Int. J. Agr. Ext. 10 (01) 2022. 261-268

DOI: 10.33687/ijae.010.01.4195

Available Online at EScience Press

International Journal of Agricultural Extension

ISSN: 2311-6110 (Online), 2311-8547 (Print)

https://esciencepress.net/journals/IJAE

GROWTH PERFORMANCE OF CARPS AT DIFFERENT PROTEIN RATIOS UNDER POLYCULTURE FARMING: IMPLICATIONS FOR FARMERS

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ARTICLE INFO

Article History

Received: November 18, 2021

Revised: January 10, 2022

Accepted: March 26, 2022

Keywords

Labeo rohita,

Catla catla,

Cirrhina mrigala,

Ctenopharyngodon idella,

Hypophthalmichthys molitrix,

Polyculture

ABSTRACT

Aquaculture is a rather recent activity in Pakistan and is still in its infancy; nevertheless, there is immense potential for the development of the sector. Freshwater carp farming is the major aquaculture activity in four of the country's five provinces. However, the main issue fish farmers face, is the availability of cheap supplementary feed with an optimized protein ratio as the protein content in the feed has a direct effect on the fish weight gain and muscle. This study examined the effects of six iso-caloric supplemental diets differing in amounts of protein (22, 24, 26, 28, 30, and 32%) on the fillet composition of rohu (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhina mrigala*), grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) raised in ponds containing natural feed. Control treatment with no supplemental feeding was also included. Mean muscle protein concentrations were significantly lower in fish from control ponds compared to ponds that received supplemental feeding, with 26% and 28% protein diets producing significantly highest muscle protein. Mean muscle lipids of 2.12% were significantly higher in muscles from fish receiving 32% protein diet compared to other treatments. Muscle ash was nearly double in fish from control ponds, where-as muscle carbohydrates were nearly double in fish from ponds receiving 22% protein diets. Among five fish species sampled, percent muscle protein was highest in rohu and lowest in silver carp, with all other species having intermediate values. Muscle lipid was greatest in grass carp and lowest in catla. Mrigal and silver carp had the greatest muscle carbohydrate, while rohu had the lowest. Supplemental feeding of diets with protein ranging from 26 to 28% is recommended during polyculture of cyprinids in ponds. Findings of the present studies ought to prove useful in the production of cost-effective and protein ratio optimized supplementary feed for the major carps and in the dissemination of high production of fish farming throughout the region.

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INTRODUCTION

Food security issues are getting more serious in a number of developing countries due mainly to rapidly growing population. This situation inevitably puts further pressure on utilization of fisheries resources. In order to ensure sustainable supply of fish while avoiding overexploitation of resources, the fisheries sector urgently needs to make a major shift in production efforts; from “fishing” to “fish farming.” Empowering fishing communities to alleviate chronic poverty requires a comprehensive approach. The efforts to promote sustainable fisheries resource management would be more effective if these are supplemented by activities that stabilize communities' livelihoods. The fisheries sector as a whole contributes to about 1 percent to the country's GDP and provides jobs for about 1percent of the country's labour force. The global increase in human nutritional demand, particularly evident in developing countries, requires an improvement in the quantity and availability of animal protein. Commercial fish production can help meet this need by providing fish meat of high biological value (Al-Ghanim *et al.*, 2015). Although having massive ability, the aquaculture sector of Pakistan is in its infancy as compared to the other food manufacturing sectors due to the lack of research and scientific expertise on fish feed requirements and quality production (Mahboob and Al-Ghanim, 2014).

The polyculture of Indian major carps and other Asian carps is well established (Noor, 2012; Sahu *et al.*, 2007), and is largely responsible for the rapid growth of aquaculture in Pakistan. Fish production in Pakistan nearly doubled from 2005 to 2015, increasing from 80,000 metric tons to over 151,000 metric tons in 2015. Indigenous Indian major carp species, such as rohu (*Labeo rohita*), catla (*Catla catla*), and mrigal (*Cirrhina mrigala*), are typically cultured with introduced grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) to maximize pond production. In addition to increasing aquaculture output, improving the nutritional content of pond-reared fish is essential to meet the food demands of Pakistan's rapidly growing human population ((Haider *et al.*, 2015).

Mrigal is among the most important commercial Indian major carps grown in earthen ponds under polyculture in Pakistan (Javed, 2015). Rohu is an herbivorous fish with high growth potential that is widely used in Pakistan (Jabeen *et al.*, 2015). Catla is the second most important Indian major carp species in Pakistan. It's high growing rate, compatibility with other Indian major carps, and surface feeding mode makes it a promising candidate for polyculture (Srivastava *et al.*, 2013). When combined with the indigenous carps, two carp species originating from China possess complementary characteristics that allow for their successful inclusion in polyculture. Silver carp are filter feeders, consuming mainly phytoplankton and detritus (Amir *et al.*, 2006). Grass carp are herbivorous, feeding on aquatic weeds and grasses that grow on pond margins (Ashraf *et al.*, 2011). In addition to consuming natural feed under extensive culture conditions, all five of these species will consume supplementary feed during semi-intensive polyculture. In Pakistan, Indian and Chinese carps are grown together in ponds a commercial scale (Chughtai *et al.*, 2015). In addition to requiring relatively low technological inputs, this polyculture also helps effectively recycle agricultural and animal wastes (Singh *et al.*, 2018). The supplemental feeding of these fish in polyculture can be negatively affected by the cost of (AOAC, 2006) higher quality ingredients in pelleted feeds (Sanusi and Danasabe, 2015). In Pakistan, typical current practices are to supplement the diets of Indian major and Chinese carps grown together in ponds with inexpensive ingredients, resulting in fish of relatively low nutritional quality (Chatta *et al.*, 2015).

When farmed fish are cultured appropriately and fed nutritionally-sound diets, their carcass composition is similar to that of their wild counterparts (Cahu *et al.*, 2004). Carcass protein levels are particularly dependent on supplemental diet protein levels (Singh *et al.*, 2008), and dietary protein in diets may adversely affect fillet quality (Grisdale-Helland *et al.*, 2008). Lipids in fish muscle are also an important consideration in human nutrition(Zehra and Khan, 2011).

The objective of this experiment was to evaluate the effects on fish muscle composition in five fish species resulting from the feeding of supplemental diets of varying protein levels during semi-intensive polyculture.

METHODOLOGY

Experimental Site

This experiment was conducted in 21, 120 m2, earthen ponds at the University of Agriculture, Fisheries Research Farm in Faisalabad, Pakistan.

Experimental Design

Each of the 21 ponds received 67 fish of five different species. The initial stocking consisted of 27 *Labeo rohita*, 13 *Hypophthalmichthys molitrix*, 10 *Catla catla*, 10 *Cirrhina mrigala*, and 7 *Ctenopharyngodon idella* with an initial mean (SD) weight of 22.71 (1.10) g. All of the ponds were fertilized daily with poultry droppings at a rate of 0.17g N/100g fish wet weight. Seven different treatments were assigned to the ponds (N = 3). No supplemental feeding occurred in the control ponds. The remaining ponds received one of six different isocaloric diets, which ranged from 22 to 32 % dietary protein

(Table 1). Feeding rates were set at 2% of total fish weight on daily basis between 8 and 10 hours and adjusted every two weeks. The study lasted 365 days.

Feed Preparation and Analysis

Six floating supplementary iso-caloric feeds (3mm pellets) having gross energy of 2500 kcal kg-1 at varying protein levels of 22, 24, 26, 28, 30 and 32% were prepared in the laboratory with Lab Extruder (Model SYSLG30-IV, China).

The proximate composition i.e. moisture, crude protein, total fats, total ash and carbohydrates of supplementary feeds were determined by following the methods of Association of Official Analytical Chemists (AOAC, 2006). The formulation and proximate composition of the supplementary feeds are presented in Table 1.

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

	Dietary Protein (%)					
Ingredient	22%	24%	26%	28%	30%	32%
Wheat flour	13	13	13	13	9	11
Starch	2	2	2	2	2	2
Rice polish	20	18	12.5	5	3	0.5
Wheat bran	15	9.5	5.5	5	2	0.5
Canola meal	1	5	10	12.5	16	25
Rape seed meal	1	1	5	4	9	7
Sunflower meal	0.5	2.5	4	5	7.5	10
Corn gluten 30%	22	22	21	22	18	5
Soybean meal	0.5	2	2	6	7	12
Fish meal	20	20	20	20	20	20
DCP	1.5	1.5	1.5	1	1	1
Soya oil	1	1	1	2	3	3.5
Vitamin& Mineral Mixture	2.5	2.5	2.5	2.5	2.5	2.5
Proximate Composition%						
Moisture	7.30	7.04	6.85	6.86	6.90	6.89
Crude Protein	22.24	24.00	26.08	28.17	30.00	32.00
Total Fats	7.25	7.43	7.48	7.37	7.91	8.33
Total Ash	7.13	6.92	6.66	6.19	6.29	5.32
Carbohydrates	56.08	54.61	52.93	51.41	48.90	47.46
Energy (Kcal/kg)	2517	2530	2500	2515	2525	2513

Vitamin-Mineral mixture (Kg^{-1} diet): Vitamin A 3,000,000 IU; Vitamin E 6000 IU; Vitamin B_1 600 mg; Nicotinic acid 12,000 mg; Calcium d. pantothenate 2400 mg; Vitamin B_{12} 8 mg; Biotin 10 mg; Dl-Methionine 30,000 mg; B.H.T. 12,500 mg; Zinc sulphate 48,000 mg; Copper sulphate 6,000 mg; Vitamin D_3 6000000 I.U.; Vitamin K_3 600 mg; Vitamin B_2 1400 mg; Vitamin B_6 800 mg; Folic acid 300 mg; Choline chloride 50% 160000 mg; L-Lysine 15000 mg; Manganese sulphate 51600 mg; Ferrous sulphate 40000 mg; Potassium iodide 400 mg. Carbohydrates were calculated by difference as 100 - (protein + lipid + ash +moisture). Energy was determined by Bomb Calorimeter.

Fish Sampling

Fish were captured biweekly from each pond and weighed to the nearest 0.01 g. Fork and total lengths to the nearest 1.0 mm were also recorded.

Monitoring of Pond Water Quality

Pond water samples were taken on fortnightly, and the resulting values were averaged on a monthly basis. Water temperature (HANNA HI-8053), pH (HI-8520), dissolved oxygen (HI-9146), and electrical conductivity (HI-8733) were recorded using meters. Light penetration (turbidity) was measured using a Secchi disc. Plankton biomass dry weights were obtained indirectly by the evaporation method (Javed, 1988) using the formula:

Planktonic biomass (mgL-1) = Total solids - Total dissolved solids

Fish Growth Parameters

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

Condition factor (K)=(W×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 (%)=(No.of recovered fish × 100)/(No.of stocked fish)

Proximate Composition

At the end of the trials, one fish was randomly selected from each pond (three fish per treatment) for muscle analysis. The head, viscera, bones, fins, scales, and tails of these fish were removed, with muscle samples taken from nape and tail. Muscle tissue was analyzed for proximate composition of moisture, protein, total lipids, ash, and carbohydrates using standard methods (AOAC, 2006).

Statistics

Data was analyzed using Statistix software, version 8.1 (Statistix, Tallahassee, Florida, USA). Dietary treatment effects were analyzed using Analysis of Variance (ANOVA), with Tukey's HSD post hoc means comparison procedure used if the ANOVA indicated significant effects. Significance was predetermined at $p<0.05$.

RESULTS

At the end of the experiment grass carp average weight, weight gain, and total length, was significantly greater than the other four species (Table 2). In comparison, catla weighed significantly less, were shorter, and had the lowest weight gain. At 1.4, condition factor was significantly highest in silver carp, followed by mrigal at 1.3, and rohu, catla, and grass carp at 1.2. Survival for all the five species of fish was 100%. At 14.83%, mean muscle protein concentrations were significantly lower in the fish from the control ponds compared to ponds that received supplemental feeding (Table 3). The supplemental diets with 26% and 28% protein produced the significantly highest protein values at 18.15% and 17.79% respectively, while muscle protein concentrations in fish from the ponds receiving the other supplemental diets were intermediate between these values and those of fish from the control treatment. Mean muscle lipid concentrations of 2.12% were significantly higher in the muscle from fish receiving the 32% protein diet compared to all of the other treatments. However, this was only slighter greater than the 1.93% lipid concentration in muscle from fish receiving the 30% protein diet. The lipid concentrations in all of the other treatments were approximately one-half that of that observed in muscle from fish fed the 30% and 32% diets. Compared to all of the other treatments, muscle ash percentages were nearly double in fish from the control ponds, where-as muscle carbohydrate concentrations were nearly double in fish from the ponds receiving the 22% protein supplemental diets. Percent moisture was relatively similar among all of the treatments.

Significant differences in muscle composition were observed among the five fish species sampled (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)	Condition Factor (K)	Survival rate
Rohu	22.71 ± 1.10a	677 ± 165c	654.29±107c	121 ± 5.0a	383 ± 42c	1.2 ± 0.13c	100 ± 0.00
Catla	22.71 ± 1.13a	406 ± 105e	383.29±103e	121 ± 5.1a	321 ± 23d	1.2 ± 0.15c	100 ± 0.00
Mrigal	22.72 ± 1.11a	764 ± 150b	741.28±111b	121 ± 5.1a	389 ± 39b	1.3 ± 0.16b	100 ± 0.00
Grass carp	22.71 ± 1.11a	793 ± 197a	770.29±132a	121 ± 5.1a	398 ± 40a	1.2 ± 0.13c	100 ± 0.00
Silver carp	22.72 ± 1.11a	478 ± 135d	455.28±113d	121 ± 5.0a	320 ± 29d	1.4 ± 0.12a	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.

	Muscle composition (% dry matter basis)				
Protein (%)	Moisture (%)	Crude protein (%)	Total fats (%)	Total ash (%)	Carbohydrates (%)
22%	79.02±1.70ab	16.70±0.69cd	1.03±0.04de	1.13±0.07b	2.12±0.18a
24%	79.26±2.12ab	17.25±0.91bc	1.07±0.14d	1.03±0.10e	1.39±0.31b
26%	78.40±1.97b	18.15±0.79a	0.98±0.52ef	1.08±0.10cd	1.21±0.31c
28%	78.74±1.57b	17.79±1.21ab	1.16±0.27c	1.12±0.11bc	1.20±0.20c
30%	79.30±1.67ab	16.55±1.10d	1.93±0.09b	1.07±0.11d	1.14±0.34d
32%	79.81±1.87ab	16.03±1.24d	2.12±0.09a	1.09±0.11cd	0.95±0.04f
0% (Control)	81.13±1.45a	14.83±0.55e	0.94±0.04f	2.10±0.09a	1.00±0.07e

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

Table 4. Mean (± 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	78.70±1.66a	17.65±1.32a	1.15±0.65e	1.23±0.40b	1.14±0.37d
Catla	79.51±2.07a	16.75±1.11b	1.28±0.48d	1.17±0.37c	1.28±0.42b
Mrigal	78.94±1.58a	17.05±1.21b	1.34±0.48c	1.28±0.37a	1.39±0.53a
Grass carp	79.59±2.05a	16.59±1.52b	1.44±0.50a	1.19±0.35c	1.20±0.40c
Silver carp	80.16±1.99a	15.75±1.18c	1.39±0.40b	1.28±0.39a	1.42±0.39a

Percent muscle protein was highest in rohu and lowest in silver carp, with the other species all having intermediate values. Percent muscle lipid was greatest in grass carp and lowest in catla. Mrigal and silver carp had the greatest muscle carbohydrate values, while rohu had the lowest. Percent moisture did not significantly differ among the species.

DISCUSSION

Supplemental feeding of the polyculture ponds obviously led to an increase in muscle protein. This is not surprising, given that supplemental feeding during polyculture has been shown to increase overall fish weight gain and pond yield (Abdelghany *et al.*, 2002; Rahman *et al.*, 2006). In addition, supplemental feeding increases nitrogen availability and retention by pond-reared fish either by direct consumption (Siddiqui and Al-Harbi, 1999) or indirectly by entering the natural food chain (Krom and Neori, 1989; Langis *et al.*, 1988). Clearly, supplemental feeding is an effective strategy to increase the nutritional value of pond-reared fish for human consumption, which is extremely important in Pakistan (Haider *et al.*, 2015).

As expected, fish muscle from all of the cultured five fish species was lean, containing a higher percentage of protein in comparison to fat. The highest muscle protein values in this study were observed in rohu. Shakir *et al.* (2013) also observed the highest accumulation of body proteins by rohu in comparison to other fish species when reared in a composite semi-intensive culture system. However, Noor (2012) observed the highest whole body protein levels in mrigal reared in polyculture with catla and rohu and receiving supplemental feeding. The muscle protein values observed in rohu in this study were slightly higher than that reported in other studies, where-as those of mrigal were higher (Sidwell *et al.*, 1974). In contrast, the muscle lipid levels in this study were lower than the literature values reported by same for rohu, and similar to those reported for catla.

Fish muscle proximate composition was significantly affected by the diets used. In general, the diets producing the highest percentage of protein in the muscle also produced the lowest percent moisture. However, the moisture content of the diets also decreased as the amount of dietary protein increased. (Ashraf *et al.*, 2011) also reported an indirect relationship between moisture content and proteins in the muscles of farmed and wild grass and silver carp. The increase of 2 to 3.5% in muscle protein observed in all of the fish species with all of the diets used in supplemental feeding was not as dramatic as the nearly 8% increase reported by (Noor, 2012) when rohu and mrigal were fed supplemental diets with 35% protein. The absolute muscle protein values reported by Khan were also greater than those observed in this study. These differences could be due to differences in the body sizes of fish used in each of these studies (Muhammad and Abir, 2011) but is more likely due to differences in the diets (Satpathy *et al.*, 2003).

Muscle lipid levels generally increased as the amount of dietary protein increased, reaching a maximum level of approximately 2% in the fish receiving the 32% protein diets. This could have been due to the slightly higher dietary lipid levels in the 30% and 32% protein diets. It could also have been due to the deamination of extra protein causing the excess carbon to be changed into the stored fat reserves (Zehra and Khan, 2011). Siddiqui and Khan (2008) also observed an increasing tendency in the fats accumulation by fish when given protein-rich diets. Among the species, grass carp had the highest muscle fat contents, which may be because of its wide food selection and better capacity for supplementary feed acceptability and digestibility as compared to other fish species (Khan *et al.*, 2004).

The relatively higher ash content in the muscle of silver carp and mrigal may indicate a heightened capability to accumulate mineral contents in their bodies (Ashraf *et al.*, 2011). Different fish species have been described to accumulate different minerals in their muscles (Mohamed *et al.*, 2010). Ash content in the fish muscle also serves as a sign of mineral availability to the fish users (Bolawa *et al.*, 2011).

The results of this study were likely influenced by the protein sources used in manufacturing each of the diets (Jobling, 2011). For example, as the amount of dietary protein increased, the amount of canola meal, sunflower meal, and soybean meal in the diets all increased. Correspondingly, the amount of rice polish, wheat bran and corn gluten all decreased. The amino acid composition and digestibility of different ingredients differs greatly (Jobling, 2011). The different protein sources used in the formulation of each diet is somewhat problematic, but likely does not invalidate the overall results of improving fish muscle protein levels with supplemental feeding during polyculture.

CONCLUSION

As the capture fisheries is decreasing day by day due to environmental pollution and toxicants in our natural waters, the focus is shifting now towards the culture fisheries. But one of the major issues in the culture farming, is the cost of supplementary feed. Major portion of this cost is due to protein sources that must be compatible with the fish for which it is used.

In conclusion, supplemental feeding of ponds undergoing carp polyculture produced the most nutritious fish for human consumption. Compared to the control ponds that did not receive any supplemental feeding, the ponds fed the 26% and 28% protein diets produced the highest muscle protein values, and the 32% diet produced fish fillets with the highest lipid content. Among the five fish species sampled, percent muscle protein was highest in rohu and lowest in silver carp, with the other species all having intermediate values. Percent muscle lipid was greatest in grass carp and lowest in catla. Supplemental feeding of diets with protein values ranging from 26 to 28% is recommended during the polyculture of cyprinids in ponds.

Present research focussed on the optimization of protein ratio in the fish feed that would help farmers towards cost effective fishing as well as reduced feed wastage that would ultimately lead to water deterioration, otherwise.

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