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EFFECT OF VITAMIN-MINERAL SUPPLEMENTED DIET AND FEEDING STRATEGIES ON REPRODUCTIVE PERFORMANCE OF *Labeo rohita* BROODSTOCK

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ABSTRACT

The present study was conducted to assess the effect of vitamin-mineral supplemented diet and feeding strategies on reproductive performance of fish (*Labeo rohita*). Two diets, with and without vitamin-mineral mixture supplementation, were fed to *Labeo rohita* yearlings for a period of 8 months (April-November) at two feeding rates i.e. 3 and 4% b.w. and two feeding frequencies i.e. once and twice day^{-1}. The study revealed that ganado-somatic index and relative fecundity were significantly higher in diet supplemented with vitamin-mineral mixture @ 4% b.w. fed twice a day during both pre-spawning (July) and post-spawning i.e. at resorption (September). Absolute fecundity and ova diameter were equally higher in control and treatment diets supplemented @ 4% b.w. twice day^{-1}. The study suggests that supplementation of vitamin-mineral mixtureat higher feeding rate and frequency improves reproductive potential of *Labeo rohita* broodstock.

Key words: Fecundity, feeding strategies, gonado-somatic index, *Labeo rohita*, vitamin-mineral supplementation

INTRODUCTION

The demand for high quality protein from aquatic sources is on rise due to the ever increasing population in India (Dey *et al.*, 2005). Nutritional feeding plays a critical role in sustainable development of aquaculture. So, fertilizers and feed resources continue to dominate aquaculture needs. Non-availability of quality feed and the increasing cost (60-70% of total production cost) are the major challenges faced by the aquaculture sector in developing countries (Munguti *et al.*, 2012).Therefore, feeding strategies with respect to different stages need to be developed, which can lower the production cost by using minimal inputs, especially feed, thereby increasing economic returns (El-Sayed 2008, Marimuthu *et al* 2010).

It is well recognized that adequate nutrition has an important role in reproductive success of all animals, including fish. Nutritional status affects several reproductive parameters, such as time of maturity, number of eggs produced (fecundity), egg size, egg quality, hatchability and larval survival (Hasan, 2001). Broodstock nutritional management must ensure supply of even micro-nutrients in proper amount and at right times, for efficient reproductive performance in terms of spawning success, maximum fecundity, gamete quality, and vertical transfer of nutrients and

biologically active substances to offspring (Izquierdo *et al.*, 2001). The production of larval fish and their subsequent growth and development along with their potential productivity as future broodstock is highly dependent on the quality of eggs (Miguad *et al.*, 2013). Since egg quality, in terms of the macro- and micro-nutrients they contain, is dependent on nutrient delivery from the female, it is vital that broodstock nutrition is optimized to ensure larval survival and early development (Izquierdo *et al.*, 2001). Thus, broodstock diets need to be formulated to fulfill all the essential nutrient requirements for the species being cultured. Adequate nutrition in terms of protein, lipids, carbohydrates, vitamins (especially A, C and E) and minerals influences the overall reproductive performance of brood fish. Some studies have been conducted in different fish species to evaluate the effects of vitamin-mineral supplementation to broodstock diets with regard to reproductive performances and egg quality (Cieres Zko and Dabrowski 2001; Gammanpila *et al*., 2007).

In most of the cultured fish species, the unpredictable and variable reproductive performance is one of the limiting factors for successful mass production of juveniles. Thus, an improvement in broodstock nutrition and feeding can significantly improve not only egg and milt quality but seed production too (Lone and Hussain, 2009; Alam and Pathak, 2010; Iqbal and Batool, 2013; Abbas *et al.*, 2014). Some trace elements and vitamins have been linked with brood fish growth and egg quality. Scanty work has been done to evaluate the effect of vitamin and mineral supplemented diets and feeding strategies (feeding rate and feeding frequency) in rohu broodstock. With the hypothesis that vitamin-mineral supplementation will improve the reproductive performance of *Labeo rohita* in terms of gonado-somatic index, fecundity and ova diameter, the present study was aimed to assess the efficacy of vitamin-mineral mixture supplementation in diet of *Labeo rohita* broodstock under different feeding regimes with respect to their reproductive potential.

MATERIALS AND METHODS

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 as well as to compensate the evaporation losses during the experimentation.

Stocking of fish

Each tank was stocked with yearlings 12-16 months age at a density of 1,000 kg ha^{-1} (16 fish per pond) in April, 2014. The fish were stocked in equal gender sex. The mature male and female fishes were differentiated on the basis of their size (females were larger in size), pectoral fin (smooth in female and rough in male), abdomen (soft and bulged in female) and vent (swollen and reddish in females and normal in male) [Thomas *et al.*, 2003]. In case of *Labeo rohit* certain percentage of both males and females reach sexual maturity in one year of age. The average size of maturity in ponds was 292-348 and 285-500 mm in males and females, respectively (Gupta *et al.*, 2007).

Feeding of fish

Fish were fed with two experimental diets (normal and vitamin-mineral mixture supplemented diets) for a period of 8 months. The 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 feed was prepared by mixing together all the required

ingredients, grinding and mixing was done by a portable mini- grinder cum pulverizer. The mixture was steamed with some water to reduce the anti-nutrient factors, if any. The mixture was cooled and 2% commercial vitamin and mineral mixture added as per treatments. The dough so prepared was processed in a portable mini-pellet machine to get desired feed pellets. The pellets were sundried and used for feeding. 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 g; phosphorus - 114.66 g; sulphur - 12.2 g). The diets were given at two feeding rates i.e. 3 and 4% fish body weight and two feeding frequencies i.e. once and twice a day as per treatments. Proximate composition of feed ingredients and experimental diets was done as per the standard methods of AOAC (2000). Feeding was done by broadcasting the pellets in the ponds as per treatments.

Observations

Maturity of female fish was recorded in terms of gonado-somatic index (GSI), fecundity and ova diameter during pre-spawning (July) and post-spawning (resorption) periods. Before dissection and removal of gonads, total body weight of fish was noted. The ovaries from each fish were carefully removed, weighed and preserved in modified Gilson's fluid. The ova were separated with the help of soft brush and the diameter of ova measured with the help of an ocular micrometer set into an eyepiece of microscope. One gram tissue was taken separately from the anterior, middle and posterior portion of each ovary and the number of eggs counted to calculate relative and absolute fecundity. Various parameters were estimated using standard formulae (Lone and Matty, 1980).

$$\text{Gonado somatic index (\%)} = \frac{\text{Weight of ovary (g)}}{\text{Total body weight (g)}} \times 100$$

Absolute fecundity (g) = $n/s \times W$

Where, s = Weight of sub-sample, n = Number of eggs in sub-sample, W = Total weight of ovary

Relative fecundity (g) = Number of eggs g^{-1} body weight

Statistical analysis

The data were analyzed using the Stat-graphic statistical software package (version 16.0. Chicago, SPSS, Inc.). The data were expressed as mean ± SE and the differences compared by one way ANOVA and Duncan's multiple range tests. A probability value of $P \leq 0.05$ indicated that the difference was statistically significant.

RESULTS AND DISCUSSION

The proximate analysis of normal and supplemented diets is given in Table 1. During both pre-spawning period and post-spawning period of *Labeo rohita* broodstock the significantly highest gonado-somatic index (GSI) was recorded in fish supplemented with vitamin-mineral mixture twice a day @ 4% body weight [b.w.] (Table 2). Absolute fecundity was significantly higher in fish fed twice a day @ 4% b.w., irrespective of the composition of diet during both pre- and post-spawning periods. Further, during pre- and post-spawning periods the relative fecundity was significantly higher in fish supplemented with vitamin-mineral mixture twice a day @ 4% b.w.

Table 1: Proximate composition (dry matter basis %) and gross energy (g $kcal^{-1}$) of diets fed to broodstock

Diets	Crude protein	Crude fiber	Ether extract	Ash	NFE**	Moisture	Gross energy
Normal	37.61	16.54	1.67	8.03	36.15	3.74	37.61
Vitamin-mineral supplemented	36.49	16.24	1.59	7.83	37.86	3.66	36.49

Table 2: Effect of formulated diets under different feeding regimes on selected reproductive parameters of *Labeo rohita* broodstock

Parameters	Period	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
		D_1 (control diet)				D_2 (diet supplemented with vitamin mineral mixture)			
Fish weight	PrS	1184^{cd}±45	1271^{bc}±41	1240^{bc}±69	1559^{a}±43	1161^{cd}±51	1322^{b}±33	1206^{bcd}±10	1071^{d}±35
	PoS	1266^{a}±106	1331^{a}±113	1360^{a}±99	1463^{a}±47	1223^{a}±147	1362^{a}±5.	1241^{a}±150	1264^{a}±46
Ovary weight	PrS	311^{b}±28.35	260^{bc}±6.08	316^{b}±10.27	432^{a}±29.20	322^{b}±19.27	240^{c}±1.60	257^{c}±3.59	341^{b}±11.49
	PoS	23^{ab}±8.09	20^{ab}±8.03	13^{b}±2.2	29^{ab}±12.47	17.50^{b}±4.8	20^{ab}±6.9	13^{b}±2.7	44^{a}±9.4
GSI	PrS	26.0^{b}±1.47	21.3^{c}±0.38	25.7^{b}±0.85	27.6^{b}±1.13	27.8^{b}±1.26	18.9^{c}±0.15	21.4^{c}±0.36	32.1^{a}±1.70
	PoS	1.79^{b}±0.48	1.37^{b}±0.45	0.94^{b}±0.14	1.95^{ab}±0.85	1.33^{b}±0.21	1.52^{b}±0.51	1.12^{b}±0.28	3.49^{a}±0.76
Absolute fecundity	PrS	502956^{b}	383433^{c}	547372^{b}	806580^{a}	569310^{b}	267779^{c}	374411^{c}	734657^{a}
	PoS	23072^{ab}	16298^{ab}	3326^{ab}	30406^{a}	9939^{ab}	16280^{ab}	468^{b}	29802^{a}
Relative fecundity (kg^{-1} ovary)	PrS	423480^{c}	312914^{d}	436818^{c}	514911^{b}	496407^{b}	210459^{e}	310921^{d}	690830^{a}
	PoS	16635^{ab}	10335^{ab}	2531^{b}	20316^{ab}	6077^{ab}	11992^{ab}	389^{b}	23660^{a}
Ova diameter (mm)	PrS	0.45^{d}±0.02	0.63^{c}±0.02	0.79^{b}±0.02	0.89^{a}±0.01	0.76^{b}±0.03	0.86^{a}±0.01	0.70^{b}±0.02	0.87^{a}±0.02
	PoS	0.49^{c}±0.04	0.39^{d}±0.03	0.43^{c}±0.06	0.47^{c}±0.01	0.53^{b}±0.01	0.46^{b}±0.09	0.59^{b}±0.02	0.67^{a}±0.04

*Solvent extracted; NFE = nitrogen-free extract

The mean values with different superscripts in a row differ significantly from each other at $P_{0.05}$;

GSI = Gonado-somatic index; PrS= pre-spawning, PoS = post-spawning;

D_1 = Control diet comprising of 30% bran, 50% groundnut oil cake & 20% fish meal; D_2 = Diet comprising of 30% bran, 50% groundnut oil cake, 18% fish meal and 2% vitamin-mineral mixture; T_1 = Feeding D_1 @ 3% once a day; T_2 = Feeding D_1 @ 3% twice a day; T_3 = Feeding D_1 @ 4% once a day; T_4 = Feeding D_1 @ 4% twice a day; T_5 = Feeding D_2 @ 3% once a day; T_6 = Feeding D_2 @ 3% twice a day; T_7 = Feeding D_2 @ 4% once a day; and T_8 = Feeding D_2 @ 4% twice a day;

During pre-spawning, the ova diameter was significantly high in fish fed twice a day @ 4% b.w., irrespective of diet composition. However, during post-spawning time, the ova diameter was high in fish fed with vitamin-mineral mixture supplemented diet twice a day @ 4% b.w. as compared to other treatment regimes. A significant decrease in GSI, absolute fecundity, relative fecundity and ova diameter from pre-spawning to post-spawning period was observed in all the treatments.

The present study revealed that diet supplemented with vitamin-mineral mixture improved the reproductive potential of *L. rohita* broodstock. Suresh *et al.* (2010) and Sudhakar *et al.* (2015) also revealed that feeding fishes with commercially available mineral-vitamin mixtures had a stimulatory effect on ovarian weight and gonado-somatic index. Female fishes fed on a diet supplemented with vitamin E produced larger eggs (Mohamed *et al.*, 2003), reduced abnormal eggs and increased fecundity (Izquierdo *et al.*, 2001) and higher gonado-somatic index (Gupta *et al.*, 1987). Furthermore, ascorbic acid (vitamin C) supplementation of broodstock feed improved hatchability and fry performance in terms of growth, food utilization and survival rate (Soliman *et al.*, 1986). Dietary vitamin A supplementation improved gonadal development and hatchability of eggs in bighead carp (Santiago and Gonzal, 2000) and increased the number of spawned eggs in zebra fish broodstock (Alsop *et al.*, 2008). Takeuchi *et al.* (1981) reported reduction in egg viability and hatching rates in rainbow trout (*Oncorhynchus mykiss*) broodstock, when fed on non-mineral supplemented diet. A decrease in fecundity, percentage of viable eggs, hatching rate, and an increase in the number of abnormal larvae were observed in Japanese sea bream (*Pagrus major*), when fed with phosphorus-deficient diets (Watanabe *et al.*, 1984a,b). In contrast, an increase in spawning percentage and total number of hatchlings was reported following zinc supplementation (Gammanpila *et al.*, 2007).

Along with proper nutrition in the form of sufficient macro- and micro-nutrients, feeding strategies like feeding rate and frequency also play important role in broodstock improvement. The present study revealed that fish fed at higher feeding rate (4% b.w.) as compared to low feeding rate and feeding frequency i.e. twice a day as compared to once a day resulted in improved reproductive potential of *L. rohita* broodstock. Currently no information is available regarding the effect of feeding rate on *L. rohita* broodstock cultured under controlled conditions; however, the results of

present study with respect to feeding frequency are in agreement with the previous reports about African catfish (Ani *et al.*, 2013), goldfish (Hafeez-ur-Rehman, 2015) and Siamese fighting fish (James and Sampath, 2004). James and Sampath (2003) have indicated that higher feeding frequencies in red swordtail (*X. helleri*) resulted in high growth and better reproductive success (gonad weight, GSI, breeding age, interbrood interval and fertility.

Conclusion: From the above study, it is concluded that the supplementation of vitamin-mineral mixture to formulated diet in enhanced reproductive potential of *Labeo rohita* broodstock. Further, feeding @ 4% b.w. in two split doses is recommended for efficient reproductive performance (GSI, absolute fecundity, relative fecundity and ova diameter) of *L. rohita* broodstock.

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