Evaluation of the Effects of Dietary Vitamin C, Vitamin E and Zinc supplementation on Growth and Reproductive Performances of Rohu fish, *Labeo rohita* (Hamilton, 1822)

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CERTIFICATE

This is to certify that this thesis entitled **"Evaluation of the effects of dietary vitamin C, vitamin E and zinc supplementation on growth and reproductive performances of Rohu fish, *Labeo rohita* (Hamilton, 1822)"** submitted by Mohammad Mamunur Rahman has been carried out under my complete supervision. This is further to certify that it is an original work and suitable in partial fulfillment for the degree of MS in Fisheries, University of Dhaka.

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ABSTRACT

Rohu fish (*Labeo rohita*, Hamilton1822) is a popular variety of carp fish species for its highly nourishing quality and prolonged freshness. An experiment was undertaken to evaluate the effects of dietary vitamin C, E and Zinc supplementation on Growth and Reproductive performances of Rohu fish, *Labeo rohita* (Hamilton, 1822) from 25 February, 2013 to 9 June, 2013 in the aquatic laboratory of Department of Fisheries, University of Dhaka. Use of vitamin C, vitamin E, Zinc (Zn) with different doses and locally available feed ingredients, like fish meal, soya cake, flour, mustard oil cake, salt, molasses and vitamin premix is a useful method of fish culture in the field of culture fishery. Proximate composition of the formulated fish feed and the experimental Rohu fish species has been determined as these factors influence the growth performances, feed utilization and reproductive performances of experimental fish during the rearing and feeding trial in the laboratory condition. Growth and reproductive performances using a formulated control feed, Feed A (without vitamin E, vitamin C and Zn) was compared with other three formulated fish feed titled as Feed B (100 mg/kg vitamin C and vitamin E with 10 mg/kg Zn in diet), Feed C (500mg/kg vitamin C and vitamin E with 10 mg/kg Zn in diet) and Feed D (1000 mg/kg vitamin C and vitamin E with 10 mg/kg Zn in diet) in eight respective tanks of Feed A_1 and Feed A_2, Feed B_1 and Feed B_2, Feed C_1 and Feed C_2, Feed D_1 and Feed D_2. Each tank contained 10 numbers of adult Rohu fish (*Labeo rohita*, Hamilton1822). The average initial weight of Rohu fish (*Labeo rohita*, Hamilton1822) for Feed A (Control feed), Feed B, Feed C and Feed D was 225.26±0.05 g, 225.36±0.05 g, 225.47±0.07 g and 225.34±0.02 g respectively. Growth performances and survival rate were influenced by formulated feed types. Average final weights were 365.15±0.43 g, 375.9±0.03 g, 406.6±0.52 g and 381.93±0.20 g having an average daily gain (ADG, g/day) of 1.16±0.01, 1.25±0.01, 1.50±0.005 and 1.30±0.005 with a Specific growth rate (SGR, %) of 4.11±0.01, 4.17±0.01, 4.33±0.007 and 4.21±0.00 % in Feed A, Feed B, Feed C and Feed D respectively. The above results showed a typical increasing trend of final body weight gain and Specific growth rate (SGR) for the experimental fish species along with the increase of feed protein level for 120 days study period. There were significant differences ($p<0.05$) among Feed conversion ratio (FCR) and Protein efficiency ratio (PER). Significant differences ($p<0.05$) were also observed in case of Feed efficiency (%) and Survival rate (%) during

the entire study period. Results of the current study suggest that the diet supplementation of 500 mg/kg vitamin E and vitamin C with additional 10 mg/kg Zinc has positive effects on the reproductive performance of experimental fish. The highest female GSI value (1.51±0.06%) was found for Feed C treatment while the lowest value (1.09±0.06%) was found for control treatment (Feed A).The maximum GSI value for male fish was (0.93±0.04%) which was found in Feed C treatment and the lowest value (0.39±0.05%) was found for Feed A treatment So, the study suggests that suitable protein level and sufficient amount of dietary vitamin C, E and Zinc supplementation is required for proper growth and successful reproduction of fish species.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols	**Details**
℃	Degree Celsius
g	Gram
cm	Centimeter
%	Percentage
ANOVA	Analysis of variance
mg	miligram
kg	kilogram
ADG	Average daily gain
SPSS	Statistical package for the social sciences
SGR	Specific growth rate
FCR	Feed conversion ratio
PER	Protein efficiency ratio
SR	Survival rate
A	Feed A
B	Feed B
C	Feed C
D	Feed D

Introduction

1.1 Importance of Fisheries in Bangladesh

Fish has been playing an important role in addressing nutritional and livelihood security of people in the developing countries. Fish provides 20% of animal protein intake to about 2.6 billion people globally and at least 50% of animal protein intake for over 400 million in Asia and Africa. But, in developed countries, it provides only 13% of animal protein intake (FAO 2008).

In the recent time fish is considered an indispensable part of the daily food intake. In Bangladesh, fish is not only treated as delicious food item but also constitutes a significant constituent of farming system. Due to steady rise in population in a geometric way throughout the world, considerable attention is being given to find the ways for increasing the production of food includes protein sources and enhancing the biological value of different food products. In Bangladesh fisheries sector plays an important role in supplying animal protein as well as foreign exchange. Fish being one of the major sources of animal protein in the country and have helped greatly in improving nutrition as they contain 60% digestible protein and all essential amino acids. Fishes also contain important mineral such as phosphorus, calcium and magnesium. In the last few decades, the aquaculture production has increased significantly.

I. Nutritional Contribution

Bangladesh is a small country having area of about 147570 km2. Fisheries play a vital role in our national economy. The contribution of fisheries sector in Bangladesh is 4.43% to national GDP and 22.21% in agricultural GDP and 2.73% to foreign exchange earnings by exporting fish products in 2010-11 (DoF).

Fisheries sector also plays an important role in rural employment generation and poverty alleviation. The total production value of fish in our GNP is 19567.90 crore taka. About 12.80 million people are related to fisheries activities (DoF 2009).

II. Sources of Fish Production

There are three categories of major fisheries resources, this are-

a) Inland Capture (34%)
b) Inland Culture (48%)
c) Marine Capture (18%)

1.2 Present status of fish culture in Bangladesh

Bangladesh, though rich in having around 1.5 million ponds of different sizes covering an area of about 146,955 hectares of land, only about 52% (DoF 1991) of the ponds are, at present, being utilized for fish production through extensive method. Majority of this area is used as carp rearing hatchery. Aquaculture practice is strongly carp species dominated. Out of total 510,000 ha aquaculture farms carp species cultured in 310.000 ha area. In 2004 the estimated quantity of cultured carp species was 680,253 MT, which was 32% of the total fish production from all sources and 85% of all farmed freshwater fish species (914,752 MT).

1.3 Rohu fish (*Labeo rohita*) an important carp species for aquaculture

Labeo rohita is the most important farmed fish in the world. Despite the great potential of *Labeo rohita* (Rohu fish) shortage of fry production to meet increasing global demands remains one of the major obstacles that limit the expansion of intensive culture of *Labeo rohita* (Rohu fish). An improvement in brood stock nutrition and feeding should be reflected by high quality eggs, sperms and also a quality and quantity of seed produced.

Several factors have enhanced the status of the farming of Rohu fish:

- Improvements in induced breeding and seed production, which have removed the reliance on the capture of natural riverine seed;
- Improved growout technology;
- Improvements in feeding and health management;

1.4 Role of vitamin C, vitamin E and Zinc supplementation on Growth, Body Composition and Reproductive Performance

For normal fish growth and health vitamins are necessary in the regular diet of fish feed. Most of these vitamins are not synthesized by fish body, so that must be supplied in the diet. Deficiency of each vitamin has certain specific symptoms but reduced growth rate is the most common symptom of any kind of vitamin deficiency.

Some of trace elements and vitamins have been linked with brood fish growth and egg quality (Sandnes *et al.*1984).

I. Role of vitamin C

Vitamin C is considered to be an essential component in diets for teleost fish (Halvier 1985; Dabrowski and Ciereszko 2001).

Numerous studies have shown that the dietary supplemented ascorbic acid has a positive effect on the reproductive performance of Rainbow trout (*Salmo guirrdneri*) (Sandnes *et al.* 1984), Tilapia (*Oreochromis mossambicus*) (Soliman *et al*. 1986a) and Atlantic salmon (*Salmo salor*) (Eskelinen 1989).

Ascorbic acid requirements may be correlated with fish ontogeny, for instance larval metamorphosis or gonadal maturation and also may have particular relevance to completion of reproduction and quality of gamates and fertility (Dabrowski and Clereszko 2001). Sandnes *et al.* (1984) and Soliman *et al.* (1986a) pointed out that brood fish dietary ascorbic acid is transferred to the eggs, where it is stored for supporting growth and development of the larvae until the first feed intake.

Ascorbic acid (vitamin C) is an essential nutrient in aqua-feeds, and is an indispensable nutrient required to maintain the physiological processes of different animals including fishes (Tolbert, 1979). Most of fish, including tilapia are not capable of vitamin C biosynthes is (Chatterjee 1973) due to the absence of the enzyme L-gulonolactone oxidase, which is responsible for synthesis of ascorbic acid (Wilson 1973).

At their natural environment, vitamin C would be present in natural food, but in intensive fish culture its supplementation becoming necessary. Small amount of this vitamin is

sufficient to prevent and cure scurvy; however, larger amount may be essential to maintain good health during environmental adversities, situation of physiological stress and conditions of infectious and parasitic diseases (McDowell 1989; Lim 1996).

Ascorbic acid (vitamin C) is essential for producing collagen and bone minerals, assists in metabolizing iron and helps in activation of vitamin D. It also assists in reducing the harmful effects of hormones produced by the adrenal gland during prolonged periods of stress (Lovell 1989; Navarre and Halver 1989).

Also, it has an important role in a great number of biochemical processes such as synthesis of collagen which is an intercellular protein and principal constituent of skin, scales, mucosa, cartilaginous tissues, bones and conjunctive tissue formation, which involves all the organs of the body (McDowell 1989).

Agrawal *et al.* (1978) reported that high levels of ascorbic acid are efficient to enhance tolerance to environmental stressors e. g. aldrin toxicity.

Several functions especially immune activity and resistance to toxicants and stress are affected, in aquaculture species, by dietary ascorbate deficiency and result in increased fish mortality (Marchie *et al*. 1996).

II. Role of vitamin E

Vitamin E receives the most attention for its important role as an antioxidant. Vitamin E activity is present in a group of naturally occurring closely related tocopherols. Among them, α-tocopherol has the highest vitamin E activity. DL-α-tocopherol acetate, a stable vitamin of α-tocopherol, is the most commonly used form in animal feeds (NRC 1983).

Carps fed with α-tocopherol deficient diet showed significantly lower gonadosomaic index ranged from 0.7-2.0% compared with 10-20% in control fish (Watanabe and Takashima 1977).

Vitamin E is among the most important a nutrient influencing the fish immune system, and the supply of vitamin E can reduce mortality and improve fish performance, while increasing specific and nonspecific immune responses (Wahli *et al.* 1998; Ortuno *et al.* 2001; Shiau and Hsu 2002; Puangkaew *et al.* 2004).

In addition, vitamin E is potent antioxidant that offer protection against oxidative damage to various fish tissues (Adham *et al.* 2000), enhance resistance of red blood cell membranes (Kiron *et al.* 2004), and protect leukocyte functions (Sahoo and Mukherjee 2002).

Adequate vitamin E supplementation in fish diets under intensive rearing is essential for survival and growth performance (Chagas *et al.* 2003). However, in this study after the 3 month feeding trial, Tilapia showed no significant changes on their growth among the diets they were exposed.

Similar results have been found for species such as Atlantic salmon (Hardie *et al.* 1990), coho salmon (*Oncorhynchus kisutch*) (Forster *et al.* 1988), rainbow trout (Blazer and Wolke 1984), catfish (Bai and Gatlin 1993) or seabass (*Dicentrarchus labrax*) (Stephan *et al.* 1993).

However, other authors have demonstrated the effects of vitamin E deficient diets on growth for different species, such as Atlantic salmon (Hamre *et al.* 1997), rainbow trout (Cowey *et al.* 1984), chinook salmon (Thorarinsson *et al.* 1994) and hybrid striped bass (*Morone chrysops* female *Morone saxatilis* male) (Kocabas and Gatlin 1999). The apparent difference among studies could be due to differences among species, the size of the fish used (as those studies describing effects of dietary vitamin E on fish growth or survival used fry, whereas those studies describing no effects used juvenile fish or the differences in experimental procedures and culture.

Most of the deficiency signs observed in fish, such as nutritional muscular dystrophy, fatty liver degeneration, anemia, erythrocyte hemolysis, hemorrhage, depigmentation, and reduction of fertility, are related to peroxidative damage to cellular membranes (NRC 1983).

Ali and Mahmood (2004) studied the effects of vitamin E on the growth rate, GSI, fecundity and the stocking density on larval growth and survival in *Anabas testudineus* (Koi). Hence, the present study is undertaken to study the effect of different levels of dietary vitamin E on growth, gonad weight and reproduction.

III. Role of Zinc (Zn)

Zinc is an important trace element in fish nutrition as it is involved in several metabolic pathways (Watanabe *et al.* 1997). Based on the past literature, Gatlin and Wilson (1984) suggested evaluating the Zinc essentiality for fish growth.

1.5 Taxonomic position

The taxonomic position of ***Labeo Rohita*** is given below:

Kigdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Sub-class: Neopterygii
Super order: Ostariophysi
Order: Cypriniformes
Super family: Cyprinioidea
Family: Cyprinidae
Genus: *Labeo*
Species: *L. rohita* (Hamilton 1822)

Plate no. 1 *L. rohita fish species*

1.6 Geographical range

Rohu (*Labeo rohita*) is a species of fish of the carp family, found in rivers in South Asia.It is a popular white fish in Bangladesh, Thailand, northern India and Pakistan.

Plate no. 2 Main producer countries of *Labeo rohita* (FAO Fishery Statistics 2006)

1.7 Seed Production

Induced breeding of Rohu has been catering for almost the entire seed requirements in all the countries where it is cultured, although riverine collection from is still forms the sole seed source in certain small areas. When induced breeding through hypophysation has been the common practice since the development of the technology in 1957, several synthetic commercial formulations of purified salmon gonadotropin and dopamine antagonists such as Ovaprim, Ovatide and Wova-FH have also been successfully used in recent years. When pituitary extract is used, females are injected with a stimulating dose of 2-3mg/kg BW followed by a second dose 5-8 mg/kg after a lapse of six hours; males are given a single dose 2-3mg/kg at the time of second injection of the female. When synthetic formulations are used, a single dose of 0.4-0.5ml/kg body weight (females) or 0.2-0.3ml/kg (males) is administered.

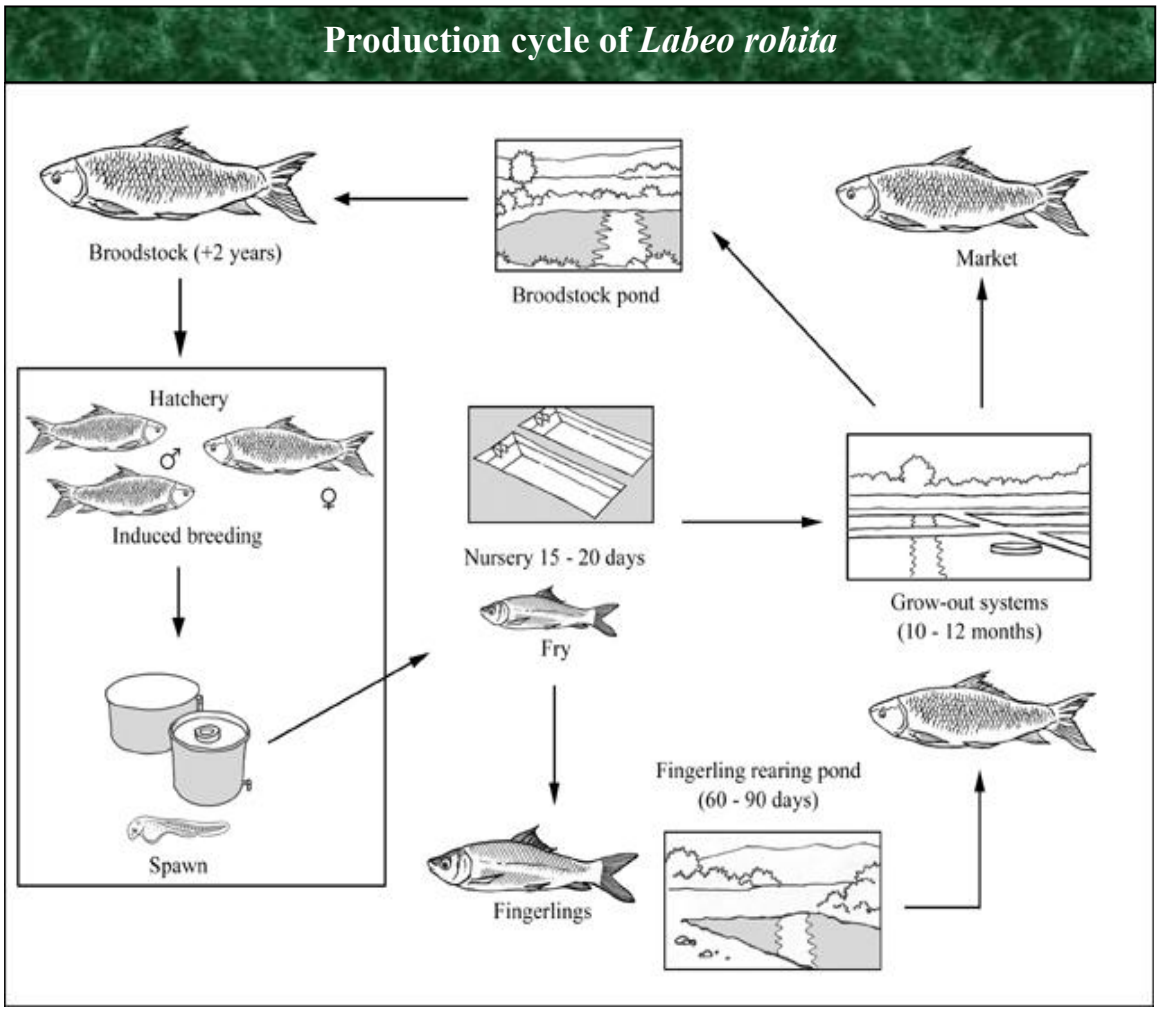

Fig. 1 Production cycle of *Labeo rohita* (FAO Major carp production)

1.8 Effects of Feeds on Growth and Survival

In intensive rearing of larvae of fishes and prawns, feeding constitutes a major factor since the fish obtain their entire nutritional requirement (except part of mineral requirement) through the food they consume (Pillay 1990).

Since live feed is rich in proteins, carbohydrates and fats along with various types of vitamins and minerals, it is always preferable to have a regular supply of live feed (Singh *et al.* 1994). Thus "live feed" serves as "living capsules" of nutrition (Tiwari 1986) and their nutritional status can be further enhanced by using technique known as "bio-enrichment" so that nutritional status of the fishes, prawns and shrimps feeding on them could be increased.

Mustafa *et al*. (2004) concluded that Anabas testudineus did not differ significantly in survival rates ($p>0.05$). Fish fed different diets showed significant differences in growth performance and feed utilization efficiency ($p<0.05$).

Doolgindachabapom (1994) recommended the following analysis for fry of *Anabas testidineus*

- Feed containing 30.6 % protein as the best feed formula in terms of growth and mortality;
- 27% protein feed showed the best performance;
- The optimum stocking density was 20 fish larval per liter.

Watanabe and Takashima (1977) concluded that the adult carp receiving diet deficient α-tocopherol results significantly less weight gain than diet higher in α-tocopherol.

1.9 Gonado-Somatic Index (GSI)

Environmental changes greatly influence the production of eggs varies not only among different species but also within the same species. This depend supon the length and weight of the gonads (Barmanh and Saikia 1995). Maturity determination by gonadosomatic ratio has proved to be a significant tool in the life of fishes. Gonads undergoing regular seasonal cyclic changes in weight, particularly in females which help to indicate the spawning season (Dadzie *et al.* 2000).

The method of studying the spawning season is to follow the seasonal changes in gonadal weight in relation to body weight which is expressed as the gonadosomatic index (Ahirrao 2002). Gonadosomatic Index (GSI) is one of the important parameters of the fish biology which gives the detail idea regarding the fish reproduction and reproductive status of the species and help in ascertaining breeding period of fish (Shankar and Kulkarni 2005).
The gonadosomatic index measures the cyclic changes in gonad weight in relation to weight and can be used to determine spawning periods (Smith 2008).

Changes in (water) temperature and photoperiod have been shown to correlate well with gonadal weights and therefore with gonado-somatic index (Lam 1983; Mananos *et al*. 1997; Mylonas and Zohar 2007). Gupta *et al.* (1991) studied common carp (Cyprinus carpio) fed with vitamin E diets and concluded following results

- The higher gonadosomatic index (GSI);
- Larger and bigger ova;
- More eggs than control group;

Gunwant *et al.* (2013) concluded from their investigation that the GSI of freshwater major carp *Labeo rohita* was maximum during spawning season whereas decrease during post spawning season.

1.10 Brood Fish Feed Formulation

High quality fish feed and rich in nutrient standard is prerequisite for proper brood fish nutrition. Bhuiyan *et al*. (1989) have conducted a survey on the potential fish feed ingredients of Bangladesh on the basis of their availability and biochemical composition for suitable fish feed. A nationwide survey was conducted to identify potential fish feed ingredients. The survey covered materials that are being traditionally used and also non-conventional items such as kitchen waste, processed waste from the food or fish industry, aquatic weed etc. Results of the survey indicate high potential of most of the enlisted conventional and non-conventional ingredients that can be economically used for the manufacture of quality fish feed.

The most wanted protein percentage found was encouraging and can be summarized in the following way:

- Less than 10% protein in 24 ingredients;
- 10-30% protein in 42 ingredients;
- 30-50% protein in 8 ingredients;
- More than 50% protein 9 ingredients;

As many as 35% ingredients were potential enough to be classified as protein supplementary feed. Ingredients classifiable as energy supplement numbered 28. Twenty four (24) ingredients were classified as roughages. Most of the enlisted ingredients are abundantly available in the country and prices are also affordable to aqua farmers.

Locally available indigenous raw materials may serve as important ingredients for the formulation and development of cheaper and quality fish feed. Another study done by Megh *et al.* (2006) concluded that quality of feed materials produced by feed manufacturer should be required to maintain optimum standard before and after reaching to the farmers.

1.11 Nutritional Parameters

Some researchers have been given emphasis to investigations with data for treatment, which is not only curative but also prophylactic (Gabaudan *et al.* 1992), such as the case of use of vitamins on the supplementation of commercial diets.

1.12 Objectives

The study was undertaken to investigate the effects of vitamin C, vitamin E and their interactive effects on brood fish and seed quality of Rohu fish *(Labeo rohita)*. Additionally, supplementation of higher level of Zinc on seed quality was also evaluated.

Specific objectives:

I. To investigate the effects of vitamin C (Ascorbic acid).
II. To evaluate the effects of dietary vitamin E (α-tocopherol).
III. To investigate their interactive effect with additional Zinc supplementation on brood fish growth, survival and gonadosomatic index.

Materials and Methods

2.1 Experimental Location

The study was conducted for 4 months in a static indoor water system in the aquatic laboratory, Department of Fisheries, University of Dhaka, Dhaka.

2.2 Experimental System

A static indoor rearing system consisting of eight (08) plastic tanks each tanks containing about 750L water was employed for the study. Female and male *Labeo rohita* (mean size range 225 g) was stocked and each tank consisted of 10 fishes. The batch weights of male and female in each container was also recorded to the nearest gram before stocking in the tank. Tap water was the source of water supply in the tanks during experimental period. An adequate level of oxygen in each tank was maintained through artificial aeration by using air pump.

2.3 Experimental Diet

The experimental diets were formulated with four levels of vitamin C (0.0, 100, 500 and 1000 mg/kg in the diet) and four levels of vitamin E (0.0, 100, 500 and 1000 mg/kg in the diet) and a combination of Zinc sulphate at the rate of 10 mg/kg. Fish were fed three times a day with experimental feeds for 4 months at a rate of 5% of the body weight per day during the whole experimental period.

2.4 Experimental Feed Composition

Feed ingredients used in this experiment were collected from local market of Kataban, University market. Feed was made from fish meal, soybean meal, wheat flour, vegetable oil, soybean oil, salt, trash fish and vitamin mineral premix. Four types of formulated diet (Feed A: Control diet; Feed B: Diet with 100 mg/kg vitamin E and Vitamin C, consecutively with a combination of 10 mg/kg Zinc sulphate; Feed C: Diet with 500 mg/kg vitamin E and Vitamin C, consecutively with a combination of 10 mg/kg Zinc sulphate; Feed D: Diet with 1000 mg/kg vitamin E and Vitamin C, consecutively with a combination of 10 mg/kg Zinc sulphate) were used during this experiment.

The proximate compositions of each of the diet are represented in the following table:

Table 1 Proximate composition of the experimental diets (%)

Sl.No.	Treatment Name	Crude protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Carbohydrate (%)
1	Feed A	36.01±0.11	10.18±0.03	11.6±0.01	11.70±0.05	30.51±0.14
2	Feed B	35.60±0.15	10.10±0.20	11.65±0.15	12.00±0.10	30.65±0.30
3	Feed C	36.45±0.08	9.80±0.15	11.35±0.07	11.20±0.10	31.20±0.33
4	Feed D	36.06±0.16	10.05±0.01	11.50±0.04	11.65±0.10	30.74±0.29

2.5 Sample Species Collection and Acclimation

Healthy adult species of Rohu (*Labeo rohita*) were collected from a local hatchery at B,baria. Adults were kept in two circular tanks with adequate aeration and sufficient water. The average size of each species was 225 g having an average length of 20 cm. After receiving at the laboratory, the sample species were transferred in two clean circular water tanks of 750L capacity as stocking tank. The fishes were then given a prophylactic treatment with 0.5% NaCl for 30 minutes. They were then allowed for acclimation of one week in three acclimation tanks with sufficient aeration through aerators. Total stocks of about 120 fish species were considered for the experiment. During acclimation, a maintenance feed ration of about 5% of body weight was allowed to experimental fish in acclimation tank for one week.

2.6 Experimental Protocol

The experimental feeding trial was scheduled for a period of 4 months. There were four treatments selected with two replications. The acclimatized fishes were reared for four months feeding trial in 8 experimental tanks. The uniform sized sample species of Rohu (*Labeo rohita*) from same stock were randomly distributed at the rate of 10 fishes per tank with a mean initial weight of 225 g.

About two-third water in each tank was changed every day morning after removal of dirty particles and feces from the aquarium manually by using outlet pipe.

2.7 Experimental Procedure

For the experiment with Rohu (*Labeo rohita*) four treatments were selected to see the responses of scheduled feeding trial on growth and survival during the experimental period. Sample species from the stocking tanks were weighted by electronic balance and ten adult species were randomly selected for each of replicates of four treatments.

Plate no. 3 A. Experimental Design, **B.** Water quality measurement, **C.** Experimental unit, **D.** Feeding process, **E.** Dissection of *Labeo rohita* **F.** Gonad of *Labeo rohita*

No feeding was allowed on the day of sampling. The feeding was made twice a day at 9.00 a.m. and 4.00 p.m. Careful observation was made so that no uneaten feed remains in the tanks. Feces in the tanks were cleaned every day morning by outlet before feeding commenced without creating any disturbances to fish. Routine measurement for water quality (such as Temperature, DO, pH etc.) was taken on a weekly basis. About 5 fishes from the stocking tank (same stock of fish used in experimental tanks) was sacrificed to determine the initial proximate composition in this system. At the end of the feeding schedule fishes were finally weighted and total number of individual species also calculated carefully to determine survival rate for each treatments.

Different growth parameters were calculated on the basis of feed intake, protein feed etc. For statistical analysis one way analysis of variance (ANOVA) was done followed by Tukey's b for getting the difference in treatment means at significant levels of 5% probability.

2.8 Feeding Rates

Sample fishes were fed with the prepare diets twice daily in the morning at 9.00 a.m. and afternoon at 4.00 p.m. throughout the study period and applied by dividing the ratio into several portions using feeding trays so that all the feeds allowed to fish is properly ingested. Fishes were fed at a rate of 5% of the body weight per day during the whole experimental period.

2.9 Sampling Procedure

Samplings of fishes in all treatments were done on same day at every 7^{th} day of the experimental period. Initial and final weight of fish in each tank was recorded individually. Fishes were netted by using a fine mesh scoop net and excess water was then removed from fish body by gently blotting on a soft tissue paper and then transferred to a water filled plastic container and weighted to nearest 0.01g precision on a electric balance individually. Total weight of fish for each treatment was recorded by cumulating the individual data.

2.10 Water Quality

Water quality parameters such as pH and temperature were monitored weekly throughout the experimental period. pH was measured by a pH meter and temperature was recorded by using a digital thermometer. All the parameters were observed to be within the level of acceptance of fish culture.

2.11 Waste Water Removal

To remove excreta or uneaten feed (if any) from each tank two third of water was changed every day before half an hour of feeding. Feeding tray was also cleaned on a regular basis. It was done to keep the water quality as good as possible. A good aeration was maintained in each of the tank all along the experimental period.

2.12 Analytical Methods

Fish samples were analyzed for their proximate composition according to standard procedure given in Association of Official Analytical Chemists (AOAC, 2000).

2.12.1 Analysis of Experimental Data

2.12.1.1 Nutrient analysis of diets and fish

a. Moisture (%)

Moisture content will be determined by oven drying at 100℃ for 24 hour. The percentage of moisture was than determined by the following formula

$$\textbf{\% of Moisture} = \frac{\text{Initial weight (g)} - \text{final weight (g)}}{\text{Weight of the sample}} \times 100$$

Where,

Initial weight = raw sample weight + moisture dish weight (before heating)

Final weight = dry sample weight + moisture dish weight (after heating)

Dry matter (%) = 100 – moisture content

Estimation of Moisture Factor

$$\textbf{Moisture factor} = \frac{\text{100- Moisture}}{100}$$

b. Crude Protein (%)

Crude protein level will be tested using a kjeltec machine. The protein content of the fish was determined by micro Kjeldahl method (AOAC 2000). It involves the conversion of organic nitrogen to ammonium sulphate by digestion of flesh with concentrated sulphuric acid in a micro kjeldahl flask. The digest was diluted, made alkaline with sodium hydroxide and distilled. The liberated ammonia was collected in a boric acid solution and total nitrogen was determined titrimetrically. The percentage of protein in the sample was calculated by using the following formula

$$\textbf{\% of Protein} = \frac{(S\text{-}B)\times 29\times \text{moisture factor}}{\text{Weight sample}}$$

Where,

S = Titration reading for sample

B = Titration reading for blank

c. Crude Lipid (%)

Crude lipid by using the Soxhlet system. For the estimation of fat content, the dried samples left after moisture determinations were finely grinded and the fat was extracted with chloroform and methanol mixture (AOAC, 2000). After extraction, the solvent was evaporated and the extracted materials were weighed.

The percentage of the fat content was calculated.

$$\textbf{(\%) of Fat} = \frac{\text{Final weight (g)-Flask weight (g)}}{\text{Sample weight}} \times 100 \times \text{moisture factor}$$

d. Ash (%)

Samples ash content was determined by igniting the samples in a Muffle Furnace at 550-600℃ for 20 hours.

$$\textbf{(\%) of Ash} = \frac{\text{Final weight (g) - crucible weight (g)}}{\text{Sample weight (g)}} \times 100 \times \text{moisture factor}$$

2.12.1.2 Growth Estimation

Experimental data collected during the growth trials were used to determine the following parameters:

a. Body Weight Gain

Body weight gain = $(W_t - W_o) \times N_t$

Here,

W_t = Final fish weight (g)

W_o = Initial fish weight (g)

N_t = Final numbers of fishes in each replicate

b. Specific growth rate (SGR)

Specific growth rate will be calculated from the difference between the wet weight at the beginning of the experiment and the weight on the day of calculation as

$$\textbf{SGR (\% day}^{-1}\textbf{)} = \frac{\ln(\text{final fish body weight}) - \ln(\text{initial fish body weight})}{\text{Time } (T_2 - T_1) \text{ (days)}}$$

c. Survival rate

$$\textbf{Survival rate (\%)} = \frac{\text{Final no. of fish survived}}{\text{No. of actual fish stocked}} \times 100$$

d. Condition Factor:

This is the factor through which condition of the fish is expressed in numerical terms i.e. degree of plumpness or flatness is usually estimated as the condition factor. It was calculated by the following formula

$$\textbf{Condition Factor (K)} = \frac{\text{Body weight (W) (g)}}{(\text{Standard body length})^3 \text{ cm}^3} \times 100$$

e. **Average Daily Gain (ADG)**

Average daily gain was determined by the following formula:

$$\textbf{ADG (g / f/ d)} = \frac{\text{Mean final fish weight - Mean initial fish weight}}{\text{Time } (T_2\text{-}T_1) \text{ (days)}}$$

f. Food Conversion Ratio (FCR)

The Food Conversion Ratio (FCR) is defined as the amount of dry food fed per unit live weight gain. It is calculated as follows:

$$\textbf{Food conversion ratio (FCR)} = \frac{\text{Feed supplied (g) (dry weight basis)}}{\text{Total weight gained by fish (g)}}$$

To calculate FCR, the dry weight of the food was obtained by using a correction for the analyzed moisture content of the diet. FCR is a measure of the degree of gross utilization of food for growth.

g. Protein Efficiency Ratio (PER)

Formula of calculating Protein Efficiency Ratio (PER) is given below:

$$\textbf{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain}}{\text{Dry protein intake}}$$

h. Feed Efficiency

Formula of calculating Feed Efficiency is given below:

$$\textbf{Feed efficiency} = \frac{\text{Weight gained in wet weight}}{\text{Feed intake in dry weight}} \times 100$$

2.12.1.3 Reproductive performance

Gonad Estimation

One female from each treatment will be sacrificed at 30-day intervals from the time of gonad development until the commencement of spawning. Their ovaries will be removed and weighted and the gonadosomatic index (GSI) was computed by the following formula:

$$\textbf{Gonadosomatic Index (GSI) (\%)} = \frac{\text{Gonad weight (g)}}{\text{Final body weight (g)}} \times 100$$

2.13 Statistical Analysis

Data was analyzed using one way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DMRT) to determine the differences between treatment means demonstrating a significant variation of P value of <0.05 (at 95% confidence intervals).

2.14 Water Temperature

The values of water temperature ranged from 27.6-30.9^{0}C in eight experimental tanks during the 120 days study period.

2.15 DO level (mg/L)

Dissolve oxygen (DO) was measured directly in the water column of tanks every week by using digital oxygen meter. The average value ranges from 6.9-7.8 mg/l.

RESULTS

Summarized and detailed result of the study on the proximate composition of fish, feed utilization efficiency (survival rate, growth performance, average daily gain etc.) and reproductive performance of *Labeo rohita* in large plastic tanks on four types of formulated diet (Feed A: Control diet; Feed B: Diet with 100 mg/kg vitamin E and Vitamin C, consecutively with a combination of 10 mg/kg Zinc sulphate; Feed C: Diet with 500 mg/kg vitamin E and Vitamin C, consecutively with a combination of 10 mg/kg Zinc sulphate; Feed D: Diet with 1000 mg/kg vitamin E and Vitamin C, consecutively with a combination of 10 mg/kg Zinc sulphate) as recorded carefully during the study period were presented under the following headlines:

Protein in experimental diet

During the study period protein content in experimental diet was found to be in the range of 35.6-36.45%. The highest protein content was found in Feed C (36.45±0.0.08%) treatment and lowest value was found in Feed B (35.6±0.15%) treatment.

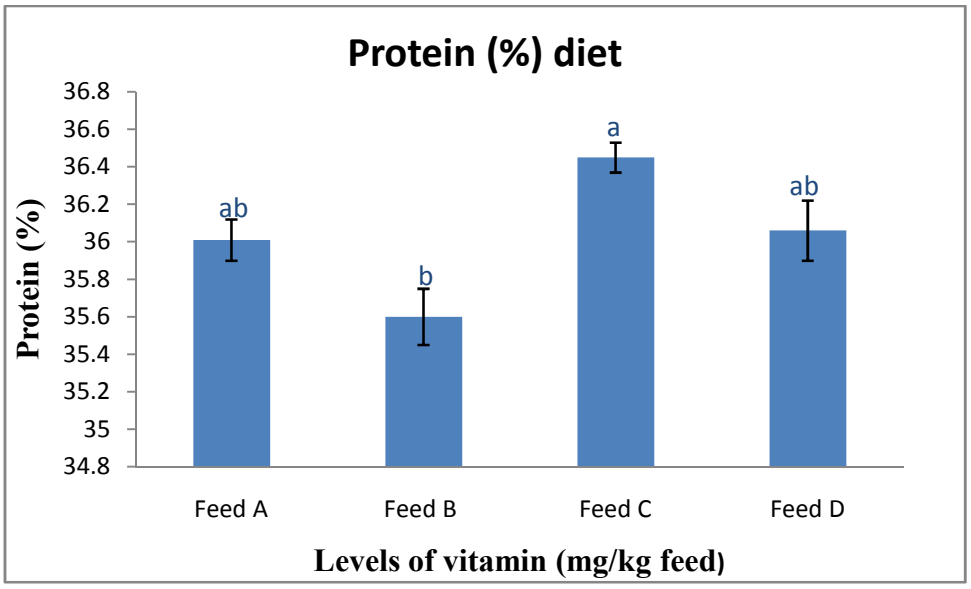

Fig. 2 Crude protein content (%) (Mean ± SEM) for experimental diets used during the study period. Bars different letters (a,b) indicate significant difference.

Proximate composition of experimental fish

Proximate compositions of the experimental fish *Labeo rohita* were determined at the initial stage of the experiment period. Same types of analysis were also carried out at the end of 120 days feeding trial. The results of proximate composition of *L. rohita* at different experimental tanks are represented in Table 2.

Table 2 Whole body proximate composition of *Labeo rohita* fed with four different types of feed (% fresh weight basis, mean ± SD)

Nutrients (%)	Treatment diets			
	Feed A	Feed B	Feed C	Feed D
Moisture	80.01±0.32	80.1±0.35	79.51±0.61	80.08±0.10
Protein	14.69±0.17	15.10±0.09	16.37±0.46	15.34±0.38
Lipid	1.46±0.06	1.32±0.36	2.17±0.18	1.71±0.76
Ash	2.1±0.12	2.03±0.43	1.33±0.27	1.44±0.02

Protein (%) in fish muscle

During the study period protein content in experimental fish species was found to be in the range of 14.69-16.37%. The highest protein content was found in Feed C (16.37±0.46%) treatment and lowest value was found in the control feed (14.69±0.17%) (Feed A). Percentage of crude protein for *L. rohita* is given in Figure 3.

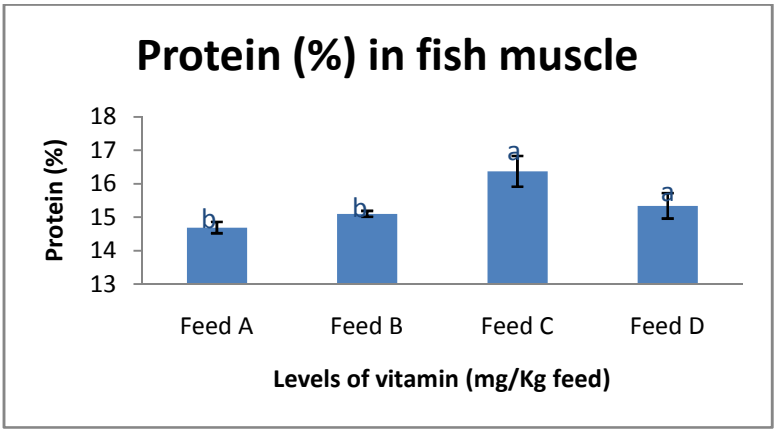

Fig. 3 Crude protein content (%), (Mean ± SEM) in *Labeo rohita* for 120 days study period. Bars different letters (a,b) indicate significant difference.

Growth performance

Table 3 Growth performance and survival rate of experimental fish species *Labeo rohita* using formulated feed is given below for 120 days experimental period.

Parameters	Treatment diets			
	Feed A (Control diet)	Feed B	Feed C	Feed D
Initial mean weight (g)	225.26±0.05	225.36±0.05	225.47±0.07	225.34±0.02
Final mean weight (g)	365.15±0.43	375.9±0.03	406.6±0.52	381.93±0.20
Average daily gain (g/day)	1.165±0.01	1.255±0.01	1.505±0.005	1.305±0.005
Percentage weight gain (%)	62.60±0.72	66.78±0.03	80.33±0.17	69.49±0.07
Survival rate (%)	85.00±1.88	90.00±.00	90.00±.00	85.00±1.88

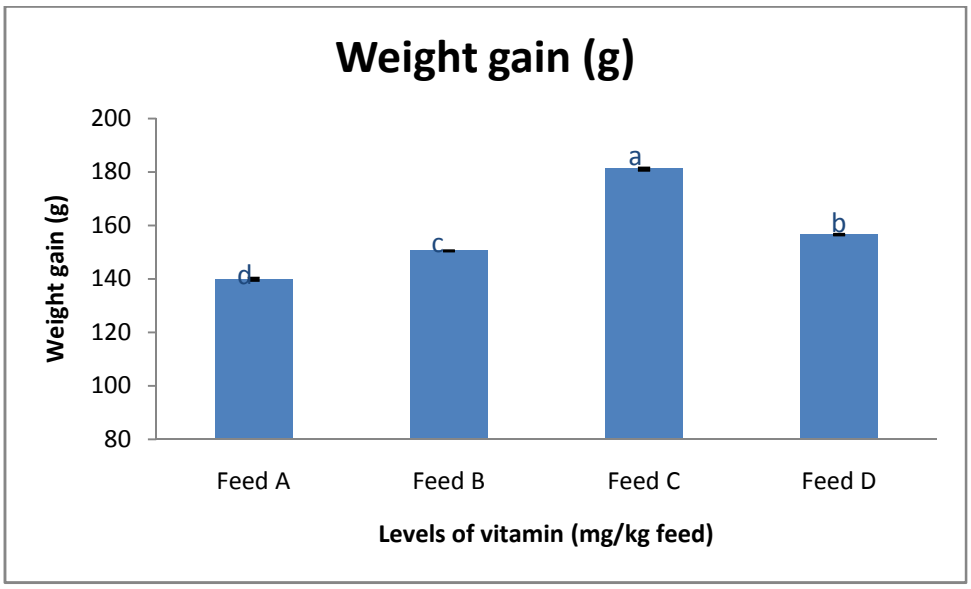

Fig. 4 Variation in weight gain (g), (Mean ± SEM) for experimental fish species during 120 days study period. Bars different letters (a,b,c,d) indicate significant difference.

Average daily gain (ADG)

The value of Average daily gain (ADG) was highest in Feed C (1.50±0.005g/d) diet while the lowest value was found in control diet (1.16±0.01g/d). The values of Average daily gain (ADG) of the experimental fish *Labeo rohita* for Feed C (1.50±0.005g/d) is significantly higher ((P<0.05) than the control diet.

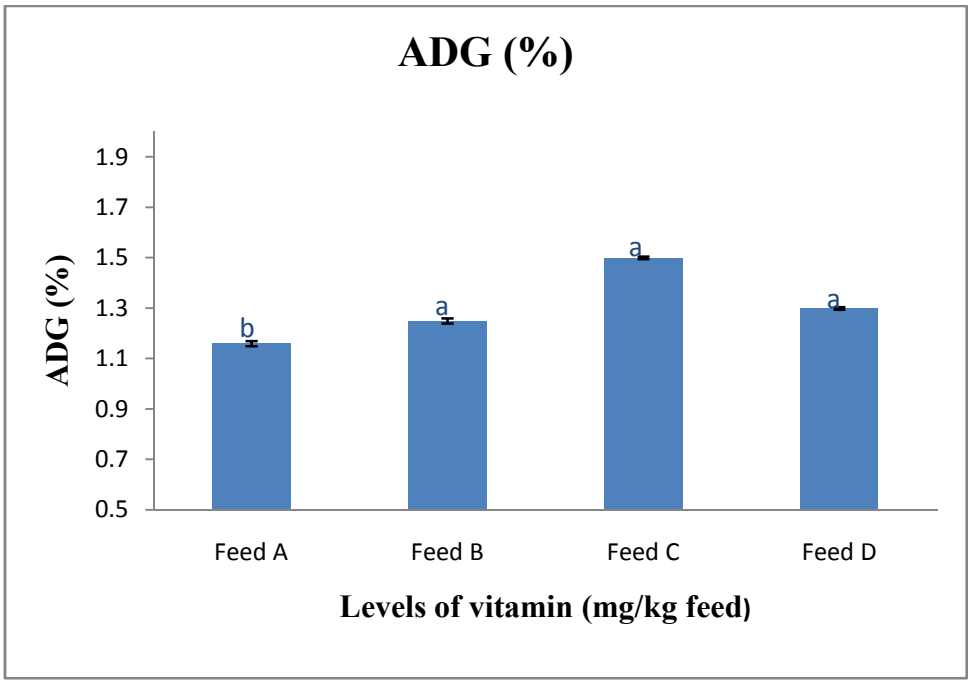

Fig. 5 Average daily gain (ADG %), (Mean ±SEM) of *Labeo rohita* cultured for 120 days study period with four experimental diets. Bars different letters (a,b) indicate significant difference.

Feed conversion ratio (FCR)

The Feed conversion ratio of *Labeo rohita* kept in eight different tanks fed four different formulated feed has been calculated after 120 days study period. The highest FCR (3.68±0.02) was found in the control feed (Feed A) while the lowest FCR (3.32±0.01) was measured in Feed C. In Feed A the value of FCR was 3.68±0.02% which is significantly higher (P<0.05) than Feed D 3.53±0.005% and Feed C (3.32±0.01) (Homogenous of FCR, Table no.48).

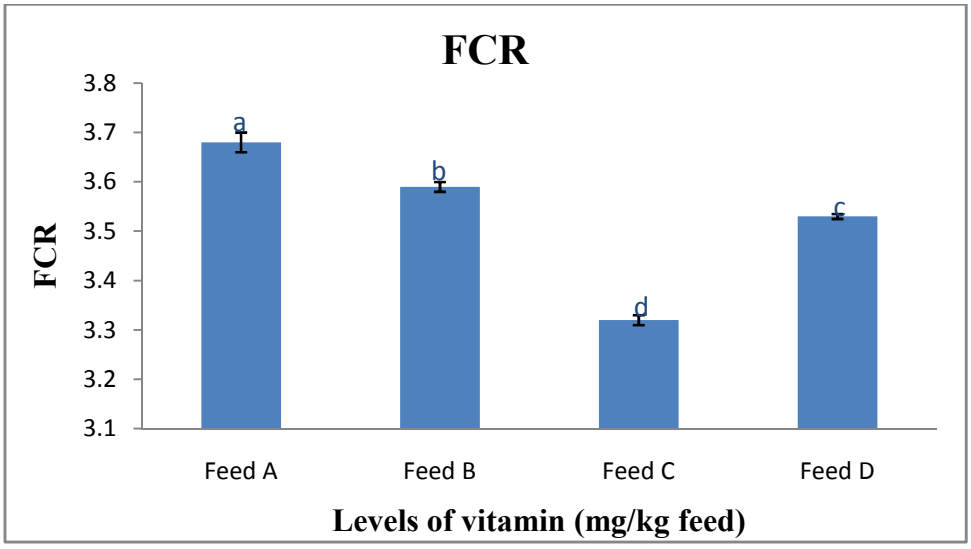

Fig. 6 Feed conversion ratio (FCR), (Mean ± SEM) of four formulated diets fed by *Labeo rohita* observed in the laboratory condition. Bars different letters (a,b,c,d) indicate significant difference.

Protein efficiency ratio (PER)

The highest PER (3.67±0.01%) was found in the Feed C treatment and lowest (2.87±0.01%) in the Feed A (Control feed) treatment. However, the PER measured in the Feed A was significantly lower ($P<0.05$) than the treatment of Feed C (Table no. 27).

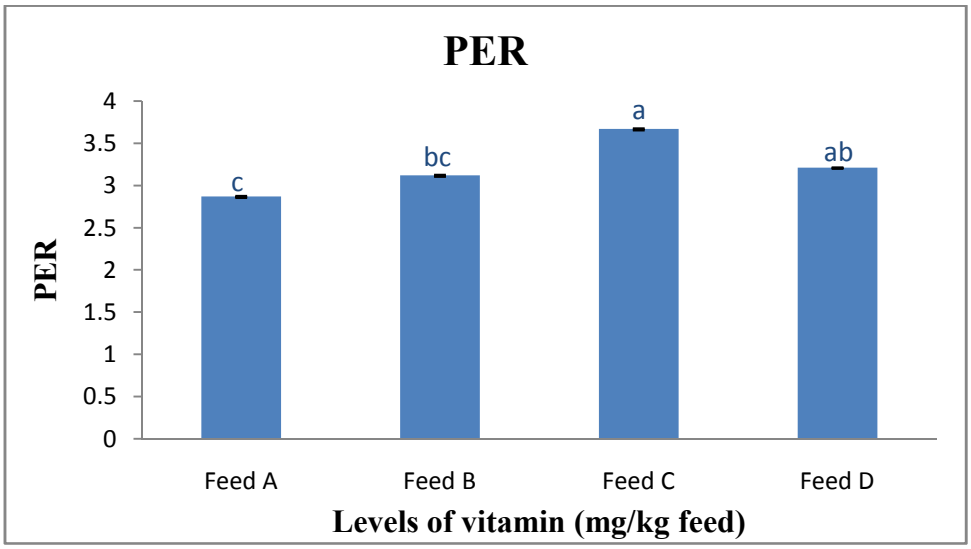

Fig. 7 Protein efficiency ratio (PER), (Mean ± SEM) of *Labeo rohita* cultured for 120 days study period with four experimental diets in a laboratory condition. Bars different letters (a,b,c) indicate significant difference.

Specific growth rate (SGR)

The values of Specific growth rate (SGR%) of the experimental fish species *L. rohita* reared in eight experimental tanks fed on four different types of formulated feed has been represented in Table no. 10. The values of Specific growth rate (SGR %) are highest for Feed C (4.33±0.007%) while the lowest value was found in Feed A (4.12±0.01%).

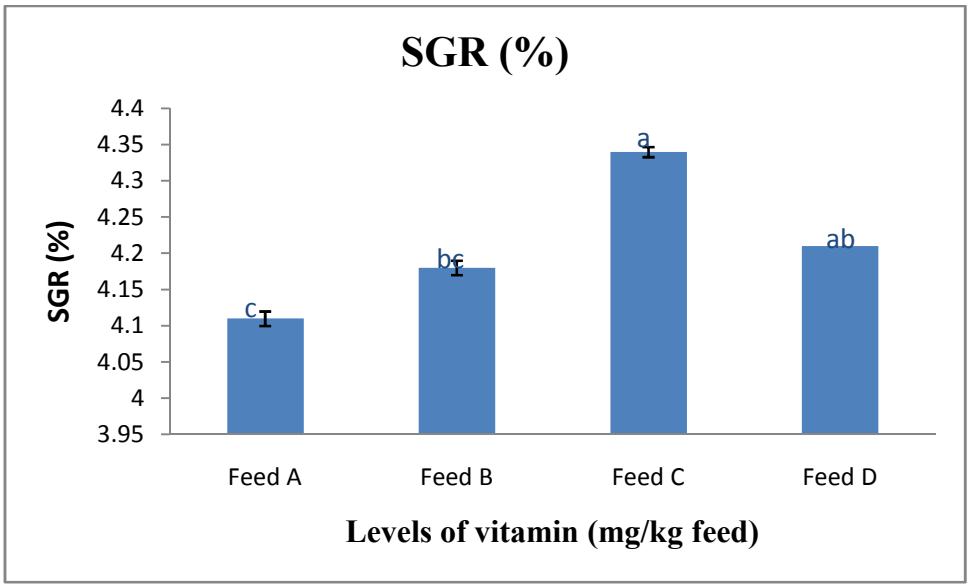

Fig. 8 Observed Specific growth rate (%), (Mean ±SEM) of *Labeo rohita* cultured for 120 days study period with four experimental diets. Bars different letters (a,b,c) indicate significant difference.

Condition factor

The values of condition factor of Rohu fish (*Labeo rohita*) reared in eight experimental tanks fed on four different types of feed has been represented in tables at the end of 120 days study period. Condition factor (K) of *Labeo rohita* was highest at Feed A (2.85±0.42%) and the lowest value was found at Feed C (2.35±0.02%) (Fig. 9).

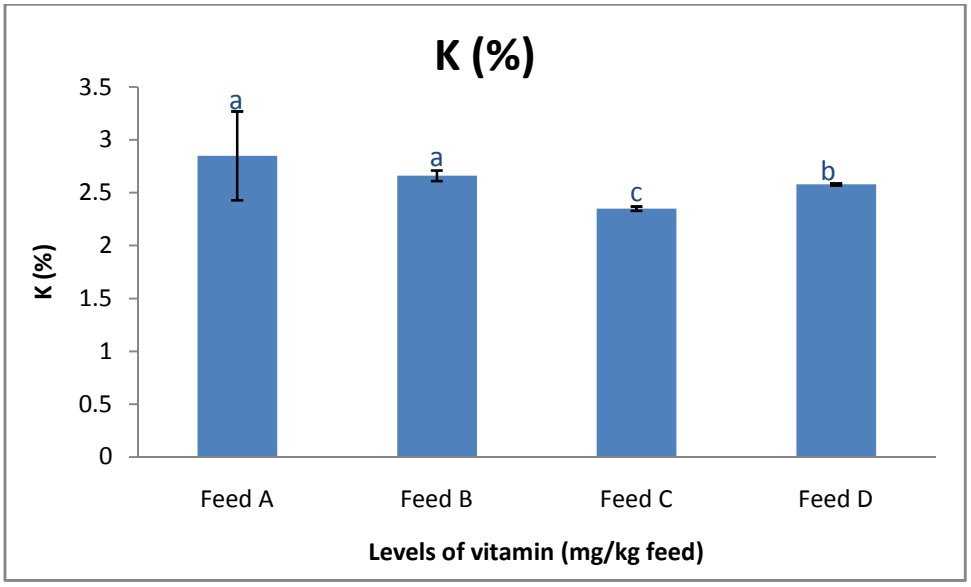

Fig. 9 Condition factor (K %) in *Labeo rohita* measured after 120 days experimental period fed with four different experimental diets.

Feed efficiency (%)

The values of feed efficiency of the experimental fish (*Labeo rohita*) reared in eight tanks fed on four different types of formulated feed has been represented in Fig.10. The highest FE (13.39±0.04%) was found in Feed C treatment and lowest (10.35±0.06%) in the Feed A (Control Feed) treatment (Fig. 10).

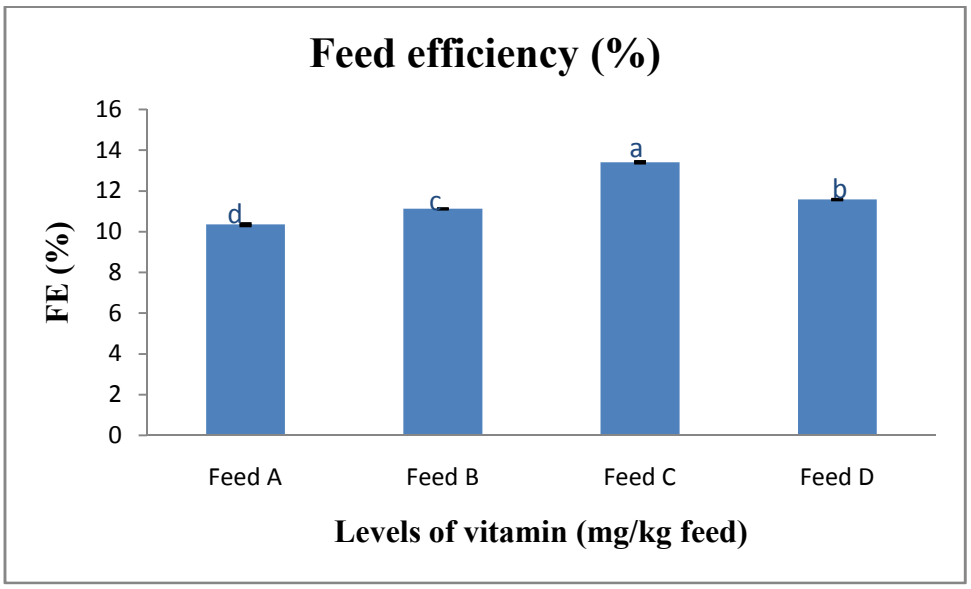

Fig. 10 Feed efficiency (FE, %), (Mean ±SEM) of *Labeo rohita* cultured for 120 days study period with four experimental diets in a laboratory condition.

Survival rate (%)

Survival rate of experimental fish species *Labeo rohita* were significantly varies among experimental diets. The control fish species had problem with feed intake, may be related with commercial feed contained in the diet that caused low palatability. The highest survival rate was found in Feed B and Feed C (90±00%) treatment and lowest survival rate was observed in Feed A and Feed D (85±1.88%) treatment.

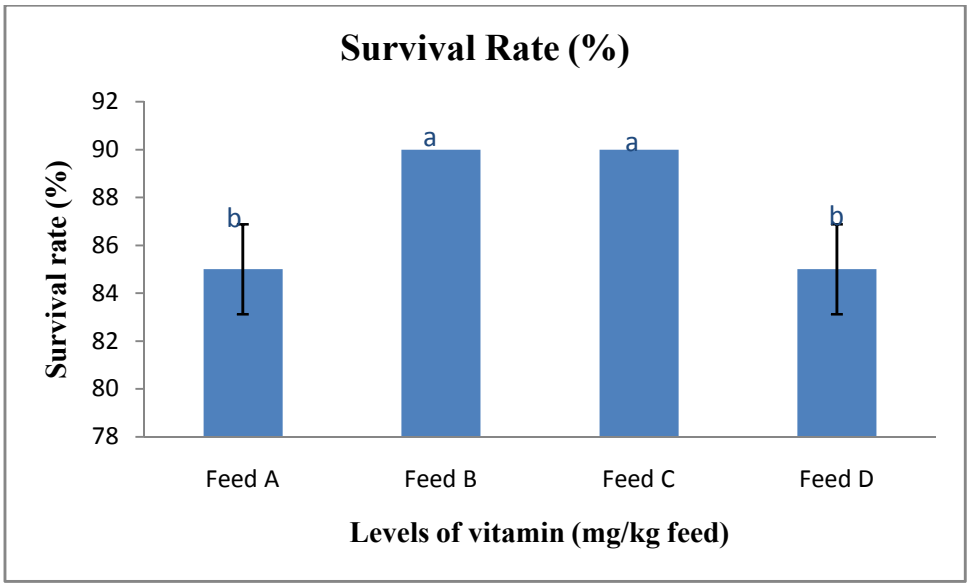

Fig. 11 Survival rate (%) of *Labeo rohita* fed on different formulated diets for 120 days study period. Bars different letters (a,b) indicate significant difference.

Reproductive performance

Gonado-somatic index (%) for male and female *Labeo rohita* species cultured for 120 days study period was measured in the laboratory condition. In this study, four types of formulated feed supplemented with and without vitamin C, E and Zinc was used. The highest female GSI value (1.51±0.06%) was found for Feed C treatment while the lowest value (1.09±0.06%) was found for control treatment (Feed A).The maximum GSI value for male fish was (0.93±0.04%) which was found in Feed C treatment and the lowest value (0.39±0.05%) was found for Feed A treatment (Fig.12 and Fig.13)

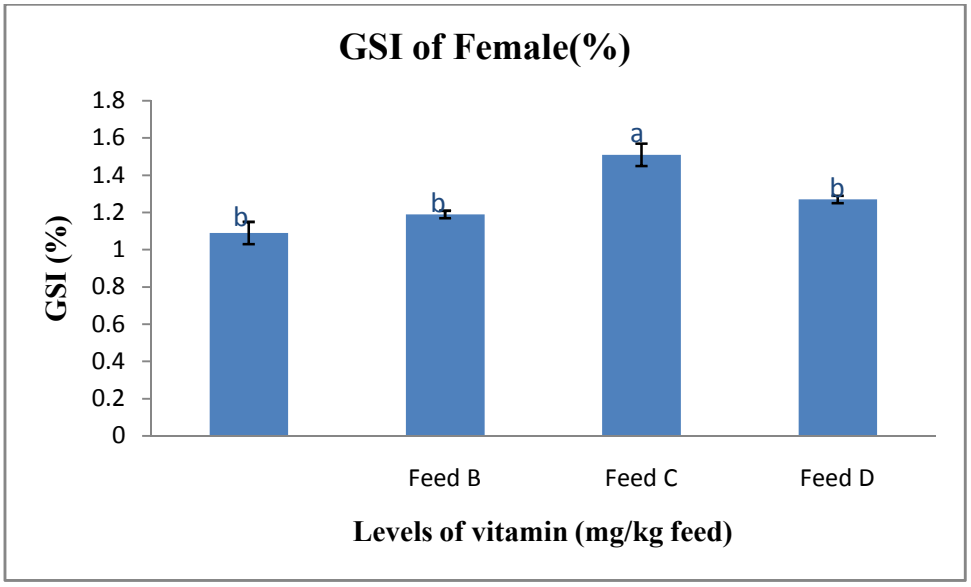

Fig. 12 Gonado-somatic Index (GSI, %), (Mean ± SEM) for female *Labeo rohita* at various culture condition for 120 days study period. Bars different letters (a,b,c) indicate significant difference.

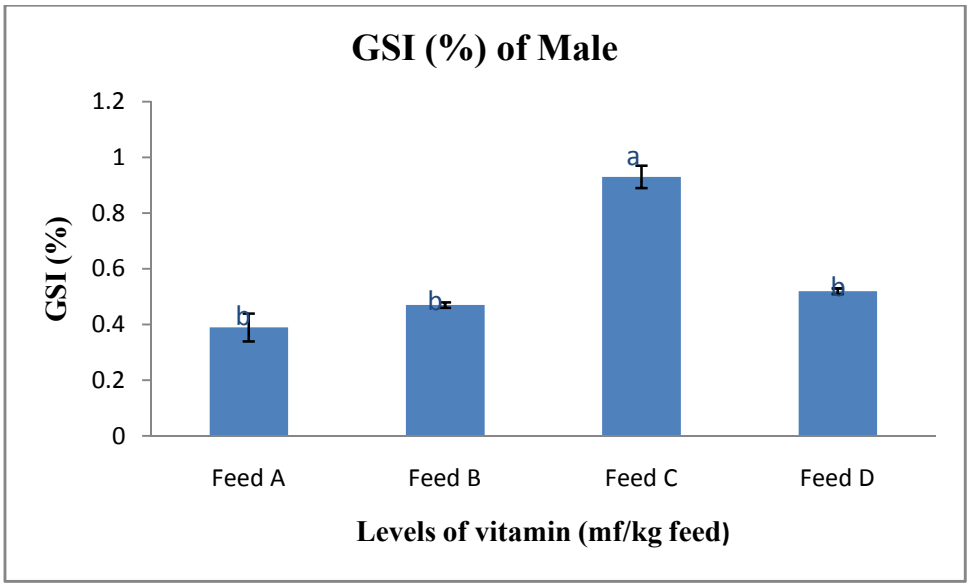

Fig. 13 Male Gonado-somatic Index (GSI, %), (Mean ± SEM) for *Labeo rohita* using four types of feed for 120 days study period. Bars different letters (a,b,c) indicate significant difference.

DISCUSSIONS

The proximate composition of the food items used during the experiment was analyzed. During the experimental period, no disease, abnormality or deformity was noticed in the experimental fish. The performance of fish in terms of growth, survival and body composition was also put on record.

Protein (%) in fish muscle

In the present study a significant amount of protein increase ($p<0.05$) is noticed among the four treatments. The highest protein level was recorded for Feed C treatment ($16.37\pm0.46\%$) while in the other three treatment Feed A, Feed B, Feed D obtained protein level was ($14.69\pm0.17\%$), ($15.10\pm0.09\%$) and ($15.34\pm0.38\%$) respectively. From the ANOVA table (Table no.23) fish muscle protein composition between groups was found to be significant and the F value found from the ANOVA table was 5.21. So, the present results suggests that sufficient amount of feed protein with optimum level of vitamin E and vitamin C (500 mg/kg feed) along with Zinc (10 mg/kg) supplementation can significantly change fish body proximate composition.

Lipid contents (%) in fish muscle

In the current experiment, highest lipid contents ($2.17\pm0.18\%$) was observed for feed C treatment while the lowest value ($1.32\pm0.36\%$) was noticed in Feed B treatment. Lipids are generally regarded as the most important constituents, which determine the quality of fish meat (Caulton and Brusell 1977; Love 1988). In their experiment, the maximum lipid contents (4.14%) were recorded in farmed *Labeo rohita* under F3. Love (1980) mentioned that moisture contents showed an inverse relationship with lipids in the meat of fatty fish and with the protein in non-fatty fish. The results of present study corroborate the findings of the abovementioned worker.

Average daily gain (ADG, g/day)

The value of Average daily gain (ADG) was highest in Feed C treatment (1.50 ± 0.005 g/d) and lowest in Feed A treatment1 (1.16 ± 0.01 g/d). The values of Average daily gain

(ADG) of the experimental fish *Labeo rohita* for Feed B treatment (1.25 ± 0.01 g/d) was lower than the Feed D treatment (1.31 ± 0.005 g/d).

Survival rate (%)

In the present study the highest survival rate was observed for both Feed B and Feed C treatment while the lowest value was observed for Feed A (Control diet) and Feed D treatment. The above results signify that higher level of vitamin can reduce growth and survival rate of fish. Several factors have also been reported to contribute to the observed variation in the growth responses of fishes. Hasan and Macintosh (1992) found that the growth performance of common carp (*Cyprinus carpio*, Cyprinidae) varied with the variation in the acceptability of diets. This result was similar for the catfish (*Clarius batrachus*, Clariidae) as revealed by Hasan *et al.* (1989).

Scientists have reported that copepods have high contents of docosa-hexanoic acid (DHA) and eicosa-pentanoic acid (EPA), which results in better larval growth rate and survival (Evjemo *et al.* 1997; Shields *et al.* 1999). Cod liver oil (CLO) and vitamin C enriched zooplanktons fed to fish larvae improved their survival and growth. Many investigators further authenticated similar observations in the case of shrimp (Kyungmin, 2000; Immanuel *et al.* 2007). These results supported the hypothesis that, stress creates increased ascorbate requirements for larval fish and crustaceans. Vitamin C (ascorbic acid) is considered to be an essential component in the diet of fishes. In this respect, body vitamin C concentration may be reflected in the survival potential more accurately than variation in the growth rate (Dabrowski 1992).

The rapid growth rate observed during this study suggested that larvae of *L. rohita* have higher vitamin C requirements. Merchie *et al.* (1996) also reported improved survival, growth performance, skeleton development, stress resistance and immune response of larvae fed vitamin C enriched diet and premeditated the larvae to have higher vitamin C requirements than the adults.

However, in contrast to this study, Kaur and Dhawan (2004) reported that dry diet resulted in higher wet weight gain and survival (90%) than live feed in case of rohu larvae.

Feed conversion ratio (FCR)

From Homogenous of FCR table (Table no.48) highly significant differences has been observed for Feed A (3.68±0.02) with Feed C (3.32±0.01), Feed D (3.53 ± 0.005) and Feed B (3.59 ± 0.01) treatments. Hasan (2003) observed lower FCR in Nile Tilapia (*Oreochromis niloticus*) when the species fed with vitamin compared to those fed formulated feed without any vitamin.

Protein efficiency ratio (PER)

The highest PER (3.67±0.01%) was found in the Feed C treatment and lowest (2.87±0.01%) in the Feed A (Control feed) treatment. The result obtained from descriptive data analysis (Table no. 14) the mean value for all treatment was 3.25 ± 0.12 while the mean lower bound and upper bound at 95% confidence level for all treatment was 2.98 and 3.53 respectively. From the ANOVA table (Table no.27) significant differences has been observed between groups for PER values.

Condition factor (K, %)

The values of condition factor were calculated during the study period at the end of 120 days. The condition factor was highest in Feed A (2.85 ± 0.42 %). However the condition factor (2.66 ± 0.05 %) in the Feed B was more or less similar with Feed D treatment (2.58 ±0.01%). From ANOVA table no.29 values of condition factor between groups was found to be significant ($p<0.05$).

Specific growth rate (SGR, %)

The values of Specific growth rate (SGR%) of the experimental fish *Labeo rohita* rearing in eighte tanks fed on four different types of fish feed were estimated and the findings were significantly different ($p<0.05$) (Table no.28). The values of SGR% highest for Feed C (4.33±0.007 %) and lowest for Feed A (4.11±0.01 %) but SGR% value of Feed B (4.17±0.01%) and Feed D (4.21±0.00%) is higher than Feed A.

Feed efficiency (%)

The Feed conversion ratio of *Labeo rohita* kept in different tanks and fed on four different types of feed have been calculated after 120 days study period . The highest FE

(13.39 ± 0.0.04 %) was found in the Feed C while the lowest (10.35 ± 0.06 %) was measured in Feed A treatment. In Feed C the value of FCR was significantly higher than all other treatments (Homogenous of FE, Table no.52). From this point of view the formulated feed C gives the best result in comparison with the formulated feed A and B.

Reproductive performance

Gonado-somatic index (%) for male and female *Labeo rohita* species cultured for 120 days study period was measured in the laboratory condition. In this study, four types of formulated feed supplemented with and without vitamin C, E and Zinc was used. The highest female GSI value (1.51±0.06%) was found for Feed C treatment while the lowest value (1.09±0.06%) was found for control treatment (Feed A).The maximum GSI value for male fish was (0.93±0.04%) which was found in Feed C treatment and the lowest value (0.39±0.05%) was found for Feed A treatment. The above findings are similar with many other authors.

Gunwant *et al.* (2013) conducted a study to determine the changes in the gonadosomatic index of major carp, *Labeo rohita*. He concluded the following results:

- The fish has only one spawning season of short duration, running from July to August as indicated by the peaks of GSI and the diameter of oocytes and testicular lobules.
- Both males and females mature simultaneously.
- Minimum GSI for female was 0.74 ± 0.12 in resting phase and maximum was observed in the spawning phase (16.49 ± 1.70).
- GSI for male was minimum in resting phase (0.087 ± 0.004) and maximum in spawning phase (2.02 ± 0.181)

Mohammad *et al.* (2010) concluded from his three years of study period that the highest value of GSI was 7.5 recorded in the month of August indicating spawning period of *Labeo rohita* and the mean value was 3.8158. The value of GSI ranged from 7.0-9.0 was reported by Ortaga-Salas and Bustamente (2006) in gold fish *Carassius auratus.*

SUMMARY

At present, *Labeo rohita* has become one of the most popular commercially important major carp species due to its high yield, high survival rate and low production cost, and many hatcheries all over the country are now producing *Labeo rohita* fry to meet up the farmers increasing demand. The investigation reported here has been carried out with the main objective to find the experimental fish growth and reproduction performances fed on four types of fish feed rearing in eight different tanks for a period of 12o days. Optimum level of vitamin E, vitamin C and Zinc in the formulated fish feed for the experimental fish species was also determined. Different biological parameters, such as average daily gain (ADG), specific growth rate, protein efficiency ratio (PER), feed conversion ratio (FCR), survival rate were measured to see the growth performances and utilization during the 120 days study period.

The study under the reported period was carried out in the aquatic laboratory, Department of Fisheries, University of Dhaka. The obtained data during the 120 days study period were analyzed statistically using SPSS 16.0.

The initial proximate composition of the sample species was determined before rearing and feeding trial. At the initial stage of the experiment the proximate composition of *Labeo rohita* kept at different tanks have been found to have variations due to difference in size (Length-weight difference) and age. After 120 days of experimental period proximate composition of *Labeo rohita* has been determined to evaluate the overall progress. The result revealed that Feed C treatment has the highest level of protein ($16.37 \pm 0.46\%$) due to its high feed protein content and at the same time presence of additional vitamin E, vitamin C and Zinc also accelerate the average daily gain for Feed C treatment to the highest value (1.50 ± 0.005 g/day). This finding suggests that the quality of the feed influence the final carcass composition of the fish species.

In another category of data collected, at the end of 120 days study period, the highest value of survival rate (90 ± 0.00) has recorded for Feed B and Feed C treatment.

Feed conversion ratio (FCR) of the *Labeo rohita* under the study period was also monitored. Results showed that Feed A treatment has the maximum FCR value in comparison with other treatment.

At the same time, feed efficiency ratio of *Labeo rohita* during the 120 days rearing period was also computed. The highest FE (13.39 ± 0.04%) was found in Feed C treatment and lowest (10.35 ± 0.06%) in the control diet.

CONCLUSION

From the above study we find dietary effect of vitamin C, vitamin E and Zinc of formulated fish feed on the body composition, growth and reproductive performances of *Labeo rohita*. During the study period ADG, SGR, PER of the rearing *Labeo rohita* showed results in favor of the use of prepared fish feed specifically diet with vitamin C and vitamin E at 500 mg/kg feed with 10 mg/kg Zinc (Feed C). So, there is a significantly positive effect on the optimum vitamin C, vitamin E and Zn level (Feed C – 500 mg/ kg of feed vitamin C, vitamin E and 10 mg/kg feed Zn) on the growth, feed utilization, body composition and reproductive performances.

The following conclusions may be drawn on the basis of the observations made during the rearing and feeding experiment of *Labeo rohita* fed on four different types fish feed with and without vitamin E, vitamin C and Zinc in the laboratory:

I. *Labeo rohita* fish is good source of protein compared to other freshwater species.

II. The formulated fish feed are found to be effective for the better growth and gonad development of Rohu fish.

III. Fish feed containing adequate amount of vitamin E, vitamin C and Zinc supplementation is the best feed for better growth and survival.

IV. For better and faster gonadal development supplementation of vitamin E, vitamin C and Zinc with additional protein is required.

RECOMMENDATIONS

1. More research needed to determine the involvement of vitamin E and vitamin C on fish growth and reproductive performances on other fish species.

2. Advance research needed to determine the effect of Zinc supplementation on fish growth and reproduction.

3. Current study is completely lab based, so future field studies are necessary to determine the original effect of vitamin E, vitamin C and Zinc supplementation for intensive fish culture.

4. Effective large scale cost benefit data analysis is required during the experimental period to initiate vitamin E, vitamin C and Zinc supplementation on commercial feed.

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APPENDICES

Proximate composition of measured data

Table 4 Descriptive Proximate composition of experimental diets

Treatment	Replication	Moisture (%)	Dry matter (%)	Crude protein (%)	Crude lipid (%)	Ash (%)	Carbohydrates (%)
Feed A (Control Feed)	A_1	11.65	88.35	35.90	10.21	11.59	30.65
	A_2	11.75	88.25	36.12	10.15	11.61	30.37
	Mean	11.70	88.3	36.01	10.18	11.6	30.51
	SEM	±0.05	±0.05	±0.11	±0.03	±0.01	±0.14
Feed B	B_1	11.9	88.10	35.75	9.90	11.5	30.95
	B_2	12.1	87.90	35.45	10.30	11.8	30.35
	Mean	12.00	88.00	35.6	10.10	11.65	30.65
	SEM	±0.10	±0.10	±0.15	±0.20	±0.15	±0.30
Feed C	C_1	11.10	88.90	36.37	9.65	11.42	31.53
	C_2	11.30	88.70	36.53	9.95	11.28	30.87
	Mean	11.20	88.8	36.45	9.8	11.35	31.2
	SEM	±0.10	±0.10	±0.08	±0.15	±0.07	±0.33
Feed D	D_1	11.55	88.45	35.90	10.06	11.46	31.03
	D_2	11.75	88.25	36.22	10.04	11.54	30.45
	Mean	11.65	88.35	36.06	10.05	11.50	30.74
	SEM	±0.10	±0.10	±0.16	±0.01	±0.04	±0.29

Table 5 Descriptive proximate composition of experimental fish muscle

Treatment	Replication	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Feed A (Control Feed)	A_1	79.69	14.52	1.40	1.98
	A_2	80.33	14.86	1.52	2.22
	Mean	80.01	14.69	1.46	2.10
	SEM	±0.32	±0.17	±0.06	±0.12
Feed B	B_1	80.45	15.19	0.96	2.46
	B_2	79.75	15.01	1.68	1.60
	Mean	80.1	15.10	1.32	2.03
	SEM	±0.35	±0.09	±0.36	±0.43
Feed C	C_1	80.12	15.91	3.25	1.60
	C_2	78.90	16.83	1.09	1.06
	Mean	79.51	16.37	2.17	1.33
	SEM	±0.61	±0.46	±1.08	±0.27
Feed D	D_1	80.18	14.96	2.57	1.42
	D_2	79.98	15.72	1.05	1.46
	Mean	80.08	15.34	1.81	1.44
	SEM	±0.10	±0.38	±0.76	±0.02

Appendix

Survival rate (%) of measured data

Table 6 Survival rate (%) of Rohu fish (*Labeo rohita*) studied at various experimental tanks at the end of 20 days study period.

Treatment	Feed A		Feed B		Feed C		Feed D	
Parameters	A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2
Initial total number of fish	10	10	10	10	10	10	10	10
Final total number of fish	8	9	9	9	9	9	9	8
Survival rate (%)	80	90	90	90	90	90	90	80

Table 7 Survival rate (%) of Rohu fish (*Labeo rohita*) studied at various experimental tanks at the end of 50 days study period.

Treatment	Feed A		Feed B		Feed C		Feed D	
Parameters	A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2
Initial total number of fish	10	10	10	10	10	10	10	10
Final total number of fish	8	9	9	9	9	9	9	8
Survival rate (%)	80	90	90	90	90	90	90	80

Table 8 Survival rate (%) of Rohu fish (*Labeo rohita*) studied at various experimental tanks at the end of 80 days study period.

Treatment	Feed A		Feed B		Feed C		Feed D	
Parameters	A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2
Initial total number of fish	10	10	10	10	10	10	10	10
Final total number of fish	8	9	9	9	9	9	9	8
Survival rate (%)	80	90	90	90	90	90	90	80

Table 9 Survival rate (%) of Rohu fish (*Labeo rohita*) studied at various experimental tanks at the end of 120 days study period.

Treatment	Feed A		Feed B		Feed C		Feed D	
Parameters	A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2
Initial total number of fish	10	10	10	10	10	10	10	10
Final total number of fish	8	9	9	9	9	9	9	8
Survival rate (%)	80	90	90	90	90	90	90	80

Appendix C

Growth performance data

Table 10 Mean value of FCR, PER, SGR (%), ADG, Feed efficiency and Condition factor (K).

Treatment	Replica	FCR	PER	SGR (%)	ADG	Feed efficiency	K (%)
Feed A (Control Feed)	A_1	3.67	2.88	4.12	1.17	10.39	3.27
	A_2	3.70	2.86	4.11	1.16	10.31	2.43
	Mean	3.68	2.87	4.115	1.165	10.35	2.85
	SEM	±0.02	±0.01	±0.01	±0.01	±0.06	±0.42
Feed B	B_1	3.59	3.12	4.17	1.25	11.12	2.71
	B_2	3.60	3.13	4.18	1.26	11.13	2.61
	Mean	3.595	3.125	4.175	1.255	11.125	2.66
	SEM	±0.01	±0.01	±0.01	±0.01	±0.007	±0.05
Feed C	C_1	3.33	3.66	4.33	1.5	13.36	2.33
	C_2	3.32	3.68	4.34	1.51	13.41	2.37
	Mean	3.325	3.67	4.335	1.505	13.39	2.35
	SEM	±0.01	±0.01	±0.007	±0.005	±0.04	±0.02
Feed D	D_1	3.54	3.21	4.21	1.30	11.57	2.63
	D_2	3.53	3.22	4.21	1.31	11.59	2.52
	Mean	3.535	3.215	4.21	1.305	11.58	2.58
	SEM	±0.005	±0.005	±0.00	±0.005	±0.014	±0.01

Appendix D

Descriptives data for analysis

Table11 Descriptives data for feed protein values.

Descriptives

Diet protein (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	36.0100	.15556	.11000	34.6123	37.4077	35.90	36.12
Feed B	2	35.6000	.21213	.15000	33.6941	37.5059	35.45	35.75
Feed C	2	36.4500	.11314	.08000	35.4335	37.4665	36.37	36.53
Feed D	2	36.0600	.22627	.16000	34.0270	38.0930	35.90	36.22
Total	8	36.0300	.35018	.12381	35.7372	36.3228	35.45	36.53

Table12 Descriptives data for fish muscle protein values.

Descriptives

Muscle protein

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	14.6900	.24042	.17000	12.5299	16.8501	14.52	14.86
Feed B	2	15.1000	.12728	.09000	13.9564	16.2436	15.01	15.19
Feed C	2	16.3700	.65054	.46000	10.5251	22.2149	15.91	16.83
Feed D	2	15.3400	.53740	.38000	10.5116	20.1684	14.96	15.72
Total	8	15.3750	.74241	.26248	14.7543	15.9957	14.52	16.83

Table13 Descriptives data for final weight gain values.

Descriptives

Weight gain

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	1.3988E2	.67175	.47500	133.8496	145.9204	139.41	140.36
Feed B	2	1.5051E2	.07071	.05000	149.8747	151.1453	150.46	150.56
Feed C	2	1.8112E2	.62225	.44000	175.5293	186.7107	180.68	181.56
Feed D	2	1.5660E2	.24749	.17500	154.3714	158.8186	156.42	156.77
Total	8	1.5703E2	16.19022	5.72411	143.4921	170.5629	139.41	181.56

Table 14 Descriptives data for various FCR values.

Descriptives

FCR

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
FeedA	2	3.6850	.02121	.01500	3.4944	3.8756	3.67	3.70
Feed B	2	3.5950	.00707	.00500	3.5315	3.6585	3.59	3.60
Feed C	2	3.3250	.00707	.00500	3.2615	3.3885	3.32	3.33
Feed D	2	3.5350	.00707	.00500	3.4715	3.5985	3.53	3.54
Total	8	3.5350	.14193	.05018	3.4163	3.6537	3.32	3.70

Table 15 Descriptives data for various PER values.

Descriptives

PER

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	2.8700	.01414	.01000	2.7429	2.9971	2.86	2.88
Feed B	2	3.1250	.00707	.00500	3.0615	3.1885	3.12	3.13
Feed C	2	3.6700	.01414	.01000	3.5429	3.7971	3.66	3.68
Feed D	2	3.3700	.22627	.16000	1.3370	5.4030	3.21	3.53
Total	8	3.2588	.32791	.11593	2.9846	3.5329	2.86	3.68

Table 16 Descriptives data for various SGR values.

Descriptives

SGR

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	4.1150	.00707	.00500	4.0515	4.1785	4.11	4.12
Feed B	2	4.1750	.00707	.00500	4.1115	4.2385	4.17	4.18
Feed C	2	4.3350	.00707	.00500	4.2715	4.3985	4.33	4.34
Feed D	2	3.8700	.48083	.34000	-.4501	8.1901	3.53	4.21
Total	8	4.1238	.25489	.09012	3.9107	4.3368	3.53	4.34

Table 17 Descriptives data for various ADG values.

Descriptives

ADG

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	1.1650	.00707	.00500	1.1015	1.2285	1.16	1.17
Feed B	2	1.2550	.00707	.00500	1.1915	1.3185	1.25	1.26
Feed C	2	1.5050	.00707	.00500	1.4415	1.5685	1.50	1.51
Feed D	2	2.7550	2.05768	1.45500	-15.7325	21.2425	1.30	4.21
Total	8	1.6700	1.03493	.36590	.8048	2.5352	1.16	4.21

Table 18: Descriptives data for various K values.

Descriptives

Condition factor

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	2.8500	.59397	.42000	-2.4866	8.1866	2.43	3.27
Feed B	2	2.6600	.07071	.05000	2.0247	3.2953	2.61	2.71
Feed C	2	2.3500	.02828	.02000	2.0959	2.6041	2.33	2.37
Feed D	2	1.9700	.93338	.66000	-6.4161	10.3561	1.31	2.63
Total	8	2.4575	.55011	.19449	1.9976	2.9174	1.31	3.27

Table 19 Descriptives data for various FE values.

Descriptives

Feed efficiency

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	10.3500	.05657	.04000	9.8418	10.8582	10.31	10.39
Feed B	2	11.1250	.00707	.00500	11.0615	11.1885	11.12	11.13
Feed C	2	13.3850	.03536	.02500	13.0673	13.7027	13.36	13.41
Feed D	2	11.5800	.01414	.01000	11.4529	11.7071	11.57	11.59
Total	8	11.6100	1.19244	.42159	10.6131	12.6069	10.31	13.41

Table 20 Descriptives data for various female GSI values.

Descriptives

Female GSI(%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	1.0900	.08485	.06000	.3276	1.8524	1.03	1.15
Feed B	2	1.1900	.02828	.02000	.9359	1.4441	1.17	1.21
Feed C	2	1.5150	.07778	.05500	.8162	2.2138	1.46	1.57
Feed D	2	1.2700	.02828	.02000	1.0159	1.5241	1.25	1.29
Total	8	1.2662	.17419	.06158	1.1206	1.4119	1.03	1.57

Table 21 Descriptives data for various male GSI values.

Descriptives

Male GSI (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Feed A	2	.3900	.07071	.05000	-.2453	1.0253	.34	.44
Feed B	2	.4700	.01414	.01000	.3429	.5971	.46	.48
Feed C	2	.9300	.05657	.04000	.4218	1.4382	.89	.97
Feed D	2	.5200	.01414	.01000	.3929	.6471	.51	.53
Total	8	.5775	.22588	.07986	.3887	.7663	.34	.97

Appendix D

ANOVA table

Table 22 ANOVA table for feed protein values.

ANOVA

Diet protein (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.725	3	.242	7.259	.043
Within Groups	.133	4	.033		
Total	.858	7			

Table 23 ANOVA table for fish muscle protein.

ANOVA

Muscle protein

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.072	3	1.024	5.212	.072
Within Groups	.786	4	.196		
Total	3.858	7			

Table 24 ANOVA table for weight gain.

ANOVA

Weight gain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1833.957	3	611.319	2.703E3	.000
Within Groups	.905	4	.226		
Total	1834.862	7			

Table 25 ANOVA table for ADG.

ANOVA

ADG

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.263	3	1.088	1.028	.470
Within Groups	4.234	4	1.059		
Total	7.498	7			

Table 26 ANOVA table for various FCR values.

ANOVA

FCR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.140	3	.047	312.000	.000
Within Groups	.001	4	.000		
Total	.141	7			

Table 27 ANOVA table for various PER values.

ANOVA

PER

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.701	3	.234	18.097	.009
Within Groups	.052	4	.013		
Total	.753	7			

Table 28 ANOVA table for various SGR values.

ANOVA

SGR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.223	3	.074	1.288	.393
Within Groups	.231	4	.058		
Total	.455	7			

Table 29 ANOVA table for condition factor.

ANOVA

Condition factor

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.889	3	.296	.963	.492
Within Groups	1.230	4	.307		
Total	2.118	7			

Table 30 ANOVA table for feed efficiency.

ANOVA

Feed efficiency

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.949	3	3.316	2.822E3	.000
Within Groups	.005	4	.001		
Total	9.953	7			

Table 31 ANOVA table for female GSI.

ANOVA

Female GSI (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.198	3	.066	17.736	.009
Within Groups	.015	4	.004		
Total	.212	7			

Table 32 ANOVA table for male GSI.

ANOVA

Male GSI (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.349	3	.116	54.039	.001
Within Groups	.009	4	.002		
Total	.357	7			

Appendix E

Post Hoc Tests

Table 33 Multiple comparisons for feed protein.

Multiple Comparisons

Dependent Variable: Diet protein (%)

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Feed A	Feed B	.41000	.18248	.088	-.0967	.9167
		Feed C	-.44000	.18248	.073	-.9467	.0667
		Feed D	-.05000	.18248	.798	-.5567	.4567
	Feed B	Feed A	-.41000	.18248	.088	-.9167	.0967
		Feed C	-.85000*	.18248	.010	-1.3567	-.3433
		Feed D	-.46000	.18248	.065	-.9667	.0467
	Feed C	Feed A	.44000	.18248	.073	-.0667	.9467
		Feed B	.85000*	.18248	.010	.3433	1.3567
		Feed D	.39000	.18248	.099	-.1167	.8967
	Feed D	Feed A	.05000	.18248	.798	-.4567	.5567
		Feed B	.46000	.18248	.065	-.0467	.9667
		Feed C	-.39000	.18248	.099	-.8967	.1167

*. The mean difference is significant at the 0.05 level.

Table 34 Multiple comparisons for fish muscle protein.

Multiple Comparisons

Dependent Variable: Muscle protein

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Feed A	Feed B	-.41000	.44328	.407	-1.6408	.8208
		Feed C	-1.68000*	.44328	.019	-2.9108	-.4492
		Feed D	-.65000	.44328	.216	-1.8808	.5808
	Feed B	Feed A	.41000	.44328	.407	-.8208	1.6408
		Feed C	-1.27000*	.44328	.046	-2.5008	-.0392
		Feed D	-.24000	.44328	.617	-1.4708	.9908
	Feed C	Feed A	1.68000*	.44328	.019	.4492	2.9108
		Feed B	1.27000*	.44328	.046	.0392	2.5008
		Feed D	1.03000	.44328	.081	-.2008	2.2608
	Feed D	Feed A	.65000	.44328	.216	-.5808	1.8808
		Feed B	.24000	.44328	.617	-.9908	1.4708
		Feed C	-1.03000	.44328	.081	-2.2608	.2008

*. The mean difference is significant at the 0.05 level.

Table 35 Multiple comparisons for fish weight gain.

Multiple Comparisons

Dependent Variable: Weight gain

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Feed A	Feed B	-10.62500*	.47558	.000	-11.9454	-9.3046
		Feed C	-41.23500*	.47558	.000	-42.5554	-39.9146
		Feed D	-16.71000*	.47558	.000	-18.0304	-15.3896
	Feed B	Feed A	10.62500*	.47558	.000	9.3046	11.9454
		Feed C	-30.61000*	.47558	.000	-31.9304	-29.2896
		Feed D	-6.08500*	.47558	.000	-7.4054	-4.7646
	Feed C	Feed A	41.23500*	.47558	.000	39.9146	42.5554
		Feed B	30.61000*	.47558	.000	29.2896	31.9304
		Feed D	24.52500*	.47558	.000	23.2046	25.8454
	Feed D	Feed A	16.71000*	.47558	.000	15.3896	18.0304
		Feed B	6.08500*	.47558	.000	4.7646	7.4054
		Feed C	-24.52500*	.47558	.000	-25.8454	-23.2046

*. The mean difference is significant at the 0.05 level.

Table 36 Multiple comparisons for ADG.

Multiple Comparisons

Dependent Variable: ADG

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	A	B	-.09000	1.02886	.934	-2.9466	2.7666
		C	-.34000	1.02886	.758	-3.1966	2.5166
		D	-1.59000	1.02886	.197	-4.4466	1.2666
	B	A	.09000	1.02886	.934	-2.7666	2.9466
		C	-.25000	1.02886	.820	-3.1066	2.6066
		D	-1.50000	1.02886	.219	-4.3566	1.3566
	C	A	.34000	1.02886	.758	-2.5166	3.1966
		B	.25000	1.02886	.820	-2.6066	3.1066
		D	-1.25000	1.02886	.291	-4.1066	1.6066
	D	A	1.59000	1.02886	.197	-1.2666	4.4466
		B	1.50000	1.02886	.219	-1.3566	4.3566
		C	1.25000	1.02886	.291	-1.6066	4.1066

Table 37 Multiple comparisons for FCR values.

Multiple Comparisons

Dependent Variable: FCR

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	A	B	.09000*	.01225	.002	.0560	.1240
		C	.36000*	.01225	.000	.3260	.3940
		D	.15000*	.01225	.000	.1160	.1840
	B	A	-.09000*	.01225	.002	-.1240	-.0560
		C	.27000*	.01225	.000	.2360	.3040
		D	.06000*	.01225	.008	.0260	.0940
	C	A	-.36000*	.01225	.000	-.3940	-.3260
		B	-.27000*	.01225	.000	-.3040	-.2360
		D	-.21000*	.01225	.000	-.2440	-.1760
	D	A	-.15000*	.01225	.000	-.1840	-.1160
		B	-.06000*	.01225	.008	-.0940	-.0260
		C	.21000*	.01225	.000	.1760	.2440

*. The mean difference is significant at the 0.05 level.

Table 38 Multiple comparisons for PER values.

Multiple Comparisons

Dependent Variable: PER

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	A	B	-.25500	.11363	.088	-.5705	.0605
		C	-.80000*	.11363	.002	-1.1155	-.4845
		D	-.50000*	.11363	.012	-.8155	-.1845
	B	A	.25500	.11363	.088	-.0605	.5705
		C	-.54500*	.11363	.009	-.8605	-.2295
		D	-.24500	.11363	.097	-.5605	.0705
	C	A	.80000*	.11363	.002	.4845	1.1155
		B	.54500*	.11363	.009	.2295	.8605
		D	.30000	.11363	.058	-.0155	.6155
	D	A	.50000*	.11363	.012	.1845	.8155
		B	.24500	.11363	.097	-.0705	.5605
		C	-.30000	.11363	.058	-.6155	.0155

*. The mean difference is significant at the 0.05 level.

Table 39 Multiple comparisons for SGR.

Multiple Comparisons

Dependent Variable: SGR

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	A	B	-.06000	.24049	.815	-.7277	.6077
		C	-.22000	.24049	.412	-.8877	.4477
		D	.24500	.24049	.366	-.4227	.9127
	B	A	.06000	.24049	.815	-.6077	.7277
		C	-.16000	.24049	.542	-.8277	.5077
		D	.30500	.24049	.274	-.3627	.9727
	C	A	.22000	.24049	.412	-.4477	.8877
		B	.16000	.24049	.542	-.5077	.8277
		D	.46500	.24049	.125	-.2027	1.1327
	D	A	-.24500	.24049	.366	-.9127	.4227
		B	-.30500	.24049	.274	-.9727	.3627
		C	-.46500	.24049	.125	-1.1327	.2027

Table 40 Multiple comparisons for Condition factor.

Multiple Comparisons

Dependent Variable: Condition factor

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	A	B	.19000	.55448	.749	-1.3495	1.7295
		C	.50000	.55448	.418	-1.0395	2.0395
		D	.88000	.55448	.188	-.6595	2.4195
	B	A	-.19000	.55448	.749	-1.7295	1.3495
		C	.31000	.55448	.606	-1.2295	1.8495
		D	.69000	.55448	.281	-.8495	2.2295
	C	A	-.50000	.55448	.418	-2.0395	1.0395
		B	-.31000	.55448	.606	-1.8495	1.2295
		D	.38000	.55448	.531	-1.1595	1.9195
	D	A	-.88000	.55448	.188	-2.4195	.6595
		B	-.69000	.55448	.281	-2.2295	.8495
		C	-.38000	.55448	.531	-1.9195	1.1595

Table 41 Multiple comparisons for Feed efficiency.

Multiple Comparisons

Dependent Variable: Feed efficiency

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	A	B	-.77500*	.03428	.000	-.8702	-.6798
		C	-3.03500*	.03428	.000	-3.1302	-2.9398
		D	-1.23000*	.03428	.000	-1.3252	-1.1348
	B	A	.77500*	.03428	.000	.6798	.8702
		C	-2.26000*	.03428	.000	-2.3552	-2.1648
		D	-.45500*	.03428	.000	-.5502	-.3598
	C	A	3.03500*	.03428	.000	2.9398	3.1302
		B	2.26000*	.03428	.000	2.1648	2.3552
		D	1.80500*	.03428	.000	1.7098	1.9002
	D	A	1.23000*	.03428	.000	1.1348	1.3252
		B	.45500*	.03428	.000	.3598	.5502
		C	-1.80500*	.03428	.000	-1.9002	-1.7098

*. The mean difference is significant at the 0.05 level.

Table 42 Multiple comparisons for female GSI.

Multiple Comparisons

Dependent Variable: Gonado-somatic index (%)

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	A	B	-.10000	.06093	.176	-.2692	.0692
		C	-.42500*	.06093	.002	-.5942	-.2558
		D	-.18000*	.06093	.042	-.3492	-.0108
	B	A	.10000	.06093	.176	-.0692	.2692
		C	-.32500*	.06093	.006	-.4942	-.1558
		D	-.08000	.06093	.259	-.2492	.0892
	C	A	.42500*	.06093	.002	.2558	.5942
		B	.32500*	.06093	.006	.1558	.4942
		D	.24500*	.06093	.016	.0758	.4142
	D	A	.18000*	.06093	.042	.0108	.3492
		B	.08000	.06093	.259	-.0892	.2492
		C	-.24500*	.06093	.016	-.4142	-.0758

*. The mean difference is significant at the 0.05 level.

Table 43 Multiple comparisons for male GSI.

Multiple Comparisons

Dependent Variable: Male GSI (%)

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	A	B	-.08000	.04637	.160	-.2087	.0487
		C	-.54000*	.04637	.000	-.6687	-.4113
		D	-.13000*	.04637	.049	-.2587	-.0013
	B	A	.08000	.04637	.160	-.0487	.2087
		C	-.46000*	.04637	.001	-.5887	-.3313
		D	-.05000	.04637	.342	-.1787	.0787
	C	A	.54000*	.04637	.000	.4113	.6687
		B	.46000*	.04637	.001	.3313	.5887
		D	.41000*	.04637	.001	.2813	.5387
	D	A	.13000*	.04637	.049	.0013	.2587
		B	.05000	.04637	.342	-.0787	.1787
		C	-.41000*	.04637	.001	-.5387	-.2813

*. The mean difference is significant at the 0.05 level.

Appendix F

Homogenous Subsets

Table 44 Homogenous of feed protein.

Diet protein (%)

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey B[a]	Feed B	2	35.6000	
	Feed A	2	36.0100	36.0100
	Feed D	2	36.0600	36.0600
	Feed C	2		36.4500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 45 Homogenous of muscle protein.

Muscle protein

	Treatment	N	Subset for alpha = 0.05
			1
Tukey B[a]	Feed A	2	14.6900
	Feed B	2	15.1000
	Feed D	2	15.3400
	Feed C	2	16.3700

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 46 Homogenous of weight gain.

Weight increase

	Treatment	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	Feed A	2	1.3988E2			
	Feed B	2		1.5051E2		
	Feed D	2			1.5660E2	
	Feed C	2				1.8112E2

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 47 Homogenous of ADG.

ADG

	Treatment	N	Subset for alpha = 0.05
			1
Tukey B[a]	A	2	1.1650
	B	2	1.2550
	C	2	1.5050
	D	2	2.7550

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 48 Homogenous of FCR.

FCR

	Treatment	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	C	2	3.3250			
	D	2		3.5350		
	B	2			3.5950	
	A	2				3.6850

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 49 Homogenous of PER.

PER

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey B[a]	A	2	2.8700		
	B	2	3.1250	3.1250	
	D	2		3.3700	3.3700
	C	2			3.6700

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 50 Homogenous of SGR.

SGR

	Treatment	N	Subset for alpha = 0.05
			1
Tukey B[a]	D	2	3.8700
	A	2	4.1150
	B	2	4.1750
	C	2	4.3350

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 51 Homogenous of Condition factor.

Condition factor

	Treatment	N	Subset for alpha = 0.05
			1
Tukey B[a]	D	2	1.9700
	C	2	2.3500
	B	2	2.6600
	A	2	2.8500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 52 Homogenous of FE.

Feed efficiency

	Treatment	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	A	2	10.3500			
	B	2		11.1250		
	D	2			11.5800	
	C	2				13.3850

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 53 Homogenous of female GSI.

Female GSI (%)

			Subset for alpha = 0.05	
	Treatment	N	1	2
Tukey B[a]	A	2	1.0900	
	B	2	1.1900	
	D	2	1.2700	
	C	2		1.5150

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 54 Homogenous of male GSI.

Male GSI (%)

			Subset for alpha = 0.05	
	Treatment	N	1	2
Tukey B[a]	A	2	.3900	
	B	2	.4700	
	D	2	.5200	
	C	2		.9300

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.