



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)

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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

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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 conditions¹. 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, respectively². 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 diet⁴. 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 vitamins⁵, lipid⁶, fatty acids⁷ and carbohydrates⁸ (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 vitamins⁹⁻¹⁴, 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 run¹⁹. 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-Berberovic²⁴ 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 digestibility²⁷ decrease blood

glucose levels in diabetic rats²⁸ and improve the histopathology of colon mucosa in rats fed with high-fat diet²⁹. 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 plants³³. 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 vitamins³⁴.

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 carps³⁵. 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 ± 0.2) g and total length 4.0 ± 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 APHA³⁶.

Collection of cow urine: Six disease free cows were selected for urine collection³⁷. 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 apparatus³⁸. 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 Ebanasar³⁹. 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 T₁ (Gir CUD), T₂ (Haryana CUD), T₃ (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 formulae⁴⁰:

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

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

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

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

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

Food utilization parameters: The food utilization parameters were calculated by using the following formulae⁴⁰:

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

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

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

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

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

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

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

Ingredients (g kg ⁻¹)	Experimental diet (kg g ⁻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 formulae⁴¹:

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

Survival rate: Mortality was recorded everyday and Survival rate is calculated by following formulae⁴¹:

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

Biochemical analysis of tissue and diets: Proximate composition of experimental diets, muscle was analyzed using standard methods⁴². 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 digestion⁴². Crude fat was estimated by Soxhlet exhaustive extraction technique using petroleum ether (40–60°C, BP) as solvent⁴². 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 difference⁴³.

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±0.007 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 T₁ (1.03±0.06 g), followed by T₂

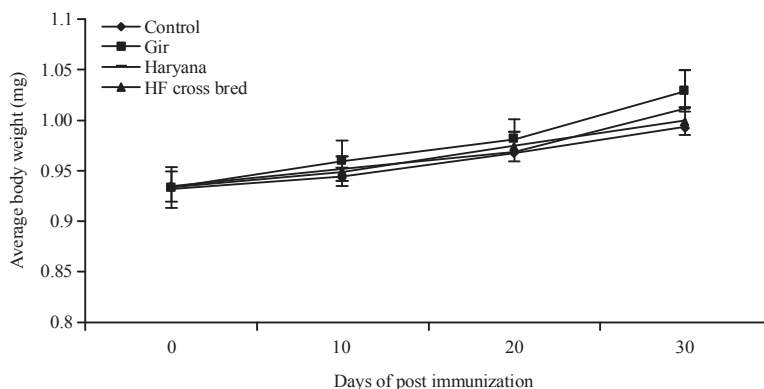


Fig. 1: Average body weight of feed additive with CUD diet on *Labeo rohita* fingerlings
(Data expressed as Mean \pm SE, n = 12)

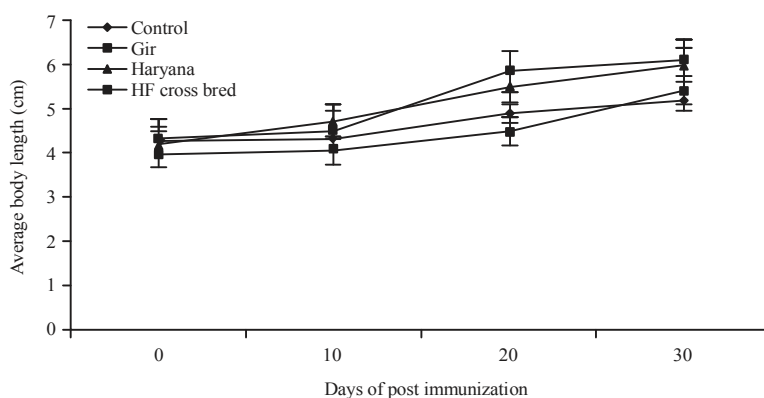


Fig. 2: Average Body length of feed additive with CUD diet on *Labeo rohita* fingerlings
(Data expressed as Mean \pm SE, n = 12)

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

Mean values in the same column with differ significantly (p<0.05). Data expressed as Mean \pm 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 \pm 0.02	0.077 \pm 0.04	0.044 \pm 0.03	0.057 \pm 0.02
Feed Conversion efficiency (%)	66.95 \pm 0.43 ^b	148.04 \pm 0.38 ^a	96.77 \pm 0.65 ^b	63.33 \pm 0.45 ^c
Absorption rate (mg/day)	0.0011 \pm 0.011 ^b	0.0027 \pm 0.02 ^a	0.0015 \pm 0.01 ^c	0.0028 \pm 0.02 ^c
Absorption efficiency (mg/day)	36.104 \pm 0.24	86.85 \pm 0.34	37.05 \pm 0.45	84.70 \pm 0.56
Gross conversion efficiency (%)	58.15 \pm 0.45	118.03 \pm 0.35	72.51 \pm 0.63	84.42 \pm 0.95
Net conversion efficiency (%)	161.08 \pm 0.66	148.66 \pm 0.76	150.07 \pm 0.54	99.81 \pm 0.765

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

(1.01 \pm 0.04 g) T₃ (1 \pm 0.07 g) and control (0.99 \pm 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

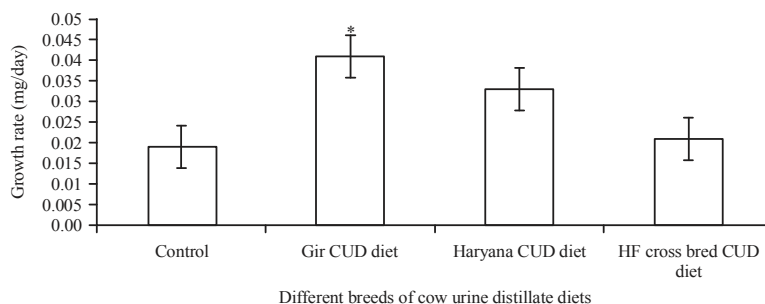


Fig. 3: Growth rate of fish fed with CUD supplemented diet on *Labeo rohita* fingerlings

*Significant $p < 0.05$. Data expressed as Mean \pm SE

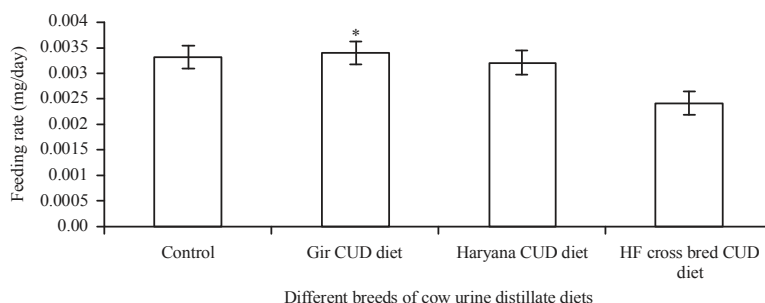


Fig. 4: Feeding rate of fish fed with CUD supplemented diet on *Labeo rohita* fingerlings

*Significant $p < 0.05$. Data expressed as Mean \pm 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).

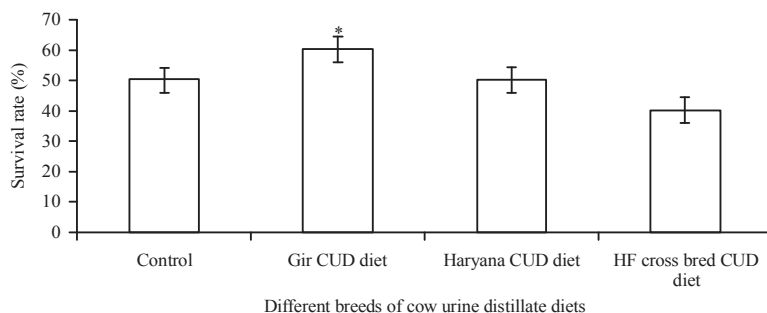


Fig. 5: Survival rate of fish fed with CUD supplemented diet on *Labeo rohita* fingerlings

*Significant $p < 0.05$. Data expressed as Mean \pm SE

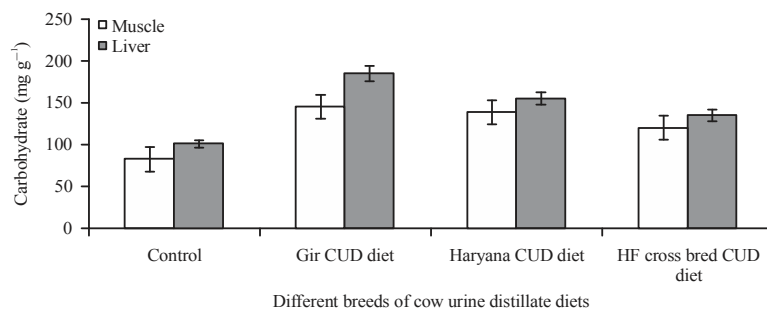


Fig. 6: Change in the total carbohydrate content (mg g^{-1} wet weight of the tissue) in different tissues of fish fed with different breeds of cow urine distillate ($n = 3$)

Data expressed as Mean \pm SE

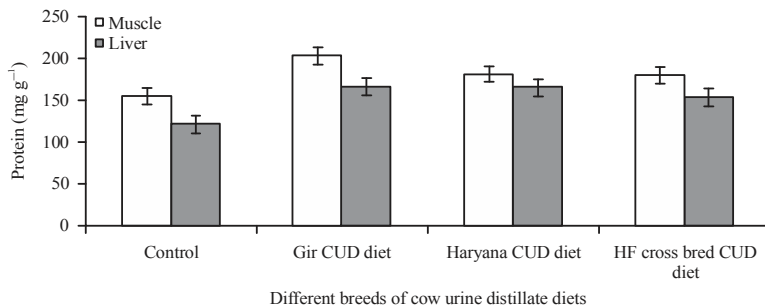


Fig. 7: Change in the total protein content (mg g^{-1} wet weight of the tissue) in different tissues of fish fed with different breeds of cow urine distillate ($n = 3$)

Data expressed as Mean \pm SE

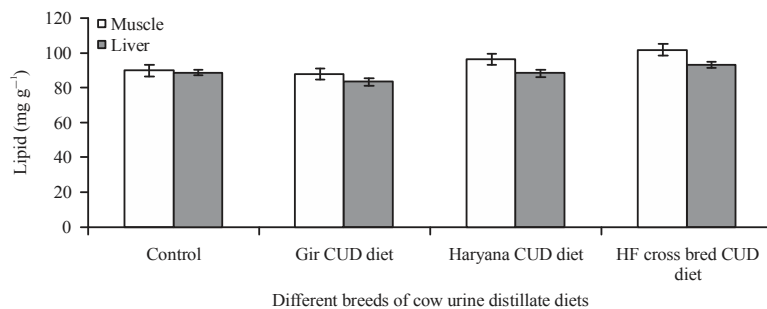


Fig. 8: Change in the total lipid content (mg g^{-1} wet weight of the tissue) in different tissues of fish fed with different breeds of Cow Urine distillate ($n = 3$)

Data expressed as Mean \pm 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 factors⁴⁴. 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 efficiency⁴⁵. 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 T₁ 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 studied⁴⁶⁻⁴⁹. Among the growth promoters, calcium plays a vital role in growth promoting as well as detoxifying⁵⁰. 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 industry⁵². Several researchers⁵³⁻⁵⁵ 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 workers⁵⁶⁻⁵⁹. 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, NH₄⁺, Ca²⁺, Cl⁻, SO₄²⁻, PO₄³⁻ and HCO₃⁻) 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, vitamins⁶⁰. Hossain and Furuichi⁵¹ reported accrued levels of Ca²⁺ 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 Ca²⁺ 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 growth⁶³. 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 agent⁶⁴⁻⁶⁶.

In the present study, T₁ 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 (T₁, T₂ and T₃), 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 microbes⁶⁸⁻⁷⁰. It is reported that the digestive organs are very sensitive to food composition and cause immediate changes in activities of the digestive enzymes⁷¹, 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 animal⁷¹.

Urea is major component in urine and is the end product of protein metabolism⁵¹. 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 chain¹⁹ 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 growth⁷². 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 organisms¹.

The complete 10 quantitative dietary essential amino acid requirements have been established for a limited number of cultured fish species⁷³. Optimum dietary arginine, lysine, methionine, tryptophan, lysine, valine, histidine, isoleucine, leucine and treonine requirements⁴⁷ 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 vitamins⁴⁷ 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 animal⁷⁴. In many fish, body lipid serves as the major sources of energy utilized during periods of starvation⁷⁵. In the present study, the maximum level of carbohydrate content was recorded in T₁ treated group fish muscle and liver which were not significantly other different breeds of CUD treatments. The total protein content increased in T₁ treated group fish muscle and liver was found to be significantly higher, when compared to control groups. The whole body lipid content decreased in T₁ treatment group muscle and liver with CUD supplemented diet and was found to be maximum level in T₃ group of fish. Dietary cow urine had an impact on all aspects of proximate composition of different fish species⁷⁶.

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.

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