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Impact of Various Dietary Protein Levels on the Growth Performance, Nutrient Profile, and Digestive Enzymes Activities to Provide an Effective Diet for Striped Catfish (*Pangasius hypophthalmus*)

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Abstract

The present study was conducted to evaluate the effect of different dietary protein levels on digestive enzyme activities, nutrient assimilation as well as growth performance in striped catfish (*Pangasianodon hypophthalmus*). Fish were fed with four experimental diets with increasing levels of protein: 30% (CP30), 35% (CP35), 40% (CP40) and 45% (CP45) for 12 weeks. At the end of the feed trial, the highest weight gain (29.88 g), specific growth rate (3.58%) and lower feed conversion ratio (1.91) were observed in fish that were fed with a 40% protein diet while CP30 treatment group had the poorest growth performance indicators ($P<0.05$). Proximate analysis revealed that fish fed with either CP40 (18.50%) or CP45 (19.33%) diets had the highest protein content in comparison to that fed CP30 (17.13%) and CP35 (17.00%) diets. Similarly, the highest body lipid content was CP35 (4.20%) treatment group and the lowest in CP40 (3.83%). The amino acid in the harvested fillets was also significantly higher ($P<0.05$) in both CP40 and CP45 dietary treatments. The increasing dietary protein level also elevated both protease and amylase activities, while lipase activity significantly decreased ($P<0.05$). The study revealed that a 40% protein level diet produced from regional ingredients in the Middle East had yielded optimal growth performance and higher muscle quality in striped catfish.

Introduction

The striped catfish (*Pangasianodon hypophthalmus*) is a commercially important freshwater fish in many East Asian countries (Nguyen, 2009). The species has attracted popularity among consumers due to its absence of intramuscular bones and high meat proportion (Cruz-Casallas et al., 2012). Along with its ease of captive rearing and high productivity rates, this species is considered an attractive and sustainable fish species for inland aquaculture production (Ali et al., 2005; Bhuyan et al., 2018). While the farming and research of this species have advanced in countries such as Thailand and Bangladesh, nations which are still developing striped catfish aquaculture such as Pakistan is considered in its infancy. Due to limited regional development, aquafeeds such as those designed for farmed trout (i.e., higher protein diets of >45%) are typically used in commercial pangasius farms. However, these diets do not fully reflect the dietary needs of

catfish and an imbalance of nutrients can lead to waste and an increase in water quality deterioration. Furthermore, the knowledge of commercial dietary constraints within a geographical context is limited, i.e., using regionally available aquafeed ingredients (Shah et al., 2014). This disparity in aquafeed ingredients is often due to cost implications. However, this can affect overall feed quality, feed intake, and nutrient digestibility in the farmed animal. Because, low-quality feed or an improperly balanced diet can lead to the animal's poor feed conversion, lower growth performance, and water quality deterioration (Sayeed et al., 2008; Yakubu et al., 2015).

Protein is a crucial macronutrient that forms a vital part of muscle composition and contributes towards the edible part of the final product for human consumption. In general, fish muscle tissue contains 11-24% of crude protein content (Strasburg & Xiong, 2017). To this effect, providing a sufficient level of dietary protein can lead to optimal growth and development, and maturation of the aquatic animal (Luo et al., 2004; Cho et al., 2005; Martinez-Palacios et al., 2007; Deng et al., 2011). A high protein-based diet can be economically costly and too much protein or an imbalance with other nutrients can subsequently lead to underutilisation and greater nitrogenous waste production (Wilson, 2003; Ozorio et al., 2006).

Studies on the dietary requirement of farmed fish species revealed that the optimal growth of catfish (*Heterobranchus longifilis)* fingerlings was ascertained on diets with 40% crude protein (Keremah & Alfred-Ockiya, 2013) and African catfish (*Clarias gariepinus*) on 35% dietary protein level (Keremah & Beregha, 2014). Khattaby et al. (2021) reported that a 35% optimum dietary protein level and 10 fish 150 L^{-1} stocking density in a tank setting could give the maximum growth rate of striped catfish while Malik & Naeem (2020) described that the best growth performance was recorded at a lower crude protein level of 30% in striped catfish fed in ponds for 90 days. While there is some knowledge of dietary protein requirements, there is still a necessity for a deeper understanding of how optimum dietary protein levels can minimize feed cost, physiological impacts, and harvest quality. Therefore, the objective of the current study is to determine how increasing levels of crude protein in practical feed formulation can influence growth performance, proximate composition, and amino acid profile in striped catfish. The study will also measure digestive performance and nutrient assimilation efficiency by quantifying gut enzyme activity. To give commercial reliance to the growing Middle East region in striped catfish farming, locally available feed ingredients were used to formulate the test diets.

Materials and Methods

Experimental Diets

Four experimental diets were prepared with increasing levels of crude protein: 30% (CP30), 35% (CP35), 40% (CP40) and 45% (CP45). All the ingredients were weighed according to formulation (Table 1) and ground to a particulate size of <1 mm and extruded (Anex model AG-3060, Warsaw, Poland) into a 1 mm diameter pellet. Finished diets were then air-dried, and the feeds were packed and stored at -20°C for later use. Amino acid profiles of the test feeds were analysed and presented in Table 2.

Table 1. Test diet formulation and proximate composition (%, dry weight).

	Experimental diets			
Ingredients	CP30	CP35	CP40	CP45
Fish meal[a]	21.00	28.00	33.00	38.00
Soybean meal[a]	20.00	20.00	20.00	20.00
Corn gluten[a]	16.00	18.00	22.00	26.00
Wheat flour[b]	20.00	15.00	10.00	5.00
Rice polish	15.00	11.00	7.00	3.00
Sunflower oil	6.00	6.00	6.00	6.00
Vitamin premix[c]	1.00	1.00	1.00	1.00
Mineral premix[d]	1.00	1.00	1.00	1.00
Proximate composition				
Moisture	8.00	8.00	8.10	8.80
Crude protein	29.60	34.10	39.70	44.90
Crude fat	10.00	9.90	10.10	10.10
Crude ash	10.00	9.10	9.40	9.70
Crude fibre	3.70	3.80	3.60	3.70
Gross energy, MJ kg^{-1}	19.50	18.20	17.50	16.90
P/E ratio, mg kJ^{-1}	16.00	19.50	21.80	24.50

[a]Fish meal (50% CP), Soybean meal (45% CP), Corn gluten (60% CP), and Rice polish, Aqua Feeds Pvt ltd, Multan, Pakistan

[b]Family Flour Mill, Pattoki, Pakistan

[c]Fivevet, Central Veterinary Medicine JSC No. 5, Ha Noi, Vietnam. Vitamin mixture: Vitamin A 3,500,000 IU kg^{-1}, vitamin B_1 3,500 mg kg^{-1}, Vitamin D_3 1,750,000 IU kg^{-1}, Zn gluconate 40 g kg^{-1}, vitamin E 3.500 mg kg^{-1}, vitamin PP (nicotinamide) 30 g kg^{-1}, sorbitol 20 g kg^{-1}.

[d]Fivevet, Central Veterinary Medicine JSC No. 5, Ha Noi, Vietnam. Mineral mixture: Ferrous sulphate 25 g kg^{-1}, calcium phosphate 397 g kg^{-1}, calcium lactate 327 g kg^{-1}, magnesium sulphate 137 g kg^{-1}, sodium chloride 60 g kg^{-1}, potassium chloride 50 g kg^{-1}, potassium iodide 150 mg kg^{-1}, manganese oxide 800 mg kg^{-1}, copper sulphate 780 mg kg^{-1}, zinc oxide 1.5 g kg^{-1}, cobalt carbonate 100 mg kg^{-1}, manganese oxide 800 mg kg^{-1}, sodium selenite 20 mg kg^{-1}.

Fish and Experimental Conditions

The striped catfish fry was acquired from the fishponds at the Department of Fisheries and Aquaculture, Research and Training Facilities (University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, District Kasur, Pakistan). Fish were stocked in 12 cages [150 (W) × 120 (L) × 90 cm (D)] that were placed into an earthen pond (half an acre with 1.3 m depth) filled with well water. Depending on the water quality in the pond, the water was partially exchanged. The rearing cages were made of synthetic green nylon mesh (mesh size 1.1 cm) that was fixed to a bamboo made frame. The top of the cages was covered with another net to prevent escape and predation. Aeration to the cages was maintained by paddle wheels. Fish (1.3 g initial weight) were stocked with 20 fish per cage and fed for 12 weeks in triplicates. Diets were assigned randomly to each cage. Fish were fed three times a day (08:00, 12:00, 17:00) at apparent satiation. Water quality parameters were measured daily, which included the water temperature (29.5±0.1°C), dissolved oxygen (5.50±0.25 mg L^{-1}), pH (7.50±0.25) and total dissolved solids (1250.2±0.1 mg L^{-1}). The procedures and methods used in the study followed the ethical guidelines of the University of Veterinary and Animal Sciences, Pakistan.

Nutrient Analysis

For proximate composition and amino acid analysis, five fish were randomly captured from each cage and anaesthetized with tricane methanesulphate (MS-222) at 150 mg L^{-1} (Yildirim-Aksoy et al., 2008) and stored at -20°C for later analysis. The proximate analysis of experimental diets and fish was performed using the Association of Official Analytical Chemists methodology (AOAC, 2006). Moisture levels were determined by heating the samples at 105 °C until a constant weight was obtained. The crude protein (CP) was assessed by the Kjeldahl apparatus (Kjeldahl digestion 60079776 and Kjeltec distillation 8100, FOSS, Hilleroed, Denmark). Crude fat was determined by Soxhlet extraction (R106S, Behr Labor-Technik, Düsseldorf, Germany) using petroleum ether. Ash contents were measured by heating the sample to 550°C for 16 hrs (Tmf-3100*,* Eyela Co*.,* Tokyo, Japan). The gross energy of experimental diets was determined by using a bomb calorimeter (Parr-1356, Parr instrument company, Illinois, USA).

Amino acids analysis was carried out using ion exchange chromatography. Samples (~3 g) ground into <500 µm particle size was treated with 5 mL formic acid to lysine-lysine acid and met-methionine sulphone which protect lysine and methionine from oxidation. Samples were then hydrolysed with 25 mL of 6 M hydrochloric acid/phenol for 24 hours at 110°C, and the pH was adjusted to 2.2. The sample solution was filtered (0.2 µm) and subjected to amino acid analysis through a Biochrom 30+ amino acid analyser (Cambridge, United Kingdom) (European Union Directive 98/64/EC, 1998).

Digestive Enzyme Assays

Three fish per treatment were randomly sampled and the proximal intestine was collected for enzyme activity measurements. Weighed sample sections (~3 g) were homogenized in Tris HCl (50 m, M, pH 7.5) buffer at a ratio of 1:2 (w/v) in an ice water bath using a tissue

Table 2. Amino acid profile of striped catfish test diets (%, dry matter).

	Experimental diets			
	CP30	CP35	CP40	CP45
EAA				
Arginine	1.78	1.82	1.66	1.87
Histidine	0.79	1.67	1.72	2.08
Isoleucine	1.19	1.34	1.98	2.15
Leucine	1.27	1.12	1.52	1.95
Lysine	2.13	3.34	3.73	3.96
Methionine	2.30	2.15	2.84	2.87
Phenylalanine	0.29	1.09	1.13	1.62
Threonine	1.90	2.17	1.82	2.04
Valine	1.68	1.89	2.06	2.45
NEAA				
Alanine	1.19	1.43	1.57	1.56
Aspartic acid +Asparagine	2.54	2.15	2.67	2.94
Cysteine	2.31	2.13	2.85	2.52
Glutamic acid +Glutamine	1.16	2.98	2.73	2.98
Glycine	1.57	1.21	1.54	1.58
Ornithine	0.21	1.06	1.56	2.13
Proline	1.84	1.92	1.21	1.83
Serine	1.67	2.56	2.96	2.97
Tyrosine	1.03	1.18	1.72	1.82
∑EAA	13.52	16.62	18.81	20.33
∑NEAA	13.33	16.59	18.46	20.99
∑Total AA	26.85	33.21	37.27	41.32
EAA/NEAA	1.01	1.0	1.02	0.97

Dietary treatments: 30% (CP30), 35% (CP35), 40% (CP40), and 45% (CP45) of the dietary protein.

homogeniser. The homogenate was then centrifuged at 4°C at 10,000 *g* for 15 min. The supernatant was then stored at -20°C for later analysis of enzyme activity (Klahan et al., 2009). The intestinal protease enzyme activity was determined by making a solution of 1% azoalbumin substrate in 50 mM (pH 7.5) of Tris-HCL. Ten microlitres of the enzyme extract were mixed with 0.5 mL of the Tris-HCL buffer (pH 7.5) and 0.5 mL substrate solution was added. The samples were incubated at 25°C for 10 min. The trichloroacetic acid (0.5 mL) was then added to the solution to cease the reaction and samples were centrifuged at 14,000 *g* for 5 min. The supernatant was measured for its absorbance at 366 nm (Gracia & Carreno, 1992). Amylase activity was measured by using 1 mL of tissue extract supernatant that was mixed with 2 mL of starch phosphate buffer and incubated at 37°C for 30 min. Three millilitres of 3,5–dinitrosalicylic acid (DNS) solution was added to the mixture and incubated for a further 30 mins until a brown color developed. The solution was diluted by adding 4 mL of water to make a final volume of 10 mL and the supernatant absorbance was read at 540 nm (Bernfeld, 1955). Lipase activity was determined by Cherry & Crandell (1932) described methodology. Fatty acids were estimated to maintain pH levels. The reaction mixture contains 1.5 mL of olive oil, substrate 1.5 mL of 0.1 M Tris-HCL buffer (8.0 pH) and 0.1 mL of crude enzyme extract added. The mixture was incubated for 24 h at 4°C and the reaction was stopped by adding 3 mL of 95% ethyl alcohol and then titrated against 0.01 N NaOH 0.9% (w/v) using phenolphthalein indicator

Calculations and Statistical Analysis

At the end of the feed trial, fish were measured, and the data was used for calculating the growth performance and feed utilization indices using the following formulae:

Weight gain (WG) = average final weight (g) - average initial weight (g)

Specific growth rate (SGR) = 100 × [ln (final wet body weight) – ln (initial wet body weight)] / duration (days)

Feed conversion ratio (FCR) = feed consumed (g) / wet weight gained (g)

Survival rate = 100 × (number of survived fish / initial number of fish)

Data were first tested for normality and homogeneity of variance. Results were presented as mean±standard deviation (n=3). One-way ANOVA was applied to the data to check the possible effects of the dietary protein levels on targeted parameters, followed by Duncan's multiple range test (SAS v9.1, Cary, North Carolina, USA) whenever the effect is significant (Steel et al., 1996). Principal component analysis (PCA) was also performed using the Unscrambler version 10.1 (CAMO, Oslo, Norway) to determine the relationship between dietary treatment feed and amino acids.

Results

Growth Performance and Feed Utilization

At the end of the 12-week feeding trial, there was no significant difference observed in the survival rate. However, increasing dietary protein inclusion had a significant impact on the growth performance of striped catfish ($P<0.05$) (Table 3). Fish fed with the CP40 diet had higher ($P<0.05$) final weights (1.22 g) and weight gain (29.88 g) in comparison to the other dietary groups. The specific growth rate was similar between fish fed with CP40 (3.58%) and CP45 (3.53%) diets but was significantly higher than those fed on CP30 (3.12%) and CP35 (3.20%) diets. The highest FCR was observed in fish fed with the CP30 diet (2.30), while the CP40 diet dietary group had the lowest value (1.91), tending to decrease with the higher dietary protein level until 40% ($P<0.05$). Overall, the measured growth indices have shown that there was a general trend of increasing dietary protein resulting in higher performance up to 40% dietary protein level and any further increase resulted in a decline of growth metrics.

Proximate Composition

Proximate composition was significantly affected by the dietary protein levels ($P<0.05$) (Table 4). Moisture content was significantly higher in fish fed CP30 (73.87%) and CP35 (73.50%) than that fed CP40 (70.25%) and CP45 (69.07%) diets. Fish fed with CP40 or CP45 diets had significantly greater crude protein

Table 3. Growth performance and feed utilisation of striped catfish fed with increasing dietary protein levels for 12 weeks (n=3).

	Experimental diets			
	CP30	CP35	CP40	CP45
Initial mean weight; g fish^{-1}	1.33±0.00	1.32±0.01	1.26±0.02	1.25±0.01
Final mean weight; g fish^{-1}	22.61±0.04^{c}	23.60±0.30^{c}	31.10±0.69^{a}	26.6±0.86^{b}
Mean weight gain; g fish^{-1}	21.26±0.04^{c}	22.28±0.31^{c}	29.88±0.67^{a}	25.50±0.87^{b}
SGR; % day^{-1}	3.12±0.00^{b}	3.22±0.01^{b}	3.58±0.0^{a}	3.53±0.04^{a}
FCR	2.30±0.01^{a}	2.09±0.02^{b}	1.91±0.04^{c}	1.99±0.03^{bc}
Survival; %	96.70±1.72^{a}	86.71±1.71^{b}	93.30±3.32^{ab}	95.00±2.90^{ab}

SGR, Specific growth rate; FCR, Feed conversion ratio. Data are means±SD. Different superscripts on the same row indicate there is a significant difference ($P<0.05$). Dietary treatments: 30% (CP30), 35% (CP35), 40% (CP40), and 45% (CP45) of the dietary protein.

content (18.50 and 19.33%, respectively) in comparison to that fed CP30 and CP35 diets (17.15 and 17.00%, respectively). The highest crude lipid and ash levels were detected in fish-fed CP35 (4.20 and 2.90%, respectively), while the lowest crude lipid and ash content was observed in CP40-fed fish (3.83 and 1.60%, respectively).

Amino Acid Profile

The muscle amino acid profile was presented in Table 5. Total amounts of essential and non-essential amino acids (EAA and NEAA, respectively) were influenced by the dietary level of protein (P<0.05). The lowest total amount of EAA was observed in the muscle of fish fed with the CP30 diet (26.16%), which was statistically similar to the CP35 dietary group (27.68%), but lower than the fish fed CP40 and CP45 diets (28.73 and 29.12%, respectively). Total NEAA concentration was highest in fish muscle fed the CP40 diet (28.64%), which was statistically similar to those fed the CP45 diet (27.46%), but higher than those fed the CP30 and CP35 diets (26.23 and 26.57%, respectively). The EAA/NEAA ratio was greater in the CP45 diet (1.06%) as compared to other dietary groups.

Individual EAA such as isoleucine, lysine and methionine levels were significantly influenced by the dietary protein levels (P<0.05) (Table 5). Isoleucine and lysine were higher in fish fed with CP40 (3.66 and 6.13%, respectively) and CP45 (3.43 and 6.24%, respectively) diets when in contrast to CP30 (2.63 and 5.42%, respectively) and CP35 (2.79 and 5.63%, respectively). The highest and lowest methionine levels were observed in fish fed with CP45 (4.47%) and CP30 (3.20%) diets, respectively.

Table 4. Proximate composition striped catfish fed with increasing dietary protein levels for 12 weeks (%, wet weight)

	Experimental diets			
	CP30	CP35	CP40	CP45
Moisture	73.87±0.55^{a}	73.50±0.36^{a}	70.25±0.28^{b}	69.07±0.38^{b}
Crude protein	17.13±0.46^{b}	17.00±0.30^{b}	18.50±0.40^{a}	19.33±0.30^{a}
Crude lipid	4.00±0.00^{b}	4.20±0.03^{a}	3.83±0.04^{c}	3.90±0.03^{ab}
Ash	2.53±0.10^{b}	2.90±0.02^{a}	1.60±0.00^{d}	2.00±0.15^{c}

Data are means±SD. Different superscripts on the same row indicate there is a significant difference (P<0.05). Dietary treatments: 30% (CP30), 35% (CP35), 40% (CP40), and 45% (CP45) of the dietary protein.

Table 5. Amino acid profile of striped catfish fed with increasing dietary protein levels for 12 weeks (% dry matter, n=3).

	Dietary treatment			
	CP30	CP35	CP40	CP45
EAA				
Arginine	1.50±0.11	1.56±0.13	1.41±0.08	1.50±0.09
Histidine	2.59±0.32	2.62±0.16	2.28±0.09	2.49±0.19
Isoleucine	2.63±0.18^{b}	2.79±0.07^{b}	3.66±0.19^{a}	3.43±0.23^{a}
Leucine	4.33±0.02	4.33 ±0.8	4.40±0.11	4.35±0.06
Lysine	5.42±0.17^{b}	5.63±0.20^{b}	6.13±0.02^{a}	6.24±0.24^{a}
Methionine	3.20±0.12^{c}	3.77±0.10^{b}	4.16±0.11^{ab}	4.47±0.14^{a}
Phenylalanine	1.39±0.09	1.54±0.09	1.53±0.14	1.44±0.14
Threonine	2.63±0.23	2.86±0.22	2.97±0.06	2.77±0.13
Valine	2.46±0.11	2.57±0.14	2.35±0.06	2.43±0.16
NEAA				
Alanine	2.10±0.27	1.82±0.36	1.87±0.25	1.69±0.08
Aspartic acid+Asparagine	4.61±0.14^{ab}	5.05±0.29^{a}	4.33±0.11^{b}	4.05±0.13^{b}
Cysteine	3.29±0.01	3.24±0.02	3.16±0.08	2.96±0.32
Glutamic acid+Glutamine	5.04±0.33^{c}	5.53±0.17^{bc}	6.69±0.18^{a}	6.00±0.15^{ab}
Glycine	3.38±0.04^{c}	3.54±0.06^{c}	4.43±0.11^{b}	4.81±0.12^{a}
Ornithine	2.24±0.20	1.92±0.37	2.36±0.02	2.47±0.20
Proline	2.30±0.09	2.22±0.13	2.54±0.23	2.27±0.10
Serine	1.54±0.11	1.39±0.08	1.54±0.15	1.48±0.22
Tyrosine	1.70±0.22	1.83±0.46	1.70±0.11	1.70±0.15
∑EAA	26.16±0.58^{b}	27.68 ±0.52 ^{ab}	28.73 ±0.38^{a}	29.12±0.41^{a}
∑NEAA	26.23 ±0.17^{b}	26.57 ±0.60^{b}	28.64±0.21^{a}	27.46±0.38^{ab}
∑AA	52.39±0.68^{c}	54.25±0.13^{b}	57.37±0.26^{a}	56.58±0.48^{a}
EAA/NEAA	1.00±0.01^{c}	1.04±0.00^{b}	1.00±0.01^{c}	1.06±0.00^{a}

Data are means±SD. Different superscripts on the same row indicate there is a significant difference (P<0.05). EAA, essential amino acids; NEAA, non-essential amino acids. Dietary treatments: 30% (CP30), 35% (CP35), 40% (CP40), and 45% (CP45) of the dietary protein.

NEAA measurements showed that aspartic acid (+asparagine), glutamic acid (+glutamine) and glycine differed by the dietary feeds (P<0.05). Fish fed with the CP35 diet had higher aspartic acid (+asparagine) (5.05%) than the CP40 (4.33%) and CP45 (4.05%) treatment groups, while CP30 fish had similar values (4.61%) with all experimental groups (P<0.05). Glutamic acid (+glutamine) content was higher in fish muscle in the CP40 group (6.69%) in comparison to CP30 (5.04%) and CP35 (5.53%) diets, while the levels were similar in the CP45 dietary group (6.00%). The highest glycine level (4.81%) was observed in fish fed CP45 while the lowest values were observed in that fed CP30 (3.38%) and CP35 (3.54%). Further differences in the amino acid profile were shown by the principal component analysis, with each dietary fish group being spatially distinct (Figure 1). The loading plots revealed specific amino acid relationships, e.g., histidine, aspartic acid, asparagine and cysteine all co-increased. The first and second principal component explains 87.50% of the sample variation.

Digestive Enzyme Activities

The measurement of digestive enzyme activity in the posterior intestine had shown there was a significant influence by the increasing dietary protein levels (P<0.05, Figure 2). The protease enzyme activity was the highest in CP40 dietary group (1.60 U mg^{-1}) and the lowest (1.15 U mg^{-1}) in CP30-fed fish. Similarly, fish-fed with either CP40 (8.22 U mg^{-1}) or CP45 (8.15 U mg^{-1}) diets had higher amylase activity in comparison to those fed CP30 (5.21 U mg^{-1}) and CP35 (5.89 U mg^{-1}) diets. Measured lipase activity in the intestine had the opposite trend. It was observed that CP30 (1.03 U mg^{-1}) and CP35 (1.04 U mg^{-1}) dietary treatments had the highest values and CP40 (0.76 U mg^{-1}) and CP45 (0.66 U mg^{-1}) possessed the lowest values.

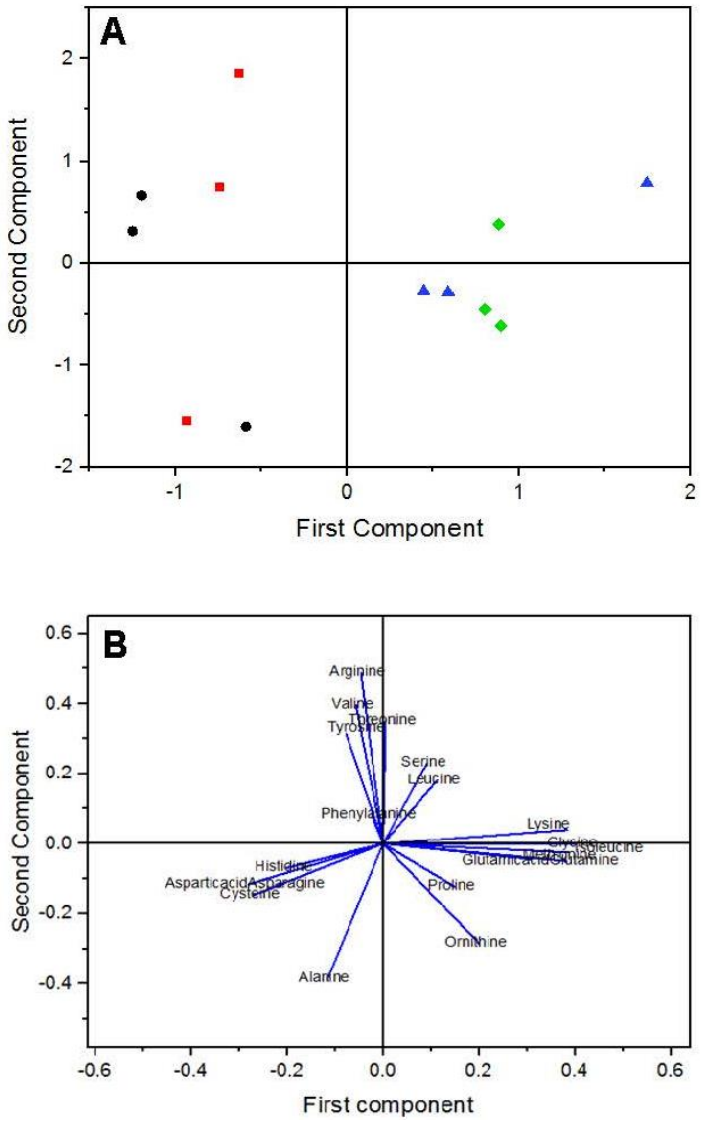

Figure 1. Principle component analysis of muscle amino acid composition. A) Score biplot and B) loading plot of the first and second principal components for amino acid between amino acid and dietary protein levels. The first and second principal component explains 87.50% of the sample variation. Dietary treatments: 30% (●, CP30), 35% (■, CP35), 40% (♦, CP40), 45% (▲, CP45) dietary protein level.

Discussion

There is a need for greater ecoefficiency in aquaculture, more specifically, using proteins such as fish meal and plant proteins more effectively to deliver more harvestable foods (Colombo et al., 2022). Newly introduced farmed fish species to a region are typically fed with a readily available aquafeed to that area. While this might avoid logistical and supply issues, such unspecific aquafeeds can in the long run cost the farmers more through feed wastage and lesser harvestable fish product. Such poor feed management can ultimately lead to a deterioration of water quality and the local environment, e.g., an increase in anoxic conditions, a decrease in biodiversity, and greater impact from eutrophication (White, 2013).

The present study has observed that there was an increasing trend in striped catfish growth performance as dietary was elevated to the point of 40% dietary crude protein content. The measured feed conversion ratio also showed feeding a 40% protein diet gave better indices than those in lower protein treatment groups. This indicates that fish fed with high protein diets can use the protein more efficiently as compared to low dietary protein diets. These results were also similar to those found in European catfish (*Silurus glanis*) that was fed 40% dietary protein yielding the highest growth performance measurements, including a higher specific growth rate (0.74) and lower FCR (0.97) in comparison to other levels. i.e., 30, 35, and 44% (Bekcan et al., 2006). In contrast, the protein requirement for South American surubim catfish (*Pseudoplatystoma* sp.) was suggested to be at 45% (Arslan et al., 2013).

The protein content requirement by striped catfish is lower than those found in generic commercial diets (i.e., sold for trout farming) being used in Pakistan's catfish farms. While past studies on striped catfish found that the protein requirement varied from 30.0-45.3% (Kader et al., 2003; Khattaby et al., 2021). It is possible that this variation in optimum growth could be the result of test design, such as strain used, age class, test ingredients used for the test diets, and rearing conditions (Sankian et al., 2017).

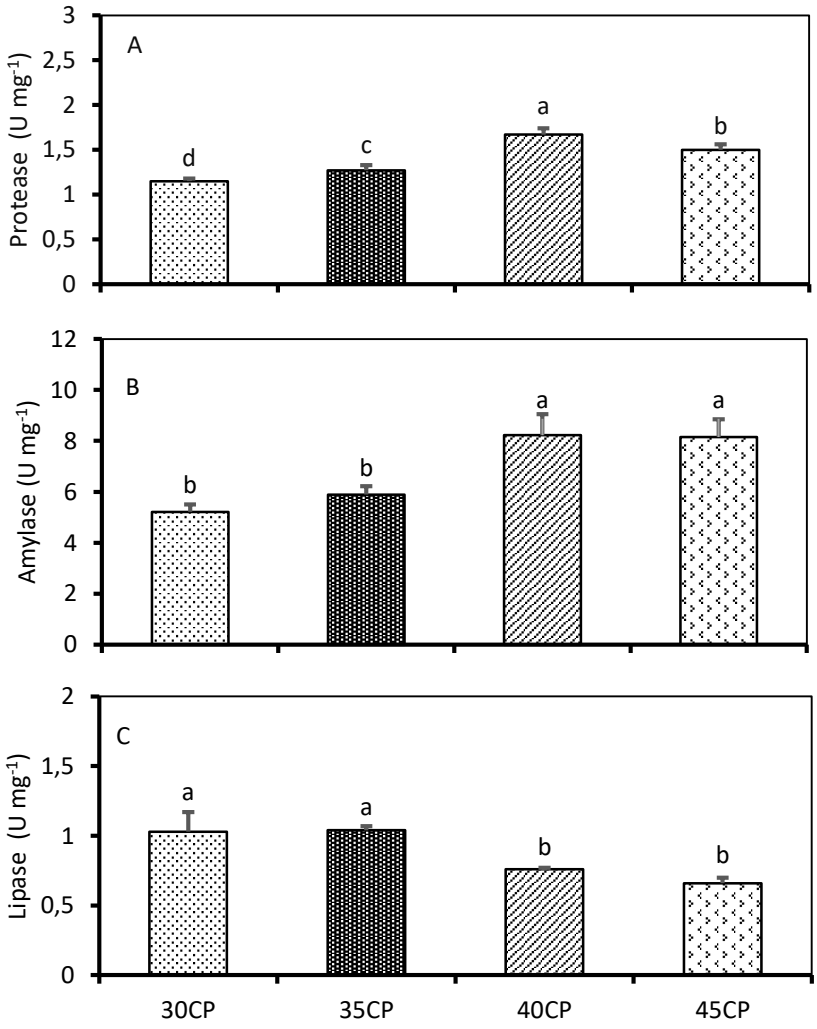

Figure 2. The effect of increasing dietary protein levels on digestive enzymes activities in striped catfish: A) protease, B) amylase, and C) lipase. Results are presented as means±SD. Bars with different superscript letters are significantly different (P<0.05). Dietary treatments: 30% (CP30), 35% (CP35), 40% (CP40), and 45% (CP45) of the dietary protein.

The increase in dietary protein beyond 45% had resulted in no additional enhancement but rather a decrease in growth metrics. In both hybrid clarias (*Clarias batrachus* × *Clarias gariepinus*, Giri et al., 2003) and black catfish (*Rhamdia quelen*, Salhi et al., 2004), dietary protein exceeding 40% led to lower growth performance indices. This issue may be due to the excessive dietary protein that can lead to higher energy expenditure due to the catabolism effect rather than the protein being accumulated and used for body growth (Phillips, 1972; Kim et al., 2002).

The results of whole body proximate analysis also showed a positive correlation between dietary protein and crude body protein content, with fish being fed with either 40% or 45% dietary protein having the highest carcass protein content. In comparison to a previous study on Wuchang bream (*Megalobrama amblycephala*), higher dietary protein levels also presented an increase in muscle protein content (Habte-Tsion et al., 2013). This may be due to the conversion and accumulation of protein with increased crude protein levels in the diet (Debnath et al., 2007). Unlike the current study, increasing dietary protein beyond 40% did not increase body protein levels in Asian stinging catfish (*Heteropneustes fossilis*, Siddiqui & Khan, 2009). This was also observed in Bagrid catfish (*Pseudobagrus fulvidraco*) with no changes in body protein content regardless of increasing protein levels in their diet (Kim & Lee, 2005). At an optimum dietary protein level, fish are most efficient at using protein for growth (Ahmed & Ahmad, 2020). However, any higher dietary protein can lead to catabolism of the protein as an energy source rather than for tissue synthesise (Hardy & Gatlin, 2002).

Phumee et al. (2009) reported that increasing dietary protein levels led to a decrease in striped catfish body lipid levels, which is consistent with the present study. This observation may be due to the low protein and high carbohydrate content in the diet and that these carbohydrates are accumulated as storage energy (Daudpota et al., 2014). On the other hand, Khan et al. (2018) reported that there was no influence on the body proximate composition of striped catfish after being fed with different protein levels. These findings are in contrast with the present study, such differences could be due to Khan et al. (2018) undertook the trial using bigger fish and harvested larger individuals, i.e., >1kg vs >1g fish.

Protein is a mandatory component in terms of meeting the nutritional demands of animal production (Tasbozan et al., 2013). Protein quality (biological value) and amount in fish feed are important factors that directly affect the productivity of the farmed species. It has been stated that there is a similarity between amino acid content in the whole body of fish species, amino acid quantity, and composition in fish diets (Mambrini & Kaushik, 1995). In the present study, both essential and non-essential amino acids in muscle tended to increase with the dietary protein increment. Individual EAAs such as isoleucine, lysine, and methionine and NEAAs such as glycine and glutamic acid increased with increasing dietary protein content. So collectively, we can infer that increasing dietary protein levels in the diet improve the amino acid profile which is agreed with the study on juvenile chu's croaker fish (*Nibea coibor,* Huang et al., 2017). The amino acid composition in the fish varies between species (Ozden & Erkan, 2011). For example, Zhao et al. (2010) reported that amino acids including lysine, leucine, glutamic acid, and aspartic acid were the most abundant amino acids in pomfret (*Pampus punctatissimus*). While the most abundant amino acids were reported as lysine, aspartic acid, glutamic acid, and leucine in Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*), white hake (*Urophycis tenui*), and Atlantic horse mackerel (*Trachurus trachurus,* Oluwaniyi et al., 2010). The abundance of these amino acids in the profile was comparable to those found in the present study. In the current study, the ratio of muscle EAA/NEAA was at its highest in the 45% protein dietary treatment group (1.06%). The overall EAA/NEAA range was at 1.00 to 1.06% between the dietary groups. Although the range was lower (1.2-1.3%) than those measured in juvenile chu's croaker fed with different levels of protein diets (36, 40, 44, 48, and 52%, Huang et al., 2017).

Digestive enzymes such as protease, amylase and lipase all aid the digestion and assimilation of nutrients (e.g., proteins lipids, carbohydrates) when the feed is transiting through the gastrointestinal tract. Although the level of these digestive enzyme activities can differ in the presence of different feed compositions (Pérez-Jiménez et al., 2009). The digestion process is known to be one of the limiting factors in feed utilization and assimilation (Sun et al., 2017), and the process can be influenced by the diet type, feeding frequency, life stage, and temperature (Hakim et al., 2006; García-Meilán et al., 2016).

In the present study, the activity of the protease enzyme found in the proximal intestine was improved with the increasing levels of dietary protein up to an optimum level of 40% crude protein, but beyond this level, the digestive enzyme activity decreases. Similarly, protease activity was found greater in fish fed with dietary protein 40% crude protein, and further increase caused a decrease in the activity of this enzyme in striped catfish (Jayant et al., 2018). While red tilapia juveniles (*Oreochromis* sp.) also exhibited a similar decrease in protease and trypsin activity after dietary protein was increased beyond 36% (Santos et al., 2020). The decrease could be explained by the presence of high protein content in the digestive tract and exhausting the production of enzymes being produced (Debnath et al., 2007). The high level of dietary protein in the diet can change the pattern of protease activity secreted in the lumen of the intestine. These changes resulted in an asynchronous availability of di-tripeptide and amino acid as a consequence of the protease specificity (García-Meilán et al., 2013).

Amylase is responsible for the digestion of carbohydrates in the gastrointestinal tract and the present study found that increasing dietary protein decreases enzyme activity. This could be attributed to the decrease in dietary nitrogen-free extract (digestible fibres) with increasing protein content as a means to balance the feed formulation. This observation was comparable to another study on striped catfish, with amylase activity being reported to decrease in the presence of elevated protein in the diet (Jayant et al., 2018). In contrast, amylase activity remained relatively unchanged when yellowhead catfish (*Pelteobagrus fulvidraco*) fingerlings were fed with different levels of dietary protein (Qin et al., 2019).

In comparison to past studies, lipase activity had decreased with the increasing amount of dietary protein, which was found to be the opposite in the study undertaken by Jayant et al. (2018). This enzyme is responsible for the breakdown and assimilation of dietary lipids. For silver catfish (*Rhamdia quelen*), intestinal lipase activity decreased with increasing dietary protein (Melo et al., 2012). It is also worth mentioning that in the current study, higher lipase activity was measured in both 30% and 35% dietary protein groups. The greater lipase activity could be explained by lipid synthesis (lipogenesis) from the presence of higher carbohydrate availability (Enes et al., 2009).

Measuring these digestive enzymes post-feed trials can give a better insight into how nutrients are assimilated, and the potential loss. While there have been previous studies on identifying the optimum levels of gross protein and energy or protein and lipid in striped catfish, it would warrant further study to examine the interactive effect between protein, lipids, and carbohydrates on digestive enzyme activity or whole-body composition (Glencross et al., 2011; Wang et al., 2011; Phan 2021a; Phan 2021b). The information gained would provide further refining in the optimal nutritional requirement and the production of more coefficient aquafeeds for striped catfish.

Conclusion

Protein is one of the most expensive constituents in commercial aquafeed, which can impact the economic feasibility of a fish farm operation. The present study serves to refine the optimum level of dietary protein requirement in striped catfish. Moreover, this study validated the protein requirement through a practical feed formulation design using feedstuffs that are typically found in Middle East nations, such as Pakistan. It was observed that 40% dietary protein had produced the highest growth performance and feed utilisation indicators. Any increase in the dietary protein beyond 40% did not enhance growth metrics but rather decreased them. This loss of growth indicates there can be an overall production loss and feed wastage, i.e., giving more feed to attain the same productivity. Past studies on dietary protein requirement in striped catfish have only determined the growth performance and feed utilisation impacts, however, this study went beyond these metrics and quantified the effect on product quality through amino acid profiling and proximate analysis. For the latter higher body protein content was observed as dietary protein increased. Furthermore, the current results suggest that digestive enzyme activities in striped catfish are modulated by the dietary protein levels. The implications of refining and meeting the dietary needs of farmed fish such as striped catfish can benefit farmers in decreasing economic loss through better nutrient assimilation efficiency. Overall, the results will have applications in the Middle East for farmers such as those in Pakistan, which will serve to sustainably grow commercial aquaculture in the region.

Ethical Statement

The procedures and methods used in the study followed the ethical guidelines of University of Veterinary and Animal Sciences.

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Author Contribution

Sheeza Bano: Formal analysis, Investigation, Writing - Original Draft. Noor Khan: Conceptualization, Methodology, Project administration, Fund acquisition, Supervision, Writing - Review & Editing. Murat Arslan: Formal analysis, Investigation, Visualization, Supervision, Writing - Review & Editing. Mahroze Fatima: Formal analysis, Investigation, Writing - Review & Editing. Anjum Khalique: Visualization, Writing - Review & Editing. Alex H.L. Wan: Formal analysis, Writing - Review & Editing.

Conflict of Interest

The authors have no conflicts of interest to declare.

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