Research Article

PERFORMANCE OF LOCALLY FORMULATED LOW COST FEEDS FOR REARING RAINBOW TROUT (*Oncorhynchus mykiss* Walbaum, 1792) FRY IN NEPAL

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ABSTRACT
Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) farming is the only promising cold water aquaculture in Nepal, but its farming is constrained by availability of quality and quantity of feed. An experiment was done to evaluate the performance of locally formulated low cost nutritious feeds for rainbow trout fry at Fisheries Research Center, Trishuli for 3 months. The experiment included four treatments i) T1- control, shrimp based feed, ii) T2 - shrimp+amino acids based feed, iii) T3 - shrimp+stinging nettle based feed, and iv) T4 - silkworm based feed. Each treatment was replicated thrice. Fry was stocked in raceways of 1 m^2 at 200 fry/m^2 density and fed at the satiation for 6 times in a day from 7.00 am to 5.00 pm. Proximate analysis showed that crude protein was 42.8% in T1, 42.0% in T2, 41.7% in T3 and 39.8% in T4 feeds. Growth of fry and food conversion ratio were significantly better ($p<0.05$) in T1, T2 and T3 than T4 due to higher crude protein content in respective feeds while feed cost was significantly ($p<0.05$) lowest in T4 due to cheaper silkworm. Based on growth, survival and gross margin T1 feed was found to be better among experimental feeds.

Key words: Shrimp, stinging nettle, silkworm, survival, feed cost

INTRODUCTION

Intensive farming of Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) in flow-through system in cold water is the only sustainable commercial fish production system in Nepal. The surge in trout production from 0.045 metric ton (t) in 1997/98 to 420 t in 2018/19 (CFPCC, 2019) shows the dramatic development of trout farming in Nepal. Rainbow trout is the second major fish after carps produced in Nepal and its production has been increasing every year due to expansion of culture area mainly driven by high market demand (CFPCC, 2019). Rainbow trout farming is constrained by availability of adequate quantity and good quality of seed and feed (Rai et al., 2008; FRD, 2013). Larval growth and survival depends mainly on the availability of quality feed that is readily consumed and efficiently digested. This provides the required nutrients to support good growth and health (Girri et al., 2002). Rainbow trout larvae in hatcheries exclusively depend on external feed for their growth from the time larvae commence first feeding (Bardach et al., 1972). Quality feed reduces the feed conversion ratio (FCR) which lowers the cost of production (Gurung et al., 2017). In Nepal, commercial feeds for rainbow trout are not widely available and on top of that such feeds are expensive. Using local ingredients in feed obviously reduces the cost of the feed (Musiba et al., 2014). Farm-made pellet feeds are also fresh, good quality and cost effective for sustainable trout farming in rural areas (Nepal et al., 2017).

Rainbow trout, being carnivore needs high protein feed (Hinshaw, 1999). To meet the protein demand, fish meal and shrimp meal have been used in trout larval feed. In Nepal, shrimp and fish meals are generally not produced and are thus imported. This indeed has increased feed cost and production cost of hatcheries (Roy et al., 1999; FRCT, 2004). Therefore, there is a need to find alternative sources of protein for trout larval feed which are cheap and locally available such as stinging nettle (*Urtica dioica*), silkworm pupae (*Bombyx mori*) etc. Efforts have been made in the past to replace shrimp and fish meal by fresh liver of buffalo, goat, chicken and egg, and had good results in specific growth rate (4.2%) and survival (99%) (Subba & Gubhaju, 2011). Though many studies have been done on nutrition and feed formulation for grow-out rainbow trout (Bista et al., 2008), least research has been done for starter feed in Nepal. This experiment therefore, attempts to formulate low cost quality feeds by using locally available ingredients for rainbow trout larvae yielding higher growth and survival.

MATERIAL AND METHODS

Experimental site and design

This experiment was conducted for 90 days from 18th March 2016 to 17th July 2016 at the Fisheries Research Center located in Trishuli, Nuwakot, Nepal. The experiment was done in 12 indoor nursing raceways of 1 m^3 (2 m□1 m□0.5 m) each with flow-through system. The experiment was conducted by using completely randomized design (CRD) with four treatments, T1 – control or shrimp based feed, T2 - control+ amino acids based feed, T3 - control+stinging nettle based feed and T4 - silkworm based feed, and 3 replications.

Stocking

Rainbow trout fries of 0.24±0.01 g obtained from Fisheries Research Centre, Nuwakot were stocked to 12 raceways. Stocking density of rainbow trout fry was 200 fry/m^2. Weight and length of 10% of the total fry population in each raceway was taken individually and rest was weighed in bulk during stocking.

Feed formulation and feeding

Prior to stocking 4 types of feed were prepared, packed in air tight plastic bags with the capacity of 1 kg and stored in a refrigerator. Proximate analysis of each type of ingredient and experimental feed was done following AOAC (1990) at the Food Research Division, Khumaltar, Lalitpur. Composition of experimental feeds is shown in Table 1.

T1 feed: Feed ingredients were shrimp, soybean, wheat flour, vitamins and minerals, which were collected from the local market of Kathmandu. Shrimp was washed and sun dried for three days then milled in a grinder. Soybean and wheat were also washed, sun dried for one day and milled separately and all powdered forms were mixed thoroughly and sieved through 0.5 mm mesh size sieve to make feed. Vitamins and minerals were added at the end.

T2 feed: T2 feed was prepared same way by adding amino acids at the end to T1 feed.
T3 feed: T3 feed was prepared by adding stinging nettle to T1 feed.

T4 feed: T4 feed was prepared by adding powder of silkworm pupae to the mixture containing soybean, wheat flour, vitamins and minerals. Silkworm pupae were collected from the silk producing farmer of Baireni, Dhading which was sun dried for four days then milled separately.

Table 1. Ingredients and composition (%) of the experimental feeds

Ingredients (%)	T 1 (Control)	T2 (Control + Amino acid)	T3 (Control + Stinging nettle)	T4 (Silkworm pupae)
Whole shrimp powder	48.0	45.0	43.2	
Silkworm pupae powder		0.0	0.0	48.0
Stinging nettle powder		0.0	4.8	0.0
Roasted soybean powder	33.6	31.5	33.6	33.6
Wheat flour	14.4	13.5	14.4	14.4
Additives				
Vitamin premix*	1.9	1.8	1.9	1.9
Mineral premix**	1.9	1.8	1.9	1.9
Vitamin ‘C’	0.2	0.2	0.2	0.2
Amino acids		0.0		
Lysine		2.7		
Methionine		2.7		
Threonine		0.8		

*Vitamins/kg premix contains: 33000 IU vitamin A, 3300 IU vitamin D3, 410 IU vitamin E, 2660 mg Vitamin BI, 133 mg vitamin B2, 580 mg vitamin B6, 410 mg vitamin B12, 50 mg biotin, 9330 mg choline chloride, 4000 mg vitamin C, 2660 mg Inositol, 330 mg para-amino benzoic acid, 9330 mg niacin, 26.6 mg pantothenic acid.
**Minerals/kg premix contains: 325 mg Manganese, 200 mg Iron, 25 mg Copper, 5 mg Iodine, 5 mg Cobalt.

Fish were hand fed at satiation level from 7 am to 5 pm daily at the interval of 2 hours. Fry were considered satiated when they began to ignore the feed. The external standpipe of each raceway was removed twice a day for approximately 10 seconds to flush away the accumulated uneaten food and fecal matter on the drainage screens to reduce the risk of blockages.

Water quality monitoring

Temperature, dissolved oxygen (DO), pH, and turbidity were recorded daily (6:00-6:30 am) at out flow of each raceway. Temperature, DO and pH were measured by using digital instrument "LABQUEST 2 Vernier" while total alkalinity, ammonia, orthophosphate and total hardness were recorded fortnightly by using instrument eXact Micro 10 Photometer at out flow of each raceway between 6:00 to 6:30 am.

Growth monitoring and harvesting

Growth of fry was determined fortnightly by sampling 10% fry of total population in each raceway. Final harvesting of fingerling was done by draining each raceway completely on 17 July 2016. On harvesting, weight and length of 10% fingerling of total population in each raceway was taken individually. Rests were counted and their batch weight was recorded.

Gross margin analysis

Gross margin analysis was done to determine gross margin of different treatments (Waibel & Setboonsarng, 1993). Variable costs included only seed and feed cost. The price was based on current local market prices for all inputs and outputs in Nepal.

Statistical analysis

Data were analyzed by using one-way analysis of variance (ANOVA) using SPSS (version 21) to find significant differences among treatments. Duncan Multiple Range Test (DMRT) was used when significant differences were found. Differences were considered significant at the 95% confidence level ($P<0.05$). All means were given with ±1 standard error (SE).

RESULTS

Experimental feed

Proximate analysis of feed ingredients and feeds are shown in Table (2) and (3). Accordingly, crude protein was highest in shrimp powder followed by silkworm pupae, soybean, stinging nettle and wheat flour. Likewise, crude fiber was highest in stinging nettle and lowest in wheat flour whereas crude fat was highest in silkworm pupae and lowest in stinging nettle. Similarly, ash was highest in stinging nettle and lowest in shrimp powder while moisture was highest in wheat powder and lowest in soybean powder (Table 2).

Table 2. Proximate analysis of major feed ingredients

Parameters	Shrimp powder	Silkworm pupae powder	Stinging nettle powder	Soybean powder	Wheat powder
Moisture (%)	6.66	5.95	9.57	3.44	10.61
Ash (%)	1.54	4.10	18.65	4.47	2.22
Crude fat (%)	2.59	27.60	1.62	12.40	1.88
Crude protein (%)	56.32	52.50	23.19	34.12	10.64
Crude fiber (%)	2.87	2.45	7.88	3.82	2.30

T1 feed had highest crude protein (CP) of 42.8% whereas Cp content of the rest of the treatments ranged from 39-42% 9Table 3). Crude fiber (CF) was highest in T2 and was lowest in T4 feed. Likewise, crude fat was highest in T4 and lowest in T2 (Table 3). Ash content was highest in T3 followed by T1 with the lowest content in T4 feed. Similarly, moisture content was ranged from 8.8% (T4) to 14.1% (T2) (Table 3).

Table 3. Proximate analysis of experimental feeds

Feed	Moisture (%)	Ash (%)	Crude fat (%)	Crude protein (%)	Crude fiber (%)
T1 feed	10.1	11.3	8.6	42.8	3.4
T2 feed	14.1	11.0	5.7	42.0	3.7
T3 feed	10.4	12.3	9.5	41.7	3.4
T4 feed	8.8	5.2	23.4	39.8	1.9

Water quality

There was no significant difference (p>0.05) in water quality parameters among different treatments during experimental period (Table 4). Since the experiment was done in flow through system, temperature, DO, pH, soluble reactive phosphorus and ammonia did not vary much during experimental period.

Table 4. Summary of water quality parameters in different treatments during experimental period (Mean ±SE)

Parameters	T1	T2	T3	T4
Temperature (°C)	16.9±0.0[a] (15.3-18.5)	16.9±0.0[a] (15.4-18.5)	16.9±0.1[a] (15.3-18.5)	16.9±0.0[a] (15.3-18.5)
Dissolved Oxygen (mg/L)	6.9±0.1[a] (6.5-7.4)	6.9±0.2[a] (6.5-7.7)	6.9±0.2[a] (6.4-7.6)	6.9±0.1[a] (6.6-7.4)
pH	7.3 (6.5-8.1)	7.3 (6.5-9.5)	7.3 (6.5-7.9)	7.3 (6.4-8.0)
Turbidity (NTU)	99.8±0.1[a] (80.0-120.0)	99.2±0.36[a] (80.0-120.0)	99.5±0.2[a] (80.0-120.0)	99.3±0.2[a] (80.0-120.0)
Total Alkalinity (mg/L)	49.0±0.8[a] (36.0-60.0)	48.9±1.9[a] (36.0-60.0)	49.6±0.9[a] (35.0-60.0)	49.0±1.3[a] (37.0-58.0)
Total Hardness (mg/L)	41.6±1.0[a] (25.0-55.0)	42.0±2.4[a] (29.0-55.0)	41.0±2.6[a] (29.0-55.0)	40.7±0.6[a] (28.0-54.0)
Orthophosphate (mg/L)	0.7±0.1[a] (0.1-0.9)	0.6±0.0[a] (0.2-0.8)	0.5±0.07[a] (0.1-0.9)	0.53±0.07[a] (0.1-0.9)
Ammonia (mg/L)	0.03±0.01[a] (0.02-0.03)	0.09±0.01[a] (0.03-0.27)	0.09±0.01[a] (0.02-0.21)	0.11±0.01[a] (0.05-0.27)

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

Growth and survival of rainbow trout fry

Growth, weight gain and survival of rainbow trout fry among different treatments are presented in Table (5). Final total weight, total weight gain and FCR were significantly higher (p<0.05) in T1 and T2 than in T4. Average length gain was also significantly higher (p<0.05) in T1 than in T4. Survival rate of fry in T1 was significantly higher (p<0.05) than in T3 (Table 5).

Table 5. Summary of growth performance of rainbow trout fry in different treatments in 90 days (Mean±SE)

Parameters	T1	T2	T3	T4
Initial mean weight (g/fish)	0.23±0.01[a]	0.25±0.02[a]	0.24±0.02[a]	0.24±0.01[a]
Initial mean length (cm/fish)	2.77±0.06[a]	2.95±0.04[a]	2.90±0.07[a]	2.90±0.07[a]
Initial total weight (g/m^2)	45.70±2.41[a]	49.20±3.59[a]	47.07±3.17[a]	47.43±1.52[a]
Final mean weight (g/fish)	4.82±0.25[a]	5.37±0.78[a]	4.87±0.09[a]	4.25±0.14[a]
Final mean length (cm/fish)	7.51±0.10[a]	7.61±0.39[a]	7.16±0.13[a]	6.89±0.08[a]
Final total weight (g/m^2)	780.5±7.2[a]	770.4±70.8[a]	666.3±39.9[ab]	606.4±31.1[b]
Mean weight gain (g/fish)	4.59±0.24[a]	5.12±0.76[a]	4.63±0.09[a]	4.01±0.15[a]
Total weight gain (g/m^2)	734.8±4.9[a]	721.2±67.3[a]	619.2±38.2[ab]	560.0±32.5[b]
Daily weight gain (mg/fish/day)	51.01±2.69[a]	56.89±8.43[a]	51.49±1.00[a]	44.54±1.62[a]
Mean length gain (cm/fish)	4.74±0.1[a]	4.66±0.3[a]	4.26±0.1[ab]	3.99±0.1[b]
Specific growth rate (%/day)	3.39±0.03[a]	3.41±0.09[a]	3.37±0.08[a]	3.21±0.07[a]
Survival (%)	81.3±3.5[a]	73.2±5.2[ab]	68.3±3.1[b]	71.3±1.8[ab]
Feed conversion ratio	1.4±0.0[a]	1.3±0.1[a]	1.7±0.1[ab]	2.0±0.1[b]

Mean values with different superscript letters in the same row are significantly different (P<0.05).

Gross margin

Feed cost and total variable costs were significantly lower (p<0.05) in T4 than rest of the treatments whereas return from fingerling was significantly higher (p<0.05) in T1 than in T3 with no significant difference (p>0.05) with T2 and T4 (Table 6).

Table 6. Gross margin analysis of different treatments (Rs./m^2) in 90 days

Treatments	T1	T2	T3	T4
Variable costs				
Fry	1000	1000	1000	1000
Feed	333±11^a	333±5^a	328±11^a	110±1^b
Total variable cost	1333±11^a	1333±5^a	1328±11^a	1110±1^b
Return				
Fingerling	2440±104^a	2195±155^{ab}	2050±92^b	2140±54^{ab}
Gross margin	1107±115^a	863±156^a	723±97^a	1030±54^a
Gross margin (Rs./m^2/yr)	4429±459^a	3450±622^a	2890±388^a	4118±214^a

Mean values with different superscript letters in the same row are significantly different (P<0.05).

DISCUSSION

Rainbow trout fry are reared in flow through system where no natural foods are available. Therefore, they need high protein and nutrient rich feeds for better growth and survival. Addition of fishmeal or shrimp meal to feed increases feed efficiency and growth through better food palatability, and enhances nutrient uptake, digestion, and absorption (Miles & Chapman, 2012). Since fishmeal and shrimp meal are expensive, it increases feed cost which ultimately increases fish production cost. Reducing the percentage of fishmeal in rainbow trout feed is a major concern of feed producers (FAO, 2020). Present experiment evaluated performance of local feed ingredients to replace shrimp meal as a protein source, either partially (T2 feed and T3 feed) or completely (T4 feed).

Rainbow trout is a carnivore, hence its protein requirement is very high (45-50%) for smaller fish (FAO, 2020). Considering this fact crude protein was found comparatively lower (39.8%) in the silkworm pupae feed because this feed lacked shrimp meal which had the highest crude protein among feed ingredients. T1, T2 and T3 feeds had 48.0%, 45.0% and 43.2% shrimp meal in the feed respectively whereas T4 feed did not have shrimp meal. In T2 feed, amino acids viz. lysine, methionine and threonine were also added with a purpose to increase protein level but it could not increase protein level (42.0%) higher than that of T1 feed (42.8%). Adding 5% stinging nettle to T3 feed increased ash content (12.3%) and crude protein level nearly to 41.7%. T4 feed lacked shrimp meal hence; crude protein level was lowest (39.8%) among experimental feeds. Amino acids, stinging nettle and silkworm pupae have increased protein level of respective feeds comparable to shrimp based feed so, these ingredients can be used to replace fish meal and shrimp meal in rainbow trout larval feed by increasing their quantities. Silkworm pupae and stinging nettle are available in Nepal and are cheaper compared to shrimp meal. Fishmeal and shrimp meal have high crude protein that favors its use in the manufacture of most animal feeds. However, its increased use in fish feed poses a challenge due to competition by alternative human needs for the same (Hua et al., 2019).

Growth and survival of fry varied with feed types. Total harvest weight, total weight gain and mean length gain of fry in T1 and T2 were higher than in T4. This might be due to higher crude protein in both feeds (>41.7%) compared to silkworm pupae feed (39.8%). Shrimp meal has well balanced amino acids profile and lacks anti-nutritional factors to enhance growth of fry (Musiba et al., 2014). Overall harvest weight and weight gain in the experiment was found to be lower which might be due to low crude protein level in the experimental feeds than required for rainbow trout fry (45-50% CP) and fingerlings (45% CP). The protein level below 40% results in the lower final body weights and higher FCR (Bista et al., 2008; Cho et al., 1976; Hinshaw, 1999). Other possible factors of poor growth might be low average temperature (16.9 ^oC) during experimental period. Nelitz et al. (2007) reported decline growth of juvenile rainbow trout at temperature below 17^o C. The water temperature was also lower than 17 ^oC during first and last month of experimental period. Fry survival was poor in T3 which can be attributed to stinging nettle in the feed. Plant proteins are relatively poor sources of essential minerals and also contain anti-nutrients such as phytic acid (FAO, 2020). Phytate binds with protein to form insoluble complexes, thus decreasing protein digestibility, utilization and minerals uptake (Kumar et al., 2012) which might have made fry vulnerable. Overall fry survival was poor in the experiment which might be due to high turbidity in water. Rainbow trout needs crystal clear running water (Huet, 1975; Nepal et al., 2002) for better growth and survival which was lacking in the experimental raceway. Turbidity ranged 80-120 NTU in raceways during experimental period which was much higher than 3-19 NTU in raceways in Kakani, Nuwakot reported by Bhagat and Barat (2015). FCR was poor in silkworm based feed fed raceways which could be due to lower crude protein of the feed (Storebakken et al., 1998).

Experimental diets did not deteriorate water quality to critical level because experiment was carried out in the flow through water systems (raceways) and feeding was done at satiation with personal visualization which avoided feed waste and consequent water quality deterioration. All water quality parameters were in permissible level except turbidity which was very high to affect growth and survival of trout fry. Turbidity was higher due to turbid source water and rainfall. Water from Trishuli River was used to supply the raceways which had high silt content. Water quality did not vary among treatments due to flowing system of water.

Feed cost and total variable costs in T4 were significantly lower than rest of the treatments because this feed used 48% silkworm pupae of total feed. Silkworm is locally available and cheaper (Rs. 157/kg) than shrimp meal (Rs. 550/kg) and amino acids (Lysine Rs. 450/kg, Methionine Rs. 850/kg, Threonine Rs. 750/kg) to reduce feed cost. Silkworm pupae based feed reduced feed cost and overall variable cost by 60% and 13%, respectively. Rest feeds used shrimp meal which increased both feed cost and overall production cost. Return from fingerling sale was higher in T1, T2 and T4 due to higher survival while gross margin was higher in T1 and T4 due to higher survival (T1) and lower feed cost (T4).

CONCLUSION

The findings of this experiment proved that shrimp containing feed is the best feed and shrimp meal is the best ingredients for rainbow trout fry feed. Between local ingredients stinging nettle and silkworm pupae, later seemed better because it gave higher survival and gross margin. Adding stinging nettle (4.8%) and silkworm pupae (48.0%) to rainbow trout fry feed resulted crude protein nearly equal to shrimp based feed indicated that both ingredients have potential to substitute shrimp and fish meal. However, there is a need to determine how much of these ingredients need to add to prepare feeds with optimum protein level for fry growth and survival. Rainbow trout farming is the only form of aquaculture in hilly regions in Nepal. Contribution of rainbow trout to total aquaculture production is though small (0.5%) at present but its role on livelihoods and income generation of farmers is commendable (CFPCC, 2019). The technology has huge potential for expansion and now rainbow trout farming is expanded to 23 districts. Trout farmers have started to produce feed in their own farm enhancing sustainability of the farming. Producing trout feed using local ingredients such as silkworm pupae and stinging nettle could be beneficial to them.

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