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Ashfauq Farooq Aga
Department of Aquaculture, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana Punjab, India

Asha Dhawan
Department of Aquaculture, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Meera D Ansal
Department of Aquaculture, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Correspondence
Ashfauq Farooq Aga
Department of Aquaculture, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Efficacy of feeding frequency, feeding rates and formulated diets on growth and survival of rohu *Labeo rohita* brood stock under intensive rearing

Ashfauq Farooq Aga, Asha Dhawan and Meera D Ansal

Abstract
An experiment was conducted to assess the efficacy of formulated diets and feeding strategies in brood stock management of *Labeo rohita* (Ham.). The experiment comprised of three factors i.e., two supplementary feeds (D1, routine diet vs. D2, diet supplemented with vitamin-mineral mixture @ 2%), two feeding rates (R1, 3% BW vs. R2 4% BW) and two feeding frequencies (F1, once-a-day vs. F2, twice-a-day feeding. There were 8 treatments regimes viz., T_1 (D_1, R_1, F_1), T_2 (D_1, R_1, F_2), T_3 (D_1, R_2, F_1), T_4 (D_1, R_2, F_2), T_5 (D_2, R_1, F_1), T_6 (D_2, R_1, F_2), T_7 (D_2, R_2, F_1), and T_8 (D2, R2, F2). The yearlings of 12-16 months age were stocked at a density of 1,000 kg ha^{-1} (16 fish/ pond), fed for a period of 8 months and the effect of different treatments on survival and growth of fish was recorded. During the culture period, 100% survival of fish was recorded in all the treatments. At the end of culture period, total body length and body weight was higher in T_8 ($P<0.05$) compared to other treatments. In addition, the percent total length growth and BW gain was maximum in T_8. Furthermore, specific growth rate was higher in T_8 ($P<0.05$) as compared to other treatments. All treatment groups had condition factor value of more than 1 indicated that the fishes were in good condition. It may be concluded that supplementary feed (with vitamin-mineral mixture), feeding rate (4% fish BW) and feeding frequency (twice-a-day) improved the growth of *L. rohita* brood stock.

Keywords: Rohu**,** body weight, length, feeding rate, feeding frequencies, vitamin mineral mixture

1. Introduction
Fish growth at different stages is largely governed by the kind of feed, feeding frequency, feed intake and its ability to absorb the nutrients. Among these, feeding frequency is an important factor for the survival and growth of fish at the early stage [10]. Optimum feeding frequency may provide maximum utilization of diet as feed cost is the largest operating cost of fish farming and often constitutes between 40-60% of the total cost of production in aquaculture [3, 24]. It is evident from earlier studies that excess feeding may lead to leaching of nutrient and limited feeding may suppress growth due to starvation.

Good feed management is the result of good feed conversion, which is the result of adequate knowledge about energetic needs of the fish, adequate distribution of feed and good feeding techniques. Therefore, a feeding strategy that uses minimal amounts of feed for increasing economic returns has the potential to lower production cost by decreasing the quantity of feed used to produce a kilogram of fish [23]. Davies *et al* [8] reported that in order to optimize production, a fish farmer has to feed the fish at a level that ensures good growth and minimal waste. The wasted feed can account for 5-30% of the feed offered and up to 50% of the total solid waste produced [6].

The ration size is a crucial factor, determining the application rate of supplementary feed [14]. Feeding rate of the species and the duration of meals (i.e. feeding frequency) should be adjusted according to the time required for feeding fish. Khan *et al*[20] reported a higher positive effect on growth (weight gain, specific growth rate, FCR, survival rate and total production) at feeding frequency of thrice a day as compared to once and twice a day feeding in pangasius catfish (*Pangasius hypophthalmus*) and silver carp (*H. molitrix*) polyculture. In addition, the higher feeding frequency resulted in greater profit and BCR (Benefit cost ratio) than the lower feeding frequencies. The ration size of 4-6% body weight per day is reported to be the best suited for *C. mrigala* in terms of FCR, SGR and PER [2, 18].

Among the dietary ingredients, vitamins and minerals have been identified in several studies as playing a role in fish growth and reproduction owing to their antioxidants potential,

protecting germ cells against DNA damage and oxidation of seminal plasma proteins with reactive oxygen radicals. The minerals are responsible for skeletal formation, maintenance of colloidal systems, regulation of acid-base equilibrium and for biologically important compounds such as hormones and enzymes. Mineral deficiencies can cause biochemical, structural and functional pathologies which depend on several factors, including the duration and degree of mineral deprivation. Fish growth was improved by an increase in dietary source of minerals in channel catfish *I. punctatus* [11], crucian carp *Carassius auratus gibelio* [30] and rainbow trout, *O. mykiss* [15]. Some trace elements and vitamins have been linked with brood fish growth and egg quality [25]. There have been numerous experiments on feeding trial with formulated diets using Indian major carp rohu, *Labeo rohita* which is among the popular and commercially cultured fish species in India and Pakistan. However, reports on experiments regarding feeding frequency of this species are very few [12]. Very meagre work has been done to evaluate the effect of vitamin and mineral supplemented diets and optimizing feeding strategies (feeding rate and feeding frequency) in carp brood stock production. Therefore, the present study was designed to develop feeding strategies, with respect to feed formulation, feeding rate and feeding frequencies, for rohu brood-stock to optimize growth and their survival.

2. Materials and Methods

The experiment was conducted at the Fish Farm of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. Proximate composition analysis of the feed ingredients, experimental diets were carried out in the Nutrition Laboratory and length and weight of fishes were taken near work field (fish ponds) on monthly basis.

3. Experimental details

3.1 Preparation of experimental tanks

The experiment was carried out in triplicate in 80 m^2 outdoor cemented tanks with 1.00-1.20 m water level. Soil layer (0.12 m) was spread at the bottom of each tank to boost the detritus food chain. All the tanks were manured with cow dung @ 20 t ha^{-1} $year^{-1}$ once during pond preparation. Well water was used to fill the tanks and to compensate the evaporation losses during experimentation.

3.2 Stocking of fish

Each tank was stocked with yearlings 12-16 months age at a density of 1,000 kg ha^{-1} (16 fish/ pond) in April, 2014. The fish of either sex were stocked in equal number. The mature male and female fishes were differentiated on the basis of their size (females were larger in size), (pectoral fin were smooth in female and rough in male), abdomen (soft and bulged in female) and vent (swollen and reddish in females and normal in male).

3.3 Feeding of fish

Each tank was stocked with yearlings 12-16 months age at a density of 1,000 kg ha^{-1} (16 fish/ pond) in April, 2014. Fish were fed with two experimental diets for a period of 8 months. Normal diet contained 30% rice bran, 50% ground nut meal and 20% fish meal and while in second case the normal diet was modified by replacing 2% fish meal with 2% vitamin mineral mixture diet [i.e. 18% fish meal + 2% vitamin mineral mixture]. The mineral mixture was supplemented in the form of Agrimin Forte® marketed by Virbac India (Animal Health Pvt. Ltd, Mumbai, India). The composition of vitamin mineral mixture per 100 g was: vitamin A - 625000 IU; vitamin D_3 - 62500 IU; vitamin E - 250 mg; nicotinamide - 1 g; copper - 312 mg; cobalt - 45 mg; iron - 1.5 g; zinc - 2.13 g; iodine - 156 mg; selenium - 10 mg; magnesium - 6 g; manganese - 1.2 g; potassium - 48.05 mg; calcium - 247.34 gm.; phosphorus - 114.66 g; sulphur - 12.2 g).

The experiment comprised of three factors i.e., two supplementary feeds (D1, routine diet vs. D2, diet supplemented with vitamin-mineral mixture @ 2%), two feeding rates (R1, 3% BW vs. R2 4% BW) and two feeding frequencies (F1, once-a-day vs. F2, twice-a-day feeding). Thus, there were 8 treatments combinations viz., T_1 (D_1, R_1, F_1), T_2 (D_1, R_1, F_2), T_3 (D_1, R_2, F_1), T_4 (D_1, R_2, F_2), T_5 (D_2, R_1, F_1), T_6 (D_2, R_1, F_2), T_7 (D_2, R_2, F_1), and T_8 (D2, R2, F2).

4. Survival and growth of fish

4.1 Morphological parameters

Fish length was measured with the help of measuring scale and fish weight was measured with the help of electronic weighing balance, fishes were netted out from ponds and placed in large tubs for weighing and measurement purpose on monthly basis, Survival of fish in each treatment during pre-spawning and post spawning was recorded at end of the experiment by completely harvesting the stock. Percent total body length gain (% TBLG), percent net weight gain (% NWG), and specific growth rate (SGR) and condition factor (K) for each treatment were calculated by using the following formulae:

% TBLG = Final total body length (cm) - Initial total body length (cm) / Initial total body length (cm) x 100

% NWG = Final BW (g) - initial BW (g) / initial BW (g) x 100

4.2 SGR (increase in weight/day) = Ln (Final body weight) – Ln (Initial body weight) / No. of culture days x100

K = Body weight (g) / (Body length, cm) ^{3} x 100

4.3 Statistical analysis

The data was statistically analyzed using Stat graphic statistical package SPSS-16 (Version 16.0. Chicago, SPSS Inc). One way ANOVA and Duncan's multiple range tests was applied to work out the effect of different diets with respect to differences during length and weight measurements. A probability value of $P \leq 0.05$ indicated that the difference was statistically significant.

5. Results

5.1 Survival

In the present study, 100% survival of fish was recorded in all the treatments, which reveals that incorporation of vitamin-mineral mixture in fish diet, feeding rate and feeding frequency had no adverse effect on the survival of fish.

6. Growth

6.1 Body length

At the end of culture period, TBL of fishes was higher ($P<0.05$) in T8as compared to other treatments ($P<0.05$) In addition, the% TLG was higher in T8as compared to other treatments (Table 1).

6.2 Body weight (BW)

At the end of culture period, BW was maximum (1.87 kg) in T8, and minimum (1.62 g) in T5. The differences among different treatments was significant ($P<0.05$). Furthermore,

the% NWG and SGR was higher in T8as compared to other treatments (Table 1).

6.3 Condition Factor

At the end of the experiment, condition factor (K value) values of female was maximum in T4 and in male fishes highest K value was recorded T5 (Table 1). Although, all treatment groups had K value of more than 1, it indicated that the fishes were in fairly good condition. Condition factor reflects length-weight relationship of fish and has been used as an index to compare growth and well-being of fish based on the principle that heavier fish of a given length are in better condition.

7. Discussion

Brood stock nutrition is one of the most poorly researched areas of finfish nutrition. An improvement in brood stock nutrition and feeding has been shown to greatly improve not only fish body mass yield but also seed production, gonadal development and fecundity. Supplementary feed is offered in the presence of natural food to augment fish growth [9]. Khan *et al*[19] while studying the effect of similar feeding regime on growth and body composition of Indian major carps (Catla, Rohu and Mrigal) reported a significant increase in the final weight gain and final body length among the fish species fed with supplemented feed. Formulation of a suitable feed for fishes has become important for potential aquaculture practice. Monomaitis *et al* [22] indicated a significant opportunity of feeding strategies for productivity and economic efficiency of rohu (*L. rohita*). Maximum conversion ratio is observed with mixed plant based diet (mustard oil cake, soyabean meal, sesame oil cake and rice bran) and vitamin premixes @ 2 times feeding/day [6, 7].

Table 4: Effect of various treatment regimes on total body weight of *L. rohita*

Parameter		Treatments							
		T1	T2	T3	T4	T5	T6	T7	T8
Body length (cm)	PrS	36.36±2.45	36.47±1.17	36.02±1.68	37.36±1.56	35.24±2.68	36.27±2.26	35.77±2.47	35.74±2.48
	PoS	47.64±4.12^{a}	48.42±3.95^{a}	48.38±4.85^{a}	49.50±4.42^{a}	46.89±4.82^{a}	48.98±3.05^{a}	48.92±4.35^{a}	56.41±4.14^{b}
% TBLG		31.10±4.22^{a}	32.53±3.59^{a}	34.32±4.21^{a}	32.50±3.24^{a}	33.06±3.73^{a}	35.03±4.28^{a}	36.77±4.38^{a}	56.81±5.66^{b}
Body Weight (kg)	PrS	0.68±0.06	0.71±0.08	0.66±0.06	0.67±0.07	0.57±0.06	0.67±0.08	0.64±0.07	0.59±0.05
	PoS	1.68±0.08^{ab}	1.80±0.07^{bc}	1.72±0.06^{ab}	1.65±0.09^{ab}	1.62±0.06^{a}	1.68±0.05^{ab}	1.70±0.06^{ab}	1.87±0.08^{c}
% NWG		147.06± 20.55^{a}	153.52± 18.54^{a}	160.61± 20.43^{a}	146.27± 15.49^{a}	182.46± 17.93^{ab}	150.75± 14.28^{a}	165.62± 15.54^{a}	216.95± 20.33^{b}
SGR (%)		0.43	0.44	0.46	0.49	0.50	0.44	0.46	0.55
Condition factor (K)		1.56	1.58	1.52	1.77	1.56	1.43	1.45	1.04

a:b:c (P<0.05) indicate significant difference across row

PrS: pre-supplementation (i.e., April, 2014); PoS: post-supplementation (i.e., November, 2014)

Our results indicated that feeding of fish with vitamin-mineral supplemented diet @ 4% BW twice a day significantly improved the body length and weight gain. The results were according to earlier reports in *L. rohita,* in which agrimin (vitamin-mineral supplement) increased the above parameters more than fishmin (minerals only) [27]. Sudhakar *et al* [26] found that the effect of selective supplementary feeds like Agrimin and Fishmin on body length, weight and gonadal somatic index of the cultivable fish species like *Hypophthalmichthys molitrix*, *Cyprinus carpio*, and *Ctenopharyngodon idella*. Supplementation of Vitamin A in Atlantic salmon increased the growth [28]. High mortality rate has been reported for salmonid and common carp fed with riboflavin deficient diet [4, 13]. Wanakowat *et al* [29] observed that feed efficiency, percent weight gain and survival rate of fish fed with B_6 free diet were significantly lower than those of supplemented (5 mg kg^{-1} diet) group. Along with vitamins, dietary minerals influence the growth and survival of many fish species. Since the minerals absorbed from water do not always meet the total metabolic requirements of fish, their supplementation through diets results in growth promotion. Diet is an important source of salts. It not only satisfies the needs of body for growth but also osmoregulation in fresh water fish. Hence provision of adequate amount of salt through supplementary feeds would spare energy used in osmoregulation and reduce stress, thereby leaving more energy for growth as observed in vitamin-mineral supplemented group.

Multiple feedings has previously been found to be stimulatory for growth, survival and feed utilization in fingerlings of Indian major carps *Catla catla, Labeo rohita* and *Cirrhinus mrigala* [7, 21]. Our results are in accordance to these workers, as fishes fed @ 4% BW and twice a day showed improvement in all the growth parameters. In pangassid catfish (*Pangasius hypophthalmus*) and silver carp (*Hypophthalmichthys molitrix*) polyculture, Khan *et al*[20]also reported a higher positive effect on growth (weight gain, SGR, feed conversion ratio (FCR), survival rate and total production) at feeding frequency of thrice a day as compared to once and twice a day feeding. In another study with stunted tilapia females, it was observed that feeding at higher rate (3% as compared to 1% and 2% BW) can compensate the growth and produced more seed through an increase in the clutch size (number of eggs per spawn) of the individual female [5]. In a comprehensive study, James and Sampath [16] reported that twice a day feeding elicited maximum growth and reproductive output in Siamese fighting fish (*B. splendens*) when compared with its counterparts (1 meal in 3 days, 1 meal in 2 days, 1 meal/day, and 3 meals/day) The same researchers studying red swordtail (*X. helleri*) indicated that higher feeding frequencies resulted in the greatest growth and better reproductive success GSI, development of gonads, breeding age, interbrood interval and fertility [17].

It may be concluded that supplementary feed (with vitamin-mineral mixture), feeding rate (4% fish body weight) and feeding frequency (twice a day) improved the growth of *L. rohita* brood stock.

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