crude fats.

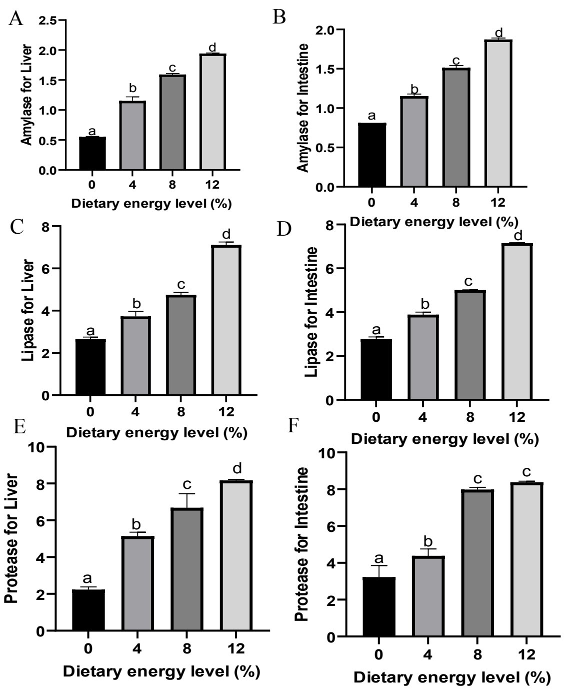

Figure 2. Digestive enzymes activities of (A &B) liver and (C and D) intestine in *C. marulius* fed varying dietary energy levels.

On the termination of experimental trial, different haematological parameters of *Channa marulius* were analysed through one-way ANOVA. White blood cells are major components of body immune system, their higher level play a significant role in providing basic defence mechanism against pathogens and foreign invaders. Present study showed significantly higher (P<0.05) number of WBCs in F12 group as compared to others. The significantly highest (P<0.05) number of RBCs and WBCs were observed in F12 followed by F8. The higher amount of haemoglobin, HCT, MCV, MCHC and PLT contents were observed in the F12 and lowest in control. The highest values of red blood cells distribution with-coefficient of variation and high standard deviation of red blood cells distribution were calculated in F12 group of fish fed with 12 % of fish oil while the control showed the lowest amount among treatments on the evaluated days (Table 4).

The activity of digestive enzymes was observed under the influence of varying energy level feeds and showed significant differences when compared to that of the F0. At the end of the trial, maximum amylase activity was observed in group of fish fed with F12 diet contained 12 % of fish oil in both the liver and intestine while the lowest activity was studied in F0 (0% fish oil). The activity of protease and lipase enzyme were found higher significantly (P<0.05) in F12 (12% fish oil) group in both liver and intestine followed by F8, F4 and significantly lower values in F0 (Table 5) (Figure 2 A-F).

4. Discussion

Lipids are the major macronutrients that yield essential fatty acids for the production of energy, growth and development of fish. The deficiency of lipid contents in the diet of fish increases consumption of protein for energy which in turn results in the excretion of ammonia. An adequate amount of lipid in fish feed can reduce feed cost and spare protein, which is significant for carnivorous fishes to effectively utilize carbohydrates as energy source. Fish oil is a crucial source of lipid used in fish feeds for its high amount of important fatty acids [21]. The results of the current study suggested that the growth performance of *Channa marulius* was improved by increasing the levels of lipid in the feed (12 % of fish oil). Best performances in growth indices were observed at the highest level of lipid (fish oil), suggesting that 12 % of fish oil could be efficiently used by *C. marulius* fingerlings. This is in agreement with the prior studies on hybrid species of snakehead fish (*Channa maculata* × *Channa argus*) which reported that body weight and rate of growth were significantly affected by increasing the lipid level in the diet [22]. Similarly, a study reported that maximum growth was achieved at 180 g/kg lipid level in *Channa striatus* [23]. According to another study, lipid level increasing from 10 % to 15 % in diets containing more than 50 % of protein did not increase growth rate in southern flounder, however, the diets that contained 45 % of protein, the weight gain of fish fed with 15 % of lipid was observed significantly higher than those fed with 10 % of lipid, indicated the occurrence of protein sparing effect where the level of protein in the diets was deficient [24]. However, this study contrasts with the study on hybrid fingerlings of snakehead (*Channa maculata* × *Channa argus*), which revealed non-significant growth by increasing varying energy levels (fish oil) in the diet [25], indicating that ability of lipid utilization is low in hybrid snakehead. Another study reported that a lipid: protein ratio of 65:450 g/kg was considered appropriate for the survival and better growth of the fry of *Channa striatus* [26]. However, in contrast to our findings, a decrease in growth performance has been observed with the increase of lipid level (fish oil) higher than 8 % in juveniles of *Solea senegalensis* [27]. This discrimination might be due to the addition of different ingredients in the diet of fish or other environmental factors.

In this study a decreasing trend was observed in the FCR fed high level of lipid in the diet while it was found higher in the control group. The decreasing rate of FCR in present study demonstrated that the highest lipid level of 12 % provided more energy to fingerlings of *Channa marulius* for better growth by utilizing high-energy feed. Earlier findings on the fingerlings of common snakehead (*C. striatus*) were in line with our study which reported that FCR was affected significantly by dietary lipid level of 14 % (cod liver oil and ground nut oil cake) [28]. The specific growth rate (SGR) was observed higher as a level of fish oil in feed F12 increased to 12 %. Lowest value of SGR was observed in the control group. The results of our study are also in line with a previous study that reported significant increase in SGR when lipid level increased from 12 % at 45 % and 48% both protein levels in feed of Northern snakehead (*C. argus).* This effect may be due to the effect of protein- sparing with higher lipid levels [4].

The whole-body proximate composition of *Channa marulius* was influenced by varying energy levels based on one way ANOVA. Moisture content displayed a decreasing trend in this study. This decreasing trend of moisture content was in accordance with the study [29], which reported the same decreasing moisture content by increasing lipid level in *Nile tilapia*. In line to present study, an earlier work reported the same decrease in moisture content by increasing lipid level in the whole body of carnivorous species [21]. The crude protein and fat content also showed a significant result which was in accordance with the results [30] when they used 7 and 9 % lipid in the feed of *Clarias batrachus* but in contrary with the study of [31] on Malawian tilapia (*Oreochromis shiranus*) in which the protein content decreased by increasing energy level and showed non-significant differences. In present study, fat content increased significantly by increasing lipid levels. The results of the study were in agreement with our study which stated that increase in dietary lipid increased whole body fat contents in juveniles of hybrid grouper [32]. Other study described that an increase lipid level in diets significantly increased the level of visceral lipid and carcass in Nile tilapia (*O. niloticus*) [33]. Whole energy and lipid contents in body increased as the lipid level increased and the results of our study were in agreement with prior studies on blunt snout bream (*Megalobrama amblycephala*) [34].

The outcomes of this study showed significant differences among treatments for amylase, protease, and lipase activities. The digestive enzymes activities were increased significantly in lipid fed diets. The development and secretion of digestive enzymes is triggered by the nature and composition of the diets by which the metabolic activities are strongly affected. For the identification of components of animal diet enzyme acts as an important tool. Similar results were observed in mahseer that protease activity is increased by highest level of sardine oil, suggesting protein sparing effect of oil [35]. Dietary lipid level of 9 %, maximum activity of protease was found for juveniles of striped catfish (*Pangasianodon hypophthalmus*) [36]. Lipase enzymes are inducible in nature and induced by lipid content in diet [37]. Bowyer described that the lipase activity of fishes fed with fish oil was higher as compared to other alternative oils which indicates that lipase activity possesses greater affinity for fish oil because of high retentions of eicosapentaenoic acid and docosahexaenoic acid present in muscle tissue. These outcomes disagree with the results in which non-significant differences was observed in protease and amylase activities in Northern snakehead *Channa argus* [4]. The modifications in digestive enzymes might be due to changes in starvation, protein concentration, feed ingredients, feeding time, food manipulations and lipid concentration.

The hematological parameters are important tools used as an effective index to determine the pathological and physiological changes in fish to identify the fish health. The effect of varying energy level diets (fish oil) significantly affected the hematology of bullseye snakehead fingerlings. The results of our study also correlate with other study that reported that different lipid sources significantly affected the hematology of vundu catfish (*Heterobranchus longifilis*). This study was in discrepancy [39] which recorded in significant differences in hematological indices of Nile tilapia trout (*O. niloticus*) served with experimental artificial formulated feed with minor addition of fish oil. Similarly, [40] studied insignificant differences in the blood protein of juvenile beluga (*Huso huso*) by increasing dietary soybean meal. Anbalagan reported that different factors alter the chemistry of blood like environmental factors, starvation, agents responsible for disease, ingredients used in feed of fish.

5. Conclusion

The results of the present study suggested that diet with high level of lipid (12 %) significantly affects growth, proximate composition, hematology, and digestive enzymes activity of *Channa marulius* fingerlings. In the light of the results of this study it can be concluded that the inclusion of lipid at the level of 12% in the diet of *Channa marulius* can improve growth, enhance the overall wellbeing and production efficiency. Therefore, it can be recommended that the fish oil in bullseye snakehead diet is essential as it serves as effective source of nutrition and can be used as the ultimate source of carbohydrates and energy.

Author contributions

NA; Formal Analysis, Investigation, Writing - Original Draft, Review & Editing, NK; Conceptualization, Methodology, Project administration, Supervision, Writing - Review & Editing., MF; Supervision, Validation, Data curation, Investigation, Visualization, Writing - Review & Editing, SB; Formal Analysis, Data curation, Investigation, Visualization, Writing - Review & Editing, AT; Formal Analysis, Visualization, Writing - Review & Editing, SA; Writing - Review & Editing, SJD; Funding acquisition, Investigation, Supervision, Writing – review & editing

Ethical approval

All experimental procedures conducted in this study adhered to the institutional guidelines of the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan (DR/682).

Conflicts of Interest

The authors report no conflicts of interest.

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Data availability statement

The data presented in this study are available on request from the corresponding author.

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