OPEN ACCESS

Journal of Fisheries and Aquatic Science

ISSN 1816-4927
DOI: 10.3923/jfas.2017.

Research Article
Cow Urine Distillate as an Ecosafe and Economical Feed Additive for Enhancing Growth, Food Utilization and Survival Rate in Rohu, *Labeo rohita* (Hamilton)

Govindharajan Sattanathan and Sournamanikam Venkatalakshmi

Centre for Animal Studies, Department of Zoology, Government College for Women (Autonomous), 612001 Kumbakonam, Tamilnadu, India

Abstract

Background and Objective: Freshwater aquaculture has made notable strides in recent years and now contributes to a third of the total fish production in India. Carp form the mainstay of Indian freshwater aquaculture. The present study assessed the efficacy of different breeds of cow urine distillate (CUD) in the diet of *Labeo rohita* fingerlings on growth, food utilization, nutritive value and survival rate parameters. **Materials and Methods:** Twelve fingerlings (9.3±2 g) were randomly distributed in four experimental groups. The experiment was conducted in glass aquaria tanks 25 L capacity each and temperature range was 28.5-30.0°C. *Labeo rohita* fed with Gir C (T_1), Haryana breed CUD diet (T_2), Holstein Friesian cross breed CUD diet (T_3) and Control diet without CUD (C). Fish were fed at 2% of wet body weight twice a day at 09.00 and 16.00 h. Mean body weight gain, mean body length gain, growth rate, specific growth rate (SGR), feed conversion ratio, survival rate and biochemical analysis were monitored in the study period of 30 days. Data were analyzed using one-way analysis of variance (ANOVA) at significance of ($p<0.05$). **Results:** At the end of the experiment, maximum growth (0.13 mg/fish), growth rate (0.0041 mg/day), percentage increase in body weight (14.07 mg), average daily growth (0.0044 mg/day) feeding rate (0.0034 mg/day) were occurred in the fish fed with T_1 diet significantly ($p<0.05$) when compared to other two experimental diets and control diet. A maximum survival rate of 60% was observed in T_1, high muscle protein (202.35 mg g^{-1}), liver (184.9 mg g^{-1}) and muscle carbohydrate (145.32 mg g^{-1}), liver (165.31 mg g^{-1}) were also observed in T_1. **Conclusion:** Hence, the present study suggested Gir CUD could be a good feed additive for higher growth and feeding efficiency, survival and nutrient value in aquaculture.

Key words: Cow urine distillate, HF cross breed, *Bos indicus*, *Labeo rohita*, food utilization, survival rate

Received: Accepted: Published:

Citation: Govindharajan Sattanathan and Sournamanikam Venkatalakshmi, 2017. Cow urine distillate as an ecosafe and economical feed additive for enhancing growth, food utilization and survival rate in rohu, labeo rohita (hamilton). J. Fish. Aquat. Sci., CC: CC-CC.

Corresponding Author: Sournamanikam Venkatalakshmi, Centre for Animal Studies, Department of Zoology, Government College for Women (Autonomous), 612001 Kumbakonam, Tamilnadu, India Tel: +91-94426 47313

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aquaculture is the farming and agriculture of economically important aquatic animals and plants under controlled conditions[1]. The culture of carps catla, rohu and mrigal are the commonest and well known species of traditional practice in India. They are considered as surface, column and bottom feeders, respectively[2]. In India, the indigenous carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) are the most important pond fishes of the country known for their excellent culture qualities viz., rapid growth rate, attainment of large size within a stipulated period, good market value, compatibility, quality, style of flesh, shopper preference and skill to tolerate the varied ecological conditions[2,3].

Advancement of aquaculture is largely dependent on availability of compatible and acceptable diet[4]. The growth of fish in the least stages is basically ruled by the type of food, ration, feeding frequency, food intake and its ability to soak up the nutrients. A simple indicator for assessing the pressure of any chemical or biological agent on fish is growth. Growth and food utilization response have important relationship with fish health and aquaculture production. The various dietary parts like vitamins[5], lipid[6], fatty acids[7] and carbohydrates[8] (as well as quality of diet have been found to contribute significantly to growth and biochemical composition of fishes. In addition to this, feed additives like vitamins[9-14], hormones[15,16] and growth promoters and also have consequential role on growth and food utilization of fishes[17,18]. An understanding of the influence of feed supplements to be used in aquaculture, on growth and food utilization of fish is essential to enhance the high production.

Organic farming has received considerable interest in recent years because of the actual fact that chemical based typical farming is harmful to the soil fertility and poses a threat to the environment in the long run[19]. This is also applicable in aquaculture. It is quite pertinent in the developing countries because of the fact that recycling of animal solid waste in fish ponds may contribute more than 50% of the total input tariff in fish culture[20,21] Studies of Afzal[22,23,1] on animal solid waste and the studies of Junge-Berberovic[24] on domestic wastewater reveal that fish yield in ponds fertile with animal excreta was 5-7 times higher than in normal fish culture pond[25,26,22].

Cows are called as "Kamdhenu" in India (the given of all wishes) as it plays significant role in rural economy, represent cattle wealth and biodiversity. Fresh cow urine has been reported to increase protein digestibility[27] decrease blood glucose levels in diabetic rats[28] and improve the histopathology of colon mucosa in rats fed with high-fat diet[29]. Use of medicinal formulations containing cow urine, butter milk and cow ghee mixed with a variety of herbs is very common in Indian Ayurvedic system for treatment of liver disorders, fever, inflammations, anemia and also as a rejuvenator[30,31]. Achliya *et al*.[32] reported the hepatoprotective activity of panchgavya against carbonate tetrachloride-induced hepatotoxicity in rats. Different preparations of cow urine and dung have been shown to prevent viral, bacterial and fungal diseases in plants[33]. Cow urine is an aqueous solution containing constituents like urea, sodium chloride, calcium, potassium, magnesium, sulphate, phosphate and some growth-promoting substances in the form of amino acids, glucose and vitamins[34].

The fish species have been chosen based on the fact that among the Indian major carps, *L. rohita* is the most preferred species and constitute about 35% of the Indian major carps production. Moreover rohu is commonly cultured, preferred and as well as high priced in all the North East region. It is the fast growing species among the cultivable carps[35]. Hence, a study was carried out to explore possibilities of using urine distillate of different breeds as feed additive to promote growth and food utilization responses, survival and nutrient values of *Labeo rohita* fingerlings.

MATERIALS AND METHODS

The study was carried out at Centre for Animal studies, Department of Zoology, Government College for Women (Autonomous), Kumbakonam, Tamilnadu, India at the period from 1.03.2017-12.04.2017.

Fish and their maintenance: Healthy and disease free fingerlings of *L. rohita* with average body weight of (1 $\pm$ 0.2 g) and total length 4.0$\pm$0.3 cm obtained from of the S.M fish farm, Kumbakonam, Tamilnadu, India were retained for acclimatization in circular plastic tanks of 70 L capacity in the wet laboratory of Centre for Animal Studies, for 2 weeks. Glass aquaria were cleaned to avoid microbial contamination and then sundried. Chlorine free water was used throughout the course of the experiment. Throughout the acclimatization period, fish were fed with formulated feed at *ad libitum*.

Water quality parameters: Water quality parameters like temperature, pH, temperature, dissolved oxygen, ammonia and nitrite were estimated by using standard methods of APHA[36].

Collection of cow urine: Six disease free cows were selected for urine collection[37]. The early morning (4.00-5.00 h) first urine of Gir, Haryana (Indigenous breeds) and HF cross bred cattle (Exotic breed) was collected from cow farm at Sri Vittal Rukminni Samsthan, Govindhapuram, Tamilnadu, India, where all the three breeds were maintained in a well ventilated shed with the provision of individual feeding and watering. Clean drinking water and feed was provided *ad libitum.* Animals were daily offered about 2 kg of green fodder and pelleted feed. All three breeds selected received same nutrition and maintenance factors. The urine was pooled and transported to laboratory in airtight sterile containers.

Cow urine distillate: Different breeds cow urine were distilled separately at 50-60°C using by glass multiple distillation apparatus[38]. Care was taken that all the three breed cow urine was distilled at same temperature and same duration simultaneously. The collected CUD was utilized on the same day without storage.

Experimental diet: The experimental diet was prepared following the method described by Venkatalakshmi and Ebanasar[39]. Ingredients compositions of the experimental diets are presented in Table 1. Thus, three experimental diets were prepared by mixing 0.1% (v/w) concentration of cow urine distillate of different breeds. The three different CUD diets were T_1 (Gir CUD), T_2 (Haryana CUD), T_3 (HF cross bred CUD) and C (Control without CUD). The required ingredients were mixed with water to make dough followed by cooking in an autoclave. After cooling CUD, vitamin and minerals were added. Finally, the dough was pressed through a hand pelletizer to get uniform size pellets (2 mm) and shade dried. The pellets were then kept in a room temperature for complete drying and then packed in clean plastic containers.

Experimental setup: After the acclimatization period, rohu with average weight 1±0.5 g and total length 4± 0.4 cm were stocked at a rate, 12/tank to have a total biomass of 9.3±2 g in each of the 4 glass aquaria tanks (20 L) to begin the experiment. Experimental tanks were constantly aerated throughout the experimental period of 30 days. The fish were fed with the experimental diet at the rate of 2% of body weight twice a day at 09.00 and 16.00 h to approximate satiation for 7 days. After the discontinuation of experimental diets, control diet was giving throughout the study. Uneaten feed and fecal matter were siphoned off and removed after each feeding, from each tank separately, oven-dried (60°C) and weighed to calculate feed intake.

Morphological growth analysis: All fish in each tank were measured for length, weight and were recorded individually at the beginning and end of the experiment to record growth performance and batch weighing was performed for every 10 days to adjust feeding level. The fishes were weighted by digital electronic balance with mg sensitivity (Systronics, India). Ruler was used to measure the total length from head and tip of caudal fin. The fingerlings were discharged in water immediately after body measurements, to avoid stress.

Growth parameters: The growth parameters were calculated by using the following formulae[40]:

$$\text{Growth} = \text{Final weight-Initial weight}$$

$$\text{Growth rate} = \frac{\text{Weight gain}}{\text{No. of days x initial weight}} \times 100$$

$$\text{Specific growth rate} = \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{No. of days}} \times 100$$

$$\text{Increase in body weight (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Average daily growth} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{No. of feeding days}} \times 100$$

Food utilization parameters: The food utilization parameters were calculated by using the following formulae[40]:

$$\text{Feeding rate} = \frac{\text{Total dry food consumed}}{\text{No. of days x Initial live weight of fish}} \times 100$$

$$\text{Food absorbed} = \text{Food consumed-faeces produced}$$

$$\text{Feed conversion rate} = \frac{\text{Wet weight gain (g)}}{\text{Dry weight of feed (g)}} \times 100$$

$$\text{Absorption rate} = \frac{\text{Total food absorbed (dry)}}{\text{No. of days x Initial live weight of fish}} \times 100$$

$$\text{Absorption efficiency} = \frac{\text{Food absorbed}}{\text{Food consumed}} \times 100$$

$$\text{Gross conversion efficiency (K1)} = \frac{\text{Growth rate}}{\text{Feeding rate}} \times 100$$

Table 1: Feed composition and proximate composition of the experimental diets fed to *L. rohita* fingerlings

	Experimental diet (kg g^{-1})			
Ingredients (g kg^{-1})	Control	T_1	T_2	T_3
Soya bean flour (SBF)*	400	400	400	400
Groundnut oil cake (GOC)*	250	250	250	250
Wheat bran (WB)*	200	200	200	200
Wheat flour (WF)*	40	40	40	40
Tapioca flour (TF)*	100	100	100	100
Vitamin and mineral mix#	10	10	10	10
CUD** (0.1% w/v)	Absent	Gir	Haryana	HF Cross bred
Proximate composition of feed* **				
Crude protein	35.32	35.54	35.53	35.42
Crude fat	98.32	99.54	97.43	98.65
Ash	72.54	70.22	73.54	72.35
Crude fibre	78.35	79.54	77.32	79.34
Nitrogen Free Extract	336.7	354.4	360.5	360.3
Metabolizable energy (kJ g^{-1})##	15.3	14.3	15.2	14.9

*Procured from local market, Proximate composition: (Halver, 1989), [SBF (522 crude protein, 10 crude fat,68 ash,65 crude fibre, 282 NFE), GOC (483 crude protein, 27 crude fat, 78 ash, 127 crude fibre, 225 NFE), WB (15.8 crude protein, 4.3 crude fat, 0.2 ash, 8.7 crude fibre, 73.4 NFE), WF (14.5 crude protein, 3.7 crude fat, 2.3 ash, 2.7 crude fibre, 64.2 NFE), TF (3.1 crude protein, 2.3 crude fat, 2.3 ash 2.0 crude fibre, 78.8 NFE)], #Composition of the mixture to supply for 1kg dry weight: (Virbac Animal health, India). Vitamin A (7,00,000 IU), Vitamin D3 (70,000 IU), Vitamin E (250 mg), Nicotinamide (1000 mg), Cobalt (150 mg), Copper (1200 mg), Iodine (325 mg), Iron (1500 mg), Manganese (1500 mg), Potassium (100 mg), Selenium (10 mg), Sodium (5,9 mg), Sulphur (0.72%), Zinc (9600 mg), Calcium (25.5%), Phosphorous (12.75%). **CUD: Cow Urine Distillate, ***Results are mean of triplicate estimations, ##Calculated using energy equivalents as proposed by Jauncey

$$\text{Net conversion efficiency (K2)} = \frac{\text{Growth rate}}{\text{Absorption rate}} \times 100$$

Gastro somatic index (GSI): The Gastro Somatic Index was calculated by using the following formulae[41]:

$$\text{Gastro somatic index (GSI)} = \frac{\text{Weight of the gut}}{\text{Weight of the fish}} \times 100$$

Survival rate: Mortality was recorded everyday and Survival rate is calculated by following formulae[41]:

$$\text{Survival rate} = \frac{\text{Initial No. of fish} - \text{Mortality}}{\text{Initial No. of fish}} \times 100$$

Biochemical analysis of tissue and diets: Proximate composition of experimental diets, muscle was analyzed using standard methods[42]. Moisture was determined by drying the sample (105°C for 24 h) to a constant weight. Crude protein was measured using the Kjeldahl method after acid digestion[42]. Crude fat was estimated by Soxhlet exhaustive extraction technique using petroleum ether (40-60°C, BP) as solvent[42]. Ash was determined by incinerating the dried sample at 550°C for 12 h. Crude fiber was estimated through 1.25% acid and subsequent 1.25% alkali digestion and incineration of the dried sample for 2 h at 550°C. Crude fibre was estimated through 1.25% acid and subsequent 1.25% alkali digestion and incineration of the dried sample for 2 h at 550°C. Nitrogen-free extract was calculated by difference[43]. The feed composition and proximate analysis of the experimental diets were presented in Table 1.

Statistical analysis: All the data collected were subjected to one-way analysis of variance (ANOVA) using SPSS (16-version) for windows software (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test applied to compare means at $p<0.05$ level of significance.

RESULTS

Water quality parameters: The basic physicochemical water parameters were measured systematically at 5 day intervals to maintain its optimal level throughout the experiment with values ranging from 26-29°C for temperature, 5.02±0.34 mg L^{-1} for dissolved oxygen, 8.27±0.81 for pH, 0.013±.007 mg L^{-1} for nitrite test and 0.103±0.014 mg L^{-1} for ammonia.

Growth performance: Fish fed with cow urine distillate supplemented diets showed better growth than the control. There were significant differences in mean body weight and mean body length between treatments with the T1 treated group having the highest mean body weight and mean body length from 7 days onwards ($p<0.05$, Fig. 1, 2). The growth response of *L. rohita* in terms of increase in body weight, growth rate, specific growth rate (SGR) are presented in Table 2. The final body weight of *L. rohita* fingerlings (Table 3) was the highest in T_1 (1.03±0.06 g), followed by T_2

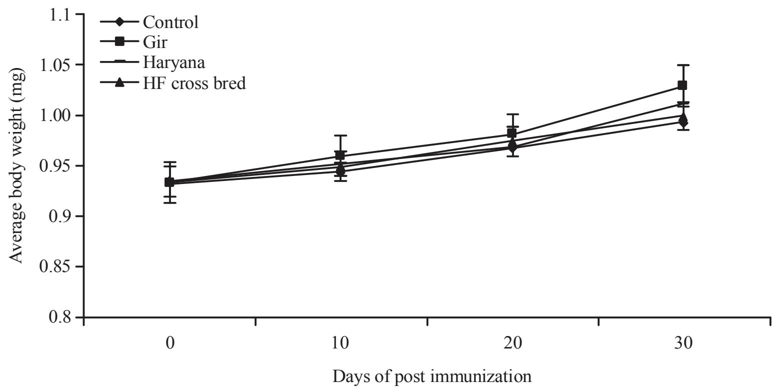

Fig. 1: Average body weight of feed additive with CUD diet on *Labeo rohita* fingerlings
(Data expressed as Mean±SE, n = 12)

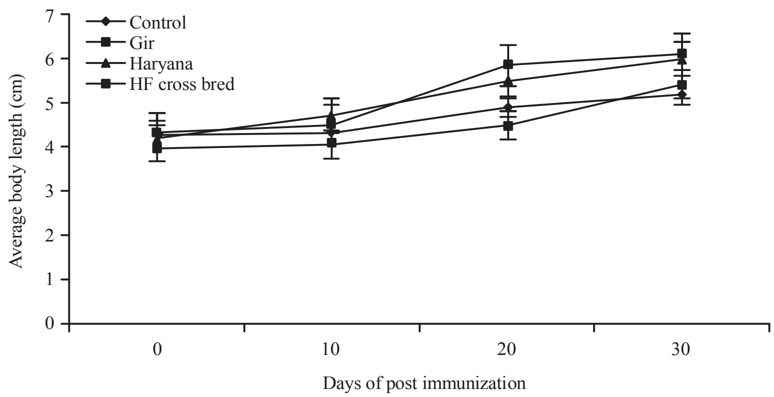

Fig. 2: Average Body length of feed additive with CUD diet on *Labeo rohita* fingerlings
(Data expressed as Mean±SE, n = 12)

Table 2: Growth parameters of *Labeo rohita* fingerlings fed with different breeds of cow urine distillate diet

Parameters	Control	T_1	T_2	T_3
Initial weight W1(g)	0.932±0.02	0.934±0.03	0.936±0.02	0.934±0.02
Final weight W2 (g)	0.994±0.05^{b}	1.030±0.06^{a}	1.012±0.07^{ab}	1.000±0.04^{b}
Initial length (cm)	4.260±0.29	4.300±0.30	4.300±0.19	4.200±0.21
Final length (cm)	5.040±0.24^{b}	5.110±0.29^{a}	5.020±0.25^{ab}	5.050±0.49^{b}
Growth W1-W2 (g)	0.062±0.02^{b}	0.132±0.01^{a}	0.076±0.02	0.066±0.04^{b}
Average Daily Growth (g/day)	0.0020±0.03^{b}	0.0044±0.02^{a}	0.0025±0.02^{ab}	0.0022±0.013^{c}
Percentage of increase in body weight (%)	6.650±0.43^{a}	14.070±0.022^{a}	8.110±0.021^{b}	7.060±0.011^{c}
Specific growth rate (%)	0.420±0.05^{a}	0.630±0.013^{ab}	0.560±0.032^{ab}	0.500±0.043^{b}
Gastro Somatic Index (%)	1.100±0.43^{b}	1.780±0.43^{a}	1.240±0.24^{ab}	1.120±0.45^{b}

Mean values in the same column with differ significantly ($p<0.05$). Data expressed as Mean±SD, n = 12

Table 3: Food utilization parameters of *Labeo rohita* fingerlings fed with different breeds of cow urine distillate diet

Parameters	Control	T_1	T_2	T_3
Food absorbed (mg/day)	0.033±0.02	0.077±0.04	0.044±0.03	0.057±0.02
Feed Conversion efficiency (%)	66.95±0.43^{b}	148.04±0.38^{a}	96.77±0.65^{b}	63.33±0.45^{c}
Absorption rate (mg/day)	0.0011±0.011^{b}	0.0027±0.02^{a}	0.0015±0.01^{c}	0.0028±0.02^{c}
Absorption efficiency (mg/day)	36.104±0.24	86.85±0.34	37.05±0.45	84.70±0.56
Gross conversion efficiency (%)	58.15±0.45	118.03±0.35	72.51±0.63	84.42±0.95
Net conversion efficiency (%)	161.08±0.66	148.66±0.76	150.07±0.54	99.81±0.76S

Mean values in the same column with differ significantly ($p<0.05$). Data expressed as Mean±SD, n = 12

(1.01±0.04 g) T_3 (1±0.07 g) and control (0.99±0.05 g), (Fig. 1). The effect of three different CUD on body weight of fish during four weeks was significant ($p<0.05$). The experiments revealed that on the 30th day, the highest

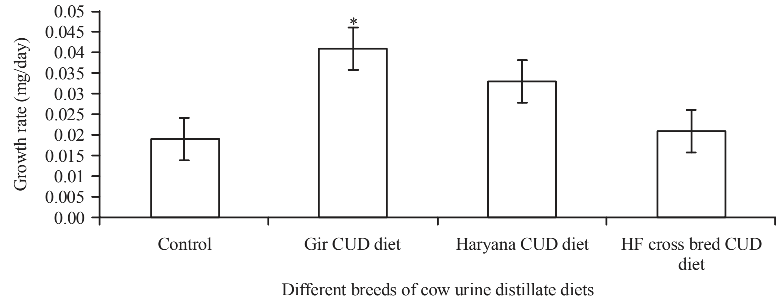

Fig. 3: Growth rate of fish fed with CUD supplemented diet on *Labeo rohita* fingerlings
*Significant p<0.05. Data expressed as Mean±SE

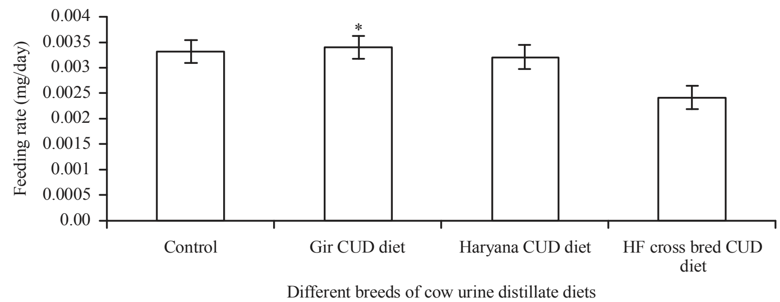

Fig. 4: Feeding rate of fish fed with CUD supplemented diet on *Labeo rohita* fingerlings
*Significant p<0.05. Data expressed as Mean±SE

growth rate was recorded in T_1. The maximum growth enhancement was recorded in T_1 with a growth rate of 0.0041 mg/day, when compared with 0.0019 mg/day in control (Fig. 3). Hence, the Gir CUD diet has a significant effect on the growth rate ($p<0.05$), (Table 3). As well as at the end of study, the maximum total length gained by the fish (Table 2) was in Gir CUD fed fish (5.11±0.24 cm), followed by Haryana CUD fed fish (5.05±0.049 cm) and HF cross bred CUD fed fish (5.02±0.025 cm). The effect of three feed ingredients on total weight and length gained by the fish during 7 days was significant ($p<0.05$).

Food utilization parameters: The effect of different breeds CUD on *Labeo rohita* fingerlings food utilization parameters like feeding rate, food absorbed, absorption rate, absorption efficiency, gross conversion efficiency and net conversion efficiency were showed in Table 3. The food utilization parameters were significantly higher in experimental fishes treated with CUD, when compared to controls. It was noted that highest feeding rate of 0.0034 mg/day was observed in T_1, which was significantly higher ($p<0.05$) when compared with control which feeding rate is 0.0033 mg/day (Fig. 4).

Gastro somatic index: The GSI of the experimental groups are summarized in Table 3. The gastro somatic index (GSI) of the experimental groups did not vary significantly ($p>0.05$).

Survival rate: The survival during the experimental study was high in T_1 and T_2 treatments when compared to control (Table 2). The mortality was recorded at 10 days interval. The highest survival rate of 60% was recorded in the T_1, which is significantly higher ($p<0.05$) than the untreated control and T_2 50% and T_3 having a least survival rate of 40% (Fig. 5).

Biochemical analysis: The proximate biochemical compositions were assayed on sample from 30th day experimental and control fishes. The maximum highest value of carbohydrate was present in T_1 muscle (145.32 mg g^{-1}) and liver (165.31 mg g^{-1}) and least value in control muscle (154.24 mg g^{-1}) and liver (120.89 mg g^{-1}) as well as highest rate of protein was present in T_1 muscle (202.35 mg g^{-1}), liver (184.9 mg g^{-1}), (Fig. 6, 7). The maximum lipid content was present in T_3 muscle (101.9 mg g^{-1}) and liver (93.1 mg g^{-1}) when compare with T_1 and T_2 and control (Fig. 8).

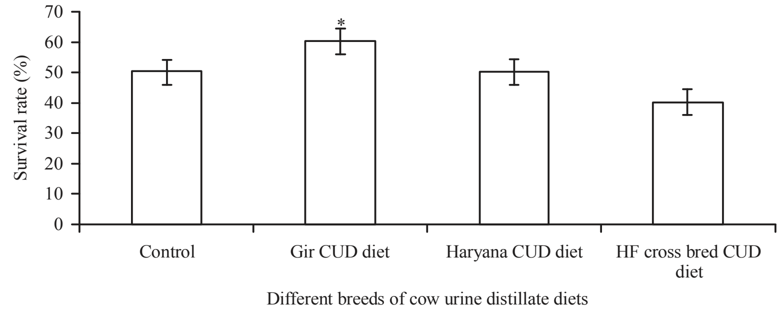

Fig. 5: Survival rate of fish fed with CUD supplemented diet on *Labeo rohita* fingerlings
*Significant $p<0.05$. Data expressed as Mean±SE

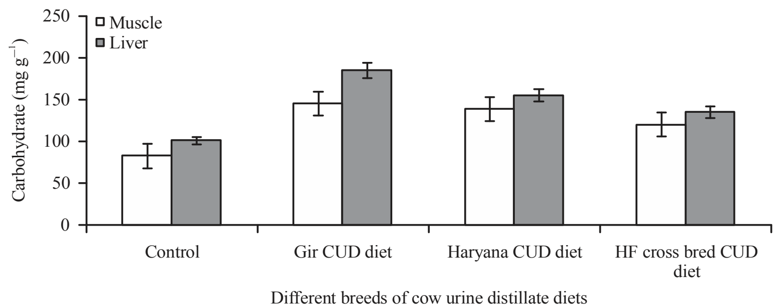

Fig. 6: Change in the total carbohydrate content (mg g^{-1} wet weight of the tissue) in different tissues of fish fed with different breeds of cow urine distillate (n = 3)
Data expressed as Mean±SE

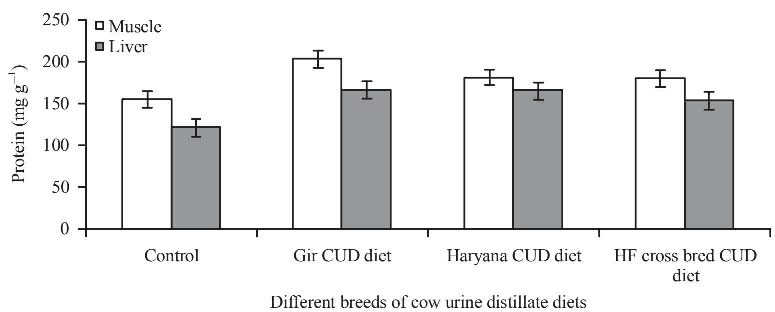

Fig. 7: Change in the total protein content (mg g^{-1} wet weight of the tissue) in different tissues of fish fed with different breeds of cow urine distillate (n = 3)
Data expressed as Mean±SE

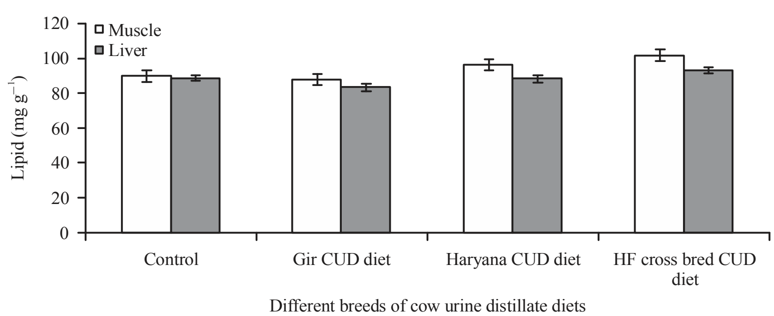

Fig. 8: Change in the total lipid content (mg g^{-1} wet weight of the tissue) in different tissues of fish fed with different breeds of Cow Urine distillate (n = 3)
Data expressed as Mean±SE

DISCUSSION

In aquaculture nutrition some feed additive, feed supplements have been also used for better growth performance. In the present study the effect of cow urine distillate (CUD) supplement diet on growth, food utilization and survival rate of *L. rohita* fingerlings. Growth is one of the most important parameters determining the economic efficiency of commercial fish culture, which is influenced by several biotic and abiotic factors[44]. Successful cultivation of fish depends on the good knowledge of suitable diet with required essential nutrients and environmental conditions. Water quality plays important role in growth and survival of aquatic organisms. It is determined by various physical, chemical and biological parameters of water body. Besides these, temperature is a major factor, which directly influences metabolism, affecting all physiological processes in ectotherms such as food intake, metabolism and nutritional efficiency[45]. In the present study, average range in pH, temperature, DO, salinity and conductivity were shown in Table 2. This results show that the fish were under stress free environment and also acted as good stress reliever.

There were significant differences in mean body weight and mean body length between treatments with the T_1 treated (Gir CUD diet) group having the highest mean body weight as well as mean body length. ($p<0.05$, Fig. 1, 2). Supplementary feeding is essential to increase the production of carp fry in ponds. The average survival of Indian major carps during the early stages is rather low (about 30% from spawn to fry) and about 50% from fry to fingerlings. This high mortality is due to lack of adequate and nutritionally balanced diets and poor management practices.

The 30th day growth rate trend indicated that the highest growth rate could be achieved within a short period of culture with Gir CUD diet treatment. It suggested that of 0.1% of Indian breeds CUD is the formulation of nutritionally adequate artificial diet for *L. rohita* fingerlings can give better production. The efficiency of CUD diet on the growth of Indian major carp *L. rohita* is clearly demonstrated in the present study. Various growth promoters like vitamins, hormones and amino acids were used as growth promoters in different fishes were well studied[46-49]. Among the growth promoters, calcium plays a vital role in growth promoting as well as detoxifying[50]. Increased levels of calcium and hardness are also found to be having positive influence over growth promoters of *Cyprinus carpio*[51]. Cow urine has been reported to contain minerals and amino acids hence, it may be the reason for its efficiency as feed additive in the promotion of growth.

Selection of feed ingredients and their costs have pronounced effect on aquaculture industry[52] Several researchers[53-55] worked on effects of various ingredients, individually and in combinations on different species and found better results when feed ingredients were combined together to formulate fish feeds. The feed conversion ratio values of various feed ingredients for carps under controlled conditions have been estimated by many workers[56-59] It is well studied and reported that the growth and conversion efficiency in various fishes were significantly influenced by the quality of food.

Cow urine is composed of eight main ionic species (Na, K, NH_4 , Ca^{2+}, Cl^-, SO_4, PO_4 and HCO_3^- with the dominance of nitrogen mainly (90%) in the forms of ammoniacal N and ammonium bicarbonate[60,61], apart from certain hormones, amino acids, glucose, vitamins[60] Hossain and Furuichi[51] reported accrued levels of Ca^+ ions and hardness is additionally found to be having positive influence over growth promoters of carp. Similar results were additionally created by Avnimelech *et al.*[62] and Muruganandham *et al.*[13] in *Catla catla* and *L. rohita* separately. Cow urine has been reported to contain Ca^+ ions and thus it should be the explanation for the promotion of growth. As the composition of cow urine reflects the average requirement of essential nutrients for plant growth[63]. It is as used as a fertilizer in agricultural production and has been reported to be highly cost-effective. Another advantage of the cow urine in agriculture or aquaculture was due to the fact that the hazardous chemical compounds or heavy metals are generally absent or low in cow urine but has high concentrations of urea which is a strong antimicrobial agent[64-66].

In the present study, T_1 showed 148.04% increase in FCR in different Gir CUD fed groups, which was significantly higher ($p<0.05$) than the fish fed diets without CUD supplementation (Control). This is in agreement with the earlier findings in *L. rohita* fingerlings[37,67]. It was reported that the CUD supplemented diet significantly increased the weight, length and SGR, percentage increase in body weight of fish than the control diet without CUD. Although we obtained higher SGR in CUD supplemented groups (T_1, T_2 and T_3), not much literature is available regarding the effect of different combinations of different breeds of cow urine distillate on SGR to compare our results. According to Jana *et al.*[19], cow dung with human urine act differently to enhance the growth and nutrient utilization of various fish species. It was also obtained similar observations in this study. Therefore, feed additive of CUD in the diet at (0.1% v/w) improves food conversion and nutrient retention, hence, leading to higher growth of fish. Cow urine has beneficial microbes[68-70]. It is reported that the digestive organs are very sensitive to food composition and cause immediate changes in activities of the digestive enzymes[71], which is finally reflected in fish health and growth. Moreover, bacteria also secrete proteases to digest the peptide bonds in proteins and therefore break down the proteins into their constituent monomers and free amino acids, which can benefit the nutritional status of the animal[71].

Urea is major component in urine and is the end product of protein metabolism[51]. The relationship between the ratio of total inorganic nitrogen to phosphate and the primary productivity of phytoplankton in different treatments led to assume that phosphorus amendment might be an alternative fertilizer therapy in the urine-fed treatment for enhancement of fish yield. The underlying mechanism was mediated through grazing food chain of fishes with phytoplankton-zooplankton fish or phytoplankton fish. Moreover, some growth promoting substances in the form of hormones, amino acids, glucose and vitamins present in cow urine were perhaps responsible for the induction of microbial based detritus food chain or the grazing food chain[19] suggested urine fed treatment as an alternative fertilizer therapy for enhancement of fish yield.

The feeding habits of cultured fish may play an important role by the way of direct consumption of remnants of cow manure, which, in turn, may determine the type of dominance in the food chain of the culture system. In the present study, rohu being herbivore might have directly consumed the remnants in manure and the bulk upon degradation induced fish yield by natural food production through autotrophic and heterotrophic pathways. Biomass of phytoplankton in the chicken manured tank was proved to produce 141% higher than that of control due to more effective food base for fish growth[72]. However, Bhakta *et al*.[1], stated that urea-nitrogen pollution originated from human urine was found to cause toxin producing flagellate blooms in aquaculture but have shown that cow urine can be profitably used for mass culture of zooplankton, algae and other economically important organisms[1].

The complete 10 quantitative dietary essential amino acid requirements have been established for a limited number of cultured fish species[73]. Optimum dietary arginine, lysine, methionine, tryptophan , lysine , valine, histidine, isoleucine, leucine and treonine requirements[47] for *Labeo rohita* have been established. Chemoprofiling of cow urine confirmed the presence of protein, urea, uric acid, amino acids, creatinine, phenol, aromatic acids, enzymes such as acid phosphatase, alkaline phosphatase, amylase and vitamins[47] Along with these, there may be some other constituents that may be responsible for the growth response. From these observations, it was clear that the better activity of using cow urine distillate may be enhancing the growth and feeding rate of *Labeo rohita*.

Carbohydrates serve as a reservoir of chemical energy required by the animal. Alteration in carbohydrate metabolism is prone to have deleterious effect on the survival of the animal[74]. In many fish, body lipid serves as the major sources of energy utilized during periods of starvation[75]. In the present study, the maximum level of carbohydrate content was recorded in T_1 treated group fish muscle and liver which were not significantly other different breeds of CUD treatments. The total protein content increased in T_1 treated group fish muscle and liver was found to be significantly higher, when compared to control groups. The whole body lipid content decreased in T_1 treatment group muscle and liver with CUD supplemented diet and was found to be maximum level in T_3 group of fish. Dietary cow urine had an impact on all aspects of proximate composition of different fish species[76].

One of the common expected problems associated with the use of fresh cow urine in aquaculture is the high concentration of ammonia and high pH that may harm the aquaculture species but cow urine distillate has less amount of ammonia compared with fresh cow urine. Despite immense nutrient potentials of cow urine as growth stimulant for aquaculture production, no studies have so far been carried out to examine the growth value of cow urine in aquaculture system. This is very pertinent in the developing countries where a safe and rational use of cow urine distillate for biological production would, perhaps, become a solution to a large section of poor farmers searching for low cost, easily available and safe fertilizer as an alternative to expensive chemical fertilizers.

CONCLUSION

The natural feed available in the pond meets the partial nutrient requirement of the animal in aquaculture. Therefore, supplementary feeding is important in semi-intensive aquaculture practices. Overall results of the present study appear to indicate that 0.1% dietary supplementation of cow urine distillate promotes weight gain, growth rate and survival percentage of *L. rohita* fingerlings. Results are also reflected with higher protein, carbohydrate and specific growth rate. Therefore, it can be concluded that cow urine distillate when incorporated at 0.1% (v/w), boosts up the growth performance and thus could be used as potent immunity to enhance aquaculture production of the *L. rohita* fingerlings.

SIGNIFICANCE STATEMENTS

This study discovers the potential of cow urine a cheaper growth and survival that cost beneficial for the fish farmers and in ecosafe aquaculture practices. This study help the researchers to uncover the new awareness in organic aquaculture and integrated farming practices.

ACKNOWLEDGMENT

The authors sincerely thank University Grants Commission, New Delhi, Government of India, for providing financial support as Major Research Project (42-561/2013(SR)) to carry out this study.

REFERENCES

1. Bhakta, J.N., D. Sarkar, S. Jana and B.B. Jana, 2004. Optimizing fertilizer dose for rearing stage production of carps under polyculture. Aquaculture, 239: 125-139.
2. Reddy, P.V., B. Gjerde, S.D. Tripathi, R.K. Jana and K.D. Mahapatra *et al*., 2002. Growth and survival of six stocks of rohu (*Labeo rohita*, Hamilton) in mono and polyculture production systems. Aquaculture, 203: 239-250.
3. Uddin, M.S., M.S. Miah and M.S. Alam, 1994. Study on production optimization through polyculture of indigenous and exotic carps. Bangladesh J. Train. Dev., 7: 67-72.
4. Ravenholt, A., 1982. Malnutrition in the Philippines. UFSI Reports South East Asia Series Issue 20, Universities Field Staff International, Hanover, NH., USA., pp: 73-84.
5. Akiyama, T.J., I. Yagisawa and T. Nose, 1981. Optimum levels of dietary crude Protein and fat for fingerling chum salmon. Bull. Natl. Res. Inst. Aquacult., 2: 35-42.
6. Bromley, P.J., 1980. Effect of dietary protein, lipid and energy content on the growth of turbot (*Scophthalmus maximus* L.). Aquaculture, 19: 359-369.
7. Paul, B.N., P.V. Rangacharyulu, S. Sarkar and P.K. Mukhopadhyay, 1996. Effect of feed color on the growth performance and activity of digestive enzymes in Rohu (*Labeo rohita*) fry. J. Aquacult., 4: 15-18.
8. Beamish, F.W.H., J.W. Hilton, E. Niimi and S.J. Slinger, 1986. Dietary carbohydrate and growth, body composition and heat increment in rainbow trout (*Salmo gairdneri*). Fish Physiol. Biochem., 1: 85-91.
9. Ebanasar, J. and V. Jayaprakas, 1995. Culture of three species of murrels with Mossambique tilapia at three predator-prey densities in earthen ponds. J. Aquacult. Trop., 10: 221-230.
10. Naik, A.T.R., H.S. Murthy and T.T. Ramesh, 1999. Effect of graded levels of G-probiotic on growth, survival and feed conversion of tilapia, *Oreochromis mossambicus*. Fish Technol., 36: 63-66.
11. Murthy, H.S. and T.R. Naik, 2002. Growth response and carcass composition of tilapia (*Oreochromis mossambicus*) to graded levels of vitamin C incorporated diets. J. Inland Fish. Soc. India, 34: 42-46.
12. Bhanja, S.K., K.D. Joshi and B.C. Tyagi, 2001. Effect of vitamin supplementation in diet on the growth and feed conversion efficiency of upland fishes. J. Aquacult., 9: 53-60.
13. Muruganandham, M., K. Jesua, A. Arokiaraj, S. Marimuthu and M.A. Haniffa, 2003. Supplementary effect of dietary 2-Tocopheral on growth and survival of fish *Channa striatus* fry. Environ. Ecol., 21: 131-132.
14. Muruganandham, M., R. Geetha and P. Ramakrishnan, 2004. Effect of Supplementary ascorbic acid on growth performance of tilapia (*Oreochromis mossambicus*) juveniles. Environ. Ecol., 22: 188-191.
15. Jayaprakas, V. and B.S. Sindhu, 1996. Effect of hormones on growth and food utilization of the Indian major carp, *Cirrhinus mrigala*. Fish. Tech., 33: 21-27.
16. Sambhu, C. and V. Jayaprakas, 1994. Effect of dietary hormone on feed utilization, growth and body composition of the pearl spot, *Etroplus suratensis* (Block) in a brackish water pond. Indian J. Mar. Sci., 24: 32-36.
17. Shambhu, C. and V. Jayaprakas, 2001. Livol (IHF-1000), a new herbal growth promoter in White Prawn, *Penaeus indicus* (Crustacea). Indian J. Mar. Sci., 30: 38-43.
18. Abraham, S., T.J. Ramesha, B. Gangadhara and T.J. Varghese, 2001. Growth response of common carp, *Cyprinus carpio* (Linn.) to varied levels of Livol, a non-hormonal growth promoter. Indian J. Fish., 48: 397-401.
19. Jana, B.B., S.K. Bag and S. Rana, 2012. Comparative evaluation of the fertilizer value of human urine, cow manure and their mix for the production of carp fingerlings in small holding tanks. Aquacult. Int., 20: 735-749.
20. Schroeder, G.L., 1980. Fish farming in manure loaded ponds. Proceedings of the ICLARM-SERCA Conference on Integrated Agriculture-Aquaculture Farming Systems, August 6-8, 1979, Manila, Philippines, pp: 73-86.
21. Dhawan, A. and H.S. Toor, 1989. Impact of organic manures or supplementary diet on plankton production and growth and fecundity of an Indian major carp, *Cirrhina mrigala* (Ham.), in fish ponds. Biol. Wastes, 29: 289-297.
22. Afzal, M., A. Rab, N. Akhtar, M.F. Khan, A. Barlas and M. Qayyum, 2007. Effect of organic and inorganic fertilizers on the growth performance of bighead Carp (*Aristichthys nobilis*) in polyculture system. Int. J. Agric. Biol., 9: 931-933.

23. Sarkar, P.D., S. Bag, C. Pradhan, S.L. Ganguly and B.B. Jana, 2011. Indian carp polyculture integrated with ducks and poultry: Ecological and economic benefits. Indian J. Anim. Sci., 81: 773-780.
24. Junge-Berberovic, R., 2000. Possibilities and limits of wastewater-fed aquacultures. Proceedings of the International Symposium on Ecosan-Closing the Loop in Wastewater Management and Sanitation, October 30-31, 2000, Bonn, Germany, pp: 113-122.
25. Nuruzzaman, A.K.M., 1991. Integrated fish farming system holds promise in Bangladesh. Published by 5/H Eastern Housing Apartment, Dhaka, Bangladesh.
26. Soliman, A.K., A.A.A. El-Horbeety, M.A.R. Essa, M.A. Kosba and I.A. Kariony, 2000. Effects of introducing ducks into fish ponds on water quality, natural productivity and fish production together with the economic evaluation of the integrated and non-integrated systems. Aquacult. Int., 8: 315-326.
27. Rastogi, S. and K. Kaphle, 2011. Sustainable traditional medicine: Taking the inspirations from ancient veterinary science. Evidence-Based Complement. Altern. Med. 10.1093/ecam/nen071.
28. Chaturvedi, P. and H. Akala, 2001. Effect of *Raphanus sativus* root extracts on glucose level in normal and diabetic rats. J. Applied Zool. Res., 12: 172-177.
29. Sipos, P., K. Hagymasi, A. Lugasi, E. Feher and A. Blazovics, 2002. Effects of black radish root (*Raphanus sativus* L. var *niger*) on the colon mucosa in rats fed a fat rich diet. Phytother. Res., 16: 677-679.
30. Shastri, A.D., 1998. Shushrut Samhita. 11th Edn., Chaukhamba Sanskrit Prakashan, Varanasi, India.
31. Bhalla, M. and G.P. Thami, 2005. Acute urticaria following 'gomutra' (cow's urine) gargles. Clin. Exp. Dermatol., 30: 722-723.
32. Achliya, G.S., N.R. Kotagale, S.G. Wadodkar and A.K. Dorle, 2003. Hepatoprotective activity of *Panchagavya ghrita* against carbontetrachloride induced hepatotoxicity in rats. Indian J. Pharmacol., 35: 308-311.
33. Sridhar, S., S. Arumugasamy, H. Saraswathy and K. Vijayalakshmi, 2002. Organic Vegetable Gardening. 1st Edn., Centre for Indian Knowledge Systems, Chennai, India.
34. Meena, M.K., M.D. Aklakur, G.M. Siddaiah, B.S. Jadhao, A.K. Pal and M.K. Chouksey, 2010. Effect of dietary zinc levels of on growth, metabolic responses and meat quality of *Labeo rohita* fingerlings. Proceedings of the Golden Jubilee National Seminar on Diversification of Aquaculture through Locally Available Fish Species, August 27-28, 2010, CIFE Kolkata Centre, India, pp: 55.
35. FAO., 1999. Aquaculture production statistics 1988-1997. FAO Fisheries Circular C815Rev.11, Food and Agriculture Organization of the United Nations, Rome, Italy, pp: 1-203.
36. APHA., 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edn., American Public Health Association, Washington, DC., USA.
37. Sattanathan, G. and S. Venkatalakshmi, 2015. The dose dependent effect of Gir Go-Ark on the growth and food utilization of *Labeo rohita* fingerlings. Life Sci. Int. Res. J., 2: 70-75.
38. Kekuda, T.P., S.P. Kumar, S.V. Suchitra, R. Kavya and R.M. Shrungashree, 2007. Studies on comparative *in vitro* antifungal activity of cow urine distillates (gomutra arka) prepared by two distilaltion processes against opportunistic fungi. Res. Rev. BioSci., 1: 208-211.
39. Venkatalakshmi, S. and J. Ebanasar, 2012. Immune enhancement of *Oreochromis mossambicus* (Peters) in relation to different doses *Lactobacillus sporogenes* given as a feed additive. J. Basic Applied Biol., 6: 58-64.
40. Petrusewicz, K. and A. MacFadyen, 1970. Productivity of Terrestrial Animals: Principles and Methods. I.B.P. Handbook No. 13, Blackwell, Oxford, UK., ISBN: 0632056703, Pages: 190.
41. Misra, C.K., B.K. Das, S.C. Mukherjee and P. Pattnaik, 2006. Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. Aquaculture, 255: 82-94.
42. Samantaray, K. and S.S. Mohanty, 1997. Interactions of dietary levels of protein and energy on fingerling snakehead, *Channa striata*. Aquaculture, 156: 241-249.
43. Khan, M.A., A.K. Jafri and N.K. Chadha, 2005. Effects of varying dietary protein levels on growth, reproductive performance, body and egg composition of rohu, *Labeo rohita* (Hamilton). Aquacult. Nutr., 11: 11-17.
44. Gupta, S.K., A.K. Pal, N.P. Sahu, R.S. Dalvi, M.S. Akhtar, A.K. Jha and K. Baruah, 2010. Dietary microbial levan enhances tolerance of *Labeo rohita* (Hamilton) juveniles to thermal stress. Aquaculture, 306: 398-402.
45. Venketramalingam, K., J.G. Christopher and T. Citarasu, 2007. *Zingiber officinalis* an herbal appetizer in the tiger shrimp *Penaeus monodon* (Fabricius) larviculture. Aquacult. Nutr., 13: 439-443.
46. Ciji, A., N.P. Sahu, A.K. Pal, M.S. Akhtar, V. Tincy, P. Mishal and P. Das, 2014. Effect of dietary vitamin E and nitrite exposure on growth and metabolic variables of *Labeo rohita* juveniles. Natl. Acad. Sci. Lett., 37: 123-129.
47. Fatma Abidi, S. and M.A. Khan, 2010. Growth, protein retention and body composition of fingerling Indian major carp, rohu, *Labeo rohita* (Hamilton), fed diets with various levels of lysine. J. World Aquacult. Soc., 41: 791-799.
48. Srijila, C.K., A.B. Rani, P.G. Babu and V.K. Tiwari, 2014. Ration restriction, compensatory growth and pituitary growth hormone gene expression in *Labeo rohita*. Aquacult. Int., 22: 1703-1710.

49. Tewary, A. and B.C. Patra, 2008. Use of vitamin C as an immunostimulant. effect on growth, nutritional quality and immune response of *Labeo rohita* (Ham.). Fish Physiol. Biochem., 34: 251-259.
50. Hossain, M.A.R. and M. Furuichi, 2000. Essentiality of dietary calcium supplement in redlip mullet, *Liza haematocheila*. Aquacult. Nutr., 6: 33-38.
51. Hossain, M.A. and M. Furuichi, 2000. Necessity of calcium supplement to the diet of Japanese flounder (*Paralichthys olivaceus*). Fish. Sci., 66: 660-664.
52. Mukhopadhyay, N., 2000. Improvement of quality of copra (dried kernel of *Cocos nucifera*) seed meal protein with supplemental amino acids in feeds for rohu, *Labeo rohita* (Hamilton) fingerlings. Acta Ichthyol. Fiscal., 30: 21-34.
53. Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete, 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev., 64: 655-671.
54. Harikrishnan, R., C. Balasundaram and M.S. Heo, 2010. *Lactobacillus sakei* BK19 enriched diet enhances the immunity status and disease resistance to streptococcosis infection in kelp grouper, *Epinephelus bruneus*. Fish Shellfish Immunol., 29: 1037-1043.
55. Ahmed, I. and M.A. Khan, 2004. Dietary lysine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). Aquaculture, 235: 499-511.
56. Bai, S.C., J.W. Koo, K.W. Kim and S.K. Kim, 2001. Effects of chlorella powder as a feed additive on growth performance in juvenile Korean rockfish, *Sebastes schlegeli* (hilgendorf). Aquac. Res., 32 : 92-98.
57. Kotzamanis, Y.P., E. Gisbert, F.J. Gatesoupe, J.Z. Infante and C. Cahu, 2007. Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota and resistance to *Vibrio anguillarum* in European sea bass (*Dicentrarchus labrax*) larvae. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., 147: 205-214.
58. Staykov, Y., S. Denev and P. Spring, 2005. The effects of mannan oligosaccharide (bio-mass) on the growth rate and immune function of rainbow trout grown in net cages. Proceedings of the International Conference on Aquaculture Europe 2005: Lesson from the Past to Optimize the Future, August 5-9, 2005, Trindheim, Norway, pp: 427-432.
59. Ghosh, K., S.K. Sen and A.K. Ray, 2005. Feed utilization efficiency and growth performance in rohu, *Labeo rohita* (Hamilton, 1822), fingerlings fed yeast extract powder supplemented diets. Acta Ichthyol. Piscat., 35: 111-117.
60. Bravo, D., D. Sauvant, C. Bogaert and F. Meschy, 2003. Quantitative aspects of phosphorus excretionin ruminants. Reprod. Nutr. Dev., 43: 285-300.
61. Yan, T., J.P. Frost, T.W. Keadya, R.E. Agnew and C.S. Mayne, 2007. Prediction of nitrogen excretion in feces and urine of beef cattle offered diets containing grass silage. J. Anim. Sci., 85: 1982-1989.
62. Avnimelech, Y., B. Weber, B. Hepher, A. Milstein and M. Zorn, 1986. Studies in circulated fish ponds: Organic matter recycling and nitrogen transformation. Aquacult. Res., 17: 231-242.
63. Akhter, N., M.F. Begum, S. Alam and M.S. Alam, 2006. Inhibitory effect of different plant extracts, cow dung and cow urine on conidial germination of *Bipolaris sorokiniana*. J. Bio-Sci., 14: 87-92.
64. Jerald, E., S. Edwin, V. Tiwari, R. Garg and E. Toppo, 2008. Antioxidant and antimicrobial activities of cow urine. Global J. Pharmacol., 2: 20-22.
65. Kumar, S., 2013. Analysis of cow's urine for detection of lipase activity and anti-microbial properties. IOSR J. Pharm. Biol. Sci., 7: 1-8.
66. Achliya, G.S., V.S. Meghre, S.G. Wadodkar and A.K. Dorle, 2004. Antimicrobial activity of different fractions of cow urine. Indian J. Nat. Prod., 20: 14-16.
67. Sattanathan, G. and S. Venkatlakshmi, 2015. Efficacy of different breeds of cow urine distillate on growth and food utilization of Indian major carp, *Labeo rohita* (Hamilton) fingerlings. History, 14: 169-185.
68. Chawla, P.C., 2010. Resorine: A novel CSIR drug curtails TB treatment. CSIR News, March 2010, pp: 60-52.
69. Mandavgane, S.A., A.K. Rambhal and N.K. Mude, 2005. Development of cow urine based disinfectant. Nat. Prod. Rad., 4: 410-415.
70. Ahirwar, R.M., M.P. Gupta and S. Banerjee, 2010. Field efficacy of natural and indigenous products on sucking pests of sesame. Indian J. Nat. Prod. Resour., 1: 221-226.
71. Dabrowski, K. and H. Guderley, 2002. Intermediary Metabolism. In: Fish Nutrition, Halver, J.E. and R.W. Hardy (Eds.). Academic Press, San Diego, CA., USA., pp: 310-367.
72. Giri, S.S., S.K. Sahoo, A.K. Sahu and P.K. Meher, 2003. Effect of dietary protein level on growth, survival, feed utilisation and body composition of hybrid Clarias catfish (*Clarias batrachus* x *Clarias gariepinus*). Anim. Feed Sci. Technol., 104: 169-178.
73. Chanda, S., B.N. Paul, K. Ghosh and S.S. Giri, 2015. Dietary essentiality of trace minerals in aquaculture-A review. Agric. Rev., 36: 100-112.
74. Murray, R.K., D.K. Granner and V.W. Rodwell, 2006. Harper's Illustrated Biochemistry. 27th Edn., Lange Medical Publications, Singapore, pp: 74-75.
75. BIS., 2013. Freshwater prawn (*Macrobrachium rosenbergii*) feed-specification. Document No. FAD 12(2340)C, Draft Indian Standard Fish Feed, Indian Standards, Manak Bhavan, New Delhi, India.
76. Padmapriya, S.S. and S. Venkatalakshmi, 2014. Effect of varying cow urine samples on growth of fish *Cirrhinus mrigala* fingerlings (Hamilton). Int. J. Fish. Aquat. Stud., 2: 26-29.