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SUPPLEMENTATION OF COW URINE DISTILLED (CUD) AS GROWTH PROMOTER IN DIET ON FINGERLINGS OF *OREOCHROMIS MOSSAMBICUS* (PETERS)

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ABSTRACT

Over last decade aquaculture fish farming has been fast developing from traditional extensive system to semi intensive and intensive culture system. Most fish farms are used formulated, pelleted feeds. Identifying the importance of supplementary feeding, the present work was carried out to enhance the growth rate in *Oreochromis mossambicus* by feeding with supplementary feed with Cow Urine Distillate. Our feed formulation using cheap ingredients enhanced the growth of fishes. In the present investigation, CUD (Cow Urine Distillated) was used as a feed additive on growth performance of *Oreochromis mossambicus* fingerlings.

KEYWORDS: CUD, Supplementation, Growth, Feed, Food utilization, Survival, *Oreochromis mossambicus*

INTRODUCTION

According to the Food and Agriculture Organization (FAO), the world harvest in 2012 consisted of 91.3 million tonnes captured by commercial fishing in wild fisheries, plus 158 million tonnes produced by fisheries. In addition, 66.6 million tons were produced by aquaculture (FAO, 2014). The average annual growth rate of over 12.47% in aquaculture sector from 1990-1996 as compared to 1.64% in capture fisheries, has not only shown its potential to meet the food security in general but also protein malnutrition in particular..Among the top ten aquaculture producing countries in the world, India is the second largest aquaculture capture both by volume and value. In India fish farming is one of the oldest systems next to agriculture, animal husbandr, and integrated form. Fish production in India registered an impressive growth of 8 times during the last 55 years. In spite of phenomenal increase in fish production during the last 6 decades, the per capita availability of fish in India continues to be low at 8 kg, against the world average of 12 kg. A person needs 11 kg of fish per year (FAO, 1999). Fish is very important dietary animal protein source in human diet. It provides 26.8% of total animal meat and has been considered as the fast growing food contribute in Asia (Deigado et al., 2002).

Indian aquaculture has shown significantly values of growth rate than that of captures fisheries during last decades. Over last decade fish farming has been express developing from traditional extensive system to semi intensive and intensive culture system by increasing the fish stocking density to get the most out of the utilization of water resources. As density exceeds the natural carrying capacity, dependence shifts from natural food to nutritionally favorable exogenous feed to achieve best growth and production (Mukhopadhyay, 1998).

Tilapia is one among the most successful largely cultured finfish species in the globe, because of their fast growth rate and ability to feed low on the aquatic food chain. Moreover, tilapia is easy to reproduce and handling is having good resistance to disease and tolerance to wide range of ecological conditions. These are being found in over 100 countries (Balarin JD, Hatton JD, 1979).With the amplification of culture methods for tilapia species during recent years, it has

become necessary to provide complete rations to meet their dietary nutrient necessities. In Tilapia production, feed cost is the major part of the changeable costs and protein is the most expensive component of the feed. Thus, reducing the amount of protein, carbohydrate in tilapia feed is one of the most significant interests of aquaculture investigators.

The medicinal usage of cow urine is practiced in india from ancient days. Hence cow urine could be expected as a good immunostimulant and water quality enhancer. A number of diseases could be cured by the use of medicines derived from the cow. Though it is a cheap resource, its benefits are very high. The laboratory analysis of cow urine shows that it contains nitrogen, sulphur, phosphate, sodium, manganese, carbolic acid, iron, silicon, chlorine, magnesium, citric, succinic, calcium salts, Vitamins A, B, D, E minerals, lactose, enzymes, creatinine, hormones and gold acids (Gowen lock, A.H and R.J. McMurray, 1988). Cow urine meets the deficiency of these micronutrients in the body and maintains the balance of these substances and cure seven the so called incurable diseases (Nelson and Dean, 2009). Hence the attempt to apply cow urine in aquaculture will bring an integrated farming practice and will have interdisciplinary relevance. Recently, cow urine and its distillate has been examined for their effect on growth promotion in aquaculture *Cirrhinus mirgala* (Hamilton) (Padmapriya S.S 2014); growth and food utilization parameters of *Labeo rohita* (Sattanathan G 2014). Cow urine distillate known as 'Kamdhenuark' exhibited many biological activities including immunomodulatory potential (R.S. Chauhan et.al 2004) and antimicrobial effect (G.S.Achliya et.al. 2004). So in the present investigation, CUD (Cow Urine Distillate) was considered for its application in aquaculture.

For supplementary diet, the continued dependence on traditional food material such as rice bran, oil cakes and fish meal has led to increase in the prices of these components, which in turn determine the profitability of aquaculture enterprises (Kumar, 2000). Hence there is a want to identify good quality, cheaper, and readily available alternative resources so as to substitute the costly ingredients in the traditional supplementary diets (Kaur and Saxena, 2003). Using formulated made feeds along with other optimum management practices, successful attempts have been made to achieve carp production of 1517 t/ha/yr. Feeding the powdered silkworm pupae, oil cake, rice and black gram is used and formulate pelleted type feeds are also practiced. Identifying the importance of supplementary feeding, the present work was designed to develop supplementary feed using cheaply available *Bos indicus* and *Bos taurus* Cow Urine Distillate as ingredients and to promote the growth of *Oreochromis mossambicus* under laboratory conditions. In the present investigation, CUD (Cow Urine Distillated) was used as feed additive on growth performance in *Oreochromis mossambicus.*

MATERIALS AND METHODS

Animal Maintenance

Fingerlings of O*reochromis mossambicus* were procured from S.M. Fish farm, Swamimalai, Thanjavur District, TamilNadu, India and were brought to the laboratory in polythene bags filled with oxygen. The fingerlings were very carefully released into the plastic tub (70 lit capacities) from polythene bags for acclimatization of the fish fingerlings. Glass aquaria were washed to avoid fungal contamination and then sundried. Healthy fishes were then transferred to glass aquaria (Vol 20 lt) containing decholrinated tap water. Fish of both sexes weighing 1.3 ± 0.2g were used in the present study. They were regularly fed with formulated feed and the medium (Tap water) was changed to remove faeces and food remnants for during acclimatization period.

Collection of Cow Urine

The present research was done to compare Cow Urine Distillates of two breeds namely *Bos indicus* and *Bos taurus*. From each breed six cows were selected after obtaining certificate from veterinary doctor stating that they are

disease free. Cow urine was collected using sterile containers. The early morning first urine from each cow was collected and then the total urine collected from six cows was pooled together for distillation. Both cow breeds selected for study are maintaining in the Gosala (cow farm) at Govindapuram, in Kumbakonam, with same nutrition and maintenance conditions (Durga *et al.*, 2015).

CUD Preparation

The collected urine samples were distillated simultaneously at 50° C - 60° C using distillation apparatus for 5 – 6 hours (Arunkumar Sathasivam *et,al 2010*). The cow urine distillate (CUD) was stored in sterile glass containers and was used for treatment on the same day without storage (Durga., *et al.*, 2015).

Experimental setup &CUD Exposure

After two weeks of acclimatization, two groups of fish were treated with *Bos indicus* and *Bos taurus* cow urine distillate supplemented feed (T_1 &T_2).The treatment CUD was added at 0.1% concentration (v/w). Fish weight was recorded to the nearest mg or sgm and the total body length was measured to the nearest 0.1 cm individually.

Feed preparation & mode of feed

Three experimental diets were formulated for the fish. Two of them contained *Bos indicus* CUD (T_1) and *Bos Taurus* CUD (T_2) at a 0.1% concentration by adding directly to mixture (v/w); the control group was without supplementation with CUD(Table 1).Each of the growth treatment was fed with formulated feed of 2% total body weight (Venkatalakshmi and Ebanasar., 2012). The experimental fish were fed twice a day for an hour between 9.00am to 10.00am and 4.00pm to 5.00pm.

Table:1 Formulation of experimental diet for fish

Ingredients / Groups	Soya powder(g)	Groundnut oil cake (g)	Wheat bean(g)	Wheat powder (g)	Vitamins and mineral(g)	Tapioca flour (g)	Supplementation
Control	400	250	200	40	10	100	1 ml of Distilled water
T1	400	250	200	40	10	100	1 ml of *Bos indicus* CUD
T2	400	250	200	40	10	100	1 ml of *Bos taurus* CUD

Morphological Growth Analysis and Food Utilization Parameters

The weight and length of individual fish were recorded individually at the initiation of experiment and then at the interval of 10 days. The fishes were weighted by digital electronic balance. Ruler was used to measure the total length from head and tip of caudal fin. The fingerlings were released in water immediately after body measurements to avoid stress. The unfed and faeces were collected and dried at 60º C in a hot air oven and weighed for calculating food utilization parameters. The growth and food utilization parameters were calculated by using the following formulae (Petursewiez and Macfutyen., 1970).

Growth Parameters were Calculated as follows

W_1 = Initial Weight, W_2 = Final Weight, WG= Weight Gain (g),GR=Growth rate (mg/day), ADG= Average Daily

Growth, SGR=Specific rate (%),PIBW= Percentage of increase in body Weight (%),T = Num of days.

$WG = W_2 - W_1$ (g)

$GR = (WG) / T \times W_1$ (g/day)

$PIBW = W_2 - W_1 / W_1 \times 100$

$ADG = W_2 - W_1$ / No of feeding days (gm/day)

$SGR = (\ln W_2 - \ln W_1) / T \times 100$

Food Utilization Parameters were Calculated as follows:

FR= Feeding rate, FA= Food absorbed, FC = Food consumed, AR=Absorption rate, AE = Absorption efficiency, GCE= Gross conversion efficiency**,** NCE=Net conversion efficiency, T = Num of days.

FR = Total dry FC / T X W_1 (mg. g/ body wt. /day)

FA = FC – faeces produced (mg. g./body wt./day)

AR = Total FA (dry) / T x W_1

AE = FA/ FC X 100

GCE (K1) = GR/ FR X 100

NCE (K2) = GR / AR X 100

Condition Factor (k)

The values of the condition factor “k” are estimated for comparative purposes to assess the impact of environmental alterations on fish performance (Clark and Fraser, 1983). 'K' factor was calculated for individual fish from the formula recommended by Schreck and Moyle (1990) as follows:

W= Weight, L= Length

$K = W / L^3 \times 100$

Survival Rate is Calculated by following Formulae

Survival rate = **(**Initial number of fish – mortality) / Initial number of fish X 100

Statistical Analysis

The test of significance was done with student’s-t test assuming unequal variance in MS-Excel.

RESULTS

Growth Performance

The growth response of *Oreochromis mossambicus* in terms of body weight, length, growth rate, specific growth rate (SGR), Average Daily Growth, Percentage of increase in body weight are presented in Table 2. The results revealed that on the 30th day, the Growth parameters were significantly higher in experimental fishes fed with CUD supplemented feed,

when compared to the control. The effect on growth rates and body length of treatment & control groups were compared in Figure1 & 2.

Table 2: Effect of CUD Supplement with fed on the Growth Parameters of *Oreochromis mossambicus* Fingerlings

Parameters	W_1(g)	W_2(g)	L_1(cm)	L_2(cm)	WG(g)	GR(mg/day)	ADG(g)	SGR%	PIBW%
Control	1.485	1.511	4.71	4.93	0.026	0.000583	0.00085	0.06	1.7508
T1	1.327	1.399	4.82	5.39	0.072	0.001808	0.00036	0.17	5.4257
T2	1.429	1.508	5.01	5.20	0.079	0.001842	0.00263	0.18	5.5283

W_1 = Initial Weight, W_2 = Final Weight,L_1 = Initial Length, L_2 =Final Length(cm),W=Growth(g),GR=Growth rate(mg/day), ADG= Average Daily Growth, SGR=Specific growth rate (%),PIBW= Percentage of increase in body Weight (%)

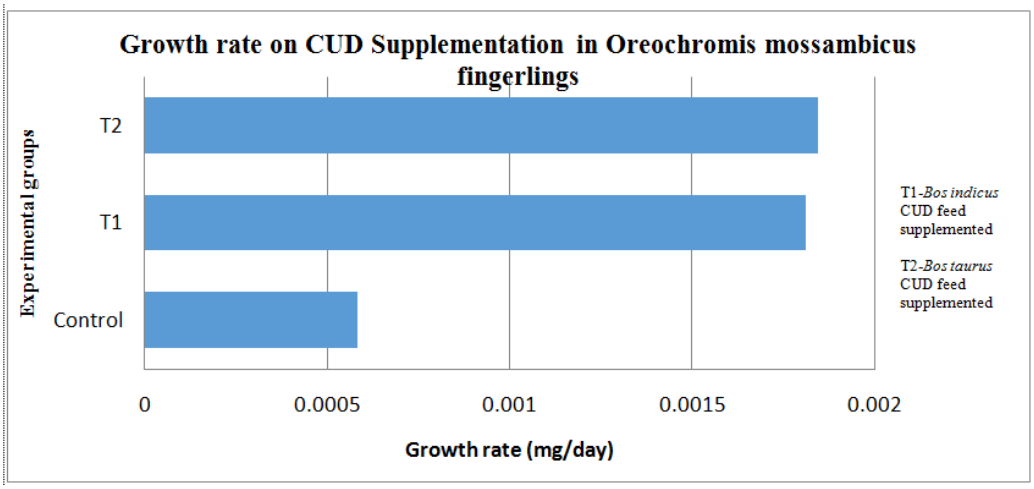

Figure 1

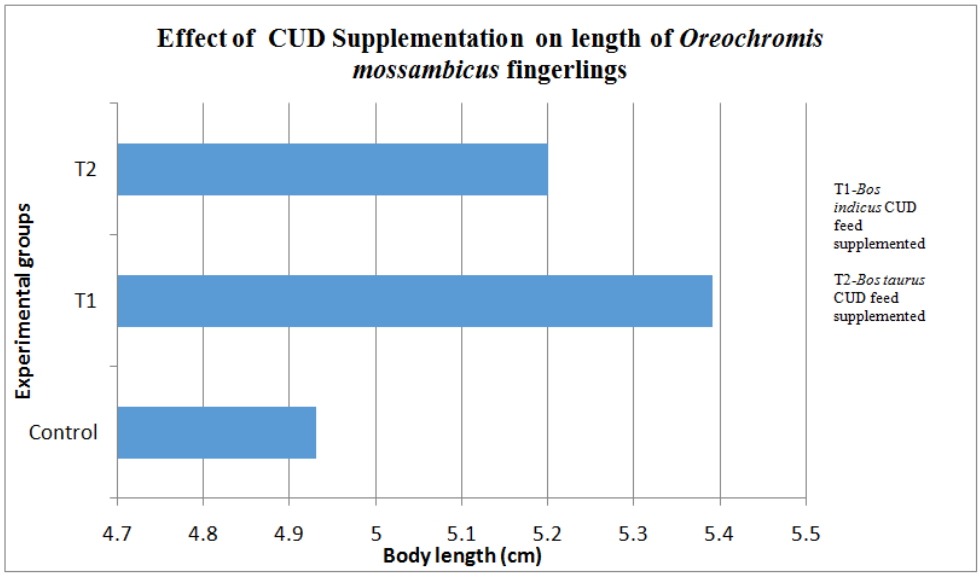

Figure 2

Food utilization Parameters

The effect of CUD supplementation on *Oreochromis mossambicus* 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 Cow Urine Distillate (CUD) supplemented groups, when compared to the control. The effect on Absorption rate is compared in Figure3.

Table 3: Effect of different Breeds of Cow Urine Distillate Supplement with Fed on the Food Utilization Parameters in *Oreochromis mossambicus* Fingerlings

Parameters	FR (mg/day)	FA (mg/day)	FAR (mg/day)	AE (mg/day)	GCE (%)	NCE (%)
Control	0.0031	0.1044	0.0023	75.542	16.129	21.739
T1	0.0022	0.0789	0.0019	83.846	81.8181	94.2648
T2	0.00222	0.0731	0.001	76.544	50.00	100.00

FR= Feeding rate, FA= Food absorbed, FAR= Food Absorption rate, AE = Absorption efficiency, GCE= Gross conversion efficiency**,** NCE=Net conversion efficiency, PER=Percentage of Feeding Rate, PAR= Percentage of Absorption Rate

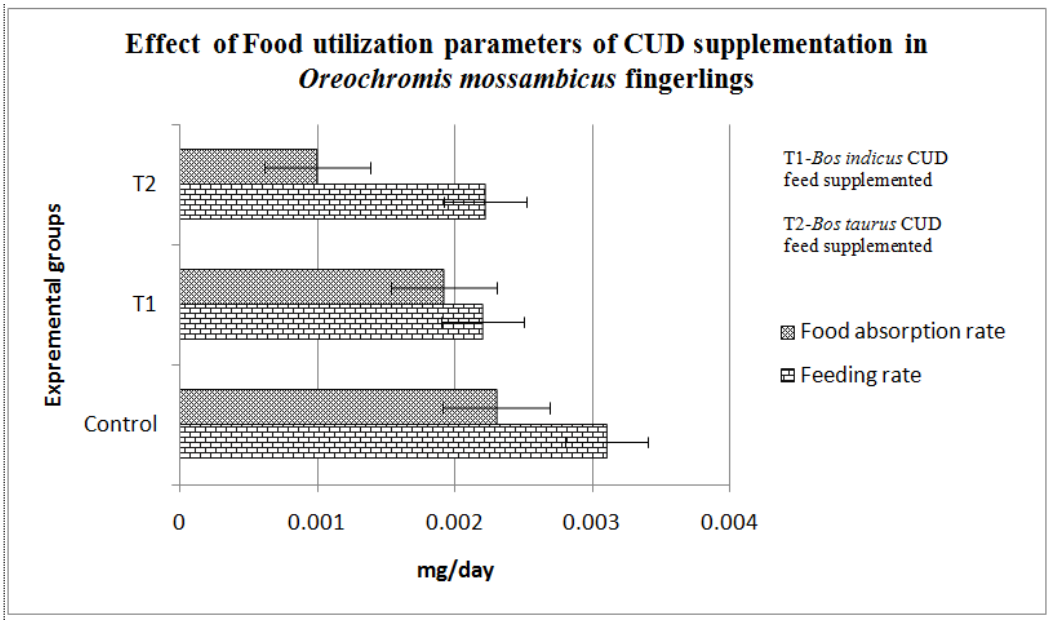

Figure 3

Survival Rate

The effect of Cow Urine Distillate supplementation fed on survival rate and mortality of *Oreochromis mossambicus* fingerlings are presented in table 4.The mortality was recorded at 10 days interval (Figure 4).

Table:4 Effect of CUD supplemented with feed on the survival of *Oreochromis mossambicus* fingerlings

Parameters	SR (%)	M (%)
C	30	70
T1	70	30
T2	60	40

SR = Survival rate, M = Mortality

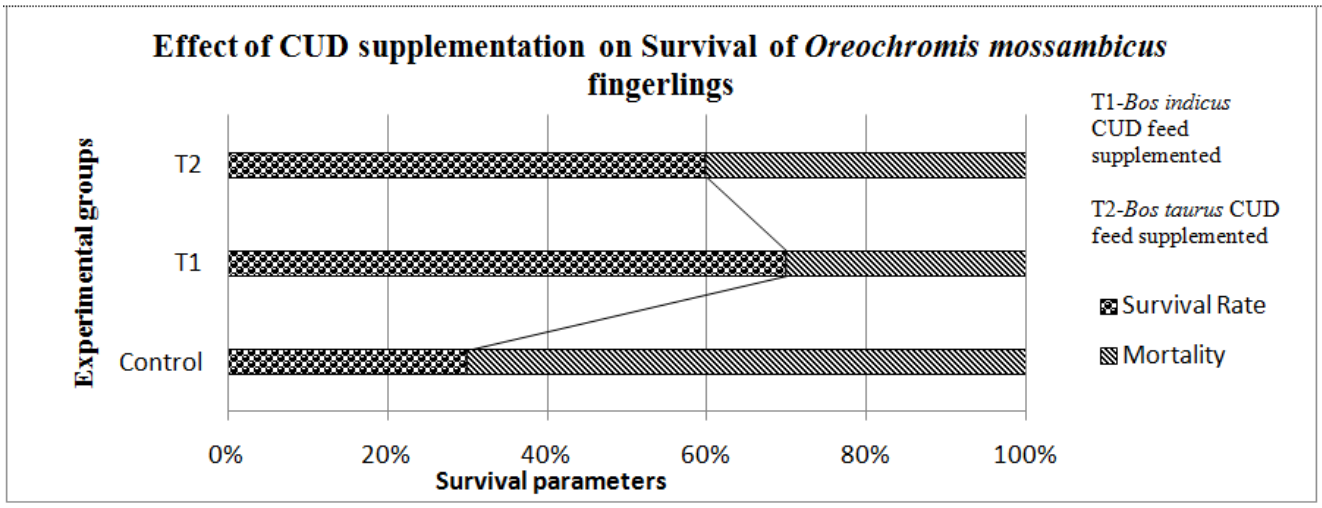

Figure 4

K Factor

In the present study, the condition factor of *Oreochromis mossambicus*, different treatment groups, the highest "k" factor recorded in T_1 group 1.26 and the lowest in value from the T_2 1.07 and control was 0.939 (Table 5).

Table 5: Effect of CUD supplemented with feed on the K factor value of *Oreochromis mossambicus* fingerlings

Parameters	K value
C	0.9397
T1	1.2610
T2	1.0724

DISCUSSIONS

The findings of present study have practical importance in maximizing the growth and survival of fingerlings by 0.1% CUD supplementation. The present study demonstrated that the CUD supplementation is efficient in *Oreochromis mossambicus* fingerlings for better growth performance at 0.1% concentration. Cow urine is well known for its medicinal properties. The investigations were undertaken to study the efficiency of cow urine distillate on growth,food utilization parameters and survival rate. The knowledge on the influence of any chemical in the environment over the growth and food utilization efficiency is essential for aquaculture practices in water bodies with such environmental conditions (Arunachalam *et al*., 1980 and Ramaneswari and Rao., 2000). Different authors reported the suitability of food components of both plant and animal origin for their ability to contribute better growth performance in cultured stocks (Sambu and Jayaprakash., 2001). Microbial probiotics are also used for enhancing growth (Ebanaser and Sheeja., 2003).

The potential of cow urine as growth enhancer has been recently studied in *Labeo rohita* (Sattanathan and Venkatalakshmi.,2015) and in *C. mrigala* (Padmapriya and Venkatalakshmi., 2014). However it has not yet been studied in feed route in *Oreochromis mossambicus*. In the present study the results confirmed that the Cow Urine Distillate supplementation with feed is capable of promoting growth and food utilization of cultured fishes as in the present experimental model of *Oreochromis mossambicus* fingerlings. Various growth promoters like vitamins, hormones and amino acids were used as growth promoters in different fishes and shrimps. Among the growth promoters, calcium plays a vital role in growth promotion as well as detoxification (Howrath and Sprague, 1978).

Increased levels of Calcium and hardness are also found to be having positive influence over growth promotion of *Cyprinus carpio* (Moni et al.,1994). Similar observations were also made by Navarathinam (1986) and Marimuthu (2003) in *Catla catla* and *L. rohita* respectively. Cow urine has been reported to contain calcium and hence it may be the reason for the promotion of growth. Cow dung is found to be an valuable source of organic fertilization, which positively influences the growth presentation of major carps of fish production (Sughra *et al*., 2003; Kanwal *et al*., 2003). Pond fertilization is a management protocol to enhance biological efficiency using both organic manure and inorganic chemical fertilizers. Evaluation of fertilizer value of different snatural manure (pig, cow,Duck, chicken and green manure) has been a subject of research in aquaculture (Green, 1990; Morissens *et al*., 1996; Yaro *et al*., 2005).

In semi-intensive polyculture system, the frequent application of organic manure, inorganic fertilizers, supplementary feed and stocking species ratio make the maintenance of production, population of natural food organism and the maximal utilization of productivity of pond ecosystem. Garg *et al*., 2005 evaluated the effect of distilled cow urine on the nutrient utilization by the white leghorn layers which showed increase in feed intake, decreased feed conversion ratio and feed efficiency ratio, digestibility of dry matter, crude protein, crude fiber and organic matter increased significantly in the cow urine treated group.

The present study is in confirmation with literature for the potential of cow urine distillate supplementation in promoting health, which was expressed as good survival rate, increased growth rate and feeding rate in *Oreochromis mossambicus* fingerlings.

Over last decade freshwater fish farming has been fast growing from traditional extensive system to semi-intensive and intensive culture method. Most semi-intensive fish farms are used formulated, pelleted feeds. Identifying the importance of supplementary feeding, the present work was carried out to enhance the growth rate in *Oreochromis mossambicus* with *Bos indicus* CUD (T_1) and *Bos taurus* CUD (T_2) supplemented diet. Our feed formulation has used cheap ingredients for enhancement of growth in fishes. For instance, the feed achieved the growth increment in terms of wet weight gain by 0.072g in *Bos indicus* and 0.079g in *Bos taurus*, as against the Control feed that produced the growth increment of only 0.026g in the *Oreochromis mossambicus* fingerlings. A similar observation was also obtained with rate of growth in terms of length. There was an increase of growth in terms of length by 5.39 cm in T_1 and 5.2 cm in T_2 fed with the formulated feed as against only 4.71 cm in the fed with Control feed. The T_2 group showed slightly modified to growth rate, food absorption rate, Gross conversion efficiency & Net conversion efficiency but low “k” value, survival rate and length when compared to the T_1 group *Bos indicus* CUD treated fishes.

The highest survival rate of 70% was recorded in the T_1, which is significantly higher ($P<0.005$) than the untreated control group having very low survival rate of 30%. T_2 shows a lesser survival rate of 50% when compared to T_1.

The values of the condition factor “k” are estimated for comparative purposes to assess the impact of environmental alterations on fish performance (Clark and Fraser, 1983). Therefore, the fluctuation in “k” may reflect the health condition of the fish as well as their lipid and protein contents (Weatherley and Gill, 1983). The obtained data are in agreement with Fletcher and White (1976) and White and Fletcher (1985) who found fluctuation in k values of fish and attributed these changes in k factors to the feeding rate, food absorption. However in the present research work *Oreochromis mossambicus* treated with *Bos indicus* CUD (T_1) is having good conditional factor value (“k” value) and survivality due to good environment conditions that lead to fish survival. Today the need of aquaculture is to produce healthily organisms in an ecologically safe and economically effective manner. Though pond fertilization with organic and

inorganic fertilizers is a very cheap and effective method of increasing productivity, their excessive use deteriorates the water quality (Boyd 1992, Garg and Bhatnagar 1996) and depletes the dissolved oxygen to detrimental level (Singh et al 2004). If cow urine is employed for promotion of growth and food utilization it will be a very cheap and effective. The method of administration through feed also has practical feasibly and cast effectiveness in increasing productivity. Hence it can be concluded that *Bos indicus* CUD can fulfill the needs of aquatic farmers to increase fish production, interms sof quality and quantity at low cost.

Thus the present study proved that the feed formulated using Cow Urine Distillate (CUD) as a cheap ingredient have better growth promoting effect than the control feed that is presently used in the experiential study.

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