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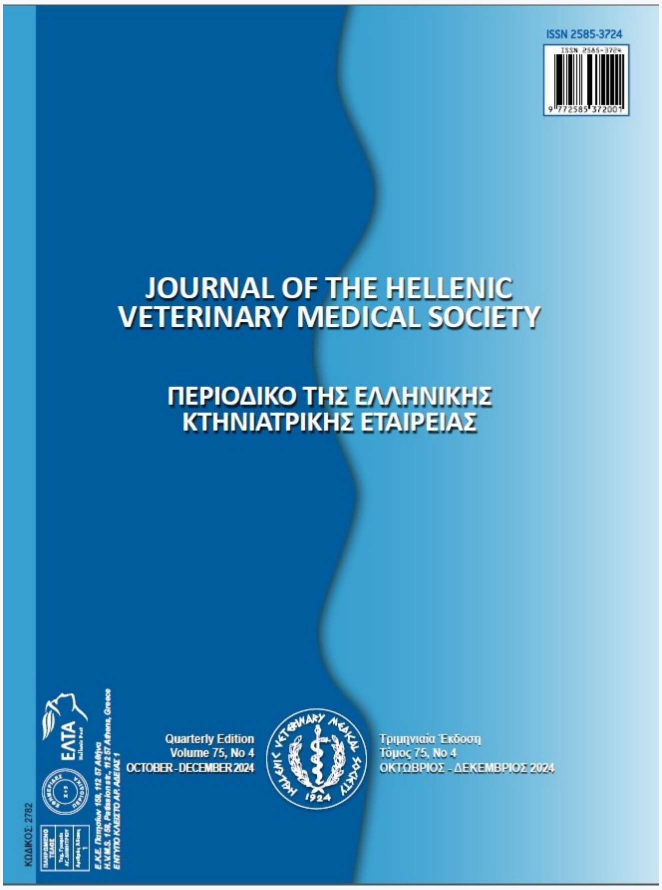

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Research article
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Varying Feeding Parameters of a Plant Based Feed Affect Growth and Blood Chemistry of *Catla catla* Fingerlings

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ABSTRACT: *Catla catla* is one of the most important and fastest growing species of current fish culture, which is practiced in most countries to date. Breeding and harvesting are a difficult job. Some of the important and critical parameters which should be kept in mind are ration size, feeding frequency and adequate stocking density. This study was carried out to optimize these critical parameters. Two hundred juvenile stock was collected from local market and was randomly divided into four groups and the groups were replicated. Artificial feed, having 30% protein, were used for assessing ration size, feed frequency and stocking density. In 1st trial, four different ration i.e. 5, 7, 9 and 11% were given while in second trial fish were offered at a frequency of 2, 4, 6 and 8 times a day. Similarly, in third trial, fish were stocked @ 10, 15, 20 and 25 fingerlings per tank. The results showed that, in trial one, with increase in feeding percentage, there was a gradual increase in weight gain until 11% of feeding, growth increase suddenly stopped. However, white blood cells and hormones level were maintained during increase feeding rate. Contradictory to increasing amount of feeding, feeding frequency deviated from feeding ration increase. A gradual increase in weight was observed from feeding twice up to feeding 6 times a day, but when feeding frequency was increased to 8th times a day, growth was suddenly declined. White blood cells and hormones remained stable up to 6th time feeding but their level dropped when feeding frequency was increased to 8th time/day. In third trial, 15 fish/tank showed optimum growth and performance. RBCs, WBCs and hormones were on the rise up to 20 fish/tank but they declined immediately when stocking density was increased. It is concluded from the above trials that 9% is the best feeding ration, 6 times is the best feeding frequency and 15 fish/tank is the best stocking density (12x4x3 feet fiberglass tank) for optimum growth and performance.

Key words**:** *Catla catla*; Feeding amount; Feeding frequency; Stocking density; Growth rate; WBCs

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INTRODUCTION

Freshwater culture is one of the oldest sources of food for human beings. Fish is one of the main freshwater component and part of food for human beings for centuries. Freshwater aquaculture is the main source of animal protein (Svanberg and Locker, 2020).

Catla catla is one of the major fast growing freshwater carps. It grows best at temperature between 25-32°C. Eggs are demersal at first and gradually become buoyant as time passes. Early stage larvae remain in surface and are strongly photo sensitive. Larvae start feeding after 3 days of hatching. As larvae increase in size, they develop organs which enable them for ingesting food items/particles (Abass *et al*., 2018).

The fry mainly feeds on zooplanktons adult fish are surface feeders but can freely move to mid waters. Along with preferred zooplanktons, they also feed on crustaceans, rotifers, insects, protozoans and algal plants. *Catla catla* matures on its second year of life. In the wild, they migrate during moon soon against the water stream where they breed in shallow marginal areas. It lays millions of eggs and the eggs flow downstream which are collected by seed collectors. Ration is an important limiting factor and ration restrictions leads to lower metabolic rate and poor gonads development. Adequate knowledge is required to take optimum performance from fish as they are very sensitive feeding regime and feeding amount (FAO, 2020). Optimum ration size (amount) plays a vital role in reduction of production cost. Another most important parameter is feeding frequency, especially in intensive farming. It plays a significant role in overall yield and production cost. Therefore, optimizing feeding strategy should be the prime consideration (Grizzetti *et al*., 2019). Low temperature suppresses optimum performance of fish, whereas a rise in temperature induces thermal stress (Fang *et al.,* 2010). Therefore, proper feeding regime and proper water temperature are the keys to successful aquaculture production (Grizzetti *et al*., 2019).

Feeding fish is very difficult as the feed must be distributed evenly in water and eaten effectively. Growth is affected by restricted feeding. Feed conversions efficiencies also change at varying feeding physical parameters. Sufficient literature is available on effect of ration, water temperature and feed utilization (Carnevali *et al*., 2006) but *Catla catla* has not been given much consideration.

Stocking density is another parameter equally important in aquaculture in terms of production. Growth is inversely related to stocking density in many fish cultures (Das et al., 2017). Suzer *et al*., (2007) suggested that stocking density is very important in economic point of view of production systems.

The present study was designed to optimize the critical parameters in fish rearing and to enhance fish farmers profitability and also to play a vital role to increase fish pond business in Pakistan.

MATERIALS AND METHODS

The current study was carried out in the Department of Fisheries and Aquaculture, UVAS, Lahore. Experimental stock was obtained from the departmental ponds and acclimatized with the environment for two days in fish hatchery facility. Ethical approval was granted from the Institutional Biosafety and Ethical Review Committee (Ethical Approval Letter No. 814/IBERC/5/2023 dated 15/03/2023).

Experimental set up

The experiment was divided in three trials. Ration size, feeding frequency and stocking density were observed in each trial and were fed 90 days. Each trial was consisted of three treatments and a control with four replicates in each treatment. Ten fish were cultured in each tank in first two trials and in third trial density was varied. Protein was fed @ 30% in each trial. Growth, length hematological parameters and T_3, T_4 and TSH were monitored. In the first trial ration level fed were 5, 7, 9 and 11% of the body weight. In second trial fish were @ frequency of 2, 3, 4 6 and time times/day. In third trail stocking density of 10, 15, 20 and 25 fish/tank were maintained.

Feed ingredients and Diet Preparation

Fish ration were consisted and prepared by mixing fish meal, soybean meal, sunflower meal, cotton seed meal, guar meal and rice polish and minerals @ 15, 20, 10, 15, 15, 24 and 1% respectively, for obtaining desired protein level. The same feed was fed and used in all trials.

Chemical analysis of the feed ingredients

Proximate analysis

Proximate analysis were performed according to the established method of AOAC (2003).

Growth Studies

Fish stock was weight at arrival, in mid of the studies and then at the end of the experiment (Espe *et al.,* 2012).

Net Weight= Final Weight - Initial Weight

$$\% \text{ Weight Gain} = \frac{\text{Final Weight - Initial Weight}}{\text{Initial Weight}} \times 100$$

Physico-Chemical parameters

Physico-chemical parameters i.e. dissolved oxygen, temperature and pH was recorded on daily basis using proper instruments for each parameter.

Survival Rate

Survival rate was recorded for all the treatment in replicates during the entire course of experiment.

Determination of Hematological Parameters

At the end of trial period, blood samples were collected accordingly and were tested for red blood cells (WBCs) and white blood cells (WBCs) under microscope and counting chamber.

Hormone analysis

Growth hormones i.e. T_3, TSH and T_4 were determined by modified method of Barlow and De Nayer (1988), RIA kit (NIADDK, NIH, USA) and enzymatic immuno-assay kits (DADE BAXTER STRATUS II) respectively.

Statistical Analysis:

ANOVA was used for analysis of collected data. Difference among variables were analyzed using Duncan multiple range tests at inferiority and superiority level of $p<0.05$.

RESULTS AND DISCUSSION

Fish weight gain increased gradually with the increase of ration size up to 9% but however, weight decreased all of a sudden when ration size was increased to 11%. No change was observed in RBCs in all the treatments. However, WBCs declined at higher ration level. No correlation was found between T_3 of fish level had different ration size. However, TSH increased from lower ration size to higher ration size (Table 1). Minabi *et al.,* (2013) observed the effect of water temperature on net biomass, growth performance, feed utilization and carcass biochemical composition. They reported a significant growth rates ($p<0.05$) at different ration sizes at different water temperature. Mean weight gain and significantly increased when water temperature was increased from 24 to 28°C and higher ($p<0.05$) at 3% ration size while there was a decline in FCE with increasing ration size from 1 to 5% at each water temperature. Our results are in line with those of presented by Maniat *et al.,* (2014). In our studies, highest growth and hematological values were recorded at 9% of ration size and then declined abruptly when ration size exceeded. This effect might be due to different fish species in the present study and reported by Maniat *et al.,* (2014) in which they used other species of fish rather than *Catla*. Growth hormones increased with increase in ration sizes (Table 1).

Feeding frequency remained the same due to similar or same water exchange protocols. DO was slightly increased as feeding frequency was increased and NO_3 level decreased but phosphate level remained variable among treatments.

Table 1. Effect of different ration level on growth, physico-chemical and hematological parameters of fish

Parameters	Ration Level			
	5%	7%	9%	11%
DO (mg/L)	9.81±0.007[b]	11.13±0.007[a]	9.11± 0.007[c]	7.51±0.007[d]
Temp (°C)	29.33±0.014[b]	26.73±0.141[d]	29.35±0.073[c]	31.70±0.141[a]
Nitrate (mg/L)	2.53± 0.707[a]	2.00± 1.414[b]	2.00± 1.412[b]	2.50±0.707[a]
Phosphate (mg/L)	1.53± 0.707[b]	1.50±0.707[b]	2.00± 1.414[a]	1.50± 0.707[b]
Weight (g)	14.00±0.633[c]	16.20±0.707[b]	20.91±0.583[a]	10.24±0.424[d]
Length (cm)	9.00±0.141[c]	9.36±0.077[b]	10.41±0.151[a]	8.245±0.134[d]
WBC (mil/mm^3)	17.10±0.431[d]	18.05±0.212[b]	19.15±0.070[a]	17.25±0.212[c]
RBC (mil/mm^3)	1.30±0.282[b]	1.25 ±0.070[c]	1.61±0.007[a]	1.20±0.007[d]
T3 (ng/ml)	1.16± 0.070[b]	0.94±0.042[c]	0.82± 0.014[d]	1.34±0.007[a]
T4 (ng/ml)	0.72± 0.084[c]	0.81±0.007[b]	0.64± 0.007[d]	1.23± 0.014[a]
TSH (ng/ml)	0.32± 0.014[c]	0.24±0.0141[d]	0.33± 0.014[b]	0.35±0.014[a]

Values are mean of triplicates ±S.E. Data figures with different superscript letters are significantly different from each other at P<0.05.

Weight gain increased with increase of feeding frequency but dropped all of a sudden when feeding frequency was increased to 8 times per day. RBCs, WBCs and growth hormones followed the similar pattern as shown in Table 2. Another study investigated the effect of feeding frequency on growth, feed utilization and size variation on *Carassius auratus gibelio*. They carried out the study in five trials i.e. two meals per day, three meals per day, four meals per day, 12 meals per day and 24 meals per day. They concluded that higher feeding frequency affect growth positively and other biochemical parameters. Our results are in line with the above investigation as feeding frequency up to six times per day had a positive impact on the growth performance.

In stocking density trial growth was variable from treatment to treatment. RBCs and WBCs followed the same trend. Growth hormones increased up to 20 fish per tank and then showed variable results. T_3 abruptly declined at a stocking density of 25 fish/tank. TSH value endured the same throughout the trial. However, TSH declined as stocking density was increased from 20 to 25 fish/tank. The results are showed in Table 3. Another study was carried out a study to evaluate growth response of *Nile tilapia* to dietary protein on different stocking density. Fish were fed a diet containing 25, 35 and 45% of crude protein @ of 4% of body weight twice daily for 70 days. The results showed that weight gain was positively affected by protein percentage and inversely affected by stocking density. Similarly, in our study, growth, hematological parameters and growth hormones increased up to 20 fish/tank and declined when the fish density was increased from 20 fish/tank except TSH. So, it can

Table 2: Effect of different feeding frequencies on growth, physico-chemical and hematological parameters of fish

Parameters	Ration Feeding Frequency			
	2 Times	**4 Times**	**6 Times**	**8 Times**
DO (mg/L)	8.17 ± 0.007^a	8.31 ± 1.124^c	7.84 ± 0.473^d	8.17 ± 0.007^b
Temp (°C)	32.50 ± 0.143^a	30.45 ± 1.62^d	32.2 ± 0.565^b	32.00 ± 0.141^c
Nitrate (mg/L)	1.50 ± 0.707^c	1.51 ± 0.707^b	2.00 ± 1.414^a	1.40 ± 0.707^d
Phosphate (mg/L)	2.50 ± 0.707^a	1.52 ± 0.707^b	1.00 ± 1.404^d	1.50 ± 0.707^c
Weight (cm)	15.55 ± 0.493^c	17.95 ± 0.77^b	20.45 ± 2.473^a	11.33 ± 0.233^d
Length (cm)	9.26 ± 0.190^c	9.65 ± 0.077^b	10.95 ± 0.353^a	8.70 ± 0.141^d
WBC (mil/mm^3)	18.60 ± 0.283^b	18.23 ± 0.03^c	20.05 ± 0.234^a	17.33 ± 0.141^d
RBC (mil/mm^3)	1.64 ± 0.021^b	1.34 ± 0.042^d	1.78 ± 0.014^a	1.56 ± 0.028^c
T3 (ng/ml)	1.34 ± 0.007^b	1.24 ± 0.043^c	1.39 ± 0.042^a	0.78 ± 0.014^d
T4 (ng/ml)	0.87 ± 0.013^d	1.25 ± 0.021^b	1.30 ± 0.028^a	0.95 ± 0.035^c
TSH (ng/ml)	0.54 ± 0.014^c	0.35 ± 0.023^d	0.78 ± 0.007^a	0.64 ± 0.007^b

Values are mean of triplicates as ±S.E. Data figures with different superscript letters are significantly different from each other at P<0.05.

Table 3: Effect of different stocking densities on growth, physico-chemical and hematological parameters of fish

Parameters	Stocking Density			
	10 Fish	**15 Fish**	**20 Fish**	**25 Fish**
DO (mg/L)	5.65 ± 0.777^d	5.85 ± 0.773^c	6.61 ± 0.268^b	6.70 ± 0.141^a
Temp (°C)	34.53 ± 0.424^a	34.25 ± 0.213^b	33.25 ± 0.212^d	33.35 ± 0.353^c
Nitrate (mg/L)	1.53 ± 0.707^c	2.00 ± 1.414^b	3.00 ± 0.007^a	1.45 ± 0.707^d
Phosphate (mg/L)	2.53 ± 0.707^a	1.50 ± 0.707^b	1.53 ± 0.007^c	1.0 ± 0.007^d
Weight (g)	15.47 ± 0.463^d	20.85 ± 0.493^a	16.35 ± 0.773^c	17.85 ± 0.770^b
Length (cm)	9.64 ± 0.346^d	10.60 ± 0.424^a	10.00 ± 0.707^c	10.10 ± 0.561^b
WBC (mil/mm^3)	18.85 ± 0.070^c	19.25 ± 0.213^b	19.89 ± 0.121^a	18.43 ± 0.028^d
RBC (mil/mm^3)	1.72 ± 0.021^b	1.42 ± 0.014^d	1.82 ± 0.084^a	1.65 ± 0.014^c
T3 (ng/ml)	1.63 ± 0.033^a	1.28 ± 0.007^c	1.43 ± 0.021^b	1.23 ± 0.0282^d
T4 (ng/ml)	0.77 ± 0.014^d	1.45 ± 0.007^a	1.43 ± 0.035^b	1.23 ± 0.028^c
TSH (ng/ml)	0.44 ± 0.021^d	0.47 ± 0.007^c	0.84 ± 0.042^b	0.875 ± 0.007^a

Values are mean of triplicates as ±S.E. Data figures with different superscript letters are significantly different from each other at P<0.05.

be concluded that our study is in line with previous studies in terms of stocking density and when they reached to a thresh-hold limit all the values decreased.

CONCLUSION

Conclusively, the present results have optimized the feeding regime and the stocking density for efficient growth of *Catla catla*. The results would likely be practiced by the fish farmers to raise the high biologic value protein yield for the masses.

AUTHORS CONTRIBUTION

Sadaf Aman conducted the experiment. Muhammad Ashraf, Javed Iqbal Qazi and Iqra Aman conceptualized, designed and Analysed the data. Rahman Ullah drafted the manuscript in its current form.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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