Asian Journal of Fisheries and Aquatic Research

Volume 24, Issue 3, Page 35-43, 2023; Article no.AJFAR.104065
ISSN: 2582-3760

Growth Performance and Muscle Composition of Carps *(Labeo rohita*, *Catla catla*, *Cirrhinus cirrhosus* and *Hypophthalmichthys molitrix)* at Different Protein Diets under Polyculture Farming

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJFAR/2023/v24i3635

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/104065

Original Research Article

Received: 29/05/2023
Accepted: 03/08/2023
Published: 12/08/2023

ABSTRACT

This study investigated the effects of three supplemental diets varying in protein percentage (T_1- 28%, T_2- 30%, and T_3- 32%) on the fillet composition of Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhinus cirrhosus*), and Silver Carp (*Hypophthalmichthys molitrix)* reared in ponds with

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supplemental diets. The control treatment with no supplemental feeding was also included to conduct the experiment. 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 significantly ($p<0.05$) higher muscle lipid concentrations (2.22%). The muscle carbohydrates were greater in fish from ponds fed 28% protein diets. The Rohu (*L. rohita*) had the highest percent of muscle protein among the three sampled fish species, while Silver carp (*H. molitrix)* had the lowest, with all other species having intermediate values. The Mrigal (*C. cirrhosus*) and Silver Carp (*H. molitrix)* had the highest carbohydrate in their muscles, while Rohu (*L. rohita*) had the least carbohydrate content. During polyculture of cyprinids in ponds, the supplemental feeding of diets with protein ranging from 28 to 30% protein can be recommended as an optimal protein diet to attain the higher growth performance of carps avoiding the wastage of feed at the farmer's level in semi-intensive polyculture farming.

Keywords: Carp Polyculture; growth performance; protein ration; muscle composition.

1. 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 stabilize the livelihoods of local communities. The fisheries industry as a whole contributes approximately 1% to the country's gross domestic product and employs approximately 1% of the labor 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. [1] stated that commercial fish production can help meet this demand by providing fish flesh with a high biological value. Due to the lack of expertise on fish feed nutrition the production of aquaculture is in its infancy [2], despite of its enormous potential.

Polyculture of Indian major carps and other Asian carps is well-established [3,4] and is primarily responsible for the rapid expansion of aquaculture in Bangladesh. The Indian carp species, such as Rohu (*L. rohita*), Catla (*C. catla*), and Mrigal (*C. cirrhosus*), are typically cultured alongside introduced Grass carp (*Ctenopharyngodon idella*) and Silver carp (*H. molitrix*) to maximize 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 Bangladesh's rapidly expanding population [5].

The Mrigal (*C. cirrhosus*) is one of the most important commercial Indian major carps cultivated in polyculture in Bangladesh in earthen ponds [6]. The Rohu is a herbivorous fish with a high growth potential that is extensively consumed in the country [7]. The Catla (*C. catla*) is the second most prominent carp species and a promising candidate for polyculture due to its rapid growth rate, compatibility with other Indian major carps, and surface feeding mode [8]. Two Chinese carp species possess complementary characteristics that enable for their successful inclusion in polyculture when combined with native carps [9]. 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 [10]. In semi-intensive polyculture, in addition to ingesting natural feed under extensive culture conditions, all four of these species will consume supplementary feed. The commercial ponds in Bangladesh cultivate both Indian and Chinese carps [11]. In addition to requiring relatively minimal technological inputs, this polyculture recycles agricultural and animal wastes effectively [12]. The cost of [13] higher quality constituents in pelleted feeds [14] can negatively impact the supplementary feeding of these fish in polyculture [13]. Current practices in the country 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 [15].

When farmed fish are adequately cultured and fed nutrient-dense diets, their carcass composition is comparable to that of their wild counterparts [16]. Dietary protein may have a negative effect on fillet quality [17]. Carcass protein levels are notably dependent on supplemental diet protein levels [18]. In terms of human nutrition, lipids in fish muscle are also crucial [19].

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

2. METHODOLOGY

2.1 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 ponds managed by farmers. The average pond size was 3.5 decimal, and its average depth was 3 feet. Each of the nine (09) ponds was stocked with 80 fishes of four distinct species (*Labeo rohita*-20, *Catla catla*-30, *Cirrhinus cirrhosus*-20, and *Hypophthalmichthys molitrix*-10). In the control ponds, no additional feeding was done, while remaining ponds were fed with diets ranging in protein percentage from 28 to 32% (Table 1). The daily feeding rate was set at 2% of total fish weight and applied for twice in a day. The feeding rate was adjusted every two weeks. Each week, fertilizer was applied to the soil. The fry 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.

2.2 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 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 Rahman [20], the liming and fertilization dosages were maintained. All carp fingerlings were transferred from nurseries to farmer-managed grow-out ponds in preparation for stocking.

2.3 Water Quality Monitoring

Important water quality parameters, including water temperature, pH, dissolved oxygen (DO), free carbon dioxide (CO_2), 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. Using a pH meter, the pH of pond water was measured. Using a dissolved oxygen meter, 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. A Secchi disc was used to measure light penetration (turbidity).

2.4 Feed Preparation and Analysis

Using a Lab Extruder, 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 proximate composition of supplementary diets is shown in Table 1.

2.5 Fish Growth Parameters

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

$$\text{Condition factor (K)}=(W\times10^5)/L^3$$

Where,

W = Fish wet weight (g)

L= Fish total length (mm)

Table 1. Proximate composition of supplemental diets used in the study

Ingredient	T_1	T_2	T_3
	28%	**30%**	**32%**
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

The survival rate was calculated by the formula:

Survival rate (%) = (No. of harvested fish × 100)/(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 × average final weight increase of fishes

2.6 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 analyze muscle tissue for its proximate composition of water, protein, total lipids, ash, and carbohydrates [13].

2.7 Data Analysis

Using SPSS (Statistical Package for the Social Sciences, version 24), one-way Analysis of Variance (ANOVA) was applied to observe the 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; [21]) at a significance level of = 0.05.

3. RESULTS

At the end of the experiment, the Mrigal had significantly (p<0.05) higher average weight, weight gain, and total length than the other three species (Table 2). Survival rates for all the species were 100%. Fish from the control ponds had substantially (p<0.05) 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 (Table 3). Mean muscle lipid concentrations of 2.22% were considerably higher in fish fed a diet containing 32% protein compared to other treatments. Nonetheless, this was only marginally higher than the 2.07% lipid concentration in muscle tissue of fish fed a 30% protein diet. Compared to all other treatments, muscle ash percentages were nearly twice as high in fish from the control ponds, while muscle carbohydrate concentrations were higher in fish from the ponds receiving the 28% protein supplemental diets. The percentage of moisture was comparable across all interventions. There were significant (p<0.05) differences in muscle composition between the sampled fish species (Table 4).

The percentage of muscle protein was highest in Rohu (*L. rohita*) and lowest in Silver carp (*H. molitrix)*, with intermediate values for all other species. The Silver Carp (*H. molitrix)* had the highest percentage of lipids in muscle, while Rohu (*L. rohita*) had the lowest. The Mrigal (*C. cirrhosus*), and Silver Carp (*H. molitrix)* had the highest carbohydrate concentrations in their muscles, while Rohu (*L. rohita*) had the lowest. The percentage of moisture did not differ significantly between species.

Table 2. Comparison of growth performance of carps in semi-intensive polyculture farming

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^{b}	664.29 ± 109^{b}	131 ± 5.2^{a}	393 ± 43^{b}	100 ± 0.00
Catla	22.81 ± 1.15^{a}	416 ± 107^{d}	393.29 ± 105^{d}	131 ± 5.3^{a}	331 ± 25^{c}	100 ± 0.00
Silver carp	22.82 ± 1.13^{a}	488 ± 137^{c}	465.28 ± 115^{c}	131 ± 5.2^{a}	330 ± 31^{c}	100 ± 0.00
Mrigal	22.82 ± 1.13^{a}	774 ± 152a	751.28 ± 113^{a}	131 ± 5.3^{a}	399 ± 41^{a}	100 ± 0.00

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

Table 3. Proximate composition of muscle from fish feeding three supplemental diets

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

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

Table 4. Proximate composition of carp species feeding with different protein percentage

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

4. DISCUSSION

The Supplemental feeding during polyculture has been shown to increase fish weight gain and pond yield [22,23]. 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 [24,25]. Clearly, supplemental feeding is an effective strategy for enhancing the nutritional value of pond-raised fish for human consumption, which is crucial for the country [5].

As anticipated, the muscle of all four cultured fish species was lean, containing a greater proportion of protein than fat. In this investigation, Rohu (*L. rohita*) had the highest concentrations of muscle protein. When raised in a composite semi-intensive culture system, Shakir et al. [26] observed that Rohu (*L. rohita*) had the highest accumulation of body proteins compared to other fish species. However, Noor [3] found the maximum levels of whole-body protein in Mrigal (*C. cirrhosis*) raised in polyculture with Catla (*C. catla*) and Rohu (*L. rohita*) and supplemented with feed. Sidwell et al. [27] found that the muscle protein values of Rohu (*L. rohita*) 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 (*L. rohita*) and comparable to those for Catla (*C. catla*).

The regimens utilized 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 moisture. However, as the quantity of dietary protein increased, the moisture content of the diets decreased as well. Also reported by Ashraf et al. [10] was an indirect relationship between moisture content and proteins in the musculature of farmed and wild Grass carp (*C. idella*) and Silver carp (*H. molitrix)*. The 2 to 3.5% 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 [3] when Rohu (*L. rohita)* and Mrigal (*C. cirrhosis*) were fed 35% protein supplemental diets. Khan et al. [28] 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 [29], but they are more likely attributable to differences in the diets [30].

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 [19].

When fed protein-rich diets, Siddiqui and Khan [31] also observed an increase in adipose accumulation in fish. Grass carp had the highest muscle fat content compared to other fish species [28]. This may be due to the Grass carp's (*C. idella*) diverse diet and greater acceptability and digestibility of supplementary feeds.

According to Ashraf et al. [10], 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. [32] distinct fish species have been described as accumulating distinct minerals in their muscles. [33] 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 [34]. The amino acid profile and digestibility of various constituents vary considerably [35]. 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 [36].

5. CONCLUSION

The fish fed a diet containing 32% protein had significantly ($p<0.05$) higher muscle lipid concentrations (2.22%). The muscle carbohydrates were higher in fish from ponds fed 28% protein diets. The Rohu (*L. rohita*) had the highest percent of muscle protein among the three sampled fish species, while Silver carp (*H. molitrix)* had the lowest, with all other species having intermediate values. The Mrigal (*C. cirrhosus*) and Silver Carp (*H. molitrix)* had the most carbohydrate in their muscles, while Rohu had the least. During polyculture of cyprinids in ponds, the supplemental feeding of diets with protein ranging from 28 to 30% protein can be recommended as an optimal protein diet to attain the higher growth performance of carps avoiding the wastage of feed at the farmer's level in semi-intensive polyculture farming. 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 wastes at the farms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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