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Efficacy of vitamin e and selenium on brood stock management and reproductive performance of rohu *Labeo rohita* (HAM.)

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Abstract
The study was conducted to assess the breeding performance of *Labeo rohita* (Ham.) by supplementing different concentration of vitamin E and selenium in the formulated diets. Test diets with 25.50% protein were developed using fish meal, ground nut oil cake, rice bran, black gram, horse gram, broken rice and maize. The diets supplemented with different concentration of Vitamin E and selenium *viz*., diet I with no supplementation of vitamin E and selenium referred as control feed, 62.5 and 125; 125 and 250; 250 and 500; 500 and 1000 mg/kg and ppm of Vitamin E and Selenium respectively and referred as diet II, III, IV and V. The Gonadal Somatic Index, fecundity, Ova diameter, fertilization, hatching and survival percentage were studied. Diet supplemented with 250 mg vitamin E and 500ppm selenium shows better result. The least performance was observed in control diet.

Keywords: *Labeo rohita,* brood fish, seed quality, vitamin E and selenium

Introduction
Fresh water aquaculture in India has made notable strides in recent years and continuing to form a major share to the aquaculture production of the country. The sector is regarded as a major option for production and supply of quality protein on a sustainable basis. With strong technological support and farmers initiatives in large scale production, fresh water aquaculture in India has been witnessing spectacular growth rate of over 6% per year over the last two decades. However, during the past five decades the research carried out in the country has certainly helped the fisheries sector in evolving several technologies from breeding to hatchery techniques to rearing of fish fry to fingerlings and culture of marketable fish and prawn. The information on protein requirement for reproduction in fish are scanty (Pathmasothy, 1985; Dahlgren, 1980; Shim *et al.*, 1989) [1, 2, 3].

Brood stock management forms the key to successful spawning. The number and quality of egg - produced brood stock is significantly affected by the conditions under which the brood stock is maintained. Literature pertaining to brood stock nutrition and its effect on reproductive performance and egg quality in cultured carp species reveals that egg production and viability are greatly affected by the nutritional quality of the diets (Watanabe *et al.*, 1984, Mokaginata *et al.*, 1998) [4, 5]. *L. rohita* is one of the most important among Indian major carps and contribute more than 20% of the total aquaculture production in the country. There is an ample scope for increasing production and productivity with respect to brood stock management. In the present study *L. rohita* brood stock was fed with different concentrations of vitamin E and selenium to know the efficacy of reproductive performance.

Methods
The study was carried out in the Government Fish Seed production center located near Bhadra Reservoir, Shivamogga. Two uniform earthen ponds of 500 M^2 with a depth of 1.5 meter was selected for maintenance of *L. rohita* brood fish from February to July month. The ponds were prepared according to Jhingran (1991) [6]. A uniform growth of brood fish weighting 750 g (±0.10 to 0.61) were stocked in the experimental brood stock ponds at the rate of 2500 kg / hr. Water samples were analyzed for the experimental ponds for temperature, pH, dissolved oxygen, free carbon dioxide, alkalinity and plankton population.

Feed

The ingredients used in the diet's formation (Table 1.) were procured from the local market. Dietary ingredients like fish meal, ground nut oil cake, rice bran, black gram, horse gram, broken rice and maize were dried pulverized and sieved. Diets were prepared following the method described by Jayaram, & Shetty (1981) [7]. Incorporation of vitamin (Himedia Laboratories, Mumbai, India and Selenium (LOB Chemical, Mumbai, India) were added after cooking and cooling of mixed ingredients except diet 1 and treated as control feed. The dough was thoroughly minced and extruded through hand palletizer of 5 mm dia. Each diet was prepared separately and dried in hot air over at 40 °C to less than 10 % moisture and stored in a plastic bag at room temperature. The fish were fed once daily in the morning at 2% body weight.

Table 1: Proportion of ingredients and proximate composition of test diets

Ingredients (%)	Diets				
	I	II	III	IV	V
Rice Bran	25	25	25	25	25
Groundnut oil cake	25	25	25	25	25
Fish meal	10	10	10	10	10
Black gram	10	10	10	10	10
Horse gram	10	10	10	10	10
Broken rice	10	10	10	10	10
Maize	10	10	10	10	10
Vitamin E(mg/kg)	-	62.5	125	250	500
Selenium (ppm)	-	125	250	500	1000
Proximate Composition (%)					
Moisture	3.80	3.80	3.80	3.80	3.80
Crude protein	25.50	25.50	25.50	25.50	25.50
Fat	3.50	3.50	3.50	3.50	3.50
Ash	5.80	5.80	5.80	5.80	5.80
Crude fiber	6.40	6.40	6.40	6.40	6.40
NFE	55.00	55.00	55.00	55.00	55.00
Energy (kj/g)	15.86	15.86	15.86	15.86	15.86

Fish Sampling

Fish sampling was done once a month and about 50 % of the stock fish was captured for taking length and weight measurements. Individual length and collective weight of fish collected from each pond were measured. On termination of brood stock maintenance, individual fish weight and length were recorded.

Breeding

Brood fish were collected by dragging from the experimental ponds. Mature male and female fish were selected based on external secondary sexual characters (6). Both female and male were injected intramuscularly with Ovaprim in a single dose simultaneously at a dose of 0.4 ml and 0.3 ml / kg respectively. The hormone injected fish were released together into a hapa already tied in a moderate water circulating Chinese hatchery. Gonadal Somatic Index (GSI), fecundity Ova diameter, rate of fertilization, hatching percentage and survival of larva up to fry stage were estimated soon after spawning of fish.

Results

L. rohita gained maximum weight (2.06 kg) in diet IV followed by diet V (2.041 kg), III (1.89 Kg), II (1.88 kg) and diet I (1.21 Kg) (Table 2.). The length of fish followed the weight of fish. (Table 2.). The Gonadal Somatic Index (GSI) of fish fed diet IV was highest (25.00), followed by fish receiving diet V (24.695) and II (24.695) and the lowest being diet I (23.70). The fecundity of *L. rohita* being the highest in diet IV (2.99 lakhs /kg) followed by III (2.30), III (2.3) II (2.20) and 2.07) in diet I. The ova diameter shown to be maximum in diet II (1.21 mm) followed by IV and V (1.I5 mm) and II (1.12mm) and 1.10 mm in diet I. The fertilization percentage varies between 97.46 in diet V and 91.50 in diet I. Brood fish fed with diet V registered higher hatching percentage (92.04) followed by IV (91.5), III (82.96), II (81.33) and lowest being diet I (75.00). The height survival of 61.54 was recorded in diet IV followed by diet V (47.25). III (44.96), II (44.04) and least in diet 1 (Table 3.)

Table 2: Growth of *L. rohita* brood stock recorded in experimental ponds

Diet	I		II		III		IV		V	
Month	Wt. (g)	L (cm)	Wt. (g)	L (cm)	Wt. (g)	L (cm)	Wt. (g)	L (cm)	Wt. (g)	L (cm)
February	752.02±0.100	38.82±0.005	753.15±0.655	38.50±0.075	751.14±0.46	39.10±0.035	752.34±0.61	39.24±0.065	752.11±0.485	39.20
March	774.04±0.400	39.78±0.055	871.06±1.08	40.42±0.035	868.95±0.005	40.10±0.005	966.35±0.58	41.8±0.025	903.80±2.02	40.55±0.02
April	91.34±0.07	40.24±0.005	973.58±0.43	42.05±0.275	990.90±0.86	42.40±0.075	1165.08±2.15	43.74±0.08	1091.49±0.17	43.98±0.01
May	891.99±0.02	42.17±0.08	1250.68±0.77	43.28±0.01	1255.2±1.01	44.31±0.075	1465.13±0.04	46.42±0.00	1282.86±2.13	46.09±0.02
June	1053.73±0.34	44.30±0.01	1452.79±0.87	45.17±0.03	1455.46±0.82	44.91±0.09	1663.22±3.05	47.1±0.03	1659.41±0.095	47.28±0.005
July	1210.93±0.14	45.44±0.14	1881.03±1.71	46.15±0.05	1891.5±0.1	46.48±0.02	2060.45±0.35	49.27±0.03	2041.15±2.085	49.12±0.005

Table 3: Response of *L. rohita* to different feeds

Diet	Gonado somatic index	Fecundity (lakhs of eggs/kg)	Average ova diameter (mm)	Fertilization %	Hatching %	Survival
I	23.70±0.11	2.07±0.002	1.101±0.002	91.25±0.37	75.00±0.44	42.50±0.36
II	24.69±0.03	2.20±0.002	1.120±0.002	91.96±0.22	81.33±0.21	44.04±0.39
III	24.69±0.03	2.30±0.010	1.210±0.002	92.75±0.23	82.96±0.31	44.96±0.21
IV	25.00±0.12	2.99±0.003	1.150±0.002	94.75±0.27	91.50±0.36	61.54±0.42
V	24.695±0.03	2.16±0.03	1.150±0.002	97.46±0.23	92.04±0.34	47.25±0.36

Discussion

Water quality parameters like temperature, pH, dissolved oxygen, free carbon dioxide, alkalinity and plankton in the brood stock pond are subjected to analyzed once in month and were within the range for fish culture.

Diet containing 25.55% protein with supplementation of vitamin E and selenium 250 mg / kg and 500 ppm respectively in the diet IV induced best growth of *L. rohita*.

Similarly, vitamin E supplemented ornamental fish brooder *Poecilia reticulata* which influenced the reproductive system and growth of fish (Mehrad *et al.,* 2010) [8]. The same diet also favored for Gonadal somatic index and fecundity of fish and survival of spawn to fry. In contrast feed with 125 mg vit E and 250 ppm selenium (Diet III) gave best result in for ova diameter (1.21 mm). Feed with 500 mg vit E and 1000 ppm selenium (Diet V) found to be better in fertilization percentage (97.46%)

Investigation on red sea bream brood stock revealed that vitamin E and Phospholipids improved the egg quality (Jayaram, & Shetty, 1981) [7]. Vitamin E lead an important role in reproductive physiology in fishes as it does in birds and manuals (Watanabe *et al.,* 1991) [9]. While Tajenchi *et al.* (1981) [10] opinioned vitamin E (tocopherol) acts on intra and intercellular antioxidant to maintain hametosis of inter and intra cellular and tissue plasma. Vitamin E in the diet of Indian major Carp and Common carp showed higher GSI bigger Ova and obtained a greater number of eggs (Halver, 1972) [11]. The findings of the present study are in line with author Halver (1972) [11] findings. Selenium appeared to expert its major effects through enzyme glutathione peroxide, while vitamin E is a membrane - bond antioxidant and/or scavenger of free radicals. Thus, the two nutrients were together to protect biological membrane against lipid oxidation Gupta *et al.* (1987) [12]. Dietary selenium and vitamin E function synergically in the prevention of oxidative damage (Tajenchi *et al.,* 1981) [10]. The present investigation combining vitamin E and selenium as additives in the diet IV was within the acceptable limits. The study reveals that in addition to egg number (or fecundity), egg quality has enhanced due to the physiological mechanism involved in Vitamin E and Selenium. The results of the study clearly indicate that vitamin E and selenium in the diet can effectively use to rise *L. rohita* brood stock in good condition. On the basis of above information further trails are to be needed with other cultivable carp species.

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