GROWTH PERFORMANCE OF *Cyprinus carpio* FRY USING FREE LYSINE INCORPORATED FEED IN AQUARIUM

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ABSTRACT

A 90-day experiment was conducted at the Institute of Agriculture and Animal Science, Paklihawa, Nepal to assess the early growth response of common carp (Cyprinus carpio L. var. communis) at varying levels of free lysine supplemented diets in glass aquaria. The basal diet comprised a mixture of rice bran, wheat bran, mustard oilcake, and soybean meal (T0). Three other treatments included basal diet along with 0.08% free lysine (T1), 0.16% free lysine (T2), and 0.24% free lysine (T3). A completely randomized design (CRD) was employed with four treatments replicated three times. Advanced fry of common carp approximately 1.4 g size were stocked in 1x2x1 cubic feet aquariums with a 45-liters water capacity at the rate of 10 fish per aquarium. Locally prepared pellet feed, maintained with 30% crude protein based on different experimental diets, were supplied at 5% of wet body weight of fish with feed adjusted fortnightly. At harvest, fry fed with lysine supplemented diets (T2 and T3) exhibited relatively better growth in mean final weight, mean weight gain, mean daily weight gain, specific growth and feed conversion ratio compared to the control (T0) and treatment T1. T2 achieved the highest weight gain of 4.56±0.07g followed by T3(3.75±0.21 g) and T1 (3.52±0.31 g), while control (T0) achieved the lowest weight gain of 3.17±0.11 g. Treatment T2 proved to be most effective, showing the highest weight gain, daily weight gain, low FCR and high SGR. Water parameters (temperature, dissolved oxygen, pH, ammonia, nitrate, phosphorous, and chloride) remained stable throughout the study.

Keywords*: soybean, production, lysine supplemented diets, weight gain, feed rations*

INTRODUCTION

Nepal is struggling with high level of hunger and poverty since very long. Poor nutrition and food insecurity have remained as challenge and barrier to development in Nepal. The value of fish as a supply of high-quality protein has recently emphasized the important role in the food security. With a view to make the country self-sufficient in aquaculture production, Nepal government implemented Prime Minister Agriculture Modernization Project (PMAMP) in 2015. To achieve the ambitious target, the current production level needs to be expanded by many folds. Aquaculture is fairly a new activity in Nepal. Carp fishes have contributed more than 95% of total fish production (Gurung & Basnet, 2003). Among all the species of the fish, common carp is the most cultivated and refined carp species throughout the world. Tacon (2001) reported that only about 3 percent of cyprinids are produced in intensive systems. However, as soon as intensive cage and tank-culture systems for producing common carp become profitable, these culture systems will rapidly spread and use of aquarium will be common. It is accordance with Japanese experience where intensive culture of common carp was developed in 1960s and peaked in 1977 with a yearly total production of 30,000 tones (Ikuta & Yamaguchi, 2005).

Demand for the fish has increased due to its highly valued texture and delicious taste. Without knowing the nutrient requirements or having commercial diets available for this fish, farmers routinely use trash fish or feed formulated for other fish species in raising the fish. But information on other nutrient requirements for preparation of suitable diets using locally available feed stuffs, is needed. Of all the available and commonly used ingredients, fish meal has been used extensively in fish feed. However, the increasing cost of fish meal and its uncertain availability has demanded alternative protein source.

Inclusion of protein in fish diet is indispensable but cost of animal protein is increasing by leaps and bounds. So, incorporation of plant protein is only solution to compensate the requirement of protein. Soybean meal (SBM) is considered as one of the most nutritive feed ingredients. Due to its balanced amino acid profile, it can be used to replace the animal protein in the fish diet (El-Saidy & Gaber, 2002). Furthermore, SBM has the advantage of being resistant to oxidation and spoilage and is free of fungi, viruses and bacteria that are harmful to fish (Dersjant-Li, 2002).

Amino acids greatly influence the growth patterns, reproductive performance and development of a fish (National Research Council, 2011). Among 20 naturally occurring amino acids, lysine is the most essential one and is required by fish for its proper growth and development. It is the second most limiting amino acid in fish (Li et al., 2007; Yu et

al., 2014; Jiang et al., 2016). It helps in absorption of calcium; maintain healthy blood vessels, produces antibodies, enzymes, collagen and repairs of tissues. However, lysine is considered as main limiting amino acid in fish diets of vegetable origin (Furuya et al., 2006; Coldebella et al., 2011). The properly processed soybean meal has been found to have less available lysine for carp which was the major factor responsible for reduced growth. Thus, lysine deficient commercial diets are commonly augmented with free lysine (Coldebella et al., 2011; Diemer et al., 2014). Dietary lysine supplementation generally improved the feeding rate of common carp (Deng et al., 2010). Therefore, free lysine has been successfully used to improve the successful growth of healthy fingerlings of common carp. However, requirement of lysine for maintenance and growth and its influence on body composition are not yet clarified for numerous species. Detailed experiments on L-lysine supplementation in different concentrations are needed to fully understand its efficiency on early growth response. Finding the optimum requirement of lysine in a feed that can influence successful growth will be a great achievement of my research. Therefore, the research is conducted with the following objectives.

- To observe the early growth performance of fish in different treatments.
- To assess the required quantity of lysine in feed for proper fish growth in early rearing phase.
- To assess water quality parameters in different treatments during experimental period.

MATERIALS AND METHODS

Experimental Site

Aquaculture laboratory of Institute of Agriculture and Animal Science, Paklihawa was purposively selected for the study for 90 days during 23rd July, 2019 to 20th October, 2019.The site is located between 27^{o} 50' N latitude and 83^{o} 45' E longitude with an elevation of 107 masl. Climate of the experimental site was subtropical and humid.

Collection and acclimatization of experimental fish

Almost two months old healthy advanced fry of common carp fish with mean initial weight (1.39±0.01 g) and mean initial length (4.56±0.02 cm) were procured from Fish research and pure line development center, Bhairahawa, Nepal. They were acclimatized in the lab condition for 10 days after dip treated in salt water (0.2 ppt). During that period, the fish were fed with locally prepared rice bran and mustard oilcake-based diet.

Experimental design and stocking of fish: The experiment was carried out in a Completely Randomized Design (CRD) with three replications of four treatment

combinations in 12 aquaria. Individual aquarium size was 1x2x1 cubic feet having 45 liters water capacity. They were washed and disinfected with potassium permanganate solution (4 ppm) thoroughly and cleaned with fresh water and dried for a week. Then, they were filled with tap water. An adequate level of dissolved oxygen was maintained through continuous aeration in each aquarium provided from compressor. The air pressure was uniformly controlled in the entire aquarium by using plastic regulator. The advanced fries were randomly distributed in 12 aquaria with 10 fish per aquarium.

Treatment description/ details

In all experimental diets, 30% crude protein was maintained and prepared accordingly. In the experimental diet, the basal diet without external supplement of free lysine was taken as control treatment (T0) whereas 0.08% free lysine supplemented in basal diet as treatment 1 (T1), 0.16% free lysine supplement supplemented in basal diet as treatment 2 (T2) and 0.24% free lysine supplement supplemented in basal diet as treatment 3 (T3) as presented in Table 1.

Table 1. The treatment description

Treatment	Description	Feeding rate
T0	Control (Rice bran + Mustard Oil cake + Wheat bran + Soybean meal)	5% body weight
T1	Control + 0.08% free lysine	5% body weight
T2	Control + 0.16% free lysine	5% body weight
T3	Control + 0.24% free lysine	5% body weight

Experimental diets

All the feed ingredients such as Rice bran, mustard oil cake, soybean meal and wheat bran were finely grinded separately using Bajaj 500-watt mixer grinder and sieved accordingly. The homogenous feed samples were sent to Central Fisheries Promotion and Conservation Center, Central fisheries Building, Balaju, Kathmandu for proximate analysis. Similarly, amount of lysine content in feed, determined in Multi-Pharmaceutical Laboratory Pvt. Ltd. located at Lalitpur. Table 2 shows the approximate amount of free lysine to be incorporated in feed in order to achieve the lysine requirement (per kg diet) in different experimental diets.

Table 2. Inclusion percentage of lysine in different ingredients in fish diet.

Ingredients	Inclusion rate (%)	% analyzed Lysine
Soybean Meal	44.68	2.85 (1.2734)
Rice bran	19	1.05 (0.1995)
Mustard Oilcake	18.12	2.63 (0.4766)
Wheat bran	16.2	0.6 (0.0972)
Premix	2	-
L-lysine HCL	-	-

The data are expressed as percentage of diet. In parenthesis, the data are percentage of analyzed lysine included in basal diet.

The amount of ingredients required to prepare the feed was calculated based on the protein content as presented in Table 3 and were thoroughly mixed at the specified amounts. The other ingredient like vitamin premix were also used in the feed as a growth promoter. Four experimental diets were prepared. One was basal diet without free lysine and other three included different concentrations of free lysine i.e., 0.08%, 0.16% and 0.24% free lysine respectively. The feeds were prepared in the form of pellets (3mm diameter) with the help of hand-operating machine. The prepared feeds were sun dried to prevent loss of moisture and preserved in air tight bottles to prevent from fungal infections.

Table 3. Formulation of experimental diet (30% crude protein level)

Ingredients	Treatments (weight in gram)			
	T0 (Control)	T1	T2	T3
Rice bran	19	19.05	19	18.92
Mustard Oilcake	18.12	18.12	18.20	18.20
Soybean meal	44.68	44.75	44.84	44.84
Wheat bran	16.2	16	15.8	15.8
Premix	2	2	2	2
Free Lysine	-	0.08	0.16	0.24
Total	100	100	100	100

Feeding rates and schedule

The fish fries were fed with experimental diets twice a day, 9:00 AM in the morning and 4:00 PM in the afternoon at the rate of 5% of the total wet body weight. Quantity of feeding was adjusted fortnightly based on the fish growth measurements.

The daily feed ration (DFR) was calculated using formula,

$$DFR = \text{Average body weight} \times \text{Number of stocks} \times \text{feeding rate}$$

Sampling procedure

The body weight and length of 50 % fish population from each replication was measured at the start of the study and for every 15 days to adjust the feed ration and monitor their weight increment. All the fish were blot dried before they were weighed using an electronic balance (KERRO Electronic Compact Scale; precision-0.01g) in each sampling day. Sampling of fish was done between 10:00 AM – 12:00 PM in each sampling day. At the end of the experiment, all the fish were harvested and length and weight of each individual fish was measured to assess the final growth of the experimental fish in indoor captive conditions.

Water quality parameters

The water quality parameters such as temperature, dissolved oxygen (DO) and pH were recorded on daily basis. The temperature and dissolved oxygen of the aquarium were determined by a DO meter (Lutron, PDO-519) while water pH was recorded by pH meter (HANNA, model HI96107, Italy). Ammonia, Nitrate and Chloride were recorded fortnightly just before exchanging water and were determined by using ENPHO water quality test kit. Guidelines provided by WHO were followed for the measurement of these parameters.

Calculations and statistical analyses

Data on initial weight, initial length, final weight, final length and feed intake were used to calculate Weight Gain (WG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and mean daily weight gain (MDWG). Calculation of these parameters were done by using following formulae.

$$\text{Mean weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Mean daily weight gain (g/fish/day)} = \frac{\text{Mean final weight (g)} - \text{Mean initial weight (g)}}{\text{Culture periods (days)}}$$

$$\text{Specific growth rate (\% per day)} = \frac{[\log \text{Final weight (g)} - \log \text{Initial weight (g)}]}{\text{Culture periods (days)}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Quantity of feed supplied (g)}}{\text{weight of fish (g)}}$$

Collected data were subjected to one-way ANOVA (Analysis of Variance) using R-Stat to see whether the influence of different treatments on the above parameters were significant or not, and $P<0.05$ was considered to be statistically significant. Duncan's

Multiple Range Test was performed for mean comparison. The water quality parameters were expressed in the form of graphs.

RESULTS AND DISCUSSION

The results, pertaining to water quality and growth of experimental fish fry, obtained during the field experiment were analyzed and presented in this chapter with the help of tables and figures wherever necessary. The obtained results are discussed with possible reasons and supporting literatures in the following headings.

Proximate composition of feed

The proximate analysis of pellet feed was carried out in the Central Fisheries Promotion and Conservation Center, Central fisheries Building, Balaju, Kathmandu using NIR analyzer (Antaris™ II FT-NIR Analyzer). The formulation and proximate composition of the control and treatment diets are given in Table 3 and Table 4 respectively. The feed was maintained with 30% crude protein. During the preparation of experimental feed, three treatments except control were prepared with the inclusion of free lysine in three different concentration levels.

Table 4. Proximate composition of feed used in experiment

Constituents	Mean ±S.E. (%)
Crude Protein	30±0.97
Crude Fat	16.75±2.60
Crude Fiber	1.84±0.36
Total Ash	8.19±1.56
Moisture	7.57±0.64

Fish growth performance in terms of weight gain, length gain, specific growth rate and Feed Conversion Ratio

Cyprinus carpio fries were sampled fortnightly to obtain their average length, mean weight, mean weight gain, specific growth rate, feed conversion ratio and total production. Stocking and harvest weight, and fish growth performance during rearing phase are presented in Table 5 and Table 6 respectively. In the present study, free lysine supplemented diets showed appreciable increase in growth in terms of weight gain, mean daily weight gain, specific growth rate, feed conversion ratio and total production.

The total harvest weight of treatment (T2) was significantly higher with 59.43±0.86g followed by 51.28±2.28g (T3) and 49.28±2.91 g (T1). The control group (T0) obtained the lowest harvest weight of 45.46±1.32 g. The similar findings were observed by Zhou et al. (2008) that lysine supplementation in diets for juvenile carp (*Cyprinus carpio*) improved the final weight and Ahmed and Khan (2004) with Indian major carp (*Cirrhinus mrigala*) showed better growth performance with lysine supplementation in diets.

Table 5. Stocking and harvesting details of common carp during experimental period of 90 days

Parameters	Treatments (Lysine levels %)				
	T0 (2.05)	T1 (2.13)	T2 (2.21)	T3 (2.29)	CV (%)
Total stock weight (g)	13.78±0.22[a]	14.03±0.19[a]	13.8±0.19[a]	13.83±0.31[a]	2.914
Total harvest weight (g)	45.46±1.32[b]	49.28±2.91[b]	59.43±0.86[a]	51.28±2.28[ab]	6.77
Average stock length (cm)	4.5±0.02[a]	4.61±0.02[a]	4.59±0.01[a]	4.54±0.01[a]	0.61
Average harvest length (cm)	6.32±0.19[b]	6.22±0.04[b]	7.06±0.14[a]	6.44±0.15[ab]	6.77

Mean values with same superscript in the same row are not significantly different (P>0.05).

Similarly, the highest final average length was recorded in treatment T2 with 7.06±0.14 cm which was significantly different from control treatment, T0 and is presented in Figure 1. The final average lengths of other treatments were 6.44±0.15 cm (T3) and 6.22±0.04 cm (T1).

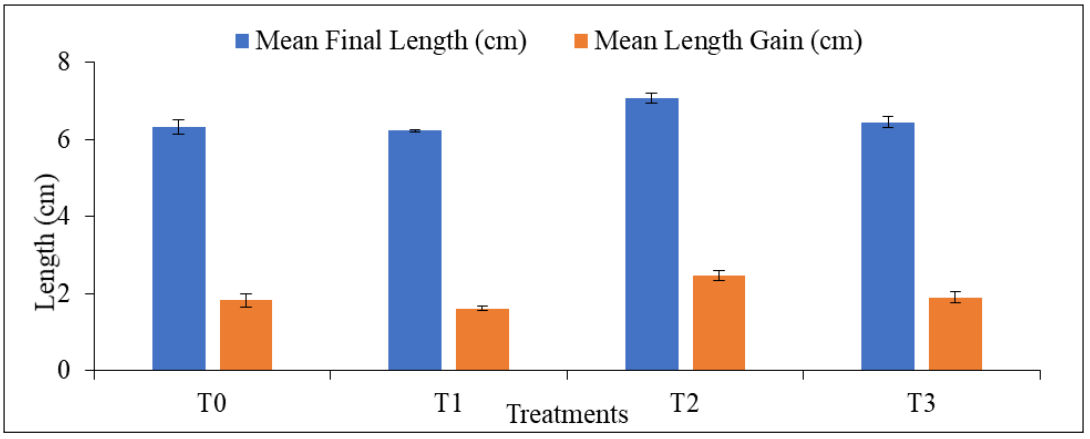

Figure 1. Final length and length gain of common carp fry in different treatments during the experimental period of 90 days

In the present study, the two treatments T2 and T3 showed significant increase in weight gain than control treatment (T0) and treatment T1 as presented in Table 6 and Figure 2. The highest average weight gain of 4.56±0.07 g was recorded in treatment (T2) followed by T3 and T1 with 3.75±0.21 g and 3.52±0.31 g respectively. The lowest average weight gain was observed in control treatment (T0) with 3.17±0.11 g. The data showed better growth of fish fry in 2.21% lysine supplemented diets as compared to control and other treatments which is within the range of result obtained by Signor *et al.* (2017).

The mean daily weight gain recorded in lysine supplemented diets of T1, T2 and T3 were 0.04±0.003g, 0.05±0.001g and 0.04±0.002g respectively. The highest mean daily weight gain of 0.05±0.001g was recorded in T2 treatment while the control treatment (T0) obtained the lowest mean daily weight gain of 0.04±0.001g only.

Table 6. Fish growth performance of common carp during experimental period.

Parameters	Treatments (Lysine levels (%))				
	T0 (2.05)	T1 (2.13)	T2 (2.21)	T3 (2.29)	CV (%)
Mean Initial weight(g)	1.38±0.03^{a}	1.40±0.02^{a}	1.38±0.02^{a}	1.38±0.03^{a}	2.914
Mean final weight (g)	4.55±0.13^{b}	4.93±0.29^{b}	5.94±0.09^{a}	5.13±0.23	6.77
Mean weight gain (g)	3.17±0.11^{b}	3.52±0.31^{b}	4.56±0.07^{a}	3.75±0.21^{ab}	9.11
Mean daily weight gain (g/fish/day)	0.04±0.001^{b}	0.04±0.003^{b}	0.05±0.001^{a}	0.04±0.002^{ab}	9.11
Survival (%)	100	100	100	100	
Specific growth rate (%/day)	0.56±0.02^{b}	0.60±0.04^{b}	0.73±0.01^{a}	0.64±0.03^{ab}	7.19
FCR	3.61±0.18^{a}	3.35±0.22^{ab}	2.78±0.07^{b}	3.13±0.18^{ab}	9.18

Data based on 0.1858 m^2 water area. Mean values with same superscript in the same row are not significantly different (P>0.05).

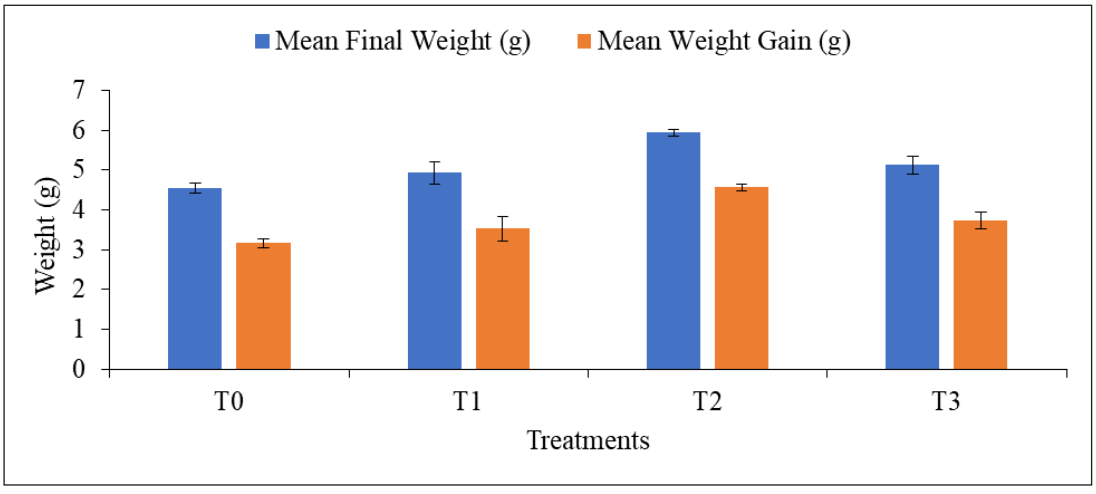

Figure 2. Final weight and weight gain of common carp fry in different treatments during the experimental period of 90 days

Box plot for weight gain of common carp stated that almost all the common carp fries gained uniform weight in treatment T2. In the case of treatments T0 and T1, almost similar weight gain was achieved, however, weight gain by common carp fry varied more widely in treatment T1 i.e., 50% of the fish fry achieved less than about 3.3 g weight which is presented in Figure 3.

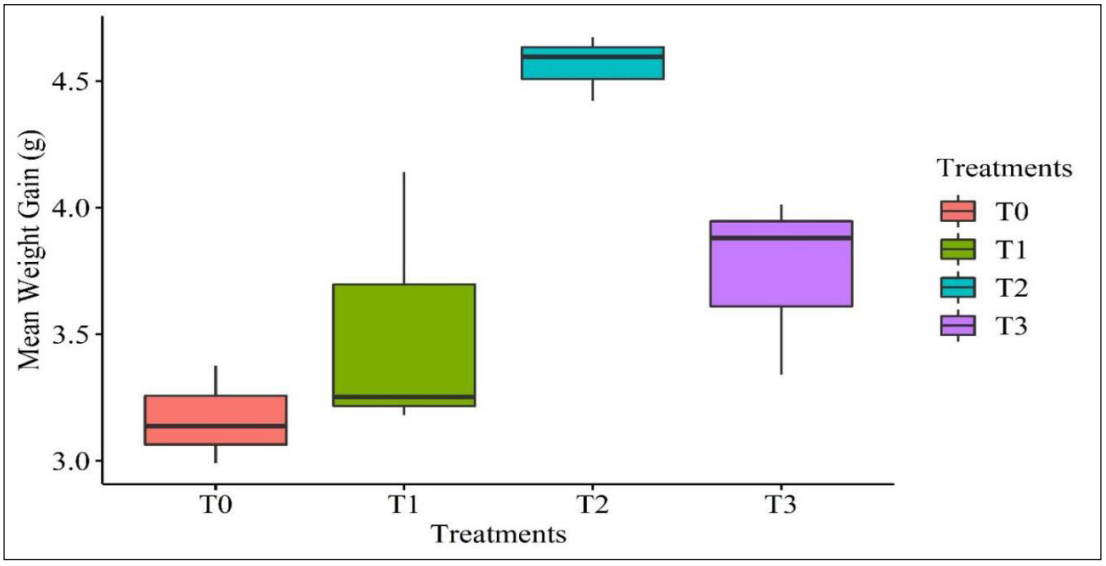

Figure 3. Box plot representation for weight gain of common carp fry in different treatments

Physico-chemical parameters of water

For desired growth of fish, water quality parameters have to be maintained at optimum levels in culture system during the experimental period. Ali et al. (1982) stated that water temperature was the most critical factor in the aquatic environment, which directly influences the metabolic rate of fish. In the present study, the average water temperatures of the experimental aquaria were 29.93±0.28 (T0), 29.88±0.29 (T1), 29.88±0.28 (T2) and 29.76±0.27 (T3) as depicted in Figure 4 which were within the desired range for growth of fish. It is also supported by Desai, A.S. and Singh, R.K. (2009) who found that the temperature ranged from 28 to 32°C was optimum for growth of common carp with ration 6% body weight/day. The statistical analysis of variance for temperature showed no significant difference ($p>0.05$) among the different treatments.

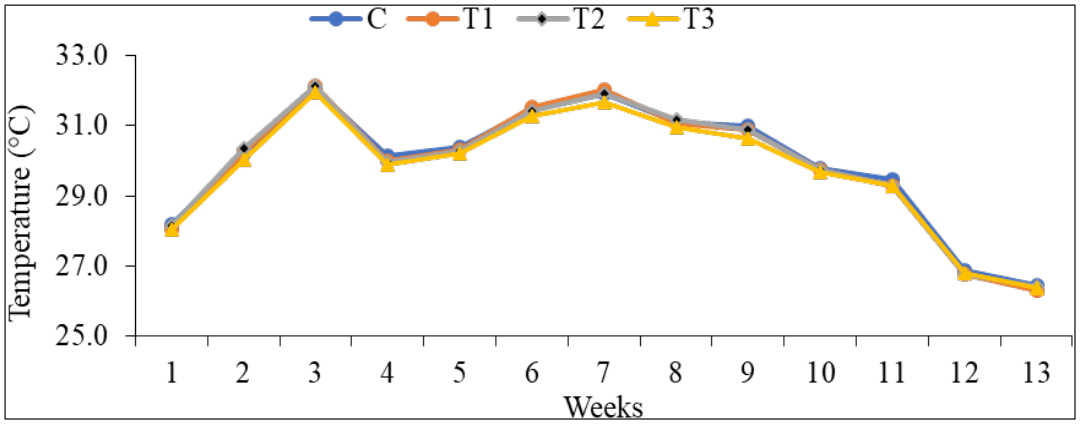

Figure 4. Mean value of water temperature (°C) during the experimental period of 90 days

Svobodova (1993) reported that cyprinids can thrive in water containing 6–8 mg/L and show signs of suffocation, only when the oxygen concentration falls to 1.5–2.0 mg/L. The average dissolved oxygen recorded in the study were 5.84±0.04, 5.89±0.03, 5.81±0.03 and 5.72±0.03 in aquaria T0, T1, T2 and T3 respectively and are presented in Figure 5. The statistical analysis of variance for dissolved oxygen showed no significant difference ($p>0.05$) among the different treatments.

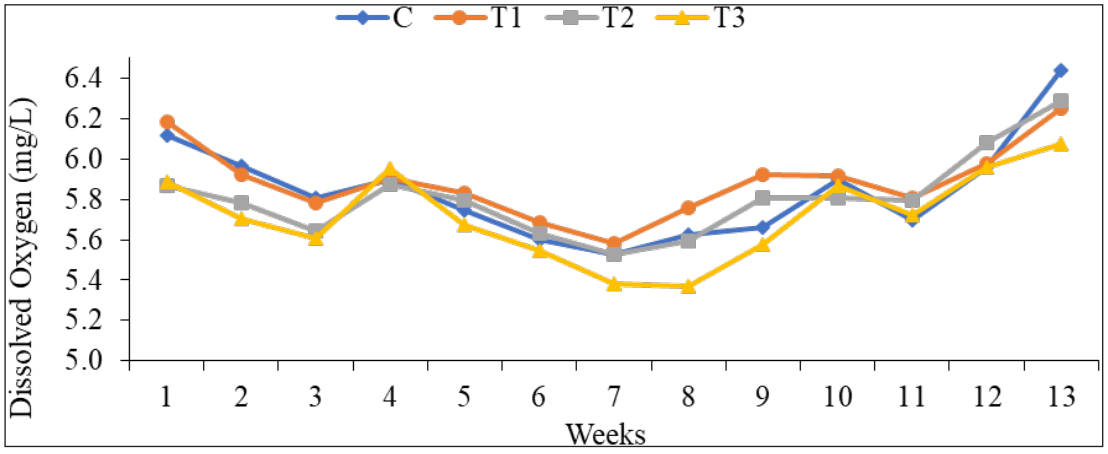

Figure 5. Mean value of dissolved oxygen (mg/L) during the experimental period of 90 days

Throughout the period of experiment, the pH of water remained moderately alkaline. The average pH values of aquaria were 8.4±0.01 (T0), 8.39±0.02 (T1), 8.34±0.02 (T2) and 8.34±0.02 (T3) as depicted in Figure 6. The study report of Swingle (1957) also suggests that pH range of 7.5 to 8.5 is suitable for optimum growth of fish which is similar with the present research findings. The statistical analysis of variance showed no significant difference ($p>0.05$) in the pH among the different treatments.

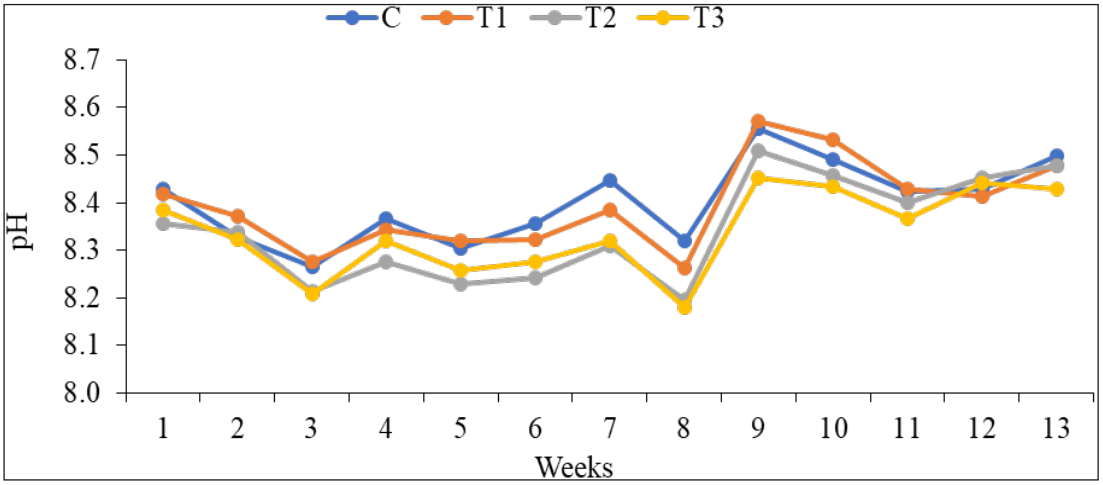

Figure 6. Mean value of water pH during the experimental period of 90 days

The major source of ammonia in aquarium water is the direct excretion of ammonia by fish (Tucker and Boyd, 1985). In the present study, the ammonia ranged from 0.2 to 0.4 mg/L (presented in Figure 7) which is within the acceptable range of ammonia reported by Stone and Thomforde (2004). The nitrate level in this study was ranged from 0 to 10 mg/l. Both free and combined chlorine is toxic to fish (Tompkins and Tsai, 1976). Therefore, if measurable concentrations of chloride residuals are present in water, the water should not be considered safe for holding fish. No significant difference was observed in ammonia, nitrate and chloride across the experimental aquarium. It indicates a constant environmental condition during the course of the study. Thus, there was no adverse effect of lysine supplemented diet on these parameters. It also confirms that the experimental fish were not subjected to any significant environmental stress.

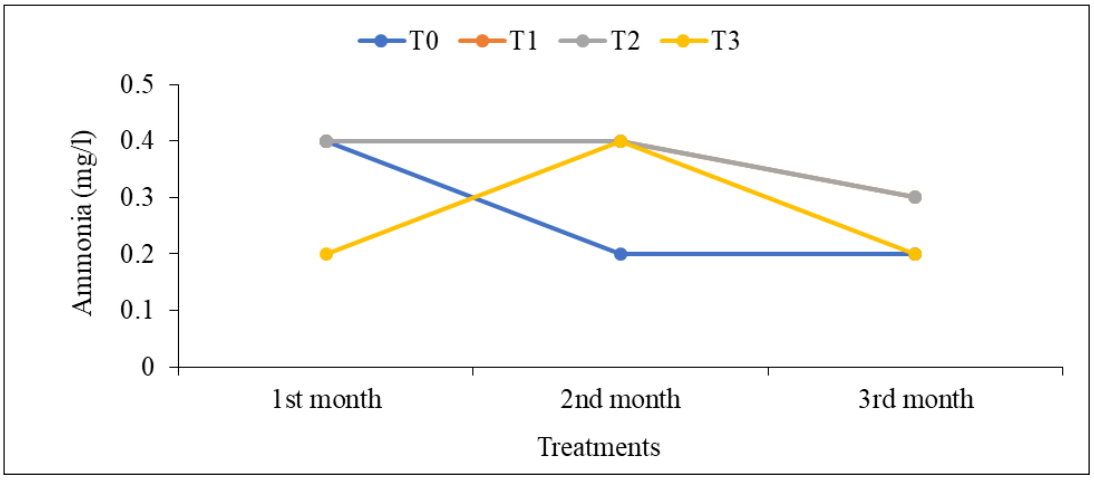

Figure 7. Mean value of ammonia during the experimental period of 90 days

CONCLUSION

The 2.21% of lysine supplementation in feed of common carp containing 30% crude protein resulted better growth performance of the fish during fry rearing phase in terms of its increased weight gain, low FCR and high specific growth rate. This optimum level supports not only efficient growth but also other factors essential for successful rearing of healthy fingerlings. Free lysine supplemented alternatives to fish meal are far more economical and are available in such higher quantities to meet the demand. Also, the soybean meal is locally and readily available anywhere at much lower prices than fish meal, it can be used effectively in aquafeed as a plant protein source in replacement of fish meal. The experiment should be carried out in other areas and need to be verified by larger scale field trials prior to adoption in fish culture practices. In order to sustain significant responses, there is further need of dose standardization also. Further research on the supplementation of lysine in aqua feeds could contribute to more economical environment friendly production.

CONFLICT OF INTEREST

The authors declare that they don't have known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENTS

I take the privilege to gratefully acknowledge and submit my hearty gratitude to Institute of Agriculture and Animal Science (IAAS), Post-graduate Campus, Kirtipur Kathmandu, Nepal for providing necessary facilities and patronage during the study period. I wish to accord my warmest thanks to University Grants Commission, Nepal for providing Masters Research Support award (MRS-76/77).

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