LS - An International Journal of Life Sciences
DOI: 10.5958/2319-1198.2016.00001.4

Application of Cow Urine Distillate for Enhancement of Nutrient Value and Water Quality of Indian Major Carp, *Labeo rohita* (Hamilton) Fingerlings

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ABSTRACT

Cow urine is known for its medicinal properties and therapeutic values in India. In Indian ancient literature, cow urine is reported to be very effective and in Ayurveda its use is described as well. Recently, there are many reports which revealed the applications of cow urine in agriculture, poultry, animal health and human health. However, there are no reports for its application in aquaculture. Hence, in the present investigation, the effect of distilled Gir cow urine (CUD) was studied on *Labeo rohita* for water quality parameters and biochemical analysis. The study revealed that water quality parameters and nutrient content were significantly increased in T1, when the fingerlings were given CUD in different concentrations 0.1%, 0.25% and 0.5%. By comparing the values on the 30th day, there was an increase in carbohydrate, protein and lipid levels in T1 and T2 when compared to control. T3 showed 100% mortality. This result clearly indicates increased growth effect of Gir CUD in *L. rohita* fingerlings at optimal dosage.

Keywords: Aquaculture, Cow urine distillate, Cow urine, Go-Ark, *Bos indicus*, *Labeo rohita*, Water quality parameters

INTRODUCTION

Indian freshwater aquaculture has evolved from being a domestic activity in eastern India to an industrial activity that presently contributes one-third of total fish production. The main farmed freshwater species are rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*) of which rohu is the dominant species because of its market demand (Mishra and Samantaray, 2004). Over the years, intensive aquaculture has been expanded and has emerged as one of the most viable and promising sectors for providing nutritional and food security to human (Mohapatra *et al.*, 2011). India ranks the third among the world freshwater fish producers (FAO, 2003).

Fish constitutes one of the major sources of protein for human beings (Bhaqowati and Rath, 1982). The nutritional value of different tissues of fish depends on their biochemical composition like protein, amino acids, vitamins, mineral contents and others. Manuring is widely practiced in fishponds for natural fish production as it is important for sustainable aquaculture and to reduce expenditure on costly feeds and fertilisers which form more than 50% of the total input cost (Edwards, 1980; Pillay, 1995; Omoyinmi e*t al*., 2012)

Gir are the hardiest of high yielders in the world. The Gir is a famous milk cattle breed of India. It has been used in the improvement of other breeds including the Red Sindhi and the Sahiwal. Cattle of the breed are famous for their tolerance to stress conditions and resistance to various tropical diseases. The breed has been exported to other parts of the world also. This is most important milk breed of country mainly kept for milk production. Under good management conditions, the Gir cow produces between 1,150 and 1,600 kg of milk lactation. The average milk production of this breed

in India is about 1,400 kg per lactation. The heritability of milk production is 0.20–0.30. Milk fat varies between 4.6 and 4.9%. Age at first calving is 45–55 month depending on the managerial practices and calving interval is 515–600 days. The body weight of Gir calf at birth is 21–23 kg, which attains about 319–327 kg at the time of calving. Bullocks are good working animals for road transport (Gaur *et al.*, 2004).

Cow urine has great pharmacological importance for its medicinal utility and has been greatly mentioned in depth in Ayurveda. Cow urine is found to be effective against reversal of certain cardiac and kidney diseases, indigestion, stomach ache, oedema, skin disease and others (Sathasivam *et al.*, 2010). The cow urine distillate (CUD) has been patented as an activity enhancer and availability facilitator for bio molecules including anti-infective and anticancer agents (Khanuja, 2002). Cow urine has certain volatile and non-volatile components which might have high antimicrobial activity (Shaw *et al.*, 2007). Nowadays, a lot of emphasis is being given on the medicinal use of cow urine in India.

Recently, the cow urine has been granted U.S. Patents (Nos. 6,896,907 and 6,410,059) for its medicinal properties, particularly for its use along with antibiotics for the control of bacterial infection and fight against cancers (Dhama *et al.*, 2005). Cow urine contains 95% water, 2.5% urea and 2.5% minerals, salts, hormones and enzymes (Bhadauria, 2002). *Gomutra* alone has got all such chemical properties, potentialities and constituents that are capable of removing all the ill effects and imbalances in the body (Chauhan *et al.*, 2001). Hence, a study was undertaken to investigate the effects of different concentration of Gir CUD to enhance water quality parameters and nutrient values of *L. rohita* fingerlings.

MATERIALS AND METHODS

Fish and Their Maintenance

Fingerlings of *L. rohita* (Hamilton) were procured from S.M. Fish farm, Swamimalai, Thanjavur District and were brought to the laboratory in polythene bags filled with oxygen. The polythene bags were kept float for 30 min in the cement tank for acclimatisation of the fingerlings before released in to the tank. Glass aquaria were washed to avoid fungal contamination and then sun dried. Healthy fishes were then transferred to glass aquaria of 20 L capacity containing dechlorinated tap water. Fish of both sexes weighing 1.0 ± 0.2g were used in the study.

Mode of Feeding

Fish were fed with formulated feed of 2% total body weight twice a day, for an hour in between 9 am and 10 am and 4 pm and 5 pm.

Collection of Cow Urine

Six disease free indigenous breeds in Goshala, Sri Vittal Rukmini Samasdan, Govindapuram were selected for urine collection. In the early morning (4.00 am), first urine of the selected Gir breed cattle (*Bos indicus*) was collected. The pooled sample of cow urine was transported to laboratory in airtight sterile container (Sattanathan and Venkatalakshmi, 2015).

Cow Urine Distillate

Gir Cow urine was distilled at 40–50°C using by glass distillation apparatus for 2–3 h (Kekuda *et al.*, 2007). The low heat was maintained to avoid foaming. The CUD was stored in sterile glass container.

Experimental Setup

After two weeks of acclimatisation, three groups of fish were treated, each with Gir CUD at 0.1% (T1), 0.25% (T2) and 0.5% (T3) concentrations. The fish treated with different concentration of Gir CUD was kept undisturbed for one week and then water was changed. A control group was maintained separately without cow urine treatment (Padmapriya and Venkatalakshmi, 2014). During this treatment period, the water quality parameters were assessed for every five days.

Biochemical Parameters

The biochemical analysis was carried out for carbohydrates, lipids and proteins in two different tissues (liver and muscle) of treated and untreated *L. rohita* fingerlings. Carbohydrate was determined by Anthrone method (Dubosis *et al.,* 1956). Protein was estimated following the method of Lowry *et al.* (1951). Lipids were extracted and estimated with phospho-vannilin as described by Folch *et al.* (1957).

Water Quality Parameters

The present investigations on the effect of CUD on water quality parameters were carried out for a period of five days interval. The water samples determine various physico-chemical parameters such as (a) water temperature, (b) pH of water, (c) dissolved oxygen (DO), (d) total dissolved solids, (e) ammonia, (f) salinity, (g) conductivity and (h) turbidity. The chemical analysis was performed by using electronic water analyzer meter (Systronics, India).

RESULT

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 T1 muscle (40.47 mg/g) and liver (72.11 mg/g), and least value in control muscle (36.52 mg/g) and liver (64.17 mg/g). As well as the highest rate of protein was present in T1 muscle (136.82 mg/g), liver (94.76 mg/g), T2 muscle (129.52 mg/g) and liver (93.47 mg/g) (Figures 1 and 2). The maximum lipid content was present in control muscle (0.94 mg/g) to compare with T1 and T2 (Figure 3). In treated fishes, level of protein and carbohydrate levels have increased when compared to control (Table 1). The results revealed that CUD has enhanced the protein and carbohydrate levels of *L. rohita* fingerlings. T3 has effected 100% mortality.

Water Quality Parameters

Measurements of (a) water temperature, (b) pH of water, (c) DO, (d) total dissolved solids, (e) ammonia, (f) salinity, (g) conductivity and (h) turbidity of water samples were collected from the aquaria tank at five days intervals from 8 December 2013 to 6 January 2014

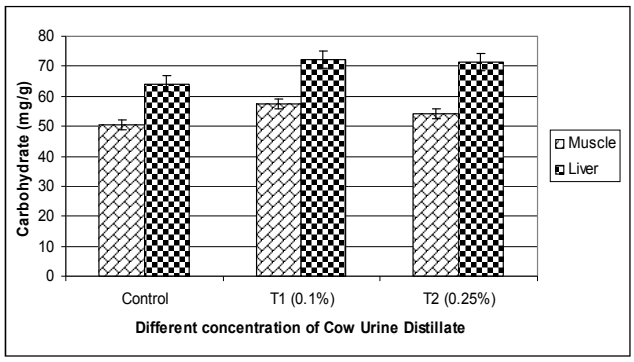

Figure 1: Change in the total carbohydrate content (mg/g wet weight of the tissue) in different tissues exposed to cow urine distillate of different concentrations

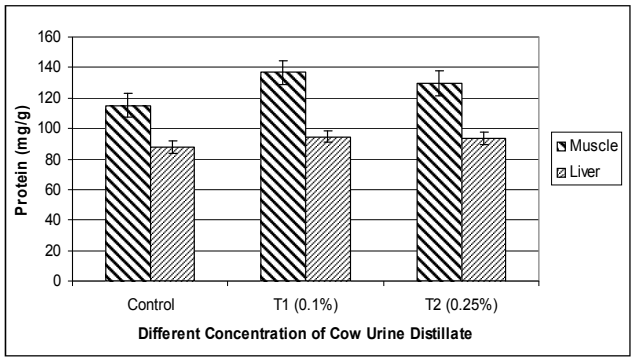

Figure 2: Change in the total protein content (mg/g wet weight of the tissue) in different tissues exposed to cow urine distillate of different concentrations

(Table 2). Temperature was maintained in acceptable range at 24.6–30.3°C. pH is an indicator of the existence of biological life as the most of them thrive in a quite narrow and critical pH range. The pH values observed in the aquaria tank during the study periods were 7.7 as minimum in and 8.9 as maximum in control. T3 treatments were 100% mortality. DO is essential for aquatic life. A low DO would indicate poor water quality and thus would have difficulty in sustaining many

Table 1: Estimation of carbohydrate, protein and lipid in the muscle and liver of control and experimental fresh water fish *Labeo rohita* fingerlings

Biochemical Analysis	Treatments / Tissues	Control	T1 (0.1%)	T2 (0.25%)
Carbohydrate(mg/g)	Muscle	36.52	40.47	40.29
	Liver	64.17	72.11	71.56
Protein (mg/g)	Muscle	115.17	136.82	129.52
	Liver	87.52	94.76	93.47
Lipid (mg/g)	Muscle	0.94	0.91	0.92
	Liver	1.25	1.28	1.33

Table 2: Physico-chemical characteristics of water analysis in control and treatments

Parameters	Initial				At the End of CUD Treatment				One Month Post CUD Treatment			
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
pH	8.5	8.5	8.5	8.5	8.6	8.2	8.1	–	8.5	8.5	8.4	–
Salinity (ppm)	2.5	2.5	2.5	2.5	2.6	2.5	2.1	–	1.1	1.0	1.5	–
Temperature (°C)	30.1	30.1	30.1	30.1	30.3	30.1	29.9	–	25.6	25.3	25.3	–
DO* (mg/L)	5.1	5.1	5.1	5.1	5.1	5.2	4.7	–	2.7	2.3	1.9	–
Turbidity (NTU)	7.5	7.5	7.5	7.5	7.9	7.1	7.2	–	6.3	7.3	6.9	–
Conductivity	152	152	152	152	252	255	254	–	358	360	362	–
TDS* (mg/L)	2.9	2.9	2.9	2.9	2.9	2.9	2.0	–	3.0	3.1	3.2	–
Ammonia (mg/L)	0.52	0.52	0.52	0.52	0.51	0.57	0.45	–	0.09	0.19	0.10	–

* DO: Dissolved oxygen; * TDS: total dissolved solids.

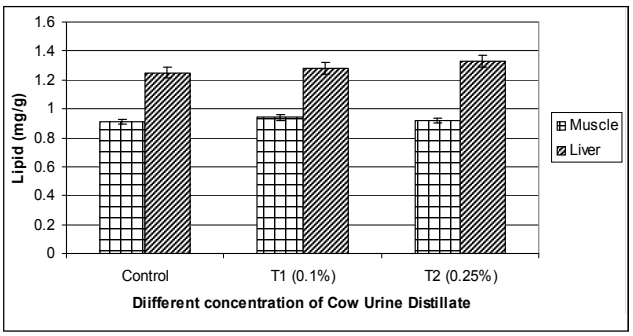

Figure 3: Change in the total lipid content (mg/g wet weight of the tissue) in different tissues exposed to Cow urine distillate of different concentrations

sensitive aquatic lives. The DO levels measured in the tank showed maximum levels T (5.2 mg/L), C (5.1 mg/L) when compared to T2 (4.7 mg/L). The ammonia levels showed at maximum in T1 (0.57 mg/L) when compared to control (0.50 mg/L); T2 (0.47 mg/L).

DISCUSSION

Fish being the major protein source for humans, the market value of fish depends on its nutrient value. Hence, finding a strategy to increase the nutrient contents will be of economical importance. In this regard, the present investigation paves a way for it in an eco-friendly manner. Macro and microelements have several significant-biological functions in living organisms, for instances serving as constituents of skeletal and soft tissue structures, necessary for transmission of nerve impulses and muscle contraction, maintenance of osmotic pressure, roles in the regulation of pH of blood and other fluids in body, and are vital for function of many enzymes, hormones, vitamins and respiratory pigments. Among the treatments, the fish T1 (0.1%) has higher carbohydrate content in liver. The highest glycogen content of liver is acceptable due to its involvement in carbohydrate and glycogen synthesis and its utilisation. Glycogen is the major storage form of carbohydrate in animals which occurs mainly in liver and muscle. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of blood glucose. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself (Maynard-Smith and Harper, 2003). In fish, the skeletal muscle glycogen is also an important store, but the concentrations found are generally an order of magnitude less than those in the liver (Heath, 1995).

Glycogen depletion in liver and muscle after toxic stress has been reported in several studies with aquatic animals (Bhavan and Geraldine, 1997; Aguiar *et al*., 2004). The significant decrease in liver, the vital organ and the site of the metabolism induces the toxicant effect, affecting the life processes, especially growth and reproduction. In other organs, it will lead to the disturbance in organ coordination and ultimately and definitely cannot lead a normal life. The earlier reports in this line of observations are Suneetha (2012); Swarnakumar *et al*. (2008), Tilak *et al*. (2003, 2005), Tilak and Marina Samuel (2001) and Susan *et al*. (1999). Hence, a high carbohydrate content in liver indicates a stress free environment and good health condition. The similar results were showed with cow urine of different breeds treated in *C. mrigala* (Padmapriya and Venkatalakshmi, 2014).

Protein being involved in the architecture and also in the physiology of the cell seems to occupy a key role in the cell metabolism. Bradbury *et al*. (1989) reported a decline

in protein level during quinalphos intoxication. The fall in protein content during stress may be due to increased proteolytic activity and deceased anabolic activity of protein. It is possible that the proteins from the tissues of the fish were utilised under stressful conditions and released into the circulatory system to meet the increased metabolic demand of the stressed fish. Moreover, the decreased protein content might also be due to tissue destruction, necrosis of disturbance of cellular fraction and consequent impairment in protein synthetic machinery (Bradbury *et al*., 1989). Tilak *et al*. (2001) reported that when the freshwater fish, *L. rohita* was exposed to sublethal concentrations of pesticide mixture of monocrotophos and fenvalerate, the protein content was decreased. The similar decreasing trend in total proteins was also reported in the liver, brain and gill tissues of *C. catla* under sublethal and lethal concentrations of fenvalerate by Susan *et al*. (1999). In contradiction, there is an increase in the protein content of the CUD treated fish. This shows that the fish were under stress free environment and also acted as good stress reliever.

The lipid content of liver and muscle was decreased (Table 1; Figure 3) due to the CUD treatments. In the present study, the lipid level is high in CUD untreated fish muscle (0.94 mg) followed by T2 (0.92 mg) and T1 (0.91 mg). In the low availability of carbohydrates, lipids sever as source of energy for supporting physiological functions of the body. Since the carbohydrate and protein are high in CUD treated fish, the lipid might be low which also indicates stress free environment. The fish with high protein, carbohydrate and low lipid content is the suitable diet for human consumption. Regarding that also, the CUD treated fish is highly beneficial.

Water quality in fish pond is affected by the interaction of several physico-chemical components that is pH, alkalinity and hardness to exhibit profound effects on pond productivity, availability of oxygen, the level of stress and ultimately on fish health. The physico-chemical characteristics of both soil and water are not static, but are dynamic and change with the introduction of fish species, provision of supplementary feeds, manures and fertilisers and other inputs. Both the soil and water qualities parameters of pond ecosystem undergo complex changes due to all these factors, as a sequence disrupting the aquatic life in pond (Ali and Salim, 2004). In aquacultural practices, water quality attributes are influenced by the various inputs regarding the requirements and biochemical conversion related to metabolism of cultured aquatic organisms (Milstein and Svirsky, 1996; Chatterjee *et al.*, 1997; Milstein *et al.*, 2003; Ali *et al*., 2001; Jha *et al*., 2004). pH values ranged between 8.1 and 8.6 during the present study of CUD treatment. No changes in CUD treatments, when compared to control.

Solubility of most metals in water is decreased as pH and salinity increase (Boyd, 2000). Water alkalinity in pond is typically influenced by pond soil and can be predicted from soil pH (Boyd and Munsiri, 1997). Water salinity plays significant roles in osmotic and ionic regulations of aquatic animals (Mateen *et al.*, 2004). During the present study, total salinity ranged between 1.0 and 2.6 mg/L for CUD treated fish. The highest salinity was observed in control (2.6 mg/L), the lowest was noted in T1 (1.0 mg/L).

Temperature is one of the most important and most changeable physical factors affecting living organisms. Temperature can affect reproductive systems, growth and development, morphological systems and the respiratory system. Respiration of *L. rohita* (Spanopoulos-Hernandez *et al.*, 2005) and *Penaeus chinensis* (Chen and Nan, 1993) increase with increasing water temperature. Lower water temperature seems to reduce the hyper osmoregulatory ability of juvenile Pacific white shrimp (Bett and Vinatea, 2009). The data in Table 1 show that higher values were recorded for the temperature in control (30.3°C) followed by T1 & T2 (30.1°C).

DO is one of the most important factors in aquaculture ponds, especially in semi-intensive and intensive culture systems, because it is essential in respiration, and its availability determines the capacity of aquaculture ponds (Boyd, 1990). A DO concentration of 5 mg/L or more is the ideal level for aquatic life. In the present study, the maximum DO was noted in T1 (5.2 mg/L) and when compared to control.

Turbidity is an important limiting factor in the productivity of a pond; it may be either due to suspended inorganic substances or due to planktonic organisms. During the present study turbidity ranged between 6.3 and 7.9 NTU. Conductivity is the ability of a substance

to conduct the electric current and depends on presence of various ions in the water. As most of the salts in water are present in the ionic forms, able to conduct current, therefore, conductivity is rapid measure of total dissolved solids. During the present study, conductivity was found to be varying between 152 and 362 mg/L. Total dissolved solids varied between 2.0 and 3.2 mg/L in CUD treatments. The highest value for total dissolved solids was noted in T2 3.2 mg/L as well as least value 2.0 mg/L noted the same groups.

Ammonia is the main nitrogenous excretory product of most aquatic animals including crustaceans (Forster and Goldstein, 1969) and is the product of the ammonification of organic matter in culture systems (Chen *et al.*, 1990). Ammonia is highly toxic to marine species (Armstrong *et al.*, 1978). Unionised ammonia is highly lipid soluble with the capability to diffuse across the cell membrane and can be detrimental to shrimp at high concentrations. Fishes are very sensitive to unionised ammonia (NH_3) and in the present study, the range is between 0.09 mg/L and 0.57 mg/L. The ammonia level in CUD treated was significantly reduced, when compared to untreated control group. The ammonia level was not increased in the treated groups when compared to control; and the results indicate the CUD is not stress for aquaculture. The present finding indicates that 0.1% concentration is optimum for the maximum growth of fingerlings of Indian major carps. Normally, in the case of high DO and high carbon dioxide, the toxicity of ammonia to fish is reduced. CUD has an enhancing growth and nutrient value increased in *L. rohita* fingerlings.

As literature reveals, the present study also confirms the potential of CUD in promoting fish health, which was expressed in good water quality and increased nutrient value. The literature records revealed that the CUD of Gir has the maximum efficiency in increasing growth rate, feeding rate and survival rate when compared to that of CUD of exotic breeds in *C. mrigala* (Padmapriya and Venkatlakshmi, 2014; Vasanthi and Venkatalakshmi, 2015) and *L. rohita* (Sattanathan and Venkatalakshmi, 2015). The native cows grown in Indian atmosphere can tolerate high temperature variation. The indigenous breed is less susceptible to the diseases. Hence, their CUD is more potent when compared to that of exogenous cows.

ACKNOWLEDGEMENT

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

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