

Original Research Article

Growth Performance of Carps at Different Protein Ratios under Polyculture Farming

Abstract

This study investigated the effects of three supplemental diets varying in protein content (T1-28%, T2-30%, and T3-32%) on the fillet composition of Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhina mrigal*), and Silver Carp (*Hypophthalmichthys molitrix*) raised in ponds with natural feed. Also included was a control treatment with no supplemental nutrition. Fish from control ponds had substantially lower mean muscle protein concentrations than fish from ponds that received supplemental feeding, with 28% and 30% protein diets producing the most muscle protein. Compared to other treatments, fish fed a diet containing 32% protein had substantially higher muscle lipid concentrations (2.22%). Muscle ash was nearly twice as high in fish from control ponds, whereas muscle carbohydrates were greater in fish from ponds fed 28% protein diets. Rohu had the highest percent of muscle protein among the three sampled fish species, while Silver carp had the lowest, with all other species having intermediate values. Mrigal and Silver carp had the most carbohydrate in their muscles, while Rohu had the least. During polyculture of cyprinids in ponds, supplemental supplementation with diets containing 30 to 32% protein is recommended. **The findings of the present studies should prove beneficial in the production of cost-effective and protein ratio-optimized supplementary feed for the major carps, as well as in the propagation of high-yield fish farming.**

Keywords: Carp Polyculture, Growth performance, Protein ration

Introduction

A number of developing nations are experiencing a deterioration in food security due primarily to their swiftly expanding populations. This circumstance will inevitably increase the pressure on fisheries resources. To assure a sustainable supply of fish while preventing the overexploitation of resources, the fisheries industry must immediately shift its production efforts from "fishing" to "fish farming." Empowering fishing

communities to reduce chronic poverty necessitates an all-encompassing strategy. Efforts to promote sustainable fisheries resource management would be more effective if complemented by activities that stabilise the livelihoods of local communities. The fisheries industry as a whole contributes approximately 1 percent to the country's gross domestic product and employs approximately 1 percent of the labour force. The global increase in human nutritional demand, especially in developing nations, necessitates an increase in the quantity and accessibility of animal protein. Al- Ghanim et al. (2015) state that commercial fish production can help meet this demand by providing fish flesh with a high biological value. Due to a lack of research and scientific expertise on fish feed requirements and quality production, the aquaculture sector of Pakistan is in its infancy compared to other food manufacturing sectors (Mahboob and Al-Ghanim, 2014), despite its enormous potential.

Polyculture of Indian major carps and other Asian carps is well-established (Noor, 2012; Sahu et al., 2007) and is primarily responsible for the rapid expansion of aquaculture in Pakistan. From 2005 to 2015, fish production in Pakistan nearly doubled, rising from 80,000 metric tonnes to over 151,000 metric tonnes. Indigenous major Indian carp species, such as rohu (*Labeo rohita*), catla (*Catla catla*), and mrigal (*Cirrhina mrigal*), are typically cultured alongside introduced grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) to maximise pond productivity. In addition to increasing aquaculture output, it is essential to increase the nutritional value of pond-reared fish to satisfy the food demands of Pakistan's rapidly expanding population ((Haider et al., 2015)).

Mrigal is one of the most important commercial Indian major carps cultivated in polyculture in Pakistan in earthen ponds (Javed, 2015). (Jabeen et al., 2015) Rohu is a herbivorous fish with a high growth potential that is extensively consumed in Pakistan. Catla is the second most prominent carp species in India in Pakistan. It is a promising candidate for polyculture due to its rapid growth rate, compatibility with other Indian major carps, and surface feeding mode (Srivastava et al., 2013). Two Chinese carp species possess complementary characteristics that enable for their successful inclusion in polyculture when combined with native carps. (Amir et al., 2006) Silver carp are filter feeders, ingesting primarily phytoplankton and detritus. Grass carp are herbivorous and graze on aquatic weeds and grasses that grow along the margins of ponds (Ashraf et al., 2011). In semi-intensive polyculture, in addition to ingesting natural feed under extensive culture conditions, all five of these species will consume supplementary feed. Commercial ponds in Pakistan cultivate both Indian and Chinese carps (Chughtai et al., 2015). In addition to requiring relatively minimal technological inputs, this polyculture recycles agricultural and animal wastes effectively (Singh et al., 2018). The cost of (AOAC, 2006) higher quality constituents in pelleted feeds (Sanusi and Danasabe, 2015) can negatively impact the supplementary feeding of these fish in polyculture (AOAC, 2006). Current practises in Pakistan

consist of supplementing the diets of Indian major and Chinese carps cultivated together in ponds with inexpensive ingredients, resulting in fish with relatively poor nutritional value (Chatta et al., 2015).

When farmed fish are adequately cultured and fed nutrient-dense diets, their carcass composition is comparable to that of their wild counterparts (Cahu and colleagues, 2004). Dietary protein may have a negative effect on fillet quality (Grisdale-Heland et al., 2008). Carcass protein levels are notably dependent on supplemental diet protein levels (Singh et al., 2008). In terms of human nutrition, lipids in fish muscle are also crucial (Zehra and Khan, 2011).

The purpose of this study was to evaluate the effects of administering supplemental diets with varying protein levels on the muscle composition of five fish species during semi-intensive polyculture.

Methodology

Study Area and Experimental design

This experiment was conducted in the Muktagacha upazila of the Mymensingh district in Bangladesh from July to December 2019 in earthen wetlands managed by farmers. The average pond size was 3.5 decimal acres, and its average depth was 3 feet. Each of the nine pools was stocked with 80 fish of three distinct species. 20 *Labeo rohita*, 30 *Catla catla*, 20 *Cirrhina mrigala*, and 10 *Hypophthalmichthys molitrix* comprised the initial inventory. In the control sites, no additional feeding occurred. The remaining ponds were fed one of three diets ranging in protein content from 28 to 32% (Table 1). The daily feeding rate was set at 2% of total fish weight and varied between 8 and 10 hours. The feeding rate was adjusted every two weeks. Using a thermometer, Secci disc, pH measuring kit, and dissolved oxygen measuring kit, water quality parameters such as water temperature, transparency, pH, and dissolved oxygen were measured at 15-day intervals at the pond's edge. Each week, fertilizer was applied to the soil. The larvae were obtained from a commercial hatchery. For monitoring fish growth, monthly samples were taken. In each sampling, 10% of the stocked fishes of each species were captured with a seine net from each pond in order to examine the growth performance of fishes.

Pond preparation and stocking

Before conducting the experiment, all ponds were hand-cleared of aquatic vegetation. Through repetitive netting (seine net, 12.5 mm mesh size), undesirable fish and other species were extracted. In addition, liming (CaO at a rate of 247 kg ha⁻¹ as a base dose and 120 kg ha⁻¹ month⁻¹ as a periodic dose) was performed to preserve the

Comment [nW1]: Should mention feeding time /day

Comment [nW2]: This paragraph should be in water quality monitoring section

Comment [nW3]: Larvae or fingerlings

quality of the water. Urea (basal dose, 50 kg ha⁻¹; periodic dose, 25 kg ha⁻¹ month⁻¹) and Triple Super Phosphate (basal dose, 50 kg ha⁻¹; periodic dose, 25 kg ha⁻¹ month⁻¹) are applied to increase the production of natural feed. After three days of liming, the soil was fertilized at its base. After Karim and Rahim (2013), the liming and fertilization dosages were maintained. All carp fingerlings were transferred from nurseries to farmer-managed grow-out ponds in preparation for stocking. The stocking was completed in the wee hours of the morning.

Comment [nW4]: Not include in reference list

Water quality monitoring

Important water quality parameters, including water temperature, water clarity, pH, dissolved oxygen (DO), free carbon dioxide (CO₂), alkalinity, and ammonia-nitrogen, were monitored monthly between 8:00 and 10:00 a.m. The water temperature was recorded using a centigrade thermometer within the range of 0–120 °C. A Secchi disc was used to measure the water's opacity in centimeters. Using a pH metre (YSI Model 60/10FT, USA), the pH of pond water was measured. Using a dissolved oxygen meter (YSI MODEL 58, USA), the dissolved oxygen content (mg L⁻¹) of the pond water was measured. Pond water's free carbon dioxide (mg L⁻¹), alkalinity (mg L⁻¹), and ammonia-nitrogen (mg L⁻¹) concentrations were determined via digital titration using a HACH reagent (FF-2, USA).

Feed Preparation and Analysis

Using a Lab Extruder (Model SYSLG30-IV, China), three floating supplementary feeds (3mm pellets with protein levels of 28, 30, and 32%) were manufactured in the laboratory. Following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2006), the approximate composition of supplementary feeds, including moisture, crude protein, total lipids, total ash and carbohydrates, was determined. The formulation and approximate composition of supplementary diets are shown in Table 1.

Table 1. Formulation and proximate composition of supplemental diets used in the study.

Ingredient	28%	30%	32%
Wheat flour	13.0	9.0	11.0
Starch	2.0	2.0	2.0
Rice polish	5.0	3.0	0.5
Wheat bran	5.0	2.0	0.5
Canola meal	12.5	16.0	25.0
Rape seed meal	4.5	9.0	7.0
Sunflower meal	5.0	7.5	10.0
Corn gluten 30%	22.0	18.0	5.0
Soybean meal	6.0	7.0	12.0
Fish meal	20.0	20.0	20.0
DCP	1.0	1.0	1.0
Soya oil	2.0	3.0	3.5
Vitamin& Mineral	2.5	2.5	2.5
Mixtur			
Moisture	6.86	6.90	6.89
Crude Protein	28.17	30.00	32.00
Total Fats	7.37	7.91	8.33
Total Ash	6.19	6.29	5.32
Carbohydrates	51.41	48.90	47.46
Energy (Kcal/kg)	2515	2525	2513

Monitoring of Pond Water Quality

Samples of pond water were collected every two weeks, and the resulting values were averaged monthly. Metres were used to measure water temperature (HANNA HI-8053), pH (HI-8520), dissolved oxygen (HI-9146), and electrical conductivity (HI-8733). A Secchi disc was used to measure light penetration (turbidity). The dry weights of plankton biomass were derived indirectly via the evaporation method (Javed, 1988) using the following formula:

$$\text{Planktonic biomass (mgL-1)} = \text{Total solids} - \text{Total dissolved solids}$$

Fish Growth Parameters

Condition factor (Carlander 1970) was calculated by the formula:

Condition factor (K) = $(W \times 10^5) / L^3$ Where,

W = Fish wet weight (g)

L = Fish total length (mm)

The survival rate was calculated by the formula:

Survival rate (%) = $(\text{No. of recovered fish} \times 100) / (\text{No. of stocked fish})$

Initial weight (g) = Weight of fish at stock Final weight (g) = Weight of fish at harvest

Weight gain (g) = Mean final weight (g) - Mean initial weight (g)

Production = No. of fishes harvested \times average final weight increase of fishes

Proximate Composition

At the end of the experiments, one fish was chosen at random from each reservoir (three per treatment) for muscle analysis. The cranium, viscera, bones, fins, scales, and tails of these fish were removed, and samples of their nape and tail muscles were taken. Standard procedures were used to analyse muscle tissue for its approximate composition of water, protein, total lipids, ash, and carbohydrates (AOAC, 2006).

Data analysis

Using SPSS (Statistical Package for the Social Sciences, version 24), one-way Analysis of Variance (ANOVA) was applied to data on water quality parameters, fish growth, and yield of carp polyculture under various treatments. Before analysis, data normality was examined. Also, the mean values were compared using the Duncan Multiple Range Test (DMRT; Gomez and Gomez, 1984) at a significance level of = 0.05.

Comment [nW5]: Not in reference list

RESULTS

At the end of the experiment, the Mrigal had substantially greater average weight, weight gain, and total length than the other three species (Table 2). Survival rates for all five fish species were 100%. Fish from the control ponds had substantially lower mean muscle protein concentrations than fish from ponds that received supplemental feeding (Table 3). The supplemental diets containing 28% and 30% protein resulted in significantly higher protein values of 17.89% and 16.65%, respectively, while muscle protein concentrations in fish from ponds receiving the other supplemental diets were intermediate between these values and those of

the control group. Mean muscle lipid concentrations of 2.22 percent were considerably higher in fish fed a diet containing 32 percent protein compared to all other treatments. Nonetheless, this was only marginally higher than the 2.07% lipid concentration in muscle tissue of fish fed a 30% protein diet. The lipid concentrations observed in the muscle of fish fed the 30% and 32% diets were approximately half of those observed in the other regimens. Compared to all other treatments, muscle ash percentages were nearly twice as high in fish from the control ponds, while muscle carbohydrate concentrations were greater in fish from the ponds receiving the 28% protein supplemental diets. The percentage of moisture was comparable across all interventions. There were significant differences in muscle composition between the five sampled fish species (Table 4).

Table 2. Comparison of growth indices of five fish Species under composite semi-intensive pond culture conditions.

Fish Species	Initial Average Weight (g)	Final Average Weight (g)	Weight gain (g)	Initial Average Total length (mm)	Final Average Total length (mm)	Survival rate
Rohu	22.81 ± 1.12 ^a	687 ± 167 ^c	664.29±109 ^c	131 ± 5.2 ^a	393 ± 43 ^c	100 ± 0.00
Catla	22.81 ± 1.15 ^a	416 ± 107 ^c	393.29±105 ^c	131 ± 5.3 ^a	331 ± 25 ^d	100 ± 0.00
Silver carp	22.82 ± 1.13 ^a	488 ± 137 ^d	465.28±115 ^d	131 ± 5.2 ^a	330 ± 31 ^d	100 ± 0.00
Mrigal	22.82 ± 1.13 ^a	774 ± 152 ^b	751.28±113 ^b	131 ± 5.3 ^a	399 ± 41 ^b	100 ± 0.00

Means with different letters are significantly different (p<0.05).

Table 3. Mean (±SD) proximate composition components of muscle from fish receiving one of six supplemental diets or non-supplemental feeding at all.

Protein (%)	Muscle composition (% dry matter basis)				
	Moisture (%)	Crude protein (%)	Total fats (%)	Total ash (%)	Carbohydrates (%)
28%	78.84±1.59 ^b	17.89±1.23 ^{ab}	1.26±0.29 ^c	1.22±0.13 ^{bc}	1.30±0.22 ^c
30%	79.40±1.69 ^{ab}	16.65±1.12 ^d	2.07±0.11 ^b	1.17±0.13 ^d	1.24±0.36 ^d
32%	79.91±1.89 ^{ab}	16.13±1.26 ^d	2.22±0.11 ^a	1.19±0.13 ^{cd}	1.05±0.06 ^f
0% (Control)	81.23±1.47 ^a	14.93±0.57 ^c	1.06±0.06 ^d	2.20±0.11 ^a	1.10±0.09 ^e

(Means±SD) values with different alphabets in the same column are significantly different (p<0.05).

Comment [nW6]: Three supplement diets?

Table 4. Mean (\pm SD) proximate composition components of muscle from five different species of fish receiving supplemental diets.

Individual fish muscle composition (% dry matter basis)					
Fish Species	Moisture%	Crude protein%	Total fats%	Total ash%	Carbohydrates%
Rohu	78.80 \pm 1.68a	17.75 \pm 1.34a	1.25 \pm 0.67e	1.33 \pm 0.42b	1.24 \pm 0.39d
Catla	79.61 \pm 2.09a	16.85 \pm 1.13b	1.38 \pm 0.50d	1.27 \pm 0.39c	1.38 \pm 0.44b
Silver carp	80.26 \pm 2.01a	15.85 \pm 1.20c	1.49 \pm 0.42b	1.38 \pm 0.41a	1.52 \pm 0.41a
Mrigal	79.06 \pm 1.60a	17.15 \pm 1.23b	1.44 \pm 0.50c	1.38 \pm 0.39a	1.49 \pm 0.55a

Comment [nW7]: Four species?

The percentage of muscle protein was highest in rohu and lowest in silver carp, with intermediate values for all other species. Grass carp had the highest percentage of lipids in muscle, while catla had the lowest. Mrigal and silver carp had the highest carbohydrate concentrations in their muscles, while rohu had the lowest. The percentage of moisture did not differ significantly between species.

Comment [nW8]: Silver carp?

DISCUSSION

The Supplemental feeding during polyculture has been shown to increase fish weight gain and pond yield (Abdelghany et al., 2002; Rahman et al., 2006). Therefore, this is not unusual. In addition, supplemental feeding increases the availability and retention of nitrogen by pond-reared fish either directly or indirectly by infiltrating the natural food chain (Krom and Neori, 1989; Langis et al., 1988). Clearly, supplemental feeding is an effective strategy for enhancing the nutritional value of pond-raised fish for human consumption, which is crucial in Pakistan (Haider et al., 2015).

As anticipated, the muscle of all five cultured fish species was lean, containing a greater proportion of protein than fat. In this investigation, rohu had the highest concentrations of muscle protein. When raised in a composite semi-intensive culture system, Shakir et al. (2013) observed that rohu had the highest accumulation of body proteins compared to other fish species. However, Noor (2012) found the maximum levels of whole-body protein in mrigal raised in polyculture with catla and rohu and supplemented with food. Sidwell et al. (1974) found that the muscle protein values of rohu were marginally higher than those reported in other studies, while those of mrigal were higher. In contrast, the muscle lipid levels in this study were lower than those reported in the literature for rohu and comparable to those for catla.

The regimens utilised had a substantial effect on the proximal muscle composition of fish. The diets that produce the highest proportion of protein in muscle also generate the lowest proportion of water. However, as

the quantity of dietary protein increased, the moisture content of the diets decreased as well. Also reported by Ashraf et al. (2011) was an indirect relationship between moisture content and proteins in the musculature of farmed and wild grass and silver carp. The 2 to 3.5 percent increase in muscle protein observed in all fish species with all supplemental diets was not as dramatic as the nearly 8 percent increase reported by (Noor, 2012) when rohu and mrigal were fed 35% protein supplemental diets. Khan also reported higher absolute muscle protein values than those observed in this study. These differences could be attributable to differences in the body proportions of the fish used in each study (Muhammad and Abir, 2011), but they are more likely attributable to differences in the diets (Satpathy et al., 2003).

Comment [nW9]: Khan et al.,2004

In general, muscle lipid levels increased as the quantity of dietary protein increased, reaching a maximum of approximately 2% in fish fed diets containing 32% protein. This may have been caused by the marginally higher levels of dietary lipids in the 30% and 32% protein diets. It may also have resulted from the deamination of excess protein, which caused the excess carbon to be converted into adipose reserves (Zehr and Khan, 2011). When fed protein-rich diets, Siddiqui and Khan (2008) also observed an increase in adipose accumulation in fish. Grass carp had the highest muscle fat content compared to other fish species (Khan et al., 2004). This may be due to the grass carp's diverse diet and greater acceptability and digestibility of supplementary feeds.

According to Ashraf et al. (2011), the relatively higher ash content in the muscle of silver carp and mrigal may indicate a greater capacity to accumulate mineral content in their bodies. (Mohamed et al., 2010) distinct fish species have been described as accumulating distinct minerals in their muscles. (Bolawa et al., 2011) found that ash content in the fish muscle is also an indicator of mineral availability to the fish consumers.

The results of this study were likely affected by the protein sources utilized in the production of each diet (Jobling, 2011). As the quantity of dietary protein increased, for instance, so did the amount of canola meal, sunflower meal, and soybean meal in the diets. The amount of rice polish, wheat bran, and maize gluten decreased proportionally. The amino acid profile and digestibility of various constituents vary considerably (Jobling, 2011). Although the use of various protein sources in the formulation of each diet is somewhat problematic, it is unlikely to invalidate the overall results of increasing fish muscle protein levels with supplemental feeding during polyculture.

CONCLUSION

The focus is currently shifting towards cultural fisheries as the capture fisheries continue to see a steady decline

as a direct result of environmental contamination and the presence of toxicants in our natural waters. However, one of the most significant challenges that culture farming has is the expense of supplemental feed. The majority of this price is attributable to the protein sources, which have to be suitable for the fish in order for it to be employed. In conclusion, providing supplementary food to ponds that were undergoing carp polyculture resulted in the production of the most nutrient-dense fish that could be consumed by humans. When compared to the control ponds, which did not receive any additional feeding, the ponds that were given the diets containing 28% and 30% of protein generated the highest muscle protein values, while the diet containing 32% protein produced fish fillets with the highest lipid content. The sample consisted of five different fish species, and the rohu had the highest percentage of muscle protein out of all of them. On the opposite end of the spectrum was the silver carp, which had the lowest proportion. The grass carp had the highest percentage of lipid in its muscles, whereas the catla had the lowest. During the process of polyculture of cyprinids in ponds, it is recommended that supplementary feeding of diets with protein values ranging from 26 to 28% be performed. The current research focuses on optimizing the protein-to-carbohydrate ratio in fish feed in order to assist farmers in achieving more cost-effective fishing practices and a reduction in the amount of feed wasted, which would otherwise ultimately lead to the worsening of water quality.

Comment [nW10]: Silver carp?

Comment [nW11]: 30-32%

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