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Original Article

Performance evaluation of the commercial aquafeeds available in the market of Pakistan on *Channa marulius* (Sole)

Avaliação de desempenho dos alimentos aquáticos comerciais disponíveis no mercado do Paquistão em *Channa marulius* (Sole)

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Abstract

The present study aimed to determine the effect of different levels of protein on the growth, body composition, amino acid profile and serology of *Channa marulius* fingerlings. The experiment was conducted in ten happas installed in earthen ponds, each stocked with 10 fishes for 90 days. Four commercial fish feeds having 25%, 30%, 32% and 40% crude protein (CP) levels were fed to fish at 3% of their wet body weight three times a day. The results of the study revealed that highest weight gain, feed conversion ratio and survival rate were observed in 30% protein feed. Meanwhile, moisture content was higher in fish fed with 30% CP feed while highest crude protein was recorded in 40% CP fed fish. Lowest fat content was observed in 32% CP feed. Amino acid profile of fish revealed better results in 30% CP feed. Total protein, glucose and globulin were also highest in fish feeding 30% CP feed, while albumin was highest in 40% CP feed. It is concluded that 30% CP feed showed better results in terms of growth, amino acid profile and serological parameters without effecting fish body composition.

Keywords: crude protein, amino acids, *Channa marulius*, body composition, serology.

Resumo

O presente estudo teve como objetivo determinar o efeito de diferentes níveis de proteína sobre o crescimento, composição corporal, perfil de aminoácidos e sorologia de alevinos de *Channa marulius*. O experimento foi conduzido em dez happas instalados em tanques de terra, cada um abastecido com 10 peixes, por 90 dias. Quatro alimentos para peixes comerciais com níveis de 25%, 30%, 32% e 40% de proteína bruta (PB) foram dados aos peixes com 3% de seu peso corporal úmido três vezes ao dia. Os resultados do estudo revelaram que maior ganho de peso, taxa de conversão alimentar e taxa de sobrevivência foram observados em 30% de proteína alimentar. Enquanto isso, o conteúdo de umidade foi maior em peixes alimentados com 30% de PB, enquanto a proteína bruta mais alta foi registrada em peixes alimentados com 40% de PB. O menor conteúdo de gordura foi observado em rações com 32% de PB. O perfil de aminoácidos dos peixes revelou melhores resultados na ração com 30% de PB. Proteína total, glicose e globulina também foram maiores em peixes alimentados com ração com 30% de PB, enquanto a albumina foi mais alta com 40% de PB. Conclui-se que a ração com 30% de PB apresentou melhores resultados em termos de crescimento, perfil de aminoácidos e parâmetros sorológicos sem afetar a composição corporal dos peixes.

Palavras-chave: proteína bruta, aminoácidos, *Channa marulius*, composição do corpo, sorologia.

1. Introduction

Aquaculture production has been increased since last three decades to meet the protein demand across the globe (Cruz-Cervantes et al., 2018). Aquaculture industry plays a significant role to meet the demand of meat and also recognized as fastest food processing industry (Baruah et al., 2004; Iqbal et al., 2014). Protein is one of the most essential components to enhance growth performance of fish. To maintain body energy and to synthesize body protein, dietary protein provides essential and non- essential amino acids (Hossain et al., 2010).

The requirement of protein in animals varies with age, species, size and reproductive state and environment (NRC, 2011). The percentage of quality protein in feed results in effective growth particularly in carnivorous fish species (Lee et al., 2002). For proper growth of fish best level of protein is an important factor for feed formulation. To maintain their several life process fish require energy which it obtain from feed in the form of protein (Okorie et al., 2007) Protein deficient feed results in poor growth, by converting nitrogenous wastes into energy through oxidation of amino acids (Wu and Gatlin III, 2014).

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The rapid development and low entry barriers for feed industry has revolutionized the Pakistan fish production due to targeted feed for aquaculture. The feed engineering especially enriched with protein has been tried in a variety of fish species (Sealey et al., 2013). Add references here. Hua et al. (2019) has confirmed the reliant of aquafeed industry is on wild captured forage fish protein. Similarly, insect protein based meal were fed to the atlantic salmon to evaluate the impact on growth performance. Quality analysis of fish feed is mostly carried out on the growth rate of fish and quality benefit of fish. Amino acid is an important energy source and is essential for fish growth to achieve nutritional significance (Santos et al., 2011). The amino acids are found abundantly in fish which are essential for maintaining health (Yin et al., 2010). Secondly, serum biochemistry (protein & carbohydrate) is an important measure to determine the health status of fish (Fazio et al., 2015). Biochemical attributes like protein, albumin, glucose and globulin served as indicator of biological status of fish (Gupta, 2014).

Channa marulius (Hamilton, 1822) locally known as 'Sol' is the largest among the murrel species. *Channa marulius* is a hardy fish and it has reasonable growth and is popular for its flesh quality (Marimithu et al., 2001). The feeding nature is carnivore but can consume and digest feed which is based on various plant origin with high level of protein (Hafeez-ur-Rehman et al., 2017).

Keeping in view the importance of artificial feed, the current study was designed to determine the effect of different levels of protein feeds on growth of *C. marulius.* As there is no specific feed of sole available in the market, this experiment was aimed to test the commercially available different CP feeds for *C. marulius* and their effect was evaluated on growth, body composition and amino acid profile of fish.

2. Materials and Methods

The trial was conducted in happas installed in earthen ponds having dimension of 20×15×10'. Average weight of the fish was 141.23±1.18 g. The design of study was completely randomized (CRD). The four commercially available diets of different CP level viz; T1 (40% CP), T2 (32% CP), T3 (30% CP), T4 (25% CP) were fed to fish in three replicates, and each treatment was stocked with 10 fish. *Channa marulius* was used as experimental fish and grouped into four treatments. The feed was fed to fish at 3% of their body weight three times a day for two months.

2.1. Growth parameters

Different parameters of growth, for example, net weight gain, percentage weight gain, feed conversion ratio (FCR) and specific growth rate (SGR%) was calculated by the following the formulae of Hopkins (1992) (Equations 1, 2 and 3).

$$Net\ weight\ gain(NWG) = Average\ final\ weight(g) - Average\ initial\ weight(g) \quad (1)$$

$$Percent\ weight\ gain = \left(\frac{Final\ weight(g) - Initial\ weight((g))}{Initial\ weight(g)} \right) / \times 100 \quad (2)$$

$$Feed\ Conversion\ Ratio(FCR) = Feed\ intake(g) / Wet\ weight\ gain(g) \quad (3)$$

2.2. Proximate analysis

Five fish from each replicate was taken and dried in oven for 24 hours at 70 °C for whole body proximate. The dry samples were grinded in pestle and mortar to make powder of fish samples and kept in zip packets and stored in refrigerator for further studying its chemical composition. After that samples were analyzed for moisture, crude protein, crude fat and ash, by following the methods of AOAC (2006). The samples were dried at 105 °C for the determination of moisture content. The crude protein was determined by estimation of nitrogen after acid digestion (N_2×6.025) by using the Kjeldhal's apparatus at Fish nutrition department of UVAS. The Soxhlet apparatus was used to determine the crude fat by ether extraction method. Crude ash was determined by flaming the sample at 660 °C (Fatima et al., 2019).

2.3. Amino acids profile

Amino acids profile of experimental fish muscle was analyzed by using amino acid analyzer (Biochrome 30$^+$ Model) available at Dr. M. Yaqoob Malik amino acid analysis laboratory at UVAS, Ravi Campus Pattoki, using ion exchange chromatography described by Official Journal of European Communities using amino acids analyser (Biochrom 30+ Model) known as gold standard for AAA (Michel et al., 2020).

2.4. Serum biochemistry and physicochemical parameters

Three fish from each treatment was randomly chosen for the collection of blood. Serum was obtained by centrifugation of sample at 1200rpm for 10 minutes. Biochemical parameters in the serum samples were analyzed for total protein, albumin, glucose, and globulin using an automated chemistry analyzer following protocol described by Salze et al. (2016). Water temperature, pH, dissolved oxygen, total dissolved solid and electrical conductivity of water was recorded on daily basis. The turbidity was measured by the secchi disc.

3. Statistical Analysis

The resulting data was analyzed by one way analysis of variance (ANOVA) through SAS version 9.1. Means were compared by using Duncan's Multiple Range Test with a significance level $P \leq 0.05$ (Steel et al., 1996).

4. Results

4.1. Growth parameters

At the end of trial, the results of growth performance were analyzed by one way ANOVA and presented in Table 1. The growth parameters were observed maximum in diet containing 30% CP, while minimum growth was observed in the diet containing 25% CP.

4.2. Body composition

The body composition of fish showed non-significant ($p \geq 0.05$) results among all the treatments and shown in

Table 1. Growth parameters of *Channa marulius* among different treatments.

Treatments	T1 40% CP	T2 32% CP	T3 30% CP	T4 25% CP
Initial Weight (g)	140.37±2.30	141.30±1.42	142.87±2.57	140.36±1.48
Final Weight (g)	165.5±2.57^{c}	167.50±0.70^{c}	184.97±0.54^{a}	174.5±1.62^{b}
Net Weight Gain (g)	25.13±0.43^{c}	26.20±1.79^{c}	42.10±2.29^{a}	32.14±0.51^{b}
Percent weight gain	17.85±0.27^{c}	18.43±1.45^{c}	29.57±2.09^{a}	24.28±0.41^{b}
FCR[a]	5.83±0.10^{a}	4.64±0.30^{b}	3.05±0.25^{c}	3.70±0.07^{c}
SGR %[b]	0.23±0.03^{d}	0.29±0.02^{c}	0.43±0.03^{a}	0.36±0.01^{b}
Survival %	90±5.7^{b}	80± 10^{c}	100±5.77^{a}	100±15.27^{a}

The date represents the mean of three replicates. [a]FCR = Feed conversion ratio; [b]SGR (%) = Specific growth rate.

Table 2. Proximate analysis of *Channa marulius* among different treatments.

Treatments	T1 40% CP	T2 32% CP	T3 30% CP	T4 25% CP
Moisture (%)	61.25±1.67^{c}	62.38±0.78^{c}	69.93±2.28^{a}	63.17±1.07^{b}
Crude Protein (%)	31.29±0.98^{a}	29.28±1.63^{a}	21.02±1.85^{c}	23.12±0.90^{b}
Crude Fat (%)	2.42±0.26^{b}	2.29±0.36^{b}	2.49±0.21^{b}	4.32±0.09^{a}
Ash (%)	4.71±0.92^{a}	4.58±1.44^{b}	4.03±0.84^{b}	4.78±0.07^{a}
NFE (%)[a]	0.33±0.35^{b}	0.57±0.26^{a}	0.53±0.12^{a}	0.61±0.04^{a}

The date represents the mean of three replicates. [a]NFE (%) = Nitrogen free extract.

Table 3. The Amino acid profile of *C. marulius* among different treatments.

	TREATMENT	T1 40%CP	T2 32%CP	T3 30%CP	T4 25%CP
Essential amino acid (%of dry matter)	**Methionine**	1.33±0.07^{b}	1.30±0.04^{b}	3.08±0.08^{a}	0.55±0.08^{c}
	Threonine	2.28±0.05^{b}	2.32±0.19^{a}	1.77±0.12^{c}	2.39±0.09^{a}
	Valine	2.15±0.05^{b}	2.29±0.21^{a}	1.71±0.09^{c}	2.31±0.16^{a}
	Isoleucine	3.69±0.09^{a}	3.76±0.28^{a}	2.91±0.07^{b}	3.99±0.23^{a}
	Phenylalanine	2.01±0.07^{b}	2.06±0.08^{b}	1.58±0.30^{c}	2.19±0.12^{a}
	Histidine	1.05±0.01^{a}	1.1± 0.03^{a}	0.81±0.04^{b}	1.11±0.06^{a}
	Lysine	4.93±0.07^{b}	5.32±0.05^{a}	4.09±0.09^{c}	5.29±0.18^{a}
	Cysteine	0.58±0.05^{a}	0.62±0.06^{c}	0.35±0.06^{c}	0.55±0.01^{b}
Non-Essential amino acid (%of dry matter)	**Aspartic acid + Asparagine**	3.52±0.02^{b}	3.35±0.01^{c}	3.08±0.04^{d}	3.65±0.19^{a}
	Serine	2.25±0.04^{b}	2.3±0.08^{a}	1.76±0.12^{c}	2.34±0.09^{a}
	Glutamic acid +Glutamine	6.98±0.01^{b}	6.85±0.06^{b}	6.04±0.29^{c}	8.05±0.81^{a}
	Glycine	4.12±0.02^{a}	4.16±0.08^{a}	3.52±0.19^{b}	4.3±0.04^{a}
	Alanine	3.33±0.11^{b}	3.36±0.23^{b}	2.77±0.16^{c}	3.54±0.17^{a}
	Tyrosine	1.19±0.20^{b}	1.28±0.05^{b}	0.97±0.07^{b}	1.39±0.13^{a}
	Arginine	0.92±0.07^{b}	0.81±0.03^{c}	0.83±0.04^{c}	1.07±0.21^{a}
	Proline	2.65±0.08^{b}	2.8±0.41^{a}	2.18±0.28^{b}	2.78±0.06^{a}
	Ornithine	3.53±0.05^{a}	3.65±0.12^{a}	2.9±0.20^{b}	3.64±0.12^{a}

The data represents the mean of three replicates.

Table 2. The maximum moisture % was observed in the diet having 30% CP while the crude protein and ash % was minimum in this diet. The crude fat and nitrogen free extract was maximum in the diet containing 25% CP.

4.3. Amino acid profile

At the end of experimental trial, the fish were analyzed for amino acid profile. The fluctuating results were observed among all the essential and non-essential amino acids in the diets containing different CP levels and shown in Table 3. 25% CP showed increase level of valine, threonine, isoleucine, phenylalanine, histidine, lysine, aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine, arginine, proline and ornithine. While 32% CP showed increase level of threonine, valine, isoleucine, histidine, lysine, serine, glysine, proline, and ornithine.

4.4. Serum parameters and physicochemical parameters

The serum parameters were analyzed from each treatment and shown in Table 4. The total protein, globulin and glucose content was highest in the 30% CP diet while the albumin content was highest in 40% CP diet. The significantly different results were observed among all treatments. Meanwhile, all the physicochemical parameters were showed non-significant results in all treatments and shown in Table 5.

Table 4. Comparison of serum parameters among different treatment.

Treatments	T1 40%CP	T2 32% CP	T3 30% CP	T4 25% CP
Total protein (gl^{-1})	3.37±0.12[c]	3.51±0.02[b]	3.89±0.11[a]	3.12±0.05[d]
Albumin (gl^{-1})	1.19±0.01[a]	1.03±0.01[b]	1.03±0.04[b]	1.08±0.01[b]
Glucose (mmol/L)	55±2.82[c]	60±3.53[b]	75±2.12[a]	72±1.41[a]
Globulin (gl^{-1})	2.18±0.11[c]	2.48±0.01[b]	2.86±0.07[a]	2.04±0.04[c]

The data represents the mean of three replicates.

Table 5. Comparison of water quality parameters among different treatments.

Treatments	T1 40% CP	T2 32% CP	T3 30% CP	T4 25% CP
Temperature (°C)	20.55±2.54	21.00±2.53	21.14±2.53	21.14±2.45
pH	7.97±0.38	7.97±0.44	7.83±0.44	7.80±0.44
TDS (mg/l)[a]	1412.08±160.91	1413.51±196.62	1434.01±224.12	1425.78±223.16
DO (mg/l)[b]	3.97±0.24	3.99±0.26	4.01±0.26	4.02±0.26
EC (µS/cm)[c]	3757.41±87.56	3783.00±66.43	3796.88±69.68	3792.35±72.73
Turbidity(cm)	10.66±1.10	10.68±1.20	10.81±1.21	10.82±1.31

The date represents the mean of three replicates. [a]TDS = Total dissolved solid; [b]DO = Dissolved oxygen; [c]EC = Electrical conductivity.

5. Discussion

In our study the highest growth rate of fish was recorded in 30% protein diet, after which a decrease in growth was observed. Usually, fish growth increase when level of the protein in diet increases (NRC,1993). Though, growth of the fish increase at a certain level, beyond that level growth of the fish not increase by increasing protein in diet or may become the cause of decrease in growth (Gunasekera et al., 2000; Kim and Lall, 2001; Hossain et al., 2010). In this experiment, specific growth rate and weight gain enhanced in the 30% supplemented protein diet as the then, growth performance was decreased. The maximum growth at 30% in our study was observed may be due to the bigger initial weight of fish, as in most of the requirement studies fish fingerling of smaller sizes and weights are used. The protein requirements of some other fish species were also investigated, such as protein requirement of black sea bass was 45.3% (Shah Alam et al., 2008), European sea bass was 45% (Perez et al.,1997) and olive flounder was 46.4% (Kim et al., 2002). The results from this study, though, are less than the protein requirements of grouper, (47.8%; Chen and Tsai, 1994) and silver pomfret (49%; Hossain et al., 2010) and higher than those of hybrid striped bass (40%; Gatlin III et al., 1994) and white bass (41%; Rudacille and Kohler 1998), however, fish used in these studies were of smaller sizes.

The protein content in fish body was best recorded with 30% protein diet. Muscle protein is most reported protein deposition source among different parts of the fish (Ghassem et al., 2014). The quality of fish flesh was determined by the protein content (Caulton and Bursell., 1977). The lipid contents were decreased with the increase in protein level as were observed in tilapia by Jauncy and Ross (1982). In our study, inverse relationship was found between fat and protein contents. However, moisture and ash content has not shown any linear pattern when fed with different treatment levels. Similar results were also reported by Maithya (1998), in African catfish.

The amino acids are important biochemical constituent for the healing of wound and recovery as described by Westaby (1985). Amino acid profile of fish produced similar results with Jais et al. (1998). Glutamic acid showed the highest amount in the profile in *C. marulius*. This relates to the statement of Ghomi et al. (2012) who observed the highest value of glutamic acid in amino acid composition of flesh of cold water rainbow trout. The most abundant amino acid observed in *C. marulius* was glutamic acid, aspartic acid, lysine and isoleucine.

The serum biochemical parameters can reflect fish health status and nutritional metabolism as described by Lin et al. (2015). Based on the analysis, the fish which was fed with 30% diet has highest concentrations of total protein and glucose. Similar results were reported in African catfish by Abdel-Tawwab et al. (2007). In our study, the highest concentration of albumin was observed in fish which was fed with 40% protein diet.

The water quality parameters directly affect the fish metabolism and food intake efficiency as described by Mutlu et al. (2016). According to Boyd (1979) examined no adverse effects of water quality parameter on the growth and survival rate of fish. In our study, the level of turbidity increase as the quantity of feed increases which directly affect the feeding rate. Although, the strong negative correlation was existing between turbidity and feeding level which suggests that increased turbidity may reduce the feeding rate as reported by Rowe and Dean (1998).

The results of the study indicated that 30% protein diet showed best results of WG, FCR and SGR. The dry matter, crude protein, crude fat and ash content were also improved in 30% diet. Serum biochemistry i.e., total protein, glucose and globulin showed better results at 30%

protein level. In conclusion, the results of the experiment suggested that the diet containing 30% protein can support maximum growth under the experimental conditions used in this study.

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