COMPARATIVE STUDY ON THE IMMUNOMODULATORY EFFECT OF *BOS INDICUS* AND *BOS TAURUS* URINE IN *OREOCHROMIS MOSSAMBICUS* (PETERS)

Thesis submitted to Bharathidasan University, Tiruchirappalli

in partial fulfilment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY IN ZOOLOGY

By

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(Ref.No:48085/Ph.D.K7/Zoology/ Full Time/ April 2013)

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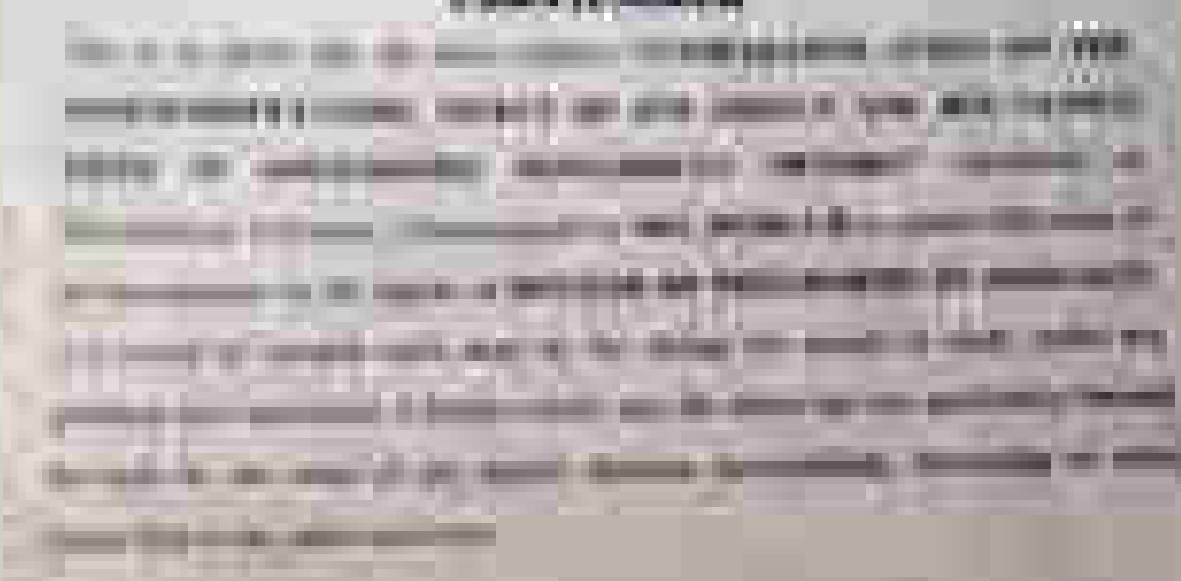
DECEMBER - 2017

Certificate



DECLARATION

I hereby declare that the thesis entitled "**COMPARATIVE STUDY ON THE IMMUNOMODULATORY EFFECT OF *BOS INDICUS* AND *BOS TAURUS* URINE IN *OREOCHROMIS MOSSAMBICUS* (PETERS)"** is a bonafide record of research work done by me and no part of the thesis has been presented earlier for any other degree, diploma, fellowship or other similar title to any other university

KUMBAKONAM,
DECEMBER, 2017

(B.DURGA)

ACKNOWLEDGEMENT

I owe sincere and earnest thanks to almighty God for his unchanging graces and blessings throughout my research tenure.

First and foremost I would like to thank, my respected Guide ***Dr. S. Venkatalakshmi, M.Sc., M.Phil., Ph.D.,*** *Head & Associate Professor of Zoology, Department of Zoology, Government College for Women (Autonomous), Kumbakonam, Tamil Nadu for her valuable suggestions, unfailing help, intellectual criticisms and moral support throughout the study. Madam, really inspired me to think systematically and logically. She had confidence in me when I doubted myself, and encouraged me and brought out the good ideas in me. I am sure that she will be remembered in all my future endeavours. I feel really blessed to work under her guidance and I am truly rewarded.*

My profound thanks to ***Dr. P. Hemalatha*** *Principal i/c,* ***Principal,*** *Government College for Women (A), Kumbakonam, Tamilnadu,* ***Mrs. V. Lakshmi,*** *Former Principal i/c,* ***Dr. A. John Merina*** *and* ***Dr.M.Revathi,*** *Former Principals, Government College for Women (A), Kumbakonam, Tamilnadu for permitting me to provide all the facilities available in the college to complete the study.*

My sincere thanks to ***Mrs. R. Sumanthi Devi,*** *Former Head of the Department and* ***Mrs. S.P. Malliga,*** *(Rtd) Associate Professor of Zoology, Government College for Women (A), Kumbakonam, Tamil Nadu for their encouragement throughout the course of my study.*

My sincere thanks to all ***Faculties of Zoology,*** *Government College for Women (Autonomous), Kumbakonam for their constant encouragement and advice throughout my study period.*

I am ever obliged to the most respected traditional healer ***Mr.Sunil Mansinkha,*** *Go-Vigyan Anusandan Kendra, Nagpur, Maharastra for his benevolent support, and gave much valuable information and references about the cow breeds and cow urine during my studies.*

My heartfelt thanks to particularly ***Sri Anna - Sri Vittaldass Maharaj****, Founder of Sri Vittal Rukmini Samsthan, Govindhapuram, Kumbakonam, Tamil Nadu for his support, permit for cow urine collection, help and good will provided at the time of my research work.*

I express my thanks to ***Miss. Kalaivani Priyadharshini****, Assistant Professor of Zoology and Advanced Biotechnology, Centre for Fish Immunology, Lady Doak College (Autonomous), Madurai who gave initial guidance on techniques in fish immunology.*

My sincere thanks to ***Mr.Sirmasth.S****, S.S.M fish feed farm for allowing me to done my field study, and also own my thanks to* ***Mr.R.Natarajan****, Amman fisheries, Ammankudi for providing fishes.*

I am very grateful for having an exceptional doctoral committee and wish to thank ***Dr.K.Ramesh Kumar****, Assistant Professor, PG & Research Department of Zoology, Rajah Serfogji Govt. College (Autonomous), Thanjavur – 613 005, Tamil Nadu and* ***Dr.G.Mathan****, Assistant Professor, Department of Bio Medical Science, Bharathidasan University, Tiruchirappalli – 620 024., Tamil Nadu for their continual support and encouragement.*

It feasts my mind to thank my research colleagues ***Mrs.G.Priya, Mrs. S.S. Padmapriya, Mr.G.Sattanathan, Mrs.S.M. Logambal, Miss. V. Praveena*** *and* ***K.Divya*** *for their timely help and kind cooperation during the entire period of my study.*

My sincere gratitude to my friends ***Dr. R.Silambarasan, Dr.E.Suresh and Dr.A.Eswar Annadurai*** *Research scholars, Centre for Advanced Study of Marine Sciences, Annamalai University, Parangipettai, Tamilnadu for allowing and giving some ideas and suggestion to my study period.*

I cherish to acknowledge the benevolence of my beloved ***parents (Mr.Bose and Mrs. Sundaraselvi Bose)*** *and my dearest brothers,* ***(Dr.Mahalingam and Mr.Chokkalingam), my father-in-law (Mr.C.Thangasamy), mother-in-law (Mrs.Selvi Thangasamy) and my sister-in-laws*** *for their prayers, blessings, encouragement, enthusiasm, co-operation and support without which I would not have been able to successfully pursue the course of my study.*

I express my indebtedness to all family members for their perpetual, unreserved love and support. Words fail to express my appreciation to my loving husband. His dedication, endurance and persistent confidence in me, has resulted in the completion of the thesis. I am really indebted to him for the motivation, patience, understanding and sacrifices made for the fulfilment of my dreams, which will always remain as my strength and shield.

In this study, direct or indirect help and suggestions were received from a large number of individuals, books and published works. Sincere efforts have been made to acknowledge or to refer them; still it is not unlikely that some of them have not been acknowledged due to inadvertence. I thank each and every soul behind the endeavor of mine.

B.DURGA

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%	Percentage
˚C	Celsius
AE	Absorption efficiency
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase activity
AST	Aspartate aminotransferase activity
cm	Centimeter
cu mm	cubic milli meter
CUD	Cow urine distilled
dL	decimal liter
DLC	Differential leucocyte count
DO	Dissolved oxygen
FA	Food absorbed
FAO	Food and aquaculture Organization
FAR	Absorption rate
FCU	Fresh Cow Urine
FR	Feeding rate
g/gm	gram
GCE	Gross Conversion efficiency
GGT	Gama Glutamyl Transferase
GSI	Gastro somatic index
h/hr/hrs	hour/hours
Hb	Haemoglobin
Hct	Heamocrit
HF	Holstein Friesians
HSI	Hepato somatic index
ICZN -	Indian Council of Zoological Nomenclature
ITIS -	Integrated Taxonomic Information System
IU -	International Unit
L	Liter
LDH	Lactate Dehydrogenase activity
MALT	Mucosa associated lymphoid tissue
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mg	milli gram
Min/mins	minutes
mm	millimeter
mmol	milli mole
MSI	Muco somatic index
N	normality
NaOH	Sodium hydroxide
NCE	Net Conversion efficiency
nm	nano meter
OD	Optimal Density
PAR	Percentage of Absorption Rate
PBS	Phosphate Buffer Saline

PFR	Percentage of Feeding Rate
PVC	Packed cell volume
RBC	Red blood corpuscle
ROS	Reactive oxygen species
rpm	Rotate per minute
SE	Standard error
SG	Specific gravity
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum glutamate pyruvate transaminase
SSI	Spleno somatic index
TDS	Total dissolved solids
TEC	Total erythrocyte count
Temp	Temperature
TLC	Total leucocyte count
TPC	Total Phenolic Content
TSI	Thymo somatic index
U	Unit/Units
v/v	volume/volume
v/w	volume/weight
µl	micro litter

Aquaculture is one of the major sources of providing food and income in several countries, which is also remaining Asians significant base for nutrition and livelihood of millions of people around the world (FAO, 2016). The countries with abundant aquaculture development could play a vital role to national economic through trade, tax revenue and license fees (World Fish Center, 2011). The aquaculture is mainly used to produce seafood for human needs, increasing wild fish, shellfish and plant stock for harvest. In India, fisheries found to be a major economic activity that is flourishing with various resources and potentials. After the independence of the country, the aquaculture has been recognized as an important sector of agriculture. The growth of sector can be identified clearly from the eleven-fold increase of production in last six decades as 0.75 million tonnes in 1950-51 to 9.6 million tonnes in 2012-13 (FAO,2014).

Fish continues to be one of the major traded food commodities around the world with more than half exported from developing countries. Production of fish through aquatic culture has transmitted the traditional method of capturing wild fish and increased number of farmed species. Especially, in 2014 marine aquatic culture fishes overtook the wild caught fishes for the contribution of human fish consumption (FAO, 2016).

Fish-including finfish and shellfish are an essential item in the human food basket (Adams, 2012). Fish contributed 17 percent of global animal-based protein supply for human consumption in 2010, and are the largest source of animal protein for approximately 1.3 billion people (Agnew *et al*., 2009). Above 75 % of fish consumption occurs in

developing countries (Alexandratos and Bruinsma, 2012). Fish contain important micronutrients like vitamin A, iron, zinc and long-chain omega-3 fatty acids that are essential for maternal health an early childhood growth, but that are frequently lacking in the diets of the poor (Allison, 2011). Almost nearly 12% of the global population depends on fisheries and aquaculture for their livelihoods, greater than 90 percent of those employed in these sectors live in developing countries (The State of World Fisheries and Aquaculture, 2014). However, the world's supply of wild-caught fish has long peaked (Aurora, 2013) and is unlikely to rise again unless over exploited stocks are rehabilitated. As global fish consumption continues to grow, fish farming has emerged to meet demand (Barrington *et al*., 2009). Already, just under half of all fish that people consume come from aquaculture, which is one of the world's fastest-developing animal food producing sectors (Belton and Little, 2011). With the source of wild-caught fish stagnant, any future growth in global fish consumption will need to be provided by aquaculture.

According to FAO the world produced 158 million tonnes (Mt) of fish in 2012. Wild-caught fisheries produced 91 Mt, which delivered 69 Mt of fish for people and 22 Mt for animal feed and other nonfood uses (Beveridge and Brummett, 2012). Aquaculture delivered another 67 Mt of aquatic product Crustaceans, Prawns and Shrimp (Beveridge *et al*., 2013). The world population is projected to reach 9.6 billion by 2050 (Bosma *et al*., 2009) and fish consumption is expected to rise in coming decades because of diet shifts resulting from increasing wealth and urbanization (Bouwman *et al*., 2013). For worldwide per capita fish feeding to rise from today's level without further pressure on wild fish stocks, aquaculture production will need to grade than double by midcentury (Bravo, 2003).

Doubling aquaculture production could significantly contribute to meet global animal protein demand in 2050. However, such a significant level of growth could also lead to substantial environmental impacts unless measures are taken to improve the sector's performance. Furthermore, the industry faces looming constraints of land, water, feed, and energy, which may limit its growth potential. It begins by examining recent trends in wild fisheries and aquaculture, along with projected aquaculture production growth to 2050.

India is one of the major countries which produce the fish through aquaculture, is home for more than ten percent of global fish diversity and presently ranks second in world's total food production after China. In last two decades India's inland fish production which includes capture and culture have registered massive growth and change. (Katiha *et al*., 2010). India's aquaculture production can be divided into two broad sectors as freshwater aquaculture and brackish water aquaculture. The freshwater aquaculture contributes to 55 percent of country's production while 45 percent from brackish water. The fishing sector has improved enormously since independence and has contributed immensely to the food basket of the nation (Ayyappan, 2003). The current population has been projected as 1.48 billion in the year 2030 leaving a wider gap between supply and demand of food; this gap has to be fulfilled by aquaculture production (Jayanthi, 2001).

The state Tamil Nadu is one of the leading states of India for fisheries development, the state is a pioneer in a country where the Department of fisheries was made during 1907 itself. Fish culture in the state had received remarkable attention as early as in 1911, subsequently by other states of India. The fisheries in the state play a major role in food security with 2,500 different species in the different aquatic environment. According to Tamil Nadu Fisheries Department, the state is having a second longest coastline in the

country with abundant natural resources for coastal aquaculture. The state contributes 10 to 12 percent of the total marine fish production in the country and total estimated potential area for brackish water aquaculture is 14,880 hectors in which only 4,455 hectors are used for culture, it shows there is vast area readily available for brackish water aquaculture.

According to state planning commission, 2014, the state fisheries sector has about 3.73 lakh hectors of water spread area with nearly 2.23 lakh inland fisherman directly depend on it for their livelihood. In the inland fishing industry, aquaculture contributes a major in increasing fish production, ensuring food security and growth of state's economy (Jayapal, 2014).

Especially, aquaculture plays a major role in rural areas to empower the livelihood through women participation (Shanthi *et al*., 2012). The state lies third place in marine fish production (Kasim and Vivekanandan , 2011) and 8th in inland fish production (State Planning Commission, 2014). The fisheries and aquaculture in the state promote rural livelihood, generates employment opportunity, complements food and nutritional security, contributes to gross domestic product and earns foreign exchange.

Even though, the aquaculture plays a positive role in providing employment, ensuring food safety and nutrient, increasing per capita income and country's economic, on the other side it creates severe environmental problems around its location. As same as food production in agriculture, aquaculture also affects the environment by modifying natural habitats, wildlife, soil, water and landscape (Dosdat, 2009). There are several studies have been done worldwide to assess the influence of aquaculture on the environment. Various type of chemicals are used during farming and operations in freshwater aquaculture as well as in marine water aquaculture, such as water/soil treatment

products, piscicides, herbicides, disinfectants, organic fertilizer, inorganic fertilizer, feed additives, aesthetics and therapeutics (Pathak *et al*., 2000).

It is estimated that around 1.5 million hectors of coastal lowlands were converted into aquaculture practice, mainly in China, Thailand, India, Indonesia, Philippines, Malaysia, Ecuador, Mexico, Honduras, Panama and Nicaragua. In some of these countries, the growth of aquaculture industries has generated several environmental problems (Paez-Osuna and Federico, 2001). Especially, the biggest issue arises when chemicals antibiotics are regularly used and many of which associated with serious health risk and potential for disrupting natural environment (Food and water watch, 2009).

The use of chemicals in the aquaculture decline the quality of water, land, surrounding environment as well as the health of fish. Therefore a natural input that can be used instead of chemicals in the aquaculture would solve the problems. There are several studies that analyzed natural nutrition for the management of aquaculture. Among this, cow urine is one of the major nutrition providers for the growth of fauna. An experiment conducted by Athithan *et al*., (2001) states that the use of cattle urine as a source of essential plant nutrients in carp production has increased the growth of carp production.

Tilapia are sometimes known as "aquatic chicken" due to their high growth rates, adaptability to a wide range of environmental conditions, and ability to grow and reproduce in captivity and feed on low trophic levels. As a result, these fishes have become leading candidates for aquaculture, especially in tropical and subtropical regions. Certainly, tilapia culture has been expanding rapidly and is now practiced in more than one hundred countries worldwide (Sayed, 2006). The total catch reported for this species to FAO for 1999 was 20,500 Mt. At present, the country ranks second in the world in total fish

production with an annual fish production of about 10.07 million metric tonnes. As the second largest country in aquaculture production, the share of inland fisheries and aquaculture has gone up from 46 percent in the 1980s to over 85 percent in recent years in total fish production (FAO 2012).The countries with the largest catches were Indonesia (18,190 Mt) and Papua New Guinea (2,310 Mt). Marketed fresh and freezing. Global tilapia production was 4,507,002 metric tons in 2012 (FAO, 2014), should exceed 4,800,000 MT in 2014 and 5,000,000 in 2015 (6% growth).

Fish diseases caused by *Aeromonas* and *Pseudomonas* are considered to be the major bacterial problems facing the aquaculture development causing mass mortalities, reduced production and low quality of aquatic organisms (Abdel Hadi *et al.,* 2009). Nevertheless, recent research has focused on understanding how the fish immune system responds to foreign agents or how natural resistance can be improved to produce a stock of fish with superior resistance (Galindo-Villegas and Hosakawa, 2004).

Many chemical entities either naturally arising or synthetic substances are known to stimulate the vertebrate immune system. Ingredients that are effective as immune stimulants in fish include chemical agents, bacterial components, polysaccharides, animal or plant extracts, nutritional factors and cytokines (Raa, 1996; Sakai, 1999 and Sealy and Gatlin, 2001, Aquaculture Science 2006; Sproul., 2000; Hashemi and Davoodi, 2012; Talmale *et al*., 2015). Immunostimulants raise resistance to infectious disease, not by enhancing innate, humoral and cellular defense mechanisms. It is well known that fish depends more heavily on nonspecific defense mechanism than do mammals (Anderson, 1992). Non-specific defence mechanisms such as the skin and scales and lytic enzymes of

the mucus and serum and cellular mechanisms like monocytes, macrophages, neutrophils and cytotoxic cells are present in fish (Secombes,1990).

To investigate the humoral and cellular factor activation it is necessary to study some biologically relevant assays such as complement activity, lysozyme production, phagocytosis, chemotaxis or the generation of reactive oxygen species (ROS). Several researchers have reviewed the importance of diet in fish immune response and found the diet to enhance disease resistance in fish culture (Landolt, 1989 and Waagbo, 1994).

Due to high resistance to some antibiotics applied in clinical practice, it is difficult to control *A. hydrophila* present in aquaculture systems (Riqueline *et al.,* 1996). The development of antibiotic resistant strains is making the use of drugs more and more ineffective. Also, transmission of resistance from resistant bacteria from aquaculture farms to bacteria of human and veterinary significance remains a major public health concern (Shariff, 1998). This bacterium can similarly be found in freshwater, marine, estuarine water. *A. hydrophila* can survive in aerobic and anaerobic environments. This bacterium can digest materials like gelatin and haemoglobin. *A. hydrophila* is very hard to kill as it is resistant to chlorine, refrigeration or cold temperatures. World demand for excellent quality protein foods has stimulated a rapid development of intensive fish culture techniques with the abundant use of chemicals.

Fish suffer from various diseases as they can carry different pathogens and parasites. Pathogens which can cause fish diseases include virus, bacteria, fungi, protozoa, water moulds etc., Fish are also exposed to various environmental pollutants, including drugs and chemicals. Common fish diseases include gill disease, dropsy, tail and fin rot, fungal infections, white spot disease, pop eye, cloudy eye, swim bladder disease, lice and

nematode worm infestations. Antibiotics are used to control bacterial diseases in fish. However, the non-specific immune functions like bacteriolytic activity and leukocyte function have been improved by some herbs (Sharma *et al.,* 2012).

In recent years, a new technique for the prevention of fish diseases is quickly emerging as a result of research into the growth of study on natural products. Fish immunology has a more current history than human and veterinary immunology as the methods used are similar. However, ways of administering the immunostimulants to fish differ and are dependent upon species, pathogen, temperature and environment.

Immunity is an important physiological mechanism in animals for defense against infectious disease agents and the maintenance of internal homeostasis (Ingram and Alexander, 1980). Oral immunization in cut throat trout against furunculosis has provided the first evidence that fish possess an immune response system (Duff, 1942). Vaccination of humans and other animals, including fish, is one of the major methods for preventing infectious disease (Potter and Baiuk, 2001). Immunization primes the immune system of the host against pathogens encountered during infections (Thompson and Adams, 2004). Fish immunization in the aquaculture production has been considered to be very important in reducing economic losses caused by disease (Ellis *et al.,* 1997; Rahman and Kawai, 2000 and Ebanks *et al.,* 2004). Several different kinds of vaccines have been investigated/developed against *A*. *hydrophila* including whole WC, OMPs, ECPs, LPS and biofilms, although currently no commercial vaccine exists (Poobalane, 2007).

However the application of vaccines in aquaculture has lot of constraints like labor, stress and economy. Livestock and fish population are affected by many infectious diseases which cause immune suppression leading to failure of vaccination or immunization against

these diseases. Despite timely vaccination by established methods, failure and breakdown of immunity have become common. Fish farming is always prone to a heavy risk of increased disease incidences leading to high mortality even after scheduled mass vaccination or immunization programmes are applied. To overcome these immune suppressive conditions, modulation of micro-environment of the immune system appears to be essential. This can be achieved by immunomodulators or immunostimulating compounds. Hence an attempt has been made to try Cow Urine Distillate as working immune modulator or immune stimulator.

Bhujel *et al.* (2010) reported the use of cow urine of as continuous pond fertilizer on a weekly basis as a splitting doze in Rainas Tar of Lamjung District in mid-hills of Nepal. A two year's project conducted to improve the empowerment of women through small-scale aquaculture used cow urine and proved it is as one of the suitable nutrients for the growth of fish.

Cow feces and urine are beneficial to sieving fish and omnivorous fishes (Kumar and Ayyappan, 1998). The collection of nitrogen-rich urine as a pond input has significant potential (Edwards *et al*., 1988). Cow urine not only provides nutrition for fish also helps for the growth of planktons in the ponds (Radheyshyam *et al*., 2012).

The cow urine has been known two US patents for its bio-enhancer properties particularly for the initial cure of tuberculosis and cancer in human beings. United States Patent and Trademark Bureau had given Patents No 6410059 and No. 6896907 to an "Indian discovery which has proved that cow urine can make antibiotics, anti-fungal agents and also anti-cancer drugs more effective". Manyother research also revealed the immunomodulatory properties of cow urine (Nair, 2002; Bhadauria, 2002; Banga *et*

*al.,*2005; Chauhan, 2004; Chauhan *et al.,* 2009; Chawla *et al.,* 2010; Tiwari, 2015 and Sunil Kumar *et al*., 2017).

Therefore, the present study has been taken in to analysis for the effect of cow urine in aquaculture, through which some significant results would be obtained for the development of healthy aquaculture in the future.

2.1 Experiment Animal: *Oreochromis mossambicus*

Taxonomy and distribution

From ITIS (2015)

Kingdom Animalia

Subkingdom Bilateria

Infrakingdom Deuterostomia

Phylum Chordata

Subphylum Vertebrata

Infraphylum Gnathostomata

Superclass Osteichthyes

Class Actinopterygii

Subclass Neopterygii

Infraclass Teleostei

Superorder Acanthopterygii

Order Perciformes

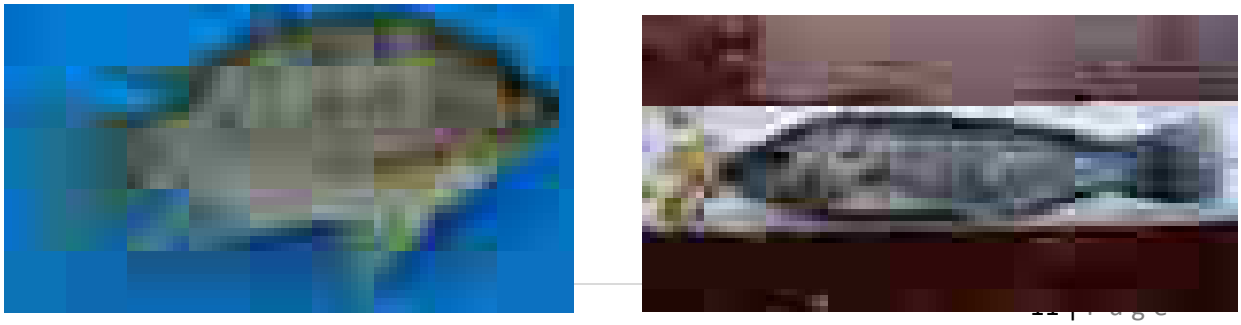
Suborder Labroidei

Family Cichlidae

Genus *Oreochromis*

Species *mossambicus* (Peters, 1852)

Plate 2.1 *Oreochromis mossambicus*



Preferred Scientific Name

Oreochromis mossambicus (Peters, 1852)

Preferred Common Name

Mozambique tilapia

Other Scientific Names

Chromis dumerilii (Steindachner, 1864); *Chromis mossambicus* (Peters, 1852); *Chromis natalensis* (Weber, 1897); *Chromis niloticus* (Linnaeus, 1758); *Chromis niloticus mossambicus* (Peters, 1852); *Chromis vorax* (Pfeffer, 1893); *Cromis mossambicus* (Peters, 1852); *Oreochromis mossambica* (Peters, 1852); *Oreochromis mozambica* (Peters, 1852); *Saratherodon mossambica* (Peters, 1852); *Sarotherodon mossambicus* (Peters, 1852); *Tilapia arnoldi* (Gilchrist & Thompson, 1917); *Tilapia dumerilii* (Steindachner, 1864); *Tilapia kafuensis* (Boulenger, 1912);*Tilapia mosambica* (Peters, 1852);*Tilapia mossambica* (Peters, 1852); *Tilapia mossambica mossambica* (Peters, 1852);*Tilapia mossambicus* (Peters, 1852);*Tilapia natalensis* (Weber, 1897);*Tilapia vorax* (Pfeffer, 1893).

Identification of fishes

Fishes were brought to the laboratory and species identification was done using the FAO species identification guide (De Bruain *et al*., 1994).

Scientific name	*Oreochromis mossambicus*
Description	Part of the Cichlidae family. Specie have established in Queensland - the Mozambique tilapia (*Oreochromis mossambicus*). *O. mossambicus* can grow to more than 36 cm and can live up to 13 years. They are usually dark grey or almost black but can be silver with 2-5 dark blotches/spots on the side. Breeding males can have red tips on their fins.
Diet	Omnivorous *O. mossambicus* feed mainly on plankton, insects and weed but will take a wide variety of other foods
Life cycle	Sexually mature at 3 years or less in favourable conditions *O. mossambicus* are able to reach sexual maturity at small sizes in poor conditions or when they are overcrowded. This is known as 'stunting' and results in large populations of mature fish with small body sizes *O. mossambicus* are mouth brooders - females protect eggs and larvae from predators by holding them in their mouths. Males build large circular breeding nests in soft silt or muddy substrate
Habitat	*O. mossambicus* are hardy fish and can survive temperatures between 8 and 42˚C, although they require temperatures of about 16˚C to remain active and feed. They can also withstand high salinities and low dissolved oxygen.
Impacts	**Environmental** Have successfully invaded and dominated many aquatic habitats due to their highly efficient reproductive strategy, simple food requirements and their ability to live in a variety of conditions have the potential to rapidly outnumber native fish and dominate aquatic communities can survive a range of environmental conditions which native fish find difficult to cope with. unlike many native freshwater fishes, tilapia are able to retreat downstream into highly saline waters during drought and move back upstream when conditions improve affect native species when competing for habitat and food, behaving aggressively and disturbing plant beds when building nests

	Social Loss of favorite fishing locations due to invasion and destruction caused by tilapia.
Control	Biosecurity Queensland advocates the ethical euthanasia protocols recommended by the 2001 ANZCCART publication: *Euthanasia of animals used for scientific purposes*. The most appropriate method may involve stunning the fish via a sharp blow to the back of the head just above the eyes. When applied correctly, this cause's brain destruction - the fish's gill covers should stop moving and its eyes should remain still. Intensive fishing may have the potential to reduce pest fish numbers in small enclosed water bodies, but it is very unlikely that fishing alone is an effective long-term control measure. **Poisoning** Poisons have been used to eradicate pest fish in ponds and small dams, but are not practical for rivers and streams as these poisons also kill native fish Tilapia is a restricted noxious fish under the *Biosecurity Act 2014*. It must not be kept, fed, given away, sold, or released into the environment without a permit.
Legal requirements	If caught these species must be immediately humanely killed and disposed of responsibly away from the water body. By law, everyone has a general biosecurity obligation (GBO) to take reasonable and practical steps to minimize the risks associated with restricted noxious fish under their control.
Taxonomic Status	Valid

Experimental fish selection

- Tilapia is often used as a good experimental model and is extensively used in genetic and physiological studies in relation to pollution, stress, or growth promoters.
- Tilapia eat a wide range of natural food organisms. It is economically and commercially important worldwide. It has resistance to stresses like transport, wintering and other causes which weaken the fish.
- It is tolerant to poor water quality, readily available in good numbers throughout the year, spawn spontaneously throughout the year and it is easily adaptable to lab conditions and it does not need much effort for maintenance.
- The very well standardized techniques of serial bleeding is available for *Oreochromis mossambicus* (Michael *et al*., 1994).

2.2 Collection and maintenance of fish

Fingerlings and adult of *Oreochromis mossambicus* were procured from S.S.M. Fish farm, Swamimalai and Amman fish farm, Ammankudi, Thanjavur District, Tamil Nadu, India were brought to the laboratory in polyethylene bags filled with oxygen. The fishes were very carefully released into the plastic tub (70 lit capacities) from polyethylene bags for acclimatization of the fish. Glass aquaria and plastic tub (70 lit capacities) were washed to avoid fungal contamination and then sun-dried. Healthy fishes were then shifted to glass aquaria (Vol 20 lit) for growth work and large 70 lit capacity tub for all other works. Fish of both sexes weighing approximate 1gm were used for growth studies and 20 - 25 gm for other parameters studied. They were regularly fed with

formulated feed and the medium (Tap water) was changed to remove faeces and food remnants during acclimatization period. Dechlorinated water was used throughout the study.

2.3 Cow Breeds Selection

The present research was done to compare Cow Urine Distillates of two breeds namely *Bos taurus* and *Bos indicus* with an intention to prove the superiority of Indian breeds and its importance in the field of aquaculture. The *Bos taurus,* the European cow, includes similar types from Africa and Asia. *Bos indicus,* the Zebu or humped cow originate in India. (ICZN, 2003). Present investigation is with Gir breed of *Bos indicus* and Holstein Friesian cross-bred of *Bos taurus* cows were selected. The average lifespan of *Bos indicus* is 20 years (Kolensnik, 1979) *Bos taurus* is 15 years.

Plate 2.2 Selected breeds for study

Bos indicus

Gir species are the hardiest of great 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. This breed is famous for their tolerance to stress

conditions and resistance to various tropical diseases.The Gir breed has been exported to other parts of the world also. It is most famous milk breed of the country mainly kept for milk production. Under good management conditions, the Gir cow produces between 1150 – 1600 kg of milk lactation. The normal milk production of this type in India is around 1400 kg per lactation period. Milk fat differs between 4.6 - 4.9%. Age at first calving is 45-55 month depending on the managemental practices and the 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. The Gir is a famous milk cattle breed of resistance to various tropical diseases. These animals contribute significantly to the total milk production of Gujarat State.

Bos taurus

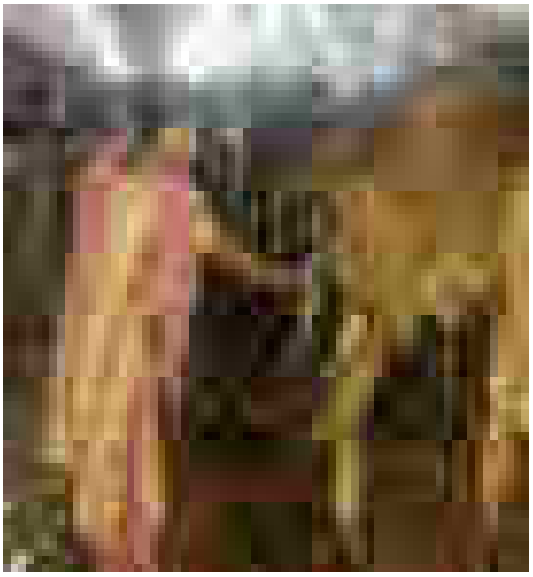
Holstein Friesians (often shortened as Friesians in Europe and Holsteins in North America) is a breed of cattle *Bos taurus* currently known as the world's highest-production dairy animals. Initiating in Europe, Friesiasns were breed in what is now the Netherlands and more specifically in the two northern provinces of North Holland and Friesland, and northern Germany, more specifically what is now Schleswig-Holstein Germany. The animals were the regional cows of the Frisians and the Saxons. The Dutch breeders bred and oversaw the development of the breed with the goal of obtaining animals that could best use grass, the area's most abundant resource. Over the centuries, the result was a high- reducing, white-and-black dairy cow. It is white and black due to artificial selection by the breeders. The breed currently averages 7655 litres/year throughout 3.2 lactations, with pedigree animals averaging 8125 litres/year over an average of 3.43 lactations. By adding, lifetime production hence stands at around 26,000

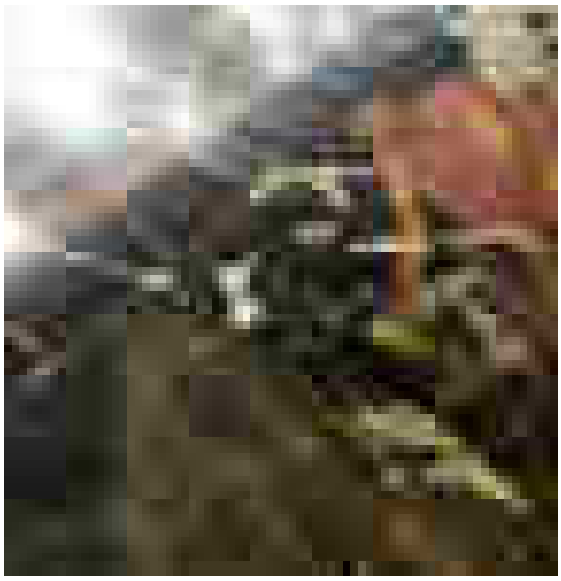
litres. In India, there are many indigenous cows crossbred with Holstein Friesian bulls and they are called HF – hybrids.

2.4 Collection of 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 a certificate from a veterinary doctor stating that they were disease free. The first few dribbles of the urine from the cow were not taken, because it is most likely to be contaminated with vaginal mucus. 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 maintaining in the Gosala (cow farm) at Govindapuram, in Kumbakonam, with same nutrition and environmental conditions. All the cows were fed with the concentrated feed and were provided clean drinking water *ad libitum*. All the animals were monitored carefully throughout the experimental period.

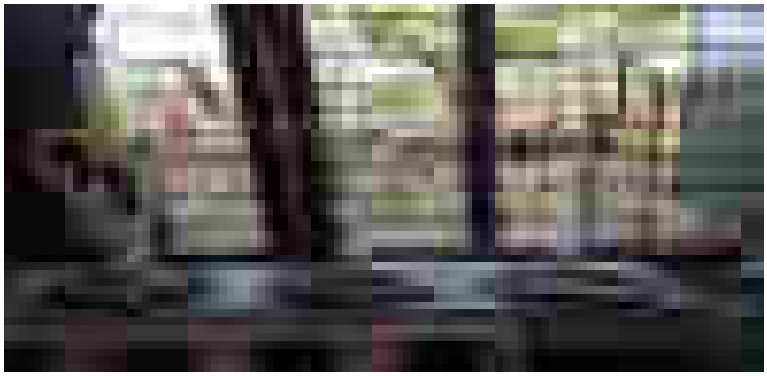
Plate 2.3 Cow Urine collection





2.5 CUD Preparation

Plate 2.4 Cow Urine Distilled (CUD) Preparation



The collected urine samples were distilled 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).

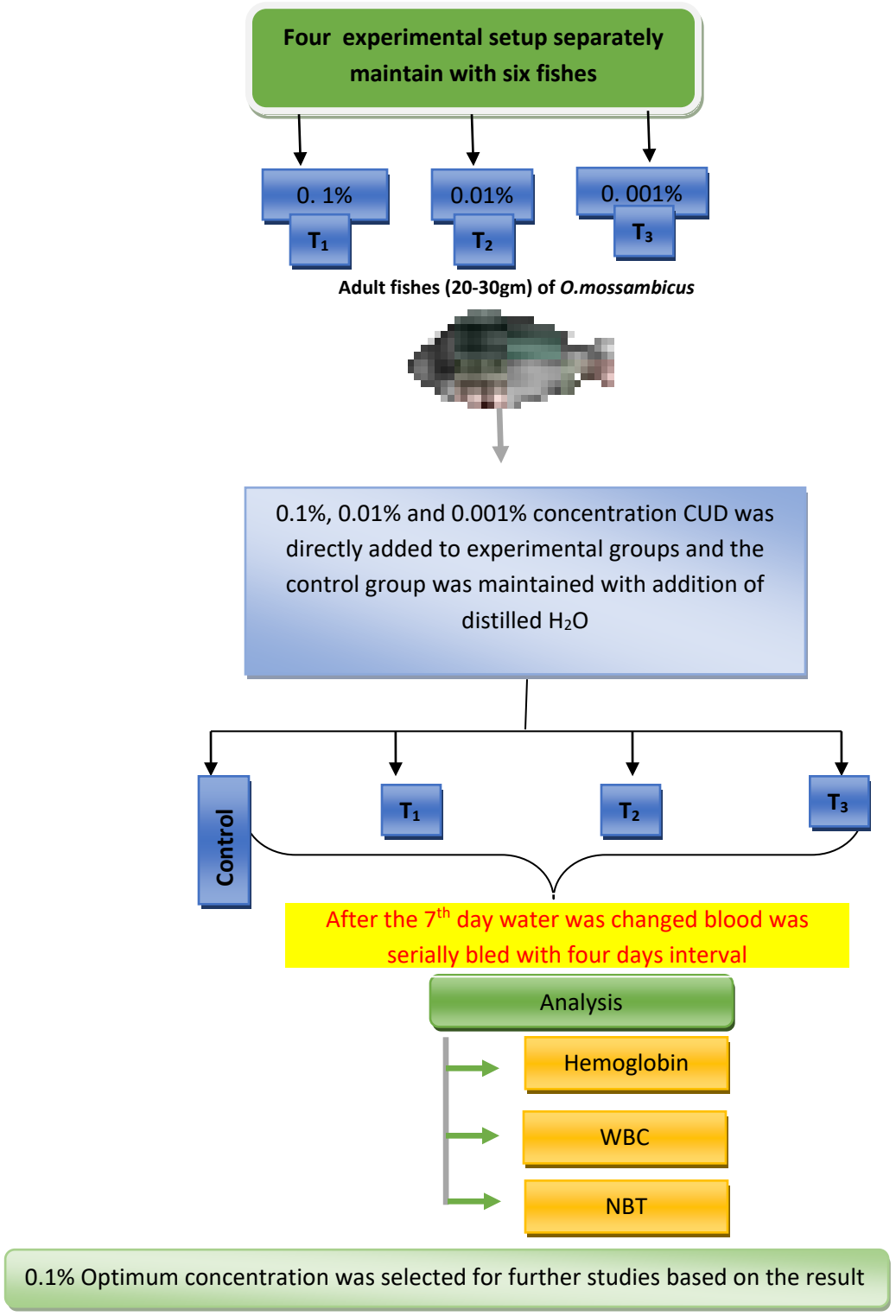
2.6 Food and feeding

Fish were fed *ad libitum* with a balanced fish diet prepared in our laboratory (Table 2.1) twice a day for an hour between 9.00 am to 10.00 am and 4.00 pm to 5.00 pm to avoid chronobiological changes. For other studies except growth and food utilization studies. For growth and food utilization studies fish were fed with a known quantity of 2% total body weight (Venkatalakshmi and Ebanasar 2012).

2.7 Dose fixation

Three groups of fish were treated with 0.1%, 0.01%, and 0.001% CUD of *Bos indicus* for one week by directly mixing CUD in water (Protocol 2.1). A control group was maintained separately. The effect of cow urine on the neutrophil activity, WBC and Hb were assessed. Based on the results the optimum concentration was selected as 0.1% (fig 2.1, fig 2.2 & fig 2.3) and the same dose was used for further studies.

Protocol 2.1: Experimental protocol for Optimal Dose fixation for CUD of *Bos indicus*



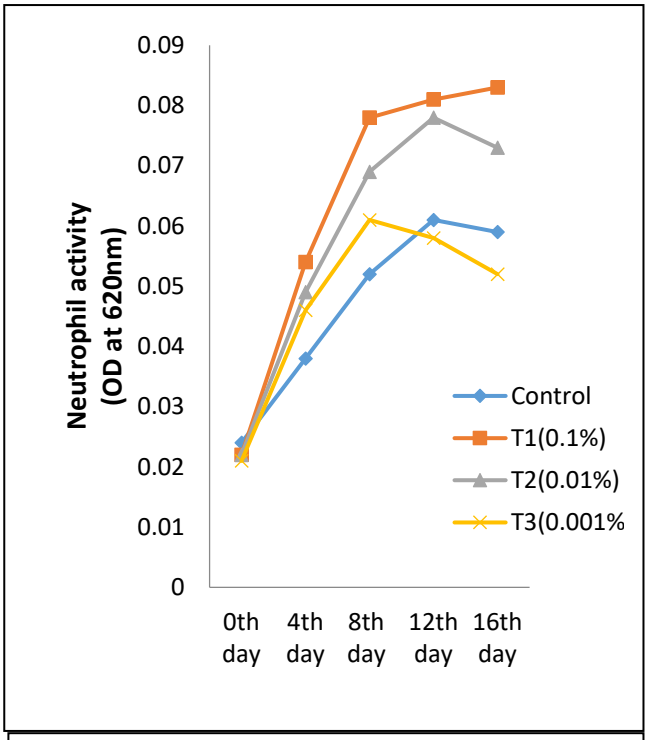


Fig 2.1. The effect of Cow Urine Distillate (CUD) on neutrophil activity of *Oreochromis mossambicus* in different concentration

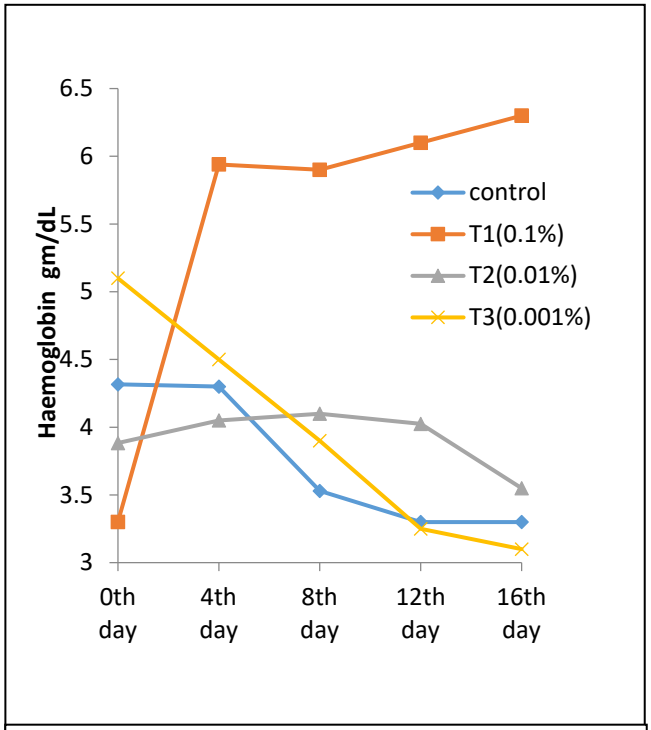


Fig 2.2. The effect of Cow Urine Distillate (CUD) on Haemoglobin level of *Oreochromis mossambicus* in different concentration

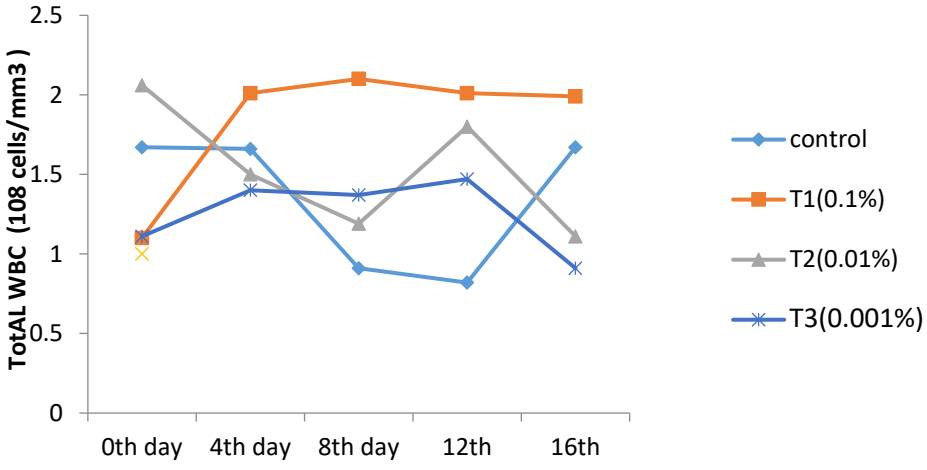


Fig 2.3. The effect of Cow Urine Distillate (CUD) on Peripheral Blood leukocyte count of *Oreochromis mossambicus* in different concentration

2.8 Administration route

The effect of CUD was analysed by administering through two different routes – Water additive and Feed additive at 0.1% concentration.

(i) Water additive

CUD is used as a water additive (i.e.) it is added directly in water at 0.1% concentration. Two groups of fish were maintained 0.1% concentration CUD of *Bos indicus* and *Bos taurus* were marked as T_1 and T_2 respectively and a control group was maintained separately without any treatments.

(ii) Feed additive

Two experimental diets were formulated. *Bos indicus* CUD (T_1) and *Bos taurus* CUD (T_2) were added to separate dough's at a 0.1% concentration (v/w), a control feed (C) was prepared without supplementation with CUD (Table 2.1).

Table 2.1 Composition of Experimental feed

Ingredients / Groups	Soya powder(g)	Groundnut oil cake(g)	Wheat bran(g)	Wheat Powder(g)	Vitamins and mineral mix* (g)	Tapioca flour(g)	Supplementation
Control	400	250	200	40	10	100	1 ml of Distilled water
T_1	400	250	200	40	10	100	1 ml of *Bos indicus* CUD
T_2	400	250	200	40	10	100	1 ml of *Bos taurus* CUD

***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 (1500mg),Maganese (1500 mg), Potassium (100 mg), Selenium (10 mg), Sodium (5,9 mg), Sulphur (0.72%), Zinc (9600 mg), Calcium (25.5%), Phosphorous (12.75%).**

The ingredients were mixed separately for various groups. Sufficient quantity of water was added to make the dough. Then the dough was steamed in a pressure cooker for 5 minutes. Then the dough was cooled sufficiently, and the 0.1% concentration CUD were added in *Bos indicus* and *Bos taurus* in the different dough that had been prepared separately. For the control feed, distillate water was added. The dough was pelletized in a hand pelletizer. Then it was dried in sunlight and room temperature. Samples of the various experimental feeds were taken. After the drying control and experimental feed were stored in airtight containers. Fish were fed with these experimental feeds supplemented with CUD for a week. Water was not changed during this period. After the treatment period, water was changed and all groups were fed with control feed for the rest of the experimental period.

2.9 Statistical Analysis

Statistical analysis of data involved Two-way analysis of variance (ANOVA) followed by Holm-Sidak multiple comparison tests. The levels of significance were expressed as p-value < 0.05. The data were expressed as a Mean ± standard error (SE) in the graph (Appendix). All statistical calculations and the graphical figure were performed using the software, Sigma Plot 13 and Ms-excel.

2.10 Experimental protocol

The work done could be categorized into six chapters.

I: Study of Immune Parameters influenced by *Bos indicus* and *Bos taurus* Urine Distillate

II: Study of Growth and Food utilization Parameters influenced by *Bos indicus* and *Bos taurus* Urine Distillate

III: Effect of Bio Chemical studies influenced by *Bos indicus* and *Bos taurus* Urine Distillate

IV: Effect of *Bos indicus* urine distillate in field

V: Comparative Study of Physical, Chemical and Microscopic characteristics of cow urine

VI: Effect of *Bos indicus* and *Bos taurus* Urine Distillate on water quality

Chapter I - 2.11 Study of Immune Parameters influenced by *Bos indicus* and *Bos taurus* Urine Distillate

2.11.1 Serial bleeding technique

The fish were bled regularly using 1 ml tuberculin syringe fitted with 24 gauge needle from the common cardinal vein situated just below the gills at operculum (Michael *et al*., 1994). The total time for bleeding a fish was around 30 seconds and the interval between two successive bleeding is four days for Haematological assay and weekly once for other Immunological assay. A control group was also bled in a similar schedule.

Plate 2.5 Serial Bleeding from common cardinal vein

2.11.2 Collection of blood

Blood was drawn directly from the common cardinal vein underneath the operculum with the help of disposable 1ml insulin syringe, containing 0.2% heparin as the anticoagulant. Great care was taken to avoid foaming when drawing the blood into the syringe as this readily resulted in hemolysis.

2.11.3 Serum separation

Blood collected in serology tubes was allowed to clot. The clot was stored overnight at 4°C and spun down at 400 g for 10 min. The separated serum was stored in sterile Eppendorf tubes at -20°C until further use.

2.11.4 Immunization

Seven days after the CUD treatment administered either as water additive or feed additive, water was changed and fish were immunized with an intraperitoneal injection of 10^9 heat-killed *A. hydrophila* cells to assess haemotological and immunological parameters.

2.11.5 Culture of *Aeromonas hydrophila*

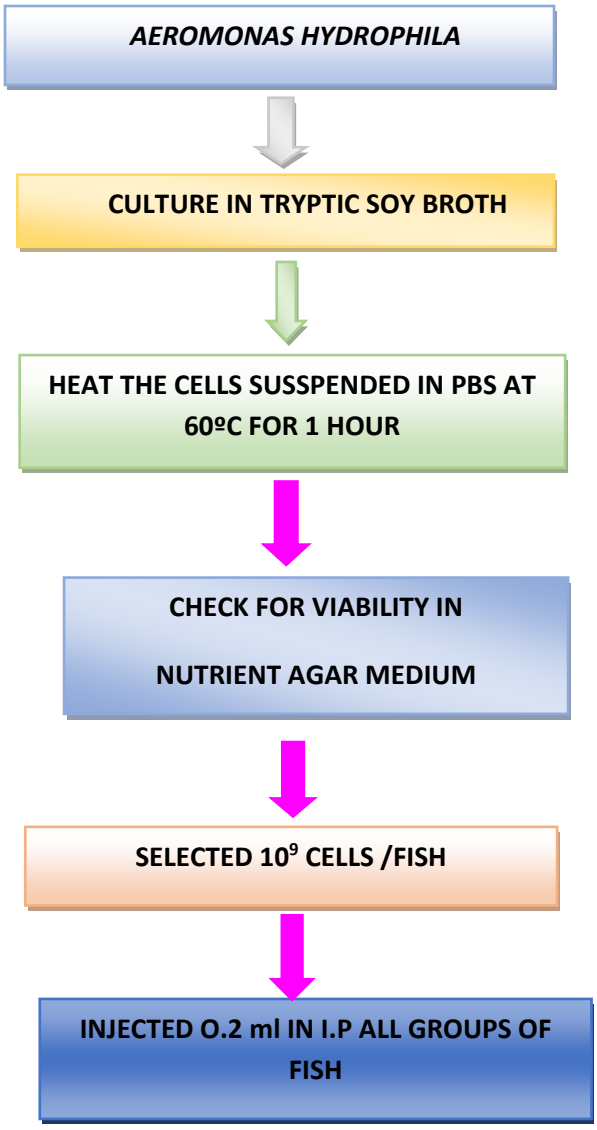
Aeromonas hydrophila is a causative agent for one of the major fish diseases in India was obtained from IMTECH, Chandigarh, India (MTCC-1729). It was cultured in Tryptic soy broth (Himedia, India) for 24 h at 37 °C in a rotary shaker for stock culture and streak plate culture was carried out for obtaining single colonies.

2.11.6 Preparation of heat-killed whole cell vaccine

A single colony of *A. hydrophila* from the agar plate was inoculated in the tryptic soy broth. After 24 hrs, the bacterial cells in the broth was washed and packed. The cells are resuspended in saline and heated to 60˚C for an hour in a water bath. The sterility was

checked by inoculating a sample on nutrient agar plates. The heat killed bacterial culture was centrifuged at 3000 rpm for 15 minutes Then the packed cells were collected and required dose (10^9cells) was prepared in PBS based on the enumeration of diluted samples in Neubauer counting chamber (protocol 2.2).

2.2. PROTOCOL FOR CULTURE OF *AEROMONAS HYDROPHILA* AND HEAT KILLED VACCINE PRODUCTION



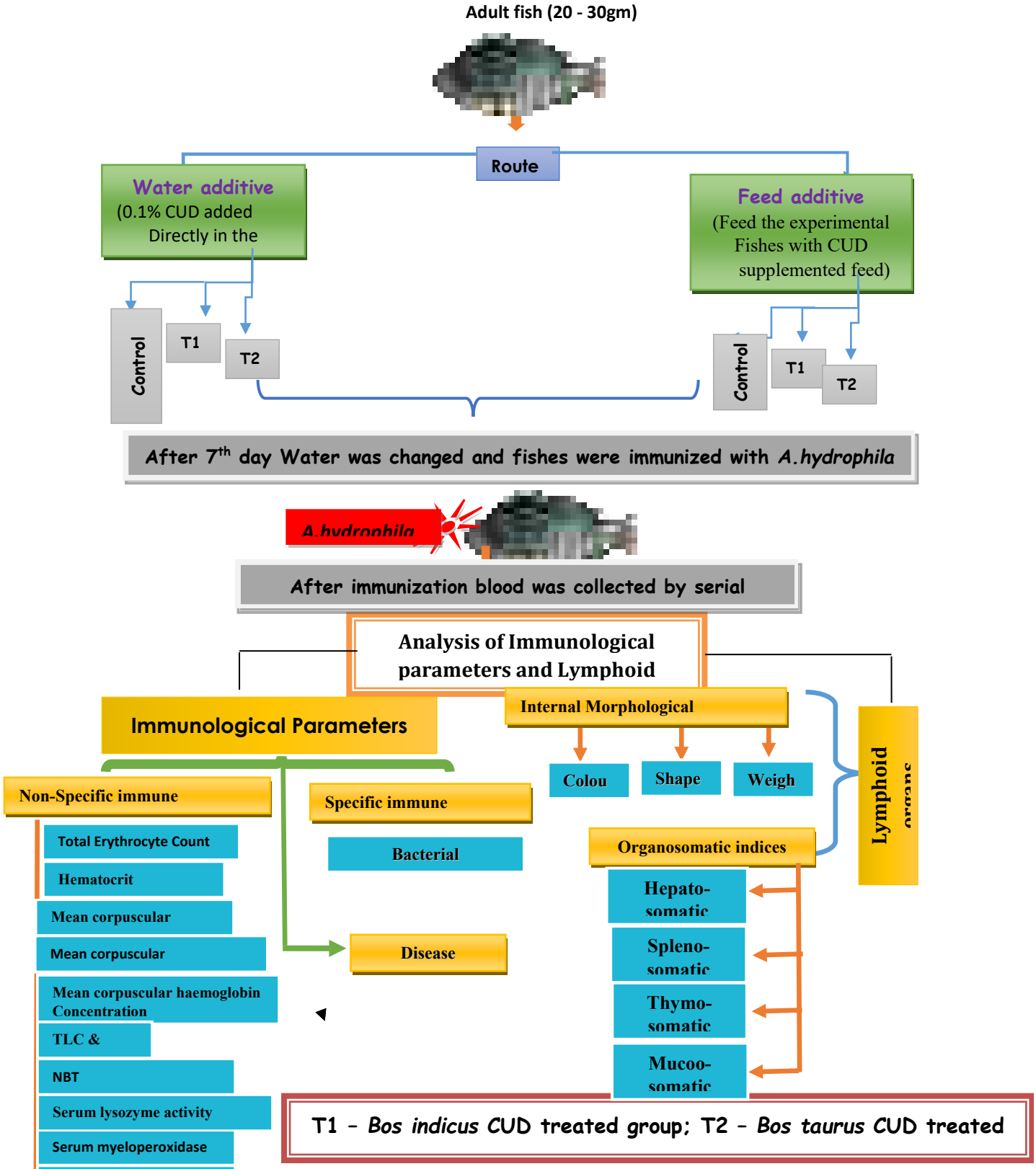
The immunological parameters of the experimental fishes were analyzed at regular intervals of four days for hematological parameters and other immunological parameters assessed seven days post immunization (Protocol 2.3).

2.11.7 Nonspecific immunological parameters

The nonspecific immune parameters assessed were

- Total Erythrocyte Count (TEC)
- Haemoglobin (Hb)
- Hematocrit (Hct)
- Mean corpuscular volume (MCV)
- Mean corpuscular haemoglobin (MCH)
- Mean corpuscular haemoglobin Concentration (MCHC)
- Total Leucocyte Count (TLC) and Differential leucocyte count
- Neutrophil activity
- Serum lysozyme activity
- Serum myeloperoxidase activity
- Serum Bactericidal activity
- Antiprotease activity

Protocol 2.3: Experimental protocol for study of CUD on Immunological parameters of Oreochromis mossambicus administrated through two different routes



a. Total Erythrocyte Count (TEC)

For RBC count, a method devised by Yokayama (1974) and later modified by Christensen *et al*., (1978) was followed. Hayems diluting fluid, which had the following composition, was used for RBC count:

Mercuric chloride	:	0.5gm
Sodium chloride	:	1.0gm
Sodium sulphate	:	5.0 gm
Distilled water	:	200ml

An improved Neubauer's counting chamber was used for counting RBC (Baker and Silverton, 1982). Using RBC pipette, the blood was drawn up to 0.5 mark and the diluting fluid to the mark 101. Although fluid is drawn to the mark 101 but the real dilution is 0.5:100 or 1:200 because the fluid in the capillary tube is discarded before the count.

The number of RBC's per sq mm was calculated as follows:

Area of a small square	:	1/400 sq mm
Depth of the counting chamber	:	1/10mm
The volume of Small Square is	:	1/4000cumm
The dilution of the blood is	:	1/200

$$\text{Total RBC} = \frac{N \times 4000 \times 200}{80} \text{ cu mm}$$

N = No of cells in 80 small squares

b. Haemoglobin percentage

0.1 N HCl taken in a tube up to the lowest mark (2% Mark). Draw blood up to 20 µl mark in the Hb-pipette and adjust the blood colum carefully without any bubble. Wipe

out excess of the blood on the sides of the pipette by using a dry piece of cotton. Transfer blood to the acid in the graduated tube, rise the pipette well and to stand the mixture for 10 min. Dilute the solution with distilled water by adding drop by drop few drops at a time carefully and mixing with reaction mixture until the color matches with the glass plate in the comparator. The matching must be done only against the natural light. Note the level of the fluid at its lower meniscus and the reading corresponding to this level on the scale is recorded in g/dl (Sahli, 1962).

c. Hematocrit (Hct):

Blood was taken by gill region inside of operculum with the help of disposable insulin syringe. Filled in the wintrobe tube with the blood with anticoagulant till the mark on top with Pasteur pipette. Ensured that, no air bubbles were left in the blood column. Centrifuged the contents of the tube for 30 minutes at 3000 rpm till packing was complete.

d. Mean corpuscular volume (MCV)

It defines the size of RBCs and is calculated by the formula and it is denoted in terms of fento liters (fl).

$$MCV = Hct \times 10/RBC\ Count$$

e. Mean corpuscular haemoglobin (MCH)

Average haemoglobin content of the single RBC is called as mean corpuscular haemoglobin. Mean corpuscular haemoglobin is calculated by the formula and its unit of measure is pg.

$$MCH = Hb \times 10/RBC\ count$$

f. Mean corpuscular haemoglobin Concentration (MCHC)

The average haemoglobin concentration in 100ml of hematocrit is called as mean corpuscular haemoglobin concentration. MCHC was obtained by the following formula and expressed in terms of percentage

MCHC = Haemoglobin × 100/ Hct

g. Total Leucocyte Count (TLC)

A white cell count (TLC) estimates the total number of white cells in a cubic millimeter of blood. WBC diluting fluid or Turk fluid contains a weak acid to lyse the red blood cells and Gentian violet stain for staining the nucleus of white blood cells.

The Turks fluid with following composition was used for TLC:

Glacial acetic acid	:	1.5ml
1% aqueous solution of Gentian violet	:	1 ml
Distilled water	:	100ml

Neubauer's hemocytometer (Baker and Silverton, 1982) was used for counting of leucocytes. Using white cell pipette, the blood was drawn up to 0.5 mark and the diluting fluid to 11 mark, thus the dilution was 1:20.

The number of RBC's per sq mm was calculated as follows:

Area of a small square	:	4 sq mm
Depth of the counting chamber	:	1/10mm
The dilution of the blood is	:	1/20

$$\text{Total WBC} = \frac{N \times 20/10}{4} \text{ cu mm}$$

N = No of cells in 80 small squares

h. Differential leucocyte count:

A tinny blood smear was prepared by spread a blood drop evenly on a clean grease free slide using a smoothly edged spreader. Giemsa's and Leishman's stains were employed for the staining of blood films. 100 leucocytes were counted by using the 40X objective high-power lens, in the blood smear.

i. Neutrophil activity

The NBT assay was assessed by following modified Stasiack and Bauman method (1996). 50µl samples of blood were taken from the experimental fish with a 1ml Tuberculin heparinized syringe. The collected blood was transfer into the flat bottom ELISA plate. The plate was incubated at 37ºC in a water bath for 1 hour (to facilitate cell adherence). The incubating plate was washed three times with PBS (100µl) to remove non-adherence cells. Add 100µl of 0.2% NBT a coloring agent and Incubate for one hr at 37º C. After incubation fix with 100% methanol (100µl) for 2-3 min after 3 min discard. Wash with 70% methanol (100µl).After washing plate was dry. Add 120 µl of 2N KOH and 140 µl of DMSO-mix properly. Take absorbance at 620 nm in ELISA reader.

j. Serum lysozyme Assay

Serum lysozyme activity was determined by using turbid metric assay described by Parry *et al*., (1965) with the microplate adaptation of Hutchinson and Manning (1996). A suspension of 0.3mg/ml Micrococcus lysodeikticus in 0.05M sodium phosphate buffer was used as the substrate. Ten microlitres of serum was added to 250µl of the bacterial suspension in 96-well microtitre plate and the reduction in absorbance at 490nm was determined after 0.5 and 4.5min of incubation at 28°C in a microplate reader (Biorad,

USA). A unit of lysozyme activity was defined as a reduction in absorbance of 0.001 min^{-1} (Ellis, 1990).

$$\frac{\text{Difference in OD x 1000 x 1}}{\text{10 x 4}}$$ (or simply) Difference in OD x 25 units/ml

k. Serum Myeloperoxidase activity

Serum Myeloperoxidase activity was measured according to Quade and Roth (1997) with partial modification (Sahoo *et al*., 2005). To 10µl of serum, 90µl of phenol red-free Hank's balanced salt solution (HBSS) containing Mg2+ and EGTA (Ethylene glycol tetraacetic acid) was added in a 96-well microtitre plate. To this mixture, 50µl of TMB (3, 3', 5, 5', -tetramethylbenzidine hydrochloride) -H_2O_2 (Genei, India) was added and incubated for 2 minutes at room temperature. 50µl of 2M H_2SO_4 was added to stop the reaction. OD was read at a microtiter plate reader at 450nm against 100µl of HBSS as blank.

l. Serum Bactericidal activity

Bactericidal activity was determined as described by Kampen *et al*., (2005) with modifications for plasma according to Welker *et al*., (2007). 20 µl of test serum, control serum and 20 µl of HBSS (without serum) respectively in 3 sets of duplicate wells in a 'U' bottom 96 well microtiter plate and incubated for 2 ½ hrs with aliquots of 20 µl 24 h culture of *A. hydrophila*. To each well, 25 µl of 3-(4, five dimethyl thiazolyl-2)- 2, 5-diphenyl tetrazolium bromide (MTT; 2.5 mg/ml) (Sigma) was added and incubated for 10 min to allow the formation of formazan. Plates were again centrifuged at 2000 ×g for 10 min, the supernatant discarded, and the precipitate dissolved in 200 µl of dimethyl

sulfoxide (DMSO). The absorbance of the dissolved formazan was read at 560 nm. Results were interpreted either as absorbance unit or as % control.

$$\text{Serum lysozyme} = \frac{\text{Sample OD}}{\text{Blank O.D}} \times 100$$

m. Antiprotease activity

Serum antiprotease assay was done as described by Bowden *et al.,* (1997). Briefly, to 10µl of serum, 20µl of trypsin (1mg/ml in 0.01M Tris HCl) was added in an eppendorf tube (A_2). The above said mixture without serum was maintained as trypsin blank (A_1). The eppendorf tubes are incubated for 5 min at room temperature. To this, 500µl of 2mM BAPNA (sodium-benzoyl- DL-arginine-p-nitroanilide HCl, SRL, India) was added. The volume was made up to 1 ml using 0.1M Tris HCl. The eppendorf tubes are incubated for 25 min at room temperature. To this mixture, 150µl of 30% acetic acid is added to stop the reaction. After that, 300µl of the mixture was taken to read the OD at 410 nm and the OD readings are used in the formula given below to express the results in terms of % Trypsin inhibition.

$$\text{Percent inhibition (\%)} = \frac{\text{Trypsin blank OD } (A_1) - \text{OD sample } (A_2)}{\text{Trypsin blank OD } (A_1)} \times 100$$

2.11.8 Specific immunity

Antibody titration

Antibacterial antisera were titrated using bacterial agglutination assay following the method of Karunasagar *et al*., (1997). It was performed in 96 well "V" bottom microtitre plates (Laxbro, Pune, India). The wells in the rows received 25µl of phosphate-buffered saline. In the first well of a row, 25µl of a sample antiserum was added and two-

fold serial dilutions were made in that row and the same was repeated in other rows with samples of other antisera. A volume of 25µl of heat-killed *A.hydrophila* suspension (prestained with 0.02% crystal violet) having a cell concentration of 10^9 cells/ml was added to each well. The microtitre plate was hand- shaken for effective mixing and incubated overnight at 37°C. The highest dilution of serum giving detectable macroscopic agglutination was expressed as a $\log_2$ antibody titre of the antiserum.

Plate 2.6 Bacterial Agglutination results

2.11.9 Disease resistance

Aeromonas hydrophila (MTCC 646) was inoculated into TSB 28°C shaking incubator at 24hrs. The culture was centrifuged at 800 × *g* for 15 min at 4°C. The packed cells were washed after the washing required dose was prepared with PBS. Fish were treated with CUD for seven days prior to vaccination with heat-killed *A. hydrophila* whole cells (10^8 cells/fish). A challenge dose of 2 x 10^6 cells/fish of virulent *A. hydrophila* always resulted in less than 50% survival in control fish group and was used as the standard challenging dose in this experiment. An untreated, vaccinated control group and an untreated, unvaccinated, saline-injected control group was maintained. Then all the groups of fish (n=6/group) were experimentally infected with the challenging dose of virulent *A. hydrophila*, four weeks after vaccination mortalities were recorded

after 96 hrs and degree of protection was assessed by calculating the relative percent survival (Knittel, 1981).

$$RPS = 1-n \frac{\% \text{ Experimental mortality}}{\% \text{ Control mortality}} \times 100$$

RPS was calculated by the formula following (Ellis, 1988).

2.11.10 Lymphoid organ

Like mammals, there are both primary and secondary lymphoid organs in fish. Primary lymphoid organs include thymus and head kidney. The secondary lymphoid organs include spleen and MALT.

1. Thymus

The thymus is the first organ to be lymphoid during histogenesis. The thymus starts developing as early as 24 hours after fertilization in tilapia species (Sahoo, 2006). Gradually the cells differentiate into thymocytes and epithelial cells. A detailed study by Sailendri and Muthukkaruppan, 1975 on the morphology and histology of thymus in *Oreochromis mossambicus* reveals that the thymus is present as a white opaque organ lying on both sides in the angle formed by the operculum and dorsolateral musculature in young adults. The thymus is essentially a lymphoepithelial organ surrounded by a thin capsule. It lies superficially in close association with the epithelial lining of the branchial cavity. It measures about 6mm x 3mm x 1mm (in fish weighing 45-50gm). It contains 11-13 x 106 thymocytes.

2. Head kidney

The head kidney is the modified pronephros at the anterior end of mesonephros. The head kidney serves as a stem cell compartment i.e. both as primary as well as a

secondary lymphoid organ (Rijkers *et al*., 1980) and appears to be the first organ that produces B-lymphocytes in teleosts (Zapata *et al*., 2006). The head kidney is an essential haematopoietic organ (Fange, 1986). The head kidney is situated above the body cavity, ventral to the vertebral column, just outside the peritoneum. In adult *Oreochromis mossambicus* (weighing 45-50gm), it is dark brown and it measures about 8-15mm in length and contains approximately 9-16 x 106 leukocytes (Sailendri, 1973).

3. Spleen

The spleen is the last organ to develop during histogenesis of the lymphoid organs of teleosts. Spleen is seen as a reddish-brown organ located as a slightly elongated flat structure lying along the left side of the stomach in close association with the pancreas. In adult *Oreochromis mossambicus* (weighing 45-50gm), it measures around 12mm in length and 4mm in breadth having 5-9 x 106 white cells (Sailendri and Muthukkaruppan, 1975). The spleen has a fibrous capsule and small trabeculae extend into the parenchyma, which divides the spleen into red and white pulps. The white and red pulps are not clearly distinct in many teleosts. The red pulp, occupies most of the part, containing all the cell types including red blood cells, lymphocytes and macrophages. The white pulp is poorly developed and contains melanomacrophages and ellipsoids. Ellipsoids are conspicuous in the spleen.

4. Mucosa-associated lymphoid tissue (MALT)

The mucosal surfaces of gut, gills and skin are protected by both humoral and cellular defence mechanisms. These tissues with their layer of mucus and array of nonspecific immune defences (Dalmo *et al*., 1997).

5. Collection of Organ

After overnight fasting, the fishes were weighed and sacrificed on 28th day post immunization. Gross pathological changes were looked for in the sacrifice fish. The lymphoid organs namely liver, kidney and spleen were collected. The organs were cleared off from the adnexal tissues using saline and placed on a blotting paper and gently pressed to remove excess saline adhering to the organ. The fish immune organs were assessed macroscopically. The weight of the organs were individually taken and based on the data recorded, organosomatic indices were calculated for all lymphoid organs. To get the organ weight to the body weight ratios of the fish as follows: Weight of the fish/ Weight of the Organ x 100. Morphological variations if any were observed in lymphoid organs.

a. Hepato - Somatic index

It is defined as the ratio of the liver weight of body weight. It is expressed as, HSI: Liver Weight/ Fish Weight x 100

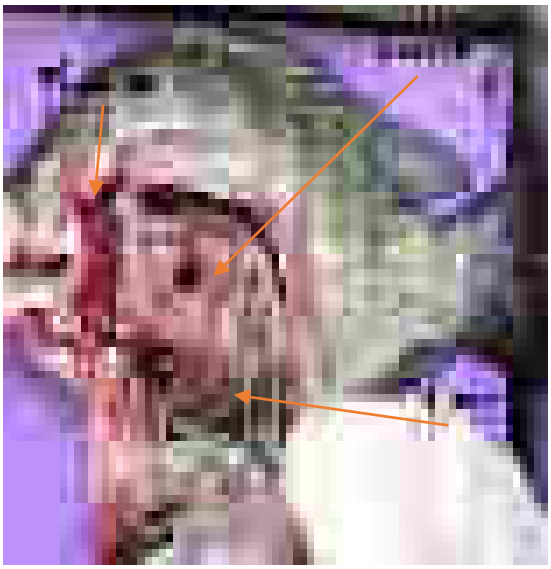
b. Spleno - Somatic index

The spleno-somatic index (SSI) is the weight of the spleen expressed as a percentage of total body weight. SSI: Spleen Weight/ Fish Weight x 100

c. Thymo - Somatic index

The Thymo - somatic index (TSI) is the weight of the Thymus expressed as a percentage of total body weight. TSI: Thymus Weight/ Fish Weight x 100

Plate 2.7 Lymphoid Organs



d. Muco - Somatic index

The muco-somatic index (MSI) is the weight of the Mucus expressed as a percentage of total body weight. MSI: Mucus Weight/ Fish Weight x 100

Chapter II- 2.12 Study of Growth and Food utilization Parameters influenced by *Bos indicus* and *Bos taurus* Urine Distillate

2.12.1 Experimental design

After two weeks of acclimatization, fish were treated with the CUD of both (*Bos indicus* and *Bos taurus* T_1 & T_2 respectively) breeds at 0.1% concentration through two different routes (water additive and feed additive). A control group was maintained separately without CUD treatment. After the treatment period of 7 days water was changed for both the experimental and Control groups (Protocol 2.4).

2.12. 2 Growth parameters

For length and weight, the fishes were measured individually at the interval of 10 days. The fishes were weighted by digital electronic balance (Systronics, India). The ruler was used to measuring the total length of the fish from head and tip of the caudal fin. The

fingerlings were released in water immediately after body measurements to avoid stress. Live weight and length of the experimental fishes were also recorded on 0^{th}, 10^{th}, 20^{th} and 30^{th} days. Based on this data following growth parameters were calculated (Petursewiez and Macfutyen, 1970).

Weight Gain = Final weight – Initial weight (mg)

$$\text{Growth Rate} = \frac{\text{Weight gain}}{\text{No of days x initial weight}} \text{ (mg. day}^{-1}\text{)}$$

$$\text{Specific Growth Rate} = \frac{\text{(Ln Final weight – Ln Initial weight)}}{\text{Num of days}} \times 100$$

$$\text{Percentage of Increase in Body weight} = \frac{\text{Final weight – Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Average Daily Growth} = \frac{\text{Final body weight – Initial body weights}}{\text{No of feeding days}} \text{ (gm day}^{-1}\text{)}$$

2.12.3 Condition factor

Condition factor of fish is expressed by relating the standard length of the fish to its weight (Beckman, 1948). 'K' factor was calculated for individual fish from the formula recommended by Schreck and Moyle (1990) as follows

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

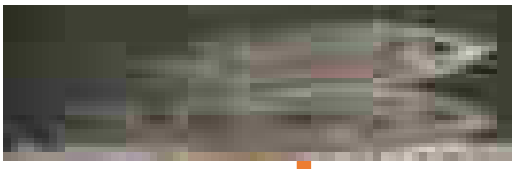
'K' is the coefficient of condition

'W' is the weight of fish (in gram)

'L' is standard length of fish (in cm)

Protocol 2.4: Experimental protocol for Growth and food utilization responses in CUD treated *Oreochromis mossambicus* fingerlings administrated through two different route

Fingerlings size 1.465±0.229gm



Routes

Water additive

(0.1% CUD added

Feed additive

(Feed the experimental Fishes with CUD supplemented feed)

Control | T1 | T2

Control | T1 | T2

Analysis

Growth Parameters

- Growt
- Growth rate
- Specific growth rate
- Average Daily growth
- Percentage increase body weight
- Survival rate

Food utilization Parameters

- Feeding rate
- Food absorbed
- Absorption rate
- Absorption efficiency
- Gross conversion efficiency
- Net conversion efficiency

T1 – *Bos indicus* CUD treated group; T2 – *Bos taurus* CUD treated group

2.12.4 Analysis of Internal Morphological Anatomy

Analysis of Morphological Anatomy was done using the following formula

a. Gastrosomatic index = Weight of the gut and contents x 100 / Weight of the fish

b. Relative length of gut = Intestine length / standard body length

c. Stomatogastric index = Total body length / Total intestine length

d. **Hepatosomatic index =** Weight of liver in x 100 / Weight of fish.

2.12.5 Food utilization parameters

i. Unfed and faeces collection

Faecal matter and uneaten feed in the experimental tanks were removed daily morning carefully using siphon method with least disturbance before the feed was given to the experimental and control fish. The unfed and faeces were dried after collection at 60º C in a hot air oven and weighed for calculating the following food utilization parameters (Petursewiez and Macfutyen, 1970).

$$\text{Feeding rate} = \frac{\text{Total dry food consumed}}{\text{No of days x initial live weight of fish}} \text{ (mg. g body wt}^{-1}\text{ day}^{-1}\text{)}$$

$$\text{Food absorbed} = \text{Food consumed – faeces produced (mg. g. body wt-}^{1}\text{ day}^{-1}\text{)}$$

$$\text{Absorption rate} = \frac{\text{Total food absorbed (dry)}}{\text{No of days x initial live wt. of fish}}$$

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

$$\text{Percentage of Feeding Rate} = \frac{\text{Total dry food consumed}}{\text{No of days x initial live wt. of fish}} \text{X } 100$$

$$\text{Percentage of Absorption Rate} = \frac{\text{Total food absorbed (dry)}}{\text{No of days x initial live wt. of fish}} \times 100$$

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

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

ii. Feed proximate analysis

Pellet stability

Pellet stability was tested by taking one gram of pellet in a wire gauze and dipping it in water for an hour. The wet content with gauze was removed without much disturbance and dried to a constant weight. All the three feed pellets (one control and two experimental) were separately pulverized, passed through 100 Micron sieve and analyzed for ash, dry matter, moisture, protein, fat, carbohydrate. Stability was expressed as a percentage.

Determination of moisture content (Loss on drying) (Harborne, 1973) **and Dry Matter**

The loss on drying test was to determine the amount of water and volatile matters in a sample that can be driven off under the condition specified (Desiccators or hot air oven). If the sample is in large crystals, then reduce the size by quick crushing to a powder. Shade-dried powdered materials weighed about 1gram were taken in a tared porcelain dish and heated at 110ºC for 5 hrs in an oven till a constant weight. Percentage moisture content and dry matter of the samples was calculated using the formula

$$Moisture\ (\%) = \frac{Loss\ in\ weight\ of\ sample}{Weight\ of\ sample} \times 100$$

$$Dry\ matter\ (\%) = 100 - Percentage\ of\ moisture$$

Determination of total ash value (The Ayurvedic Pharmacopoeia of India, 2001)

Accurately 3 gm of the air-dried powdered sample was weighed in a porcelain dish, which was previously ignited and weighed. The powdered sample was spread like a fine layer on the bottom of the plate. The dish was incinerated at a temperature 600ºC at 6 hours until free from carbon. The dish was cooled and weighed.

$$Total\ ash\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

Determination of water-soluble ash (The Ayurvedic Pharmacopoeia of India, 2001)

The ash obtained in the determination of total ash was boiled for 5 min with 25 ml of water. The insoluble substance was collected on an ashless filter paper or mesh cloth and washed with warm water. The insoluble ash was moved into a pre-weighed porcelain dish and ignited for 15 min at a temperature at not existing 450ºC. The weight of the insoluble content was subtracted from the weight of the total ash. The difference in weight was considered as the water-soluble ash. The percentage of water-soluble ash was calculated concerning the air-dried sample.

$$Water\ soluble\ ash\ (\%) = \frac{Weight\ of\ total\ ash - Weight\ of\ water\ insoluble\ ash}{Weight\ of\ sample} \times 100$$

Estimation of crude fat (Raghuramulu *et al*., 1983)

Weighed 2 gm of dry material or sample was extracted with ether to remove fat. After extraction, the calculation was done based on weight difference before and after extraction.

$$Crude\ fat(\%)\ =\ weight\ of\ the\ sample - weight\ of\ extract$$

Estimation of crude fibre (Raghuramulu *et al*., 1983)

Weighed 2 gm of dry material was ground with ether to remove fat. After extraction with ether 2 gm of dried material was boiled with 200 ml of 1.25% H_2SO_4 for 30 min with bumping chips. Filtered through a muslin cloth or filter paper and washed with boiling water until washings were free of acid. Then the residue was boiled with 200 ml of 1.25% NaOH solution for 30 min. Again it was filtered through a muslin cloth and washed with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portions of water and 25 ml alcohol. Remove the residue and transferred to an ashing dish (pre-weighed W_1 gm), dried the residue for 2 hrs at 130 ± 2ºC, cooled the dish in a desiccator and weighed (W_2 gm). Ignited the residue for 30 min at 600 ± 15ºC. Cooled in a desiccator and again reweighed (W_3 gm). The percentage of crude fibre was calculated by using following formula.

$$Crude\ fibre\ (\%) = \frac{\text{Loss in weighed on ignition (W2 } -\text{ W1) – (W3 – W1)}}{\text{Weight of sample}} \times 100$$

W_1- Pre-weighed ashing dish

W_2 - Ashing dish with dry residue

W_3 - Ashing dish with ash

2.12.6 Survival rate was calculated using the formulae:

$$\text{Survival rate} = \frac{\text{Initial number of fish – mortality}}{\text{Initial number of fish}} \times 100$$

Chapter III – 2.13 Effect of *Bos indicus* and *Bos taurus* Urine Distillate on the tissue and serum Bio chemistry

2.13.1 Effect of *Bos indicus* and *Bos taurus* urine Distillate on muscle and liver Biochemistry

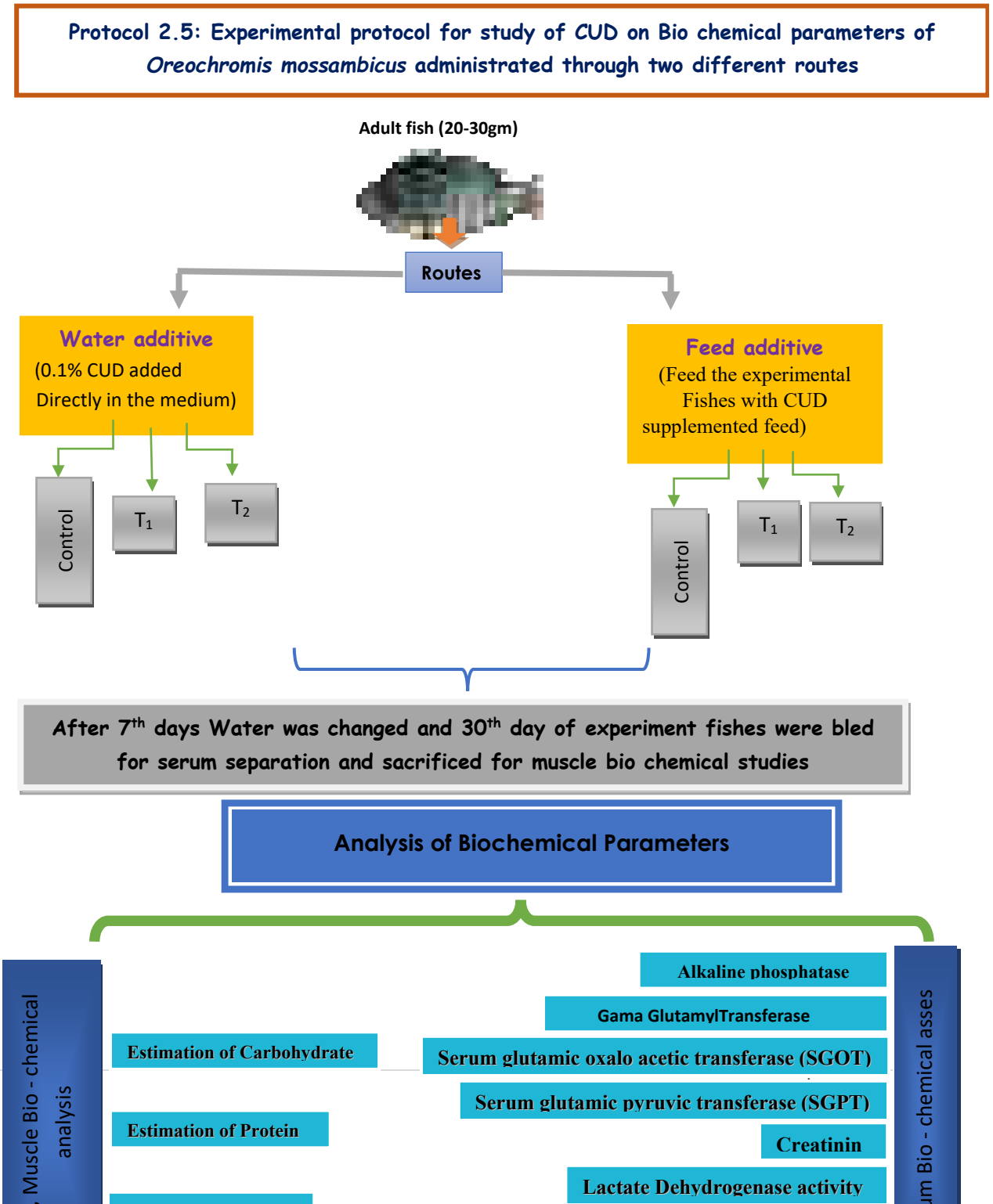
Fishes were weighed and sacrificed on day 30 experimental period was conducted on each carcass use study the biochemical composition of body and liver muscle. The same as followed both routes, water and feed supplementation administration (Protocol 2.5). **Estimation of Protein: (Lowery's method)**

Protein was estimated following the method of Lowry *et al*., (1951). Freshly weighed (100 mg) tissues were homogenized with 5% tricholoroacetic acid in a homogenizer. The homogenate was centrifuged at 3000 rpm for 10 minutes and the residue was dissolved in 0.1N NaOH. Exactly 0.2 ml of this solution was made up to 0.1 ml using 0.1N NaOH. To this 3.5 ml of Folin’s reagent was added and thoroughly mixed. After 30 minutes the optical density was measured at 670 nm in UV spectrophotometer.

a. Estimation of carbohydrate: (Anthrone Method)

Total carbohydrate was determined by Anthrone method (Roe, 1954). Freshly weighed (100 g) liver and muscle tissue were homogenized in distilled water (10 ml) and 5% tricholoroacetic acid in a homogenizer. The homogenate was centrifuged at 2500 rpm for 5 minutes. To every 1 ml of the supernatant 4 ml of anthrone reagent was added. A

standard glucose solution was also run along with the samples. The samples were boiled in a water bath (100 C) for 10 minutes for colour development. The optical density was measured in a UV spectrophotometer at 620 nm. The percentage carbohydrate content of the tissue was calculated using the OD of the unknown sample and that of standard glucose solution.



b. Estimation of Lipid: (Phospho-vanillin method)

Lipids were extracted as described by Folch *et al*., (1957), and estimated by the phospho-vanillin method of Barnes and Blackstock (1973). 50 mg of tissues were homogenized (5% w/v) in a waring blender in Chloroform-methonol mixture (2:1). The homogenate was filtered through whatman No.1 filter paper, and the residue was rehomogenised as before and then filtered. The non-lipid matter from pooled filtrate was removed by shaking vigorously with 0.88% KCl (added as one fourth of the volume). 1 ml of filtrate was taken in a test tube and evaporated under water bath (10 minutes) and 1 ml of concentrated H_2SO_4 was added boiled for 10 minutes. For estimation of total lipid, 0.2 ml of solution was taken and 5 ml of vanillin reagent was added. The developed colour was read in UV Spectrophotometer at 520 nm against reagent blank. The choloestrol was used as a standard.

2.13.2 Effect of *Bos indicus* and *Bos taurus* urine Distillate on serum Biochemistry

On the 30^{th} day of experimental period blood was collected from all group of fishes. The blood was stored and centrifuge for serum separation. Serum was separated by the same procedure as mentioned earlier (2.11.3).

a. Alkaline phosphatase

For the determination of alkaline phosphatase activity in serum following P-Nitrophenylphosphate. Kinetic, Liquid method was used with commercially available kit from €URO diagnostic systems Pvt Ltd.,

Reagent Preparation

1 mmol/L of Diethanolamine (DEA) at pH 10.4 was used as R_1 solution. 0.5 mmol/L magnesium chloride was used as buffer and 10 mmol/L of p -

Nitrophenylphosphate (pNPP) was used as R_2 Substrate.1 volume of R_2 with 4 volumes of R_1 was mixed as working reagent.

Procedure

1ml of Working Reagent, 20 µl of sample was taken in a test tube and mixed them and wait 1 minute. Read initial absorbance (A) of the sample, start the stopwatch and read absorbance at 1 minute intervals thereafter for 3 minutes at 405nm. Calculate the difference between absorbance and the average absorbance differences per minute (ΔA/min).

Calculation

ALP of U/L = ΔA/min x 2764

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L)

b. Gama Glutamyl Transferase

For the determination of γ Glutamyl Transferase activity in serum following Carboxy Substrate Method was used with Transferase kit from Coral clinical systems.

Reagent Preparation

Dissolve 1 substrate tablet in 2.2 ml of buffer reagent as working reagent.

Procedure

Two clean dry test tube were labelled as Test (T) and Control (C). 1 ml of Working Reagent was added both test tubes. After a minute 0.1ml sample was added into (T) test tube. 0.1ml of clean Distilled water was added in Control. Mix well and read the

initial absorbance A_1. Repeat the absorbance reading after 1, 2 & 3 minutes at 405nm. Calculate the mean absorbance change per minute (ΔA/ min).

Calculation

GGT Activity in U/L = ΔA/ min. x 1158

c. SGPT

For the determination of SGPT (ALAT) activity in serum following the IFCC method using the SGPT (ALAT) kit from Coral clinical systems.

Reagents Preparation

Reagents were prepared as per the directions given in the kit.

Procedure

1ml of working reagent was taken in a test tube 0.2 ml of sample was added and mixed well. At the 340nm absorbance reading was taken initially and after 1, 2 & 3 minutes. Calculate the mean absorbance change per minute (ΔA/ min).

Calculation

SGPT (ALAT) Activity in U/L = ΔA/ min. x 1746

c. SGOT

For the determination of SGOT (ASAT) activity in serum following the IFCC method using the SGOT (ASAT) kit from Coral clinical systems.

Reagents Preparation

Reagents were prepared as per the directions given in the kit.

Procedure

1ml of working reagent was taken in a test tube 0.2 ml of sample was added and mixed well. At the 340nm absorbance reading was taken initially and after 1, 2 & 3 minutes. Calculate the mean absorbance change per minute (ΔA/ min).

Calculation

SGOT (ASAT) Activity in U/L = ΔA/ min. x 174

d. Creatinine

Quantitative reaction of creatinine was estimated by using commercially available kit from €URO diagnostic systems Pvt Ltd.

Reagents Preparation

Reagents were prepared as per the directions given in the kit.

Two Point Kinetic Procedure:

Two test tubes were taken and labelled as Test (T) and Standard (S). In standard test tube 1ml of Working Reagent, 50 µl of Standard were added test tube (T) 1ml of Working Reagent, 50 µl sample were added. Mix well and read the absorbance (A) of standard (S) and test (T) against distilled water at 520 nm (505-570 nm) after 30 sec. (A_0) and 90 sec. (A_1).

Calculation

$\Delta As = A_{S1} - Aso$

$\Delta AT = A_{T1} - A_{TO}$

e. Lactate Dehydrogenase activity

For determination of Lactate Dehydrogenase activity in serum followed by IFCC method using the LDH (P L) kit from Coral clinical systems.

Reagents Preparation

Reagents were prepared as per the directions given in the kit.

Procedure

1ml of working reagent was taken in a test tube 0.2 ml of sample was added and mixed well. At the 340nm absorbance reading was taken initially and after 1, 2 & 3 minutes. Calculate the mean absorbance change per minute (ΔA/ min).

Calculation

LDH Activity in U/L = ΔA/ min. x 8095

f. Albumin

For determination of albumin in serum following the BCG method was used the commercially available kit from DIATEK.

Reagent Composition

BCG Reagent: 90 mmol/L of Succinate Buffer, 0.26 mmol/L of Bromocresol Green, 4.0 g/dl of Standard Concentration were used for preparation of BCG Reagent.

Procedure

Three test tubes were taken and labelled as blank (B), standard(S) and test (T).To these tubes 0.01 ml of distilled water, albumin standard and sample were added respectively.1 ml of BCG reagent was added to all the test tubes. The test tubes were incubate for 5 min at room temperature and absorbance were measured at 630nm against blank.

Calculation

Albumin in g/dl = Abs. T / Abs. S X 4

Globulin in g/dl = (Total Proteins) (in g/dl) - (Albumin) (in g/dl)

$$\text{A/G Ratio} = \frac{\text{(Albumin) (in g/dl)}}{\text{Globulin in g/dl}}$$

g. Total Protein

For determination of protein in serum using the commercially available kit from DIATEK.

Reagent preparation

Reagents were prepared as per the directions given in the kit.

Procedure

Three test tubes were taken and labelled as blank (B), standard (S) and test (T). To these tubes 0.01 ml of distilled water, protein standard and sample were added respectively.1 ml of Biuret reagent was added to all the test tubes. The test tubes were incubate for 10 min at room temperature and absorbance were measured at 555 nm (520-570) within 60 min against blank.

Calculation

Total Protein in g/dl = Abs.T / Abs.S

h. Cholesterol

Determination of cholesterol in serum following the CHOD-POD method was used the commercially available kit from DIATEK.

Reagents preparation

Reagents were prepared as per the directions given in the kit.

Procedure

Three test tubes were taken and labelled as blank (B), standard(S) and test (T). To these tubes 10 µl of distilled water, protein standard and sample were added

respectively.1 ml of cholestrol reagent was added to all the test tubes. The test tubes were incubate for 10 min at room temperature and absorbance were measured at 510 nm against blank.

Calculation

Cholesterol in mg/dl = Abs.of T / Abs.of S X200

i. Bilirubin Total and Direct Test

Determination of Bilirubin Total & Direct in serum followed by Diazotized Sulfanilic (DSA) Method using commercially available kit from Jeev Diagnostics Pvt.Ltd.,

Reagent Composition

Reagents were prepared as per the directions given in the kit.

Procedure

Bilirubin Total

Two test tubes were taken and to the first test tube 1ml of Bilirubin Total reagent I and 0 1ml of sample were added. In second test tube 0.8 ml of Bilirubin Total reagent I, 0.20ml of Bilirubin Total reagent II and 0.10ml of sample were taken. Test tubes were incubated for 2 min at room temperature and absorbance of all the cuvettes was read at 546 nm against a serum blank immediately.

Calculation

$$= \frac{\text{Abs Sample - Abs Blank}}{\text{Cal / Std}} \times \text{Cal / Std}$$

Chapter IV - Effect of *Bos indicus* urine distillate in field

2.14.1 Field selected

The study was carried out S.S.M. Fish farm, Swamimalai, Thanjavur District, Tamil Nadu, India.

Plate 2.8 Location for field trial

2.14.2 Study Area

Plate 2.9 Experimental Ponds

C - Untreated Pond (Control), T - CUD Treated Pond

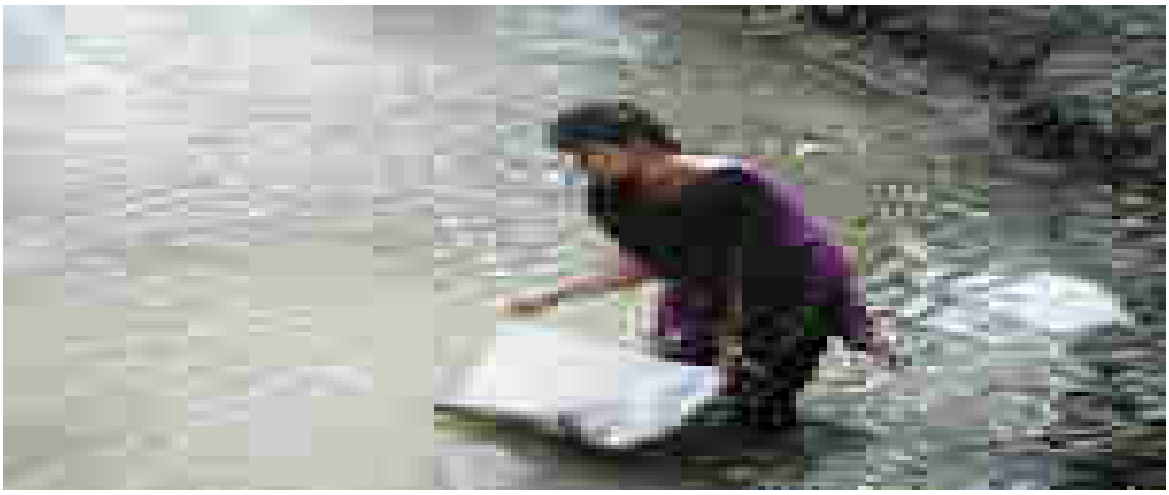
Latitude = 10 degree, 0.2 minutes North

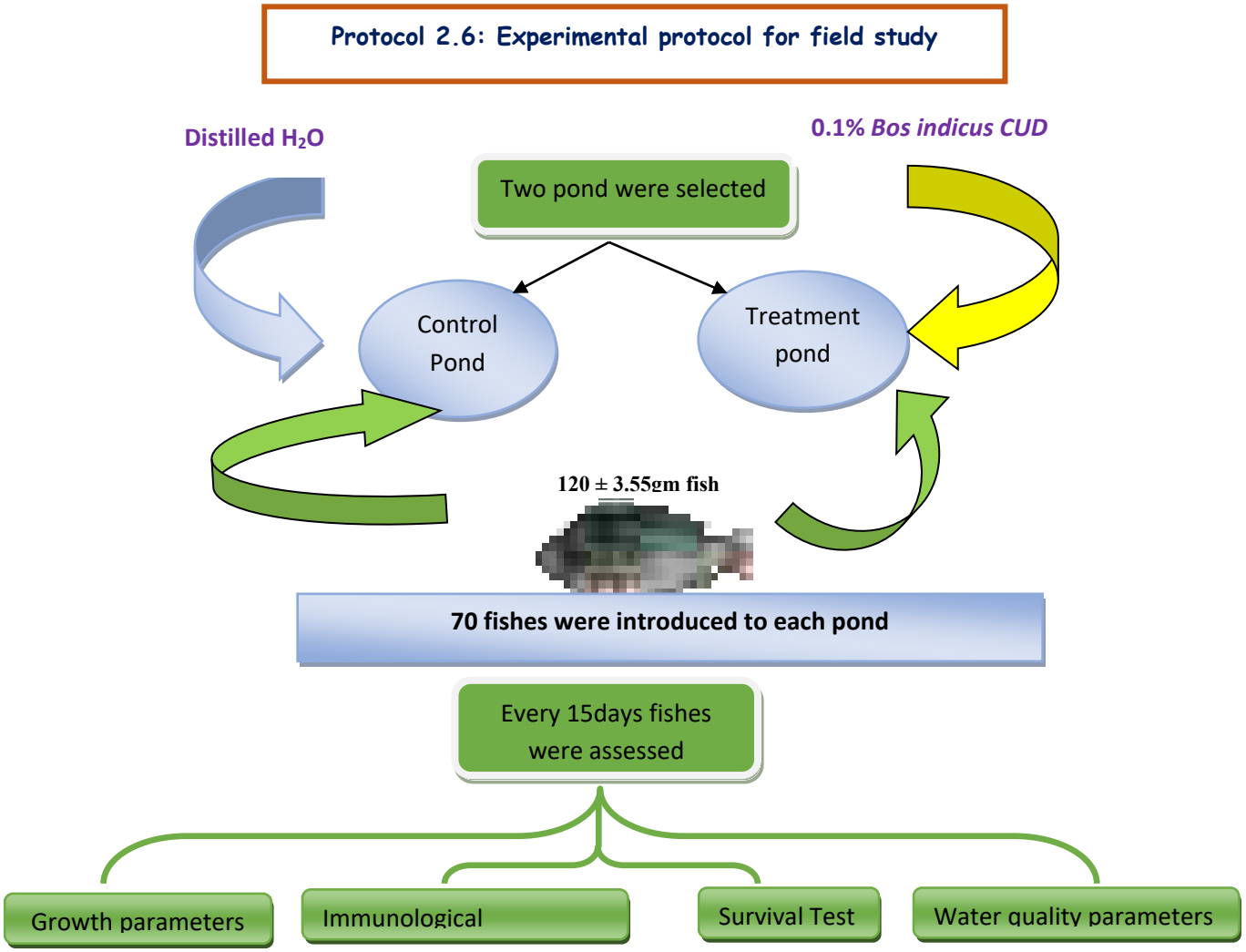
Longitude = 79 degree, 60 minutes East

2.14.3 Experimental design

Two ponds were selected, 70 fish of size 120 ± 3.55 gm were introduced in each pond and maintained in natural conditions. One pond is left untreated as control and another pond was treated with 0.1% Cow urine distillate. 15.46 l of cow urine distillate were added in 15,461 l of water to arrive 0.1% concentration. The pond volume was calculated with morphometric values. Fish were fed with a commercial diet (36 percent CP, crude protein). The two groups of fishes were maintained under same environmental and nutritional conditions. The control and treated group were assessed for growth, heamatology and immunological parameters. The study was lasted for 120 days starting with 120 gm fishes and until a commercial size of approximately 500 g was reached for harvest. Standard water quality parameters were monitored during fish acclimatization and throughout the trial (Protocol 2.6).

Plate 2.10 Introduction of Fish into Pond





2.14.4 Factors studied

The effect of CUD added in the Experimental pond was compared with that of the untreated control bond in terms of the following parameters.

2.14.5 Growth Parameters

The same procedures adopted for laboratory studies (2.12.2) were applied in field study.

Plate 2.11 Capturing fishes from field study

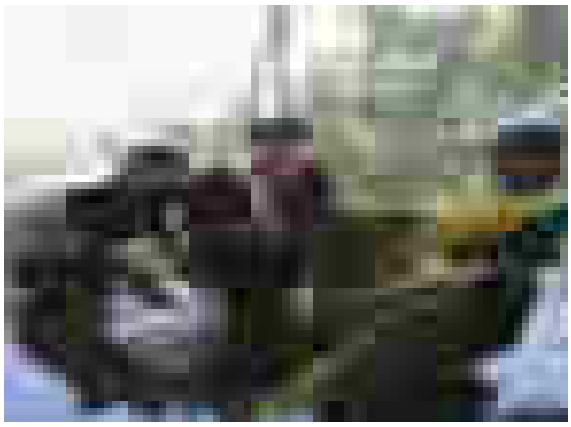


Plate 2.12 Length Measurment

Initial Length **Harvesting Length**

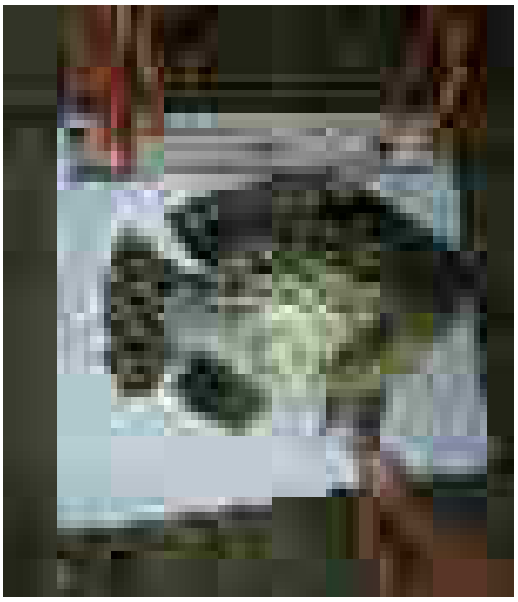
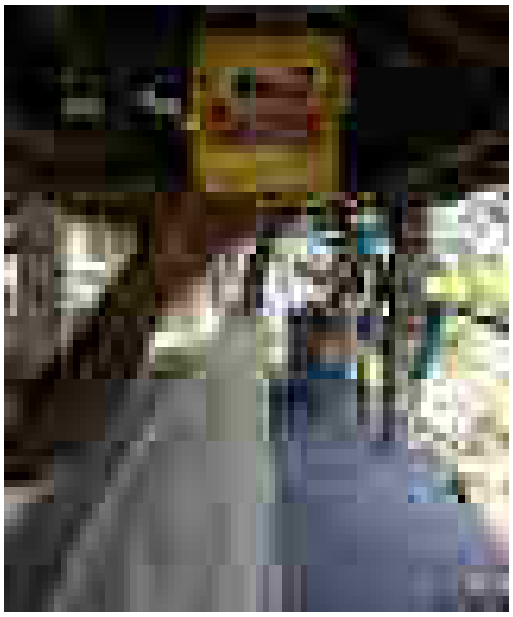


Plate 2.13 Weight Measurment

Initial weight **Harvesting time weight**



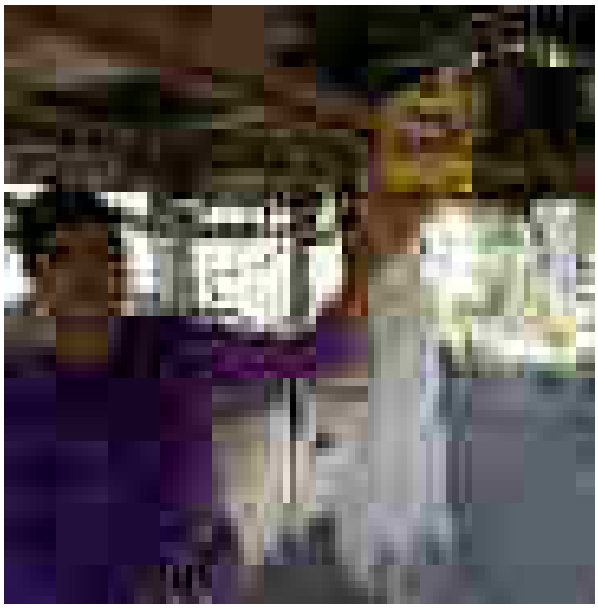


Plate 2.14 Harvesting Control Fish

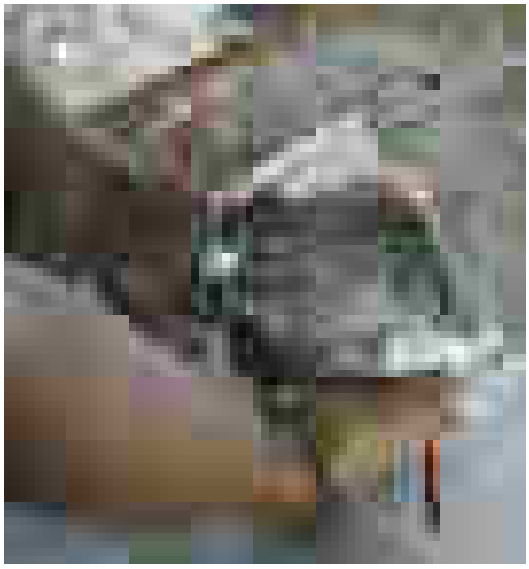
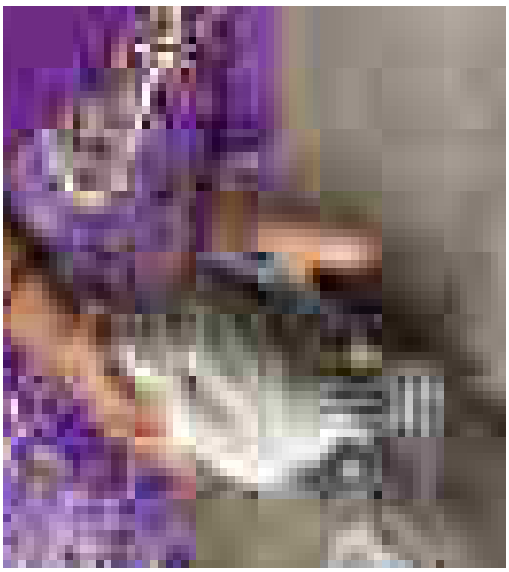


Plate 2.15 Harvesting Experimental Fish



2.14.6 Immunological parameter

The health of the fish was assessed by means of analyzing the following parameters

- Total Erythrocyte Count (TEC)
- Total Leucocyte Count (TLC)
- Haemoglobin (Hb)
- Mean corpuscular haemoglobin (MCH)
- Neutrophil activity
- Lysozyme activity
- Myeloperoxidase activity
- Bactericidal activity
- Antiprotease

The procedures were adopted for studies in laboratory condition was applid for the field study also (2.11.7a, 2.11.7b, 2.11.7c. 2.11.7f, 2.11.7i, 2.11.7j, 2.11.7k, 2.11.7l and 2.11.7m) respectively.

d. Survival Test

The survival rate was calculated by using the formula

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

Chapter V - 2.15 Comparative Study of Physical, Chemical and Microscopic characteristics of cow urin

Physical, Chemical and Microscopic characteristics were studied for both fresh cow urine (FCU) and CUD of both breeds (Protocol 2.7).

2.15.1 Physical properties

Some physical characteristics were immediately examined at the time of collection (pH, temperature and Specific gravity). Other than characteristics were examined within 6 hours in laboratory.

a. Colour

The colour of urine was recorded while observing it in a beaker. The colour was considered in association with volume of urine sample.

b. Odour and Taste

Odour and taste were determined by using common sensational observation of the sample.

c. pH

pH was measured using pen-type portable pH meter

d. Weight

Weight was measured in digital balancer with four-digit sensitivity. 1ml of the sample was weighed and was expressed as mg.

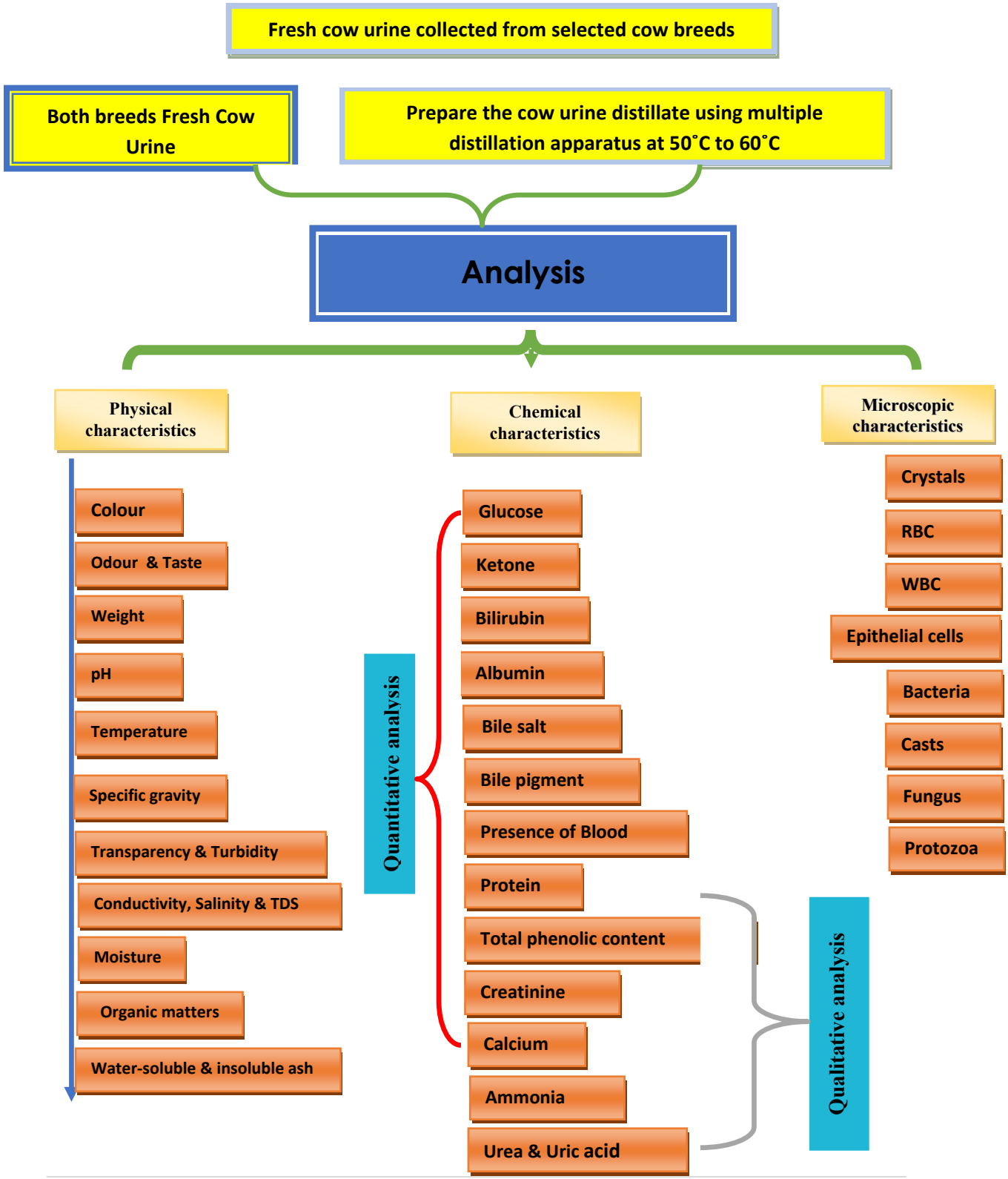
e. Temperature

Urine temperature was measured with a thermometer for fresh cow urine immediately after collection. Water analyzer (Systronics) was used for measuring temperature of CUD at lab.

f. Transparency and Turbidity

The transparency and turbidity of cow urine samples were assessed subjectively and reported using the standard descriptors: clear, slightly cloudy, cloudy, opaque, or flocculent.

Protocol 2.7: Experimental protocol for Comparative study of Physical, Chemical and Microscopic characteristics fresh cow urine and its distillate



g. Specific gravity (SG)

The Specific Gravity of Cow urine was measured by the urinometer. Urinometer cylinder was filled to about one inch from the top with urine, measure and record sample temperature. Hold the urinometer float by the top and slowly insert it into the cylinder. To avoid wetting the float stem above the liquid line and excessive wetting of the stem will cause the float to sink below the true test reading. Impart a slight spin to the float as it were released. Read the float scale at the lowest portion of the urine meniscus. Be sure to keep the float away from sides of cylinder while reading. For calibration temperature correction was necessary. For every 3˚C increase temperature, 0.001 should be added to result.

h. Conductivity, Salinity and Total dissolved solids (TDS)

Conductivity, Salinity and Total dissolved solids (TDS) were measured in water analyzer (Systronics).

i. Moisture, Organic matters and total ash

Moisture from each sample was eliminated at 110˚ C in a hot air oven for eight hours. Organic matter from this moisture free sample was eliminated at 450˚C in a muffle furnace for three hours. From the weights moisture content, organic matter and total ash contents were estimated the following formula;

Moisture = $W_1 – W_2$

Organic matters = $W_4 – W_3$

Total ash = final weight of the sample (W_4)

W_1 = weight of the sample before incubate at 110˚ C

W_2 = weight of the sample after incubate at 110˚ C

W_3 = weight of the moisture free sample

W_4 = weight of the sample after incubate at 450˚C

j. Determination of water-soluble ash (The Ayurvedic Pharmacopoeia of India, 2001)

The ash obtained in total ash was boiled for 5 min with 25 ml of water. The insoluble substance was collected on an ashless filter paper or cloth and washed with warm water. The insoluble ash was shifted into a pre-weighed porcelain dish and ignited for 15 min at a temperature not exceeding 600ºC. The weight of the insoluble substance was subtracted from the weight of the total ash. The difference in weight was considered as the water-soluble ash.

2.15.2 Chemical examination of FCU and CUD

Urine was analysed to detect and measure the level of variety of substances, including protein, glucose (sugar), ketone bodies, blood, and other substances. Qualitative analysis was done for these substance. If the result was positive, then quantitative analysis was carried out.

a. Glucose

The glucose was determined by Benedict test. 5 ml of Benedict Reagent ($CuSO_4$, sodium citrate, sodium carbonate, distilled water) was taken and eight drops of cow urine was mixed. It was heated for 5 minutes up to boiling. The solution if remains clear, blue in colour indicates negative. If the solution has precipitate of green to yellow, orange and red it indicates positive.

b. Ketone Bodies

The test for detection of ketone bodies in urine was done by Ross test. Half-inch layer of Ross reagent was placed and 5 ml of urine was added. Shake the two components and 1-2 ml of ammonium hydroxide was added. Wait for five minutes. Development of purple color ring at the junction was indicate the presence of ketone bodies.

c. Bilirubin

Bilirubin was detected by Gmelin test. A test tube was taken and 2 ml of nitric acid and 2 ml of Cow urine sample was added. The appearance of green to violet colour ring at the junction of two fluids is an indication of the presence of bilirubin in urine.

d. Albumin

The amount of albumin present in the urine sample was assessed by sulfosalicylic acid method. 2ml of urine was taken in a clear test tube and pH was checked. If urine is neutral, or alkaline a drop of glacial acidic acid was added and mixed well. Moreover, 2 to 3 drops of sulfosalicylic acid reagent was added to the test tube holding the tube against a dark background. The presence of albumin in urine is detected based on cloudiness and precipitate.

e. Bile salt

10ml of urine was taken in a test tube. A sprinkle of a little dry sulphur powder was added on the surface of the urine. Observe the sulphur particle and record. Sink or floating nature of the sulphur was noted. If sink it indicates the presence of bile salt and floating indicates the absence of bile salts.

f. Presence of blood in Urine

2 ml of glacial acetic acid was taken in a test tube and a small amount of benzidine powder was added. 1 ml of urine was added to test tube and 1 ml of fresh H_2O_2 was also added. Mix them and wait for 5 minutes. Green to blue colour formation indicates the presence of blood in urine.

g. Calcium

Sulkowitch test is used for calcium detection.

Sulkowitch reagent

The oxalic acid is 2.5 g, ammonium chloride is 2.5 g, 5ml of glacial acetic acid and finally adding 150ml distilled water.

Procedure

Take two test tubes, and to the first test tube add 5 ml urine and 5 ml distilled water . In second test tube 5 ml of Cow urine and five ml of sulkowitch reagent was taken. Compare the two test tubes in transmissible light after 2 to 10 min. The presence of white precipitations is an indication of the presence of calcium.

h. Urine Protein

Qualitative analysis

Qualitative Urine Protein was determined by Robert's reagent method.

2 ml Cow urine and its distillate was taken in the test tubes. 2 ml of Robert's reagent was over layed and allow the urine to run slowly down along the wall of the test tube. Formation of white ring at the junction of reagent and urine indicates the presence of protein.

Quantitative analysis

1.25 ml of filtered Cow urine and its distillate was taken in a test tube and 3.75 ml solution of 3% sulfosalicylic acid was added and mixed. After 5 min, read the OD with a spectrophotometer, at 670nm. To the control, 1.25 ml of distillet water and 3.75 ml of 0.9% NaCl solution.

i. Total phenolic content

Qualitative analysis

The qualitative analysis of phenolic compound was done by ferric chloride test. 2 ml of sample was taken in a test tube a few drops of alcohol and 5% ferric chloride solution were added. Bluish green or red colour indicated the presence of phenolic compounds.

Quantitative analysis

The total phenolic content (TPC) of the sample was determined using Folin-Ciocalteu reaction; Gallic acid was used as a standard. 1ml of sample was added to 5 ml of 50% Folin-Ciocalteu reagent followed by the addition of 4 ml of 7.5% Na_2CO_3 solution. After 30 minutes incubation at room temperature, the absorbance was measured at 730 nm.

j. Creatinine

Qualitative analysis

The qualitative analysis of creatinine was done by Weill test. Three to four ml of urine and three to five drops of 10% sodium hydroxide solution added in a test tube. Add a few drops of sodium nitroprusside. The colour of the solution rapidly turns from pink to yellow indicates presence of creatinine.

Quantitative analysis

Quantitative estimation of creatinine was done by Jaffe's Kinetic & Point method using commercially available kit from €URO diagnostic systems.

k. Ammonia

The ammonia in the fresh cow urine and distillate were determined calorimetrically by the use of Nessler's reagent. 5ml of the sample was taken in a test tube 0.1ml of potassium sodium tartrate and 0.1 ml of Nessler's reagent were added to the test tube. After 5 min OD was taken at 450 nm in UV-spectrophotometer. 5 ml of distilled water was used as blank.

l. Urea

Urea was determined by following Amsath *et al*., (2010) method.

Reagents

A. Acid Ferric Chloride Solution

1ml of H_2So_4 was made up to 100ml with 5% Ferric Chloride Solution containing 50gm in 1ml of water.

B. Acid reagent

Five ml of Orthophosphoric acid and 40ml of ferric chloride solution were made up to 50 ml with distilled water.

C. Colour reagent

300ml of acid reagent and 10ml of 2.5% Diacetyl monoxine were made up to 500 ml with distilled water.

D. Standard urea

The 100 mg anhydrous urea crystals were dissolved in 100 ml of distilled water.

Procedure

2 ml of the sample, 2 ml of Standard urea and 2 ml of distilled water were taken in separate test tubes. 3 ml of colour reagent was added to all test tubes. All the test tube were kept in water bath for 20 min OD was measured at 520 nm after cooling.

m. Uric acid

0.1ml of the sample, 0.1% standard uric acid and blank was taken separate test tubes. 4 ml of working reagent was added to all test tubes and its volume was made up to 10 ml with distillate water. All the test tubes were incubated for 10 mints at room temperature. 0.1ml of distillate water was considered as blank. After the incubation period absorbance was measured at 510 nm.

2.15.3 Microscopic examination of FCU & CUD

Cow urine was examined under the light microscope for the presence of red and white blood cells, epithelial cells, crystals, casts, bacteria, fungus and protozoa.

2.16 Chapter VI - Effect of *Bos indicus* and *Bos taurus* Urine Distillate on water quality

Water quality was studied throughout the experimental period on 0^{th} day, 7^{th} day and 28^{th}. Physico-chemical parameters analyzed include (a) water temperature, (b) pH of water, (c) total dissolved solids, (d) ammonia, (e) salinity, (f) conductivity and (g) turbidity. The chemical analysis was performed by using electronic water analyser (Systronics, India). Dissolved oxygen was evaluated by Winkler's method (Golterman *et al,* 1978). Ammonia concentrations were determined as described by Koroleff (1976).

The results were analyzed statistically by Two-way analysis of variance (ANOVA) with Holm-Sidak multiple comparison test using the sigma plot 13. The levels of significance were expressed as p-value less or greater than 0.05. In the text of the thesis, different levels of significance was determined by a posteriori Holm-Sidak comparison of the treated groups with the control group. It is indicated as parenthetical content. In the figures, a posterior Holm-Sidak comparison of control and treated groups and also comparisons among the treatments, are shown with different alphabets indicating significant difference ($P<0.05$) among them**.**

CHAPTER I - 3.1 STUDY OF IMMUNE PARAMETERS INFLUENCED BY *BOS INDICUS* AND *BOS TAURUS* URINE DISTILLATE

The Immune responses of *Oreochromis mossambicus* to different breeds of cow urine distillate treatments against *A.hydrophila* infection were studied by administering through two different routes.

3.1.1 Studies on the Immune parameters when cow urine distillate was administered through different routes

The immune status of *Oreochromis mossambicus* after seven days exposure to two breeds of CUD as water additive and feed additive was recorded.

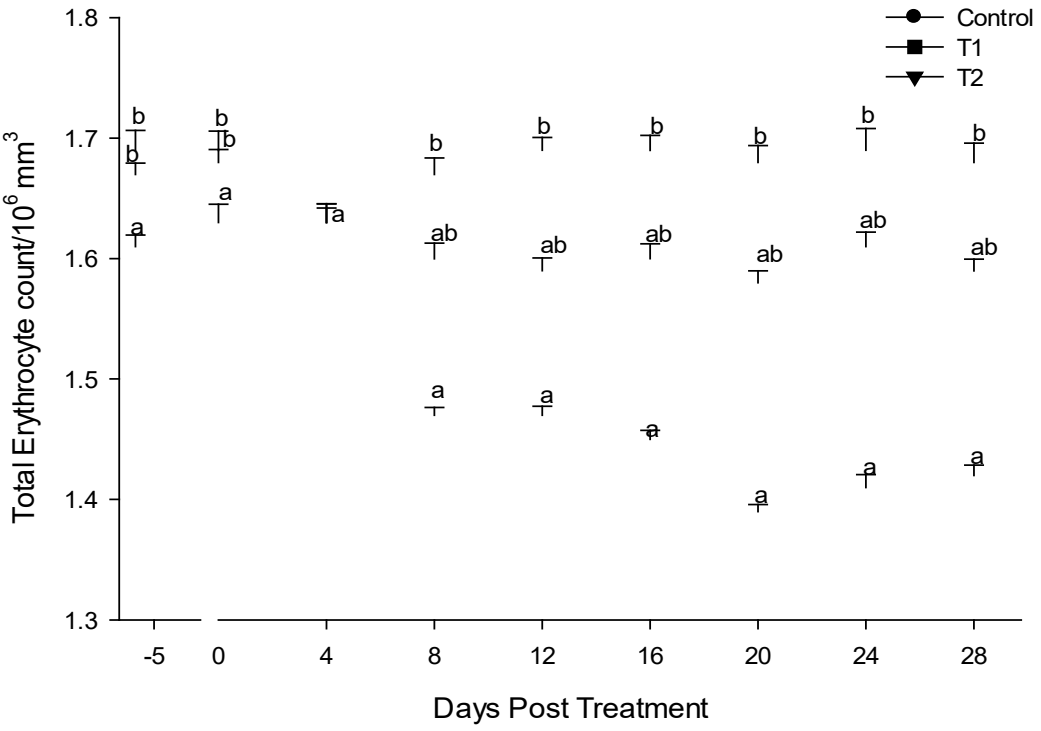
I. Non specific immune parameters

a. Total Erythrocyte Count (TEC)

Water additive route

Fig 3.1.1 reveals the variation of total erythrocytes count between the treatment groups and control due to the influence of direct administration of cow urine distillate (CUD) in *Oreochromis mossambicus.* The graph depicts total RBC of control group is significantly

Figure 3.1.1 Effect of CUD administered through water additive on Total Erythrocyte Count (TEC) in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



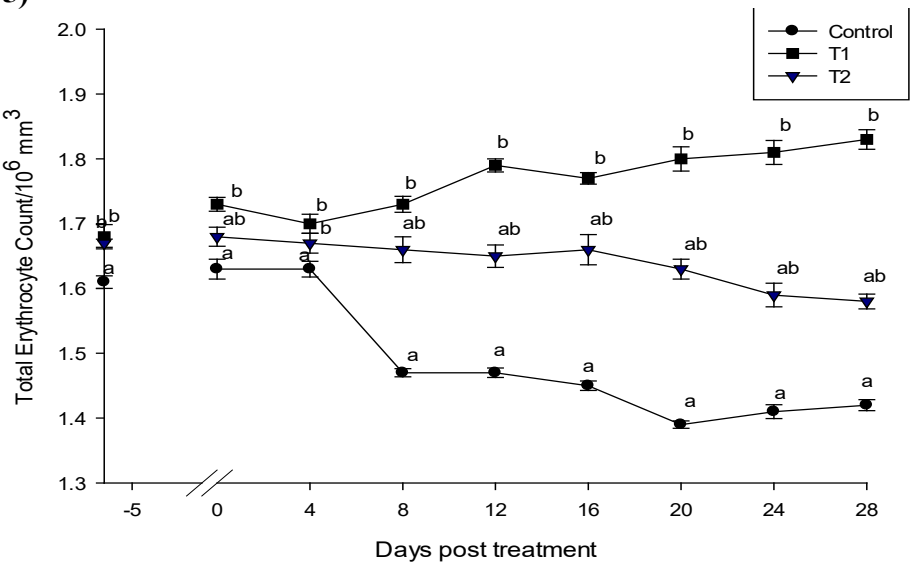
decreasing while number of days are increasing, as it was 1.61 X 10^6/mm^3 at the 0^{th} day and it decreased into 1.39 x 10^6/mm^3 on 20^{th} day. The *Bos taraus* and *Bos indicus* urine distillate treated groups showed a different trend. On the 4^{th} day of immunization there is a slight decrease in the RBC count. For T_1 it decreased from 1.69 x 10^6/mm^3 on the 0^{th} day

to 1.62 x 10^6/mm^3 on the 4th day. But again it was recovered on the 8th day to 1.69 x 10^6/mm^3 ($P<0.001$) and it was almost maintained in the same level. Similar trend was observed for T_2 with a significant lesser count of erythrocyte count ($P>0.05$). By both T_1 and T_2 are found significantly higher when compared to control ($P<0.001$).

Feed additive route

Fig 3.1.2 illustrates that effect of feed supplementation of cow urine distillate (CUD) on total erythrocytes count of *Oreochromis mossambicus.* The variation of Total RBC between *Bos taurus* and *Bos indicus* with the comparison of control group is significantly different ($P<0.001$). From the graph it is clear that, in the control group total RBC value is decreasing while number of days are getting increasing, as it was 1.69 x 10^6/mm^3 total RBC at the 0th day and it was decreased into 1.39 x10^6/mm^3 on the day 20th ($P<0.001$). When comparing control group it is clear that in *Bos taurus* urine distillate treated group, the extent of decrease is lesser than control group ($P<0.001$). The *Bos indicus*

Figure 3.1.2 Effect of CUD administered through feed additive on Total Erythrocyte Count (TEC) in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference ($P<0.05$)



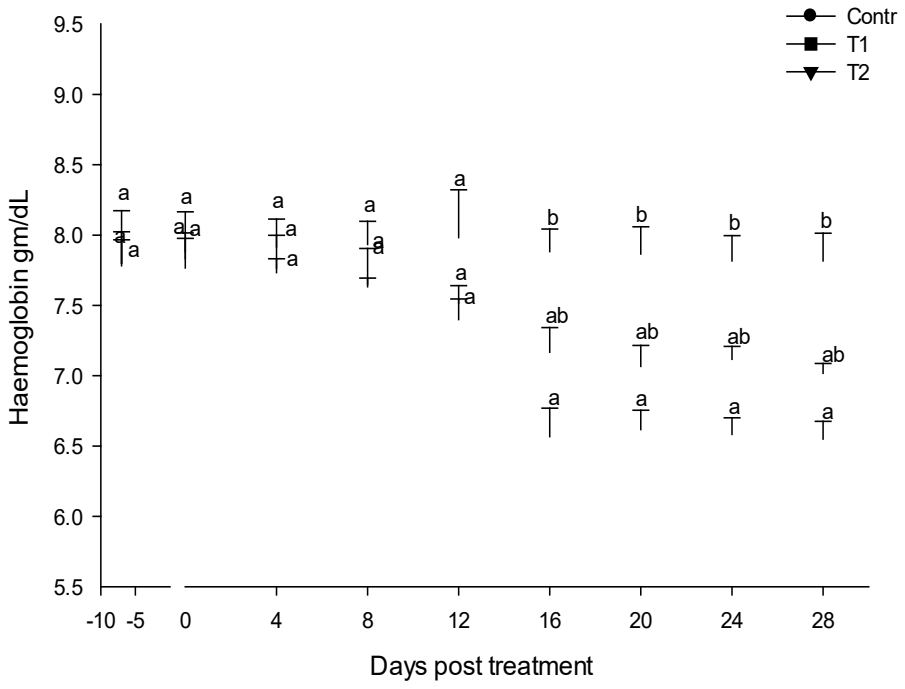
represents the increasing trend over the days, as it was between 1.7 and 1.8 x 10^6/mm^3 at the 0^{th} day and 28^{th} day respectively. It is significantly increasing the 0^{th} day to 12^{th} day and 16^{th} day to 28^{th} day ($P<0.05$) .When comparing all the three it is clear that, control and *Bos taurus* are having negative trend while *Bos indicus* is having positive trend.

b. Haemoglobin (Hb)

Water additive route

The fig 3.1.3 shows effect of direct administration of cow urine distillate (CUD) on Heamoglobin in *Oreochromis mossambicus*. It illustrates the variation between *Bos indicus* and *Bos taurus* with the comparison of control group. The blood heamoglobin was significantly ($P<0.001$) higher in T_1 group which means *Bos indicus* urine treated group. From the graph, it is clear that, all the three were having almost same heamoglobin during

Figure 3.1.3 Effect of CUD administered through water additive on Heamoglobin in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

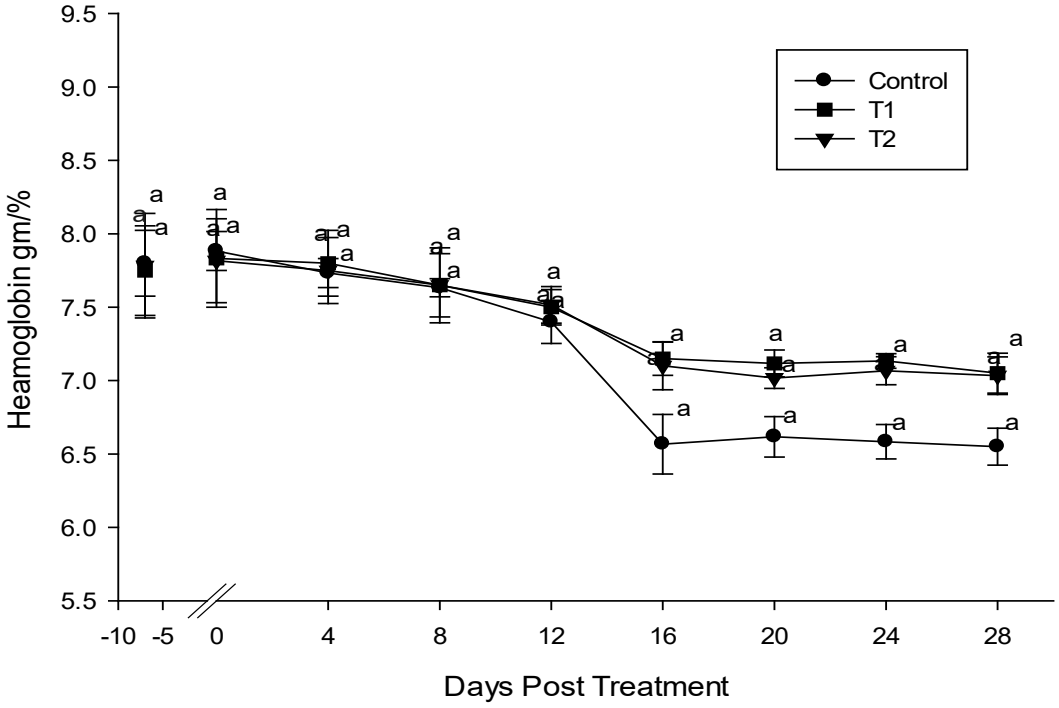


the initial days, T_2 and control groups were decreasing continuously over the days. The T_1 *Bos indicus* urine treated group maintaining same values from the initial day. The heamoglobin level of all groups significantly (P<0.5) differ from each other but the highest significance was found in the T_1 group on the 12th day of experimental period.

Feed additive route

The Fig 3.1.4 shows effect of feed supplementation of cow urine distillate (CUD) on Heamoglobin of *Oreochromis mossambicus* which illustrates the variation between *Bos taurus* and *Bos indicus* with the compared to control group. The experimental groups and

Figure 3.1.4 Effect of CUD administered through feed additive on Heamoglobin in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



control group none significantly differ (P>0.05). From the graph, it is clear that, all the three were having almost same Heamoglobin values during the initial days, and decreasing after 12th day. From the graph it is clear that, the trend of decreasing was less from 0th day

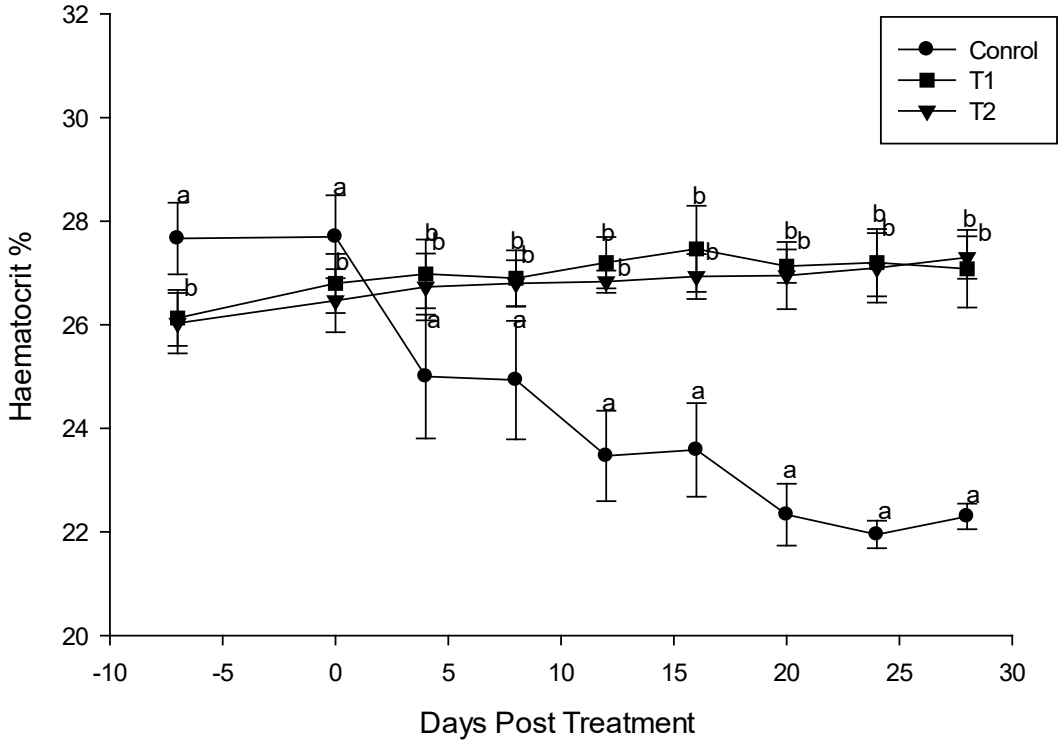
to 12th day than from 16th day to final day, whereas the decrease was high during the days between 12th to 16st is rapid in all the three. From the comparison of all the three it is visible that, heamoglobin of control group was decreased highly than other two, while in *Bos indicus* it was decreased less among the three.

c. Haematocrit (Hct)

Water additive route

The blood haematocrit was significantly (P<0.001) higher in both breed CUD treated groups as compared with the untreated control group throughout the study period as shown in figure 3.1.5. The maximum value was observed in T_1 group which is *Bos indicus* treated group. In the control group it is decreasing continuously over the days from

Figure 3.1.5 Effect of CUD administered through water additive on Haematocrit in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

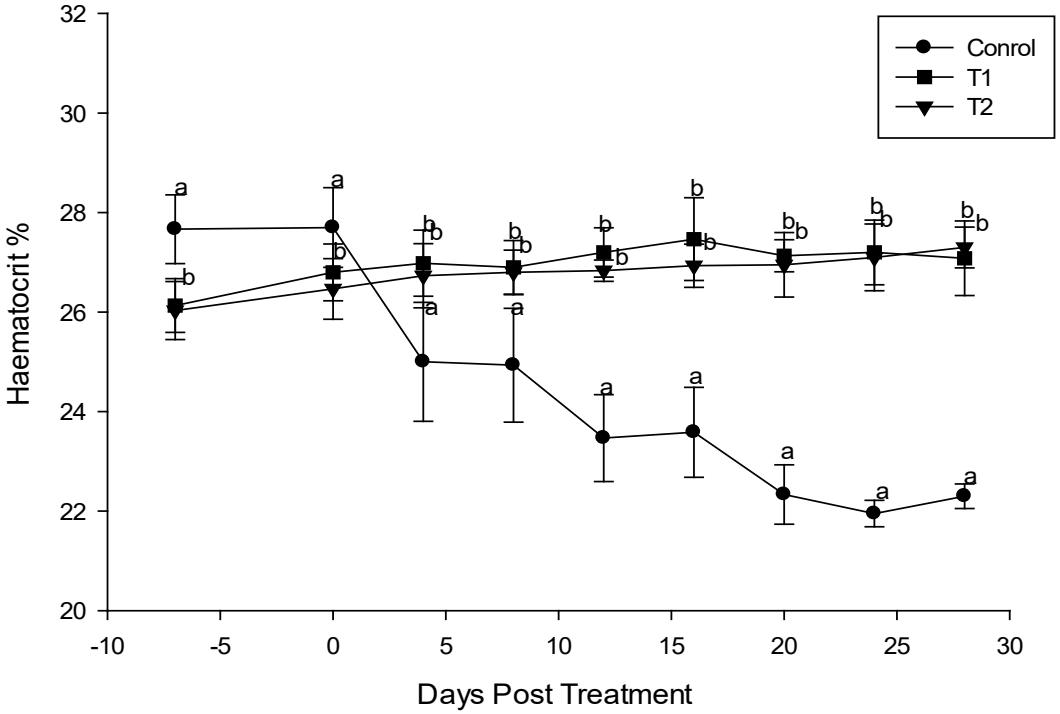


initial. Among the treatment groups and the post treatment days of experiment, the T_1 group showed highest activity on day 16-post treatment. The T_1 and T_2 group not significantly differ though a slight variation. The P value was observed in 0.065 (P>0.05).

Feed additive route

Fig 3.1.6 exhibits the effect of feed supplementation of cow urine distillate (CUD) on Haematocrit of *Oreochromis mossambicus* showing the variation between *Bos taurus* and *Bos indicus* with the compared to control group. The treatment group

Figure 3.1.6 Effect of CUD administered through feed additive on Haematocrit in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



significantly differ with control group (P<0.05). From the graph, it is clear that, from the 0^{th} day control group was decreasing continuously while other treatment groups were increasing. Notably the control group which was 27.6 % total haematocrit on the first day was decreased to nearly 22.3% total haematocrit on the final day. Through the overall

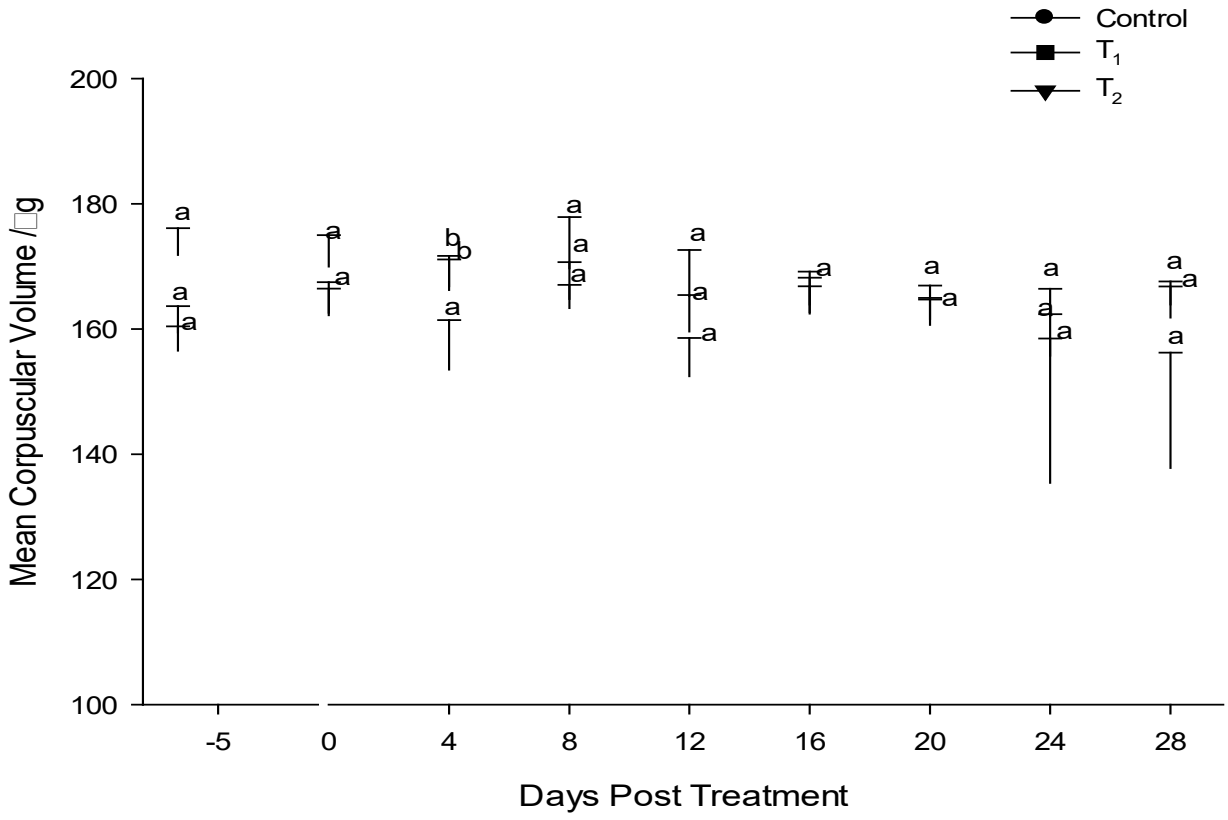
observation, it is visible that, only control group is having negative trend while the treatment groups are having positive.

d. Mean Corpuscular Volume (MCV)

Water additive route

Figure 3.1.7 clearly reveals the effect of direct administration of cow urine distillate (CUD) of *Oreochromis mossambicus* on the Mean Corpuscular Volume (MCV). Both experimental groups have no significant (P>0.05) difference between each other, in

Figure 3.1.7 Effect of CUD administered through water additive on Mean Corpuscular Volume in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



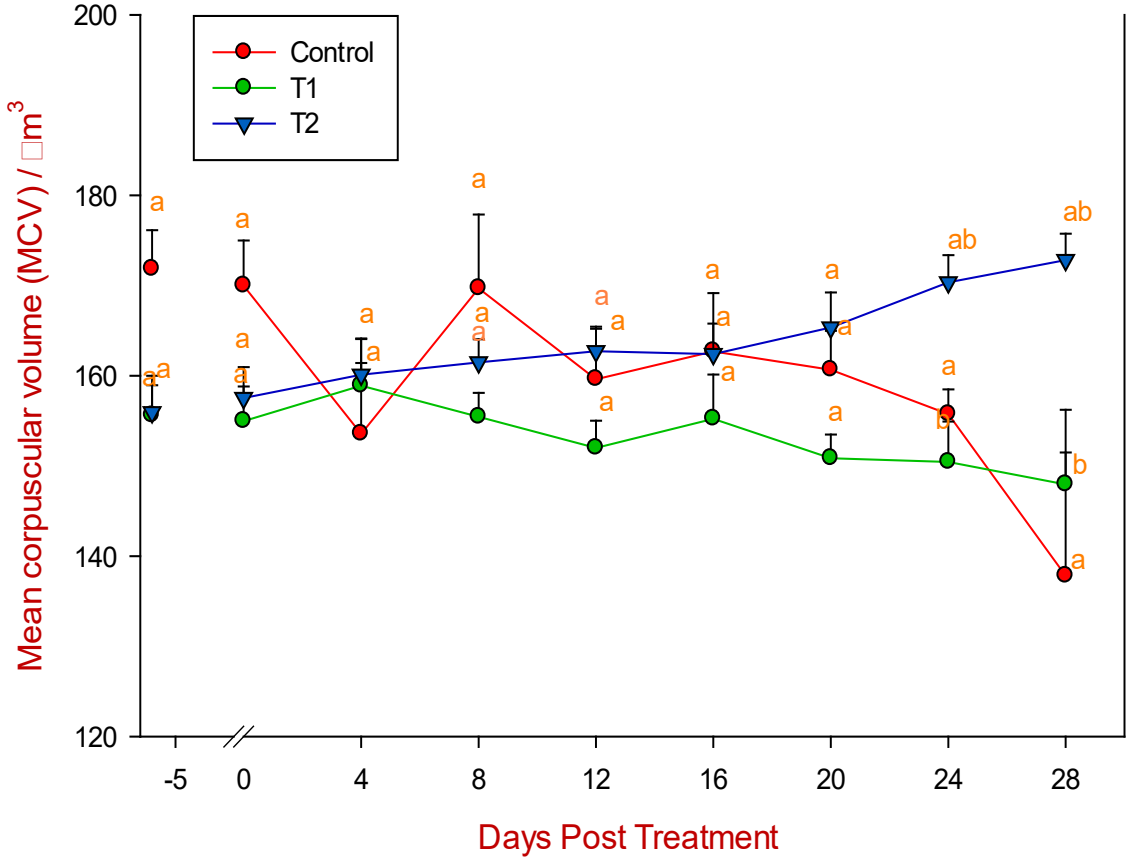
general, while the control group shows fluctuations. The treatment groups are same initially, but over the days the control and T_2 group modify from the initial value but the T_1

group don't have any negative changes and the maintains the same value, as depicted from graph.

Feed additive route

The Fig 3.1.8 reveals the effect of cow urine distillate (CUD) on mean corpuscular volume of *Oreochromis mossambicus*. It illustrates the variation of between *Bos taurus* and *Bos indicus* with the comparison of control group. The *Bos indicus* treated group (T_1)

Figure 3.1.8 Effect of CUD administered through feed additive on Mean Corpuscular Volume in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



significantly differ between each other (T_2 and control with T_1 group) (P <0.05). From the graph it is clear that, the control group MCV value was fluctuating over the days as it was decreased on 4th, 12th, 24th and 28th days and increased on 8th, 16th days. The *Bos indicus*

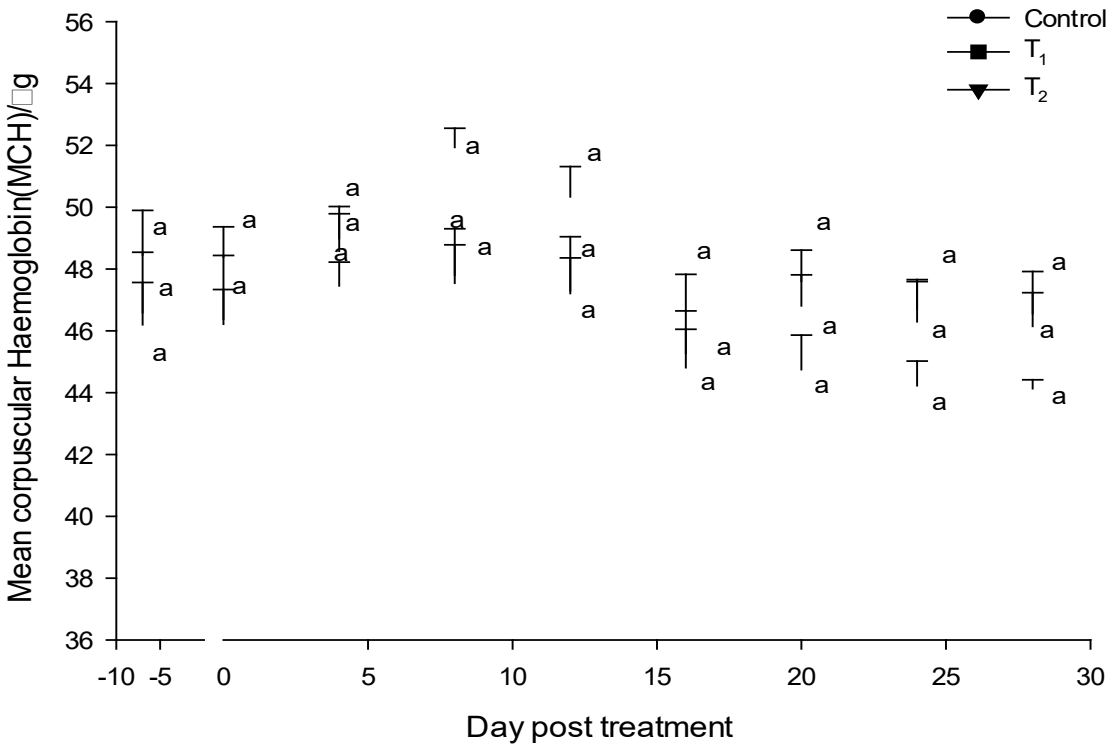
urine treated group also was having slight fluctuation over the days notably; it was increased during 4th, 16th and 24th days whereas it was decreased on 8th, 20th and 28th. The trend of *Bos taurus* showing the positive curve over the days as the value was around 160 fL on the 0th day has been increased around 175fL on the final day. In overall, it is clear that, control group and *Bos indicus* were having fluctuation over the days while *Bos taurus* was having continuous positive trend.

e. Mean Corpuscular Haemoglobin (MCH)

Water additive route

Figure 3.1.9 clearly reveals the effect of direct administration of cow urine distillate (CUD) of *Oreochromis mossambicus* on the Mean Corpuscular Haemoglobin (MCH). All

Figure 3.1.9 Effect of CUD administered through water additive on Mean Corpuscular Haemoglobin in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

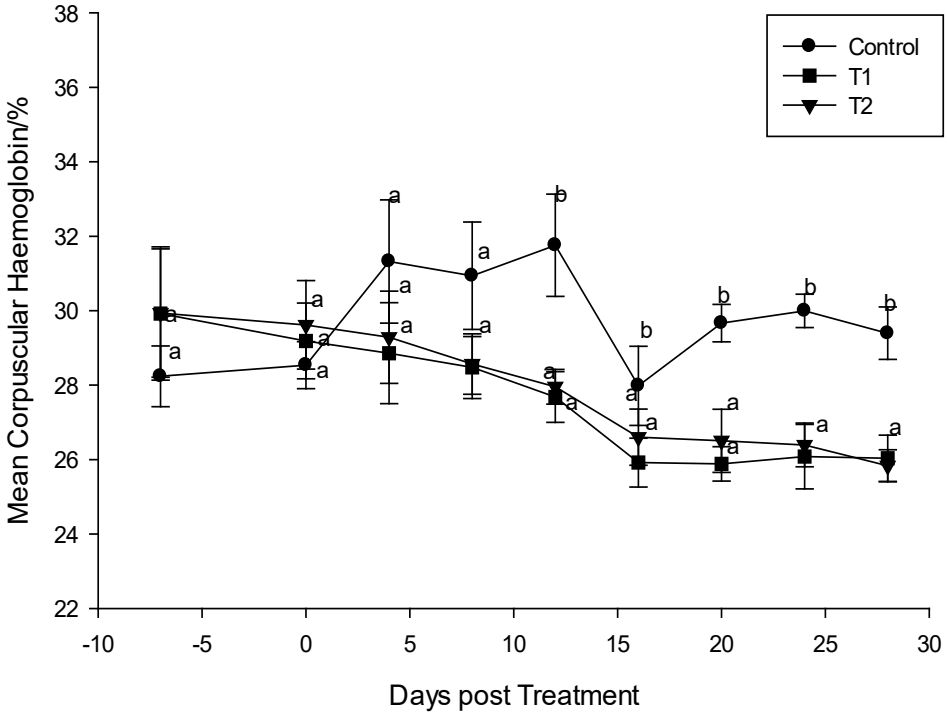


the groups have no significant (P>0.05) difference between each other. In general, both the treatment groups having almost same initially, whereas over the days the control and T_2 group were modifying from the initial value but the T_1 group didn't have any negative changes and was maintaining the same value, as depicted from graph.

Feed additive route

The figure 3.1.10 shows the effect of feed supplementation of cow urine distillate (CUD) on Mean Corpuscular Haemoglobin of *Oreochromis mossambicus*. It illustrates the variation of between *Bos taurus* and *Bos indicus* with the comparison of control group. The Mean Corpuscular Haemoglobin (MCH) significantly differ between each other (P<0.001).

Figure 3.1.10 Effect of CUD administered through feed additive on Mean Corpuscular Haemoglobin in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



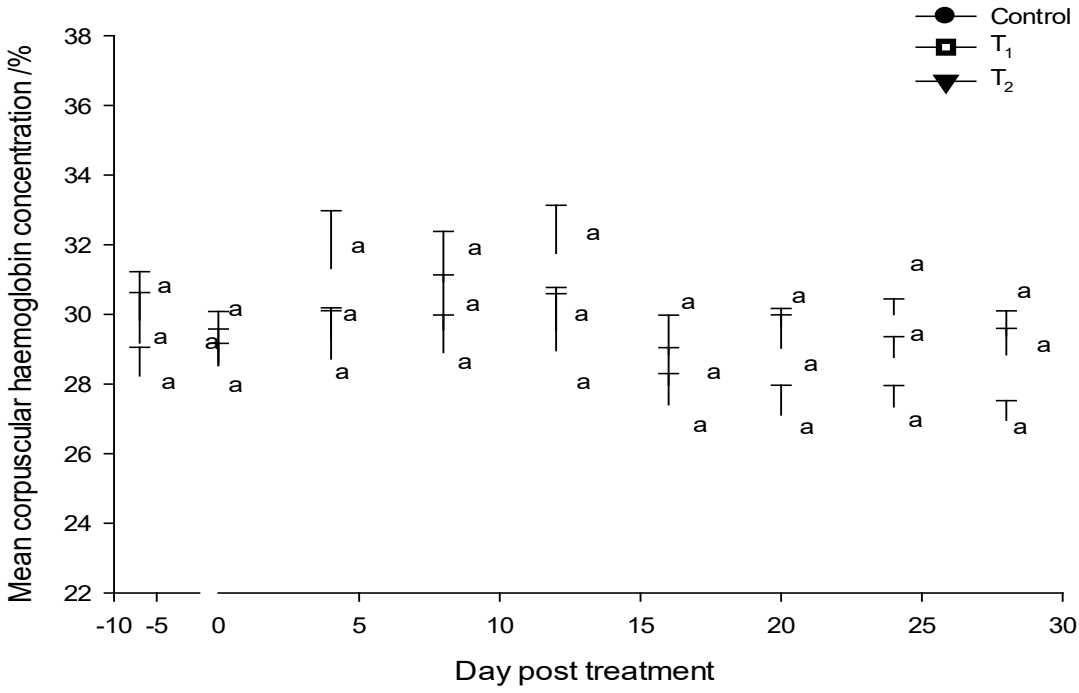
As the graph depicts, control and T_2 group were continuously decreased from the initial day and the *Bos indicus* urine treated group was having slight fluctuation over the days. Notably, it was increased during 4th, 12th and 24th days whereas it was decreased on 8th, and 16thdays.

f. Mean Corpuscular Haemoglobin Concentration (MCHC)

Water additive route

Figure 3.1.11 clearly reveals the effect of direct administration of cow urine distillate (CUD) of *Oreochromis mossambicus* on the Mean Corpuscular Haemoglobin Concentration (MCHC). All the group have no significant (P>0.05) difference between each other. In general,

Figure 3.1.11 Effect of CUD administered through water additive on MeanCorpuscular Haemoglobin Concentration in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



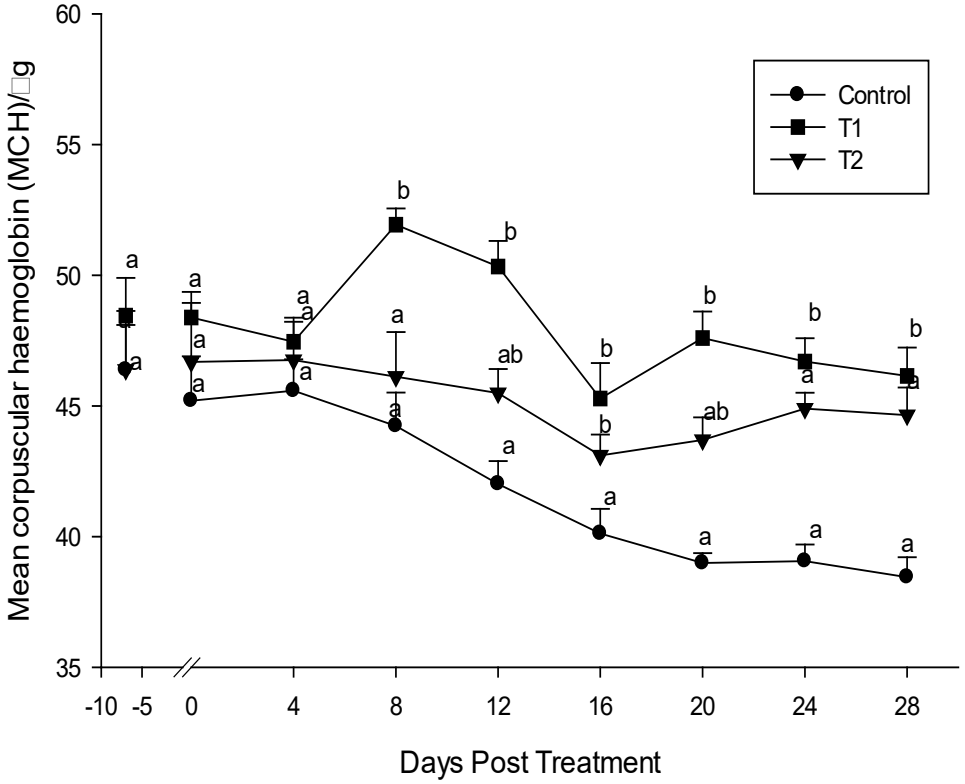
both the treatment group were having almost same values initially, while over the days the

control and T_2 group modified from the initial value but the T_1 group didn't have any negative changes and the was maintaining the same value.

Feed additive route

The figure 3.1.12 shows the effect of feed supplementation of cow urine distillate (CUD) on mean corpuscular volume of *Oreochromis mossambicus*. It illustrates the variation of between *Bos taurus* and *Bos indicus* with the comparison of control group.

Figure 3.1.12 Effect of CUD administered through feed additive on Mean Corpuscular Haemoglobin Concentration in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



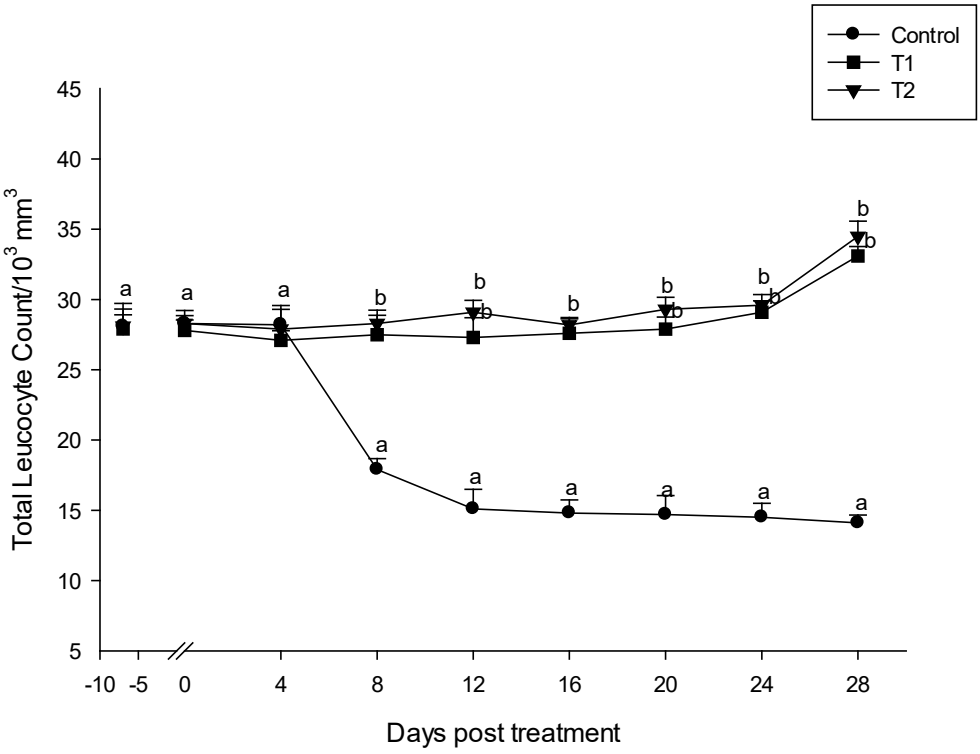
The Mean Corpuscular Haemoglobin Concentration (MCHC) the treatment group significant differ with control group (P<0.001). Control and T_2 were decreased from the 8th day of experiment period while at the same time the 8th day T_1group was increased.

g. Total Leucocyte Count (TLC)

Water additive route

From the results shown in figure 3.1.13 it is evident that there was significant ($P<0.001$) increase in the number of total circulating leucocytes in the CUD treated groups on all the days tested from 4th day when compared to control. Among the CUD treatment groups no significant difference was observed.

Figure 3.1.13 Effect of CUD administered through water additive on Total leucocytes in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

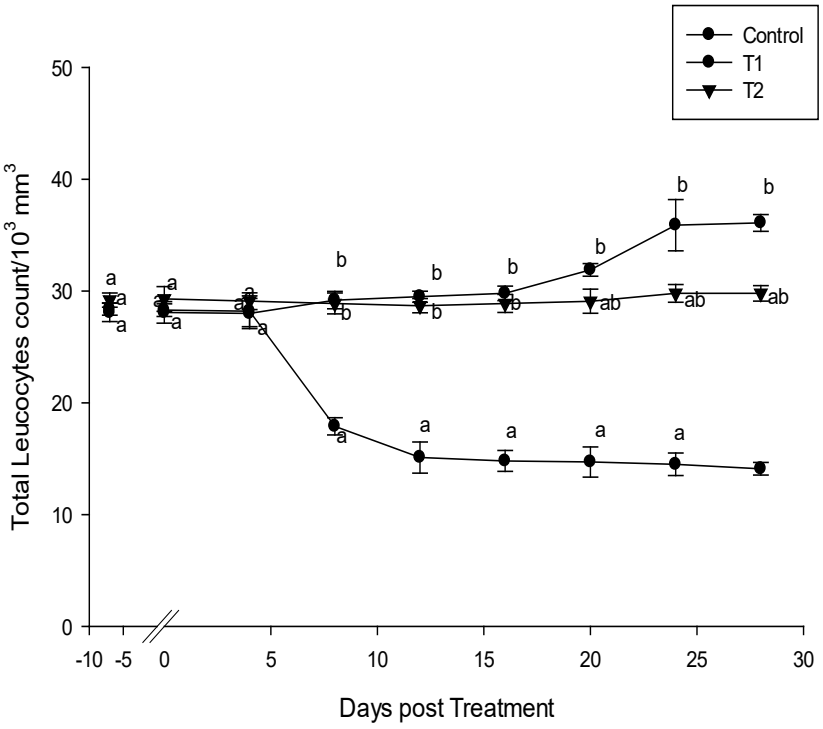


Feed additive route

The figure 3.1.14 illustrates effect of feed supplementation of cow urine distillate (CUD) on total peripheral leukocytes count of *Oreochromis mossambicus* and the variation between *Bos taurus* and *Bos indicus* with the comparison of control group. The treatment

group significantly differ with control group (P<0.001). From the graph, it is clear that, all the three were having same total WBC during the initial days, notably from the 0th day to 4thday none significantly differ (P>0.05). Control group was decreased rapidly from 28.1X10^3/mm^3 WBC on the 4th day to 14.1 X10^3/mm^3 WBC on the final day, whereas *Bos taurus* was increased from 28.1X10^3/mm^3 WBC on the 4th day to 34.5 X10^3/mm^3 WBC on the final day. The trend of *Bos indicus* also showed the slightly positive growth over the days as it was 27.9 X 10^3/mm^3 WBC on 4thday and increased into nearly 31.1 x 10^3/mm^3 WBC on the final day. Through the overall observation, it was visible that, only control group was having negative trend while treatment groups were having positive trend.

Figure 3.1.14 Effect of CUD administered through feed additive on Total leucocytes in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



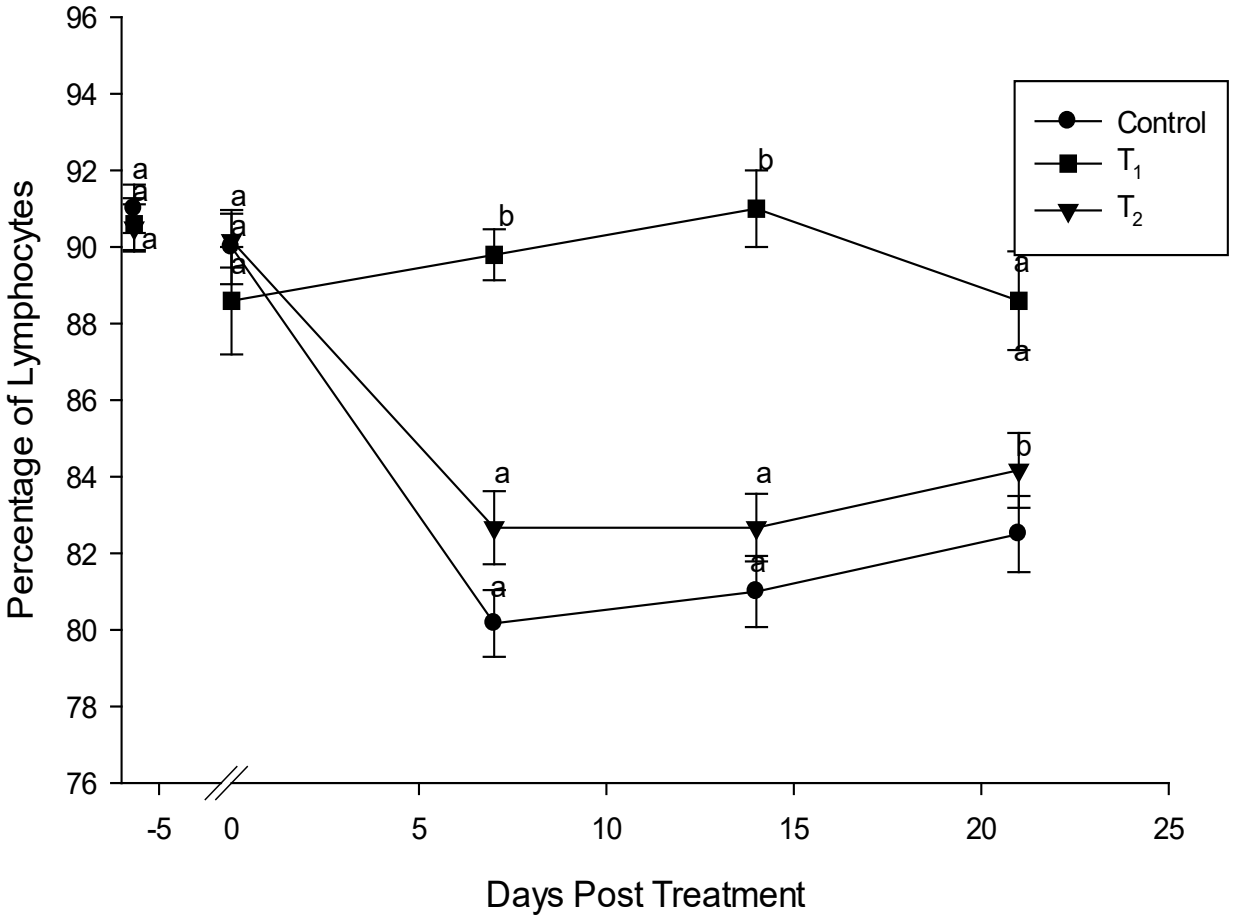
h. Differential Leucocyte Count (DLC)

Lymphocytes

Water additive route

The Figure 3.1.15 illustrates a significant increase ($P < 0.05$) in percentage of lymphocytes found in T_1 when compared to T_2 and untreated control group, in water additive administration of CUD significantly ($P < 0.05$) only on 14^{th}day in treatment group.

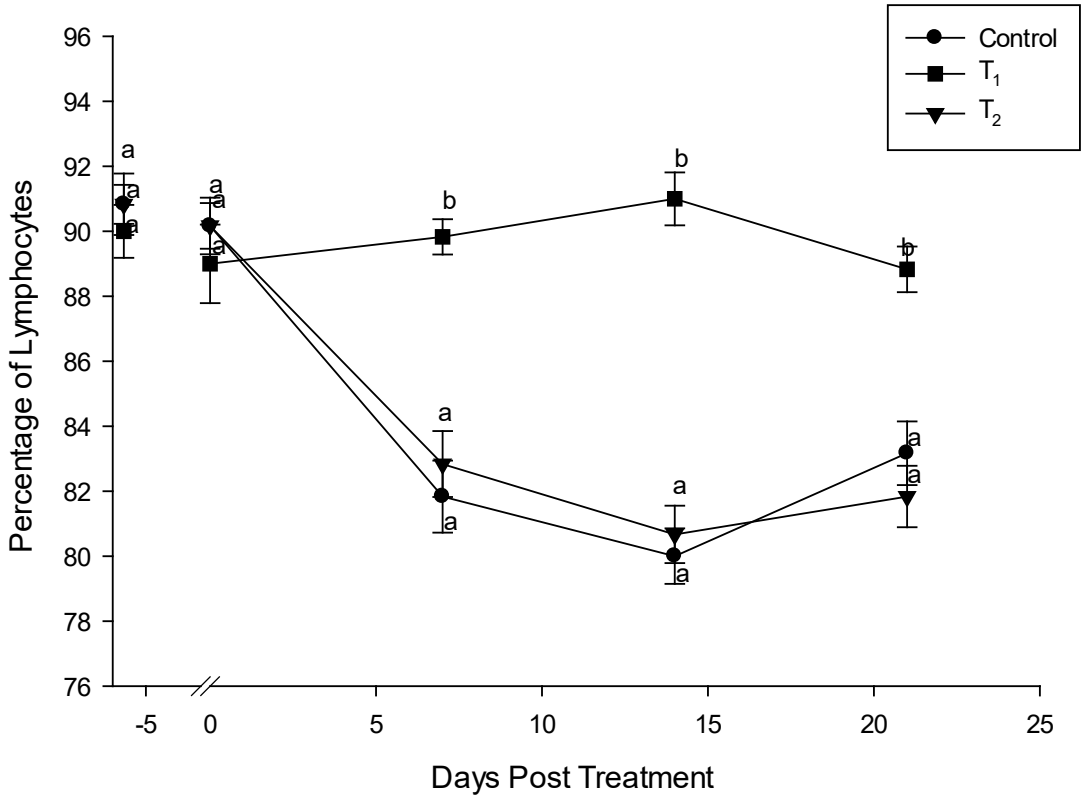
Figure 3.1.15 Effect of CUD administered through water additive on Percentage of lymphocytes in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



Feed additive route

The Figure 3.1.16 illustrates a significantly increase ($P < 0.05$) in percentage of lymphocytes found in T_1 when compared to T_2 and untreated control group, on feed additive administration of CUD significant ($P < 0.05$) only on 14thday in treatment group.

Figure 3.1.16 Effect of CUD administered through feed additive on Percentage of lymphocytes in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

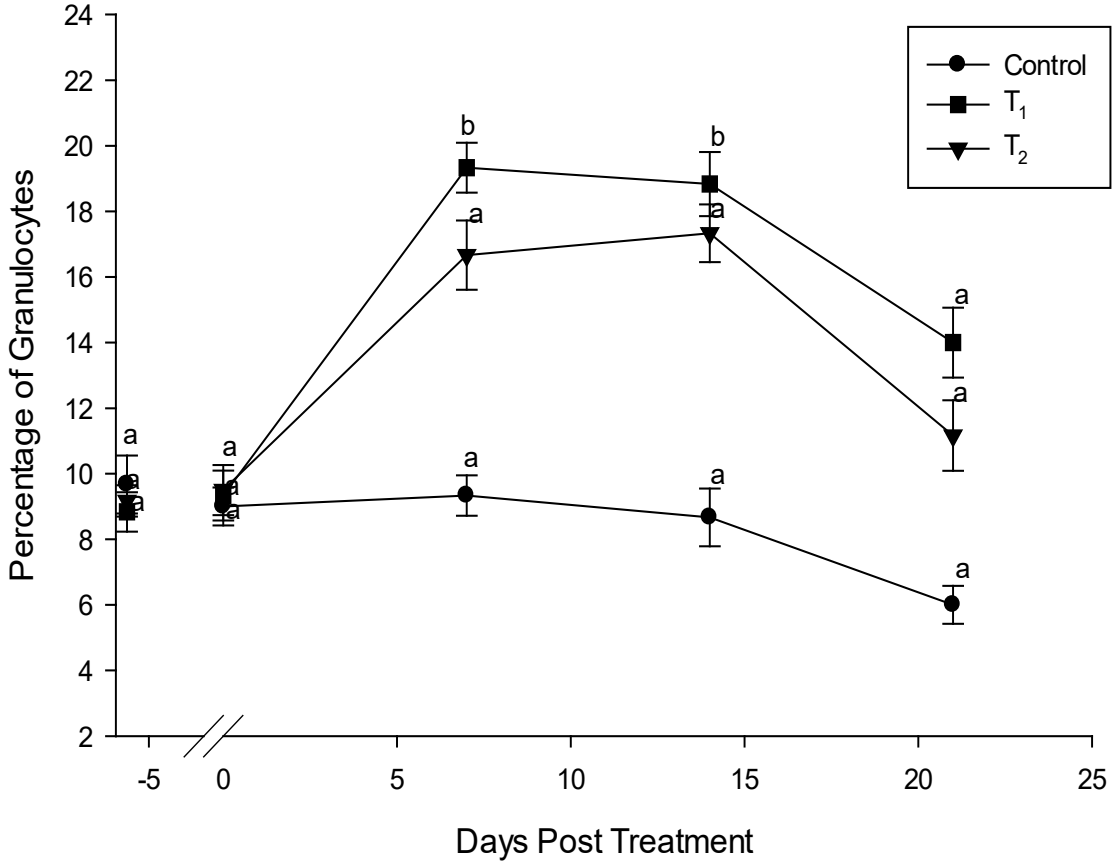


Granulocytes

Water additive route

The Figure 3.1.17 illustrates a significant increase ($P<0.05$) percentage of granulocytes found in treatment groups when compared to untreated control group, in water additive administration of CUD. It's increasing from 0^{th} day to 14^{th} day in the experiment. The T_1 group significantly ($P<0.05$) showed difference.

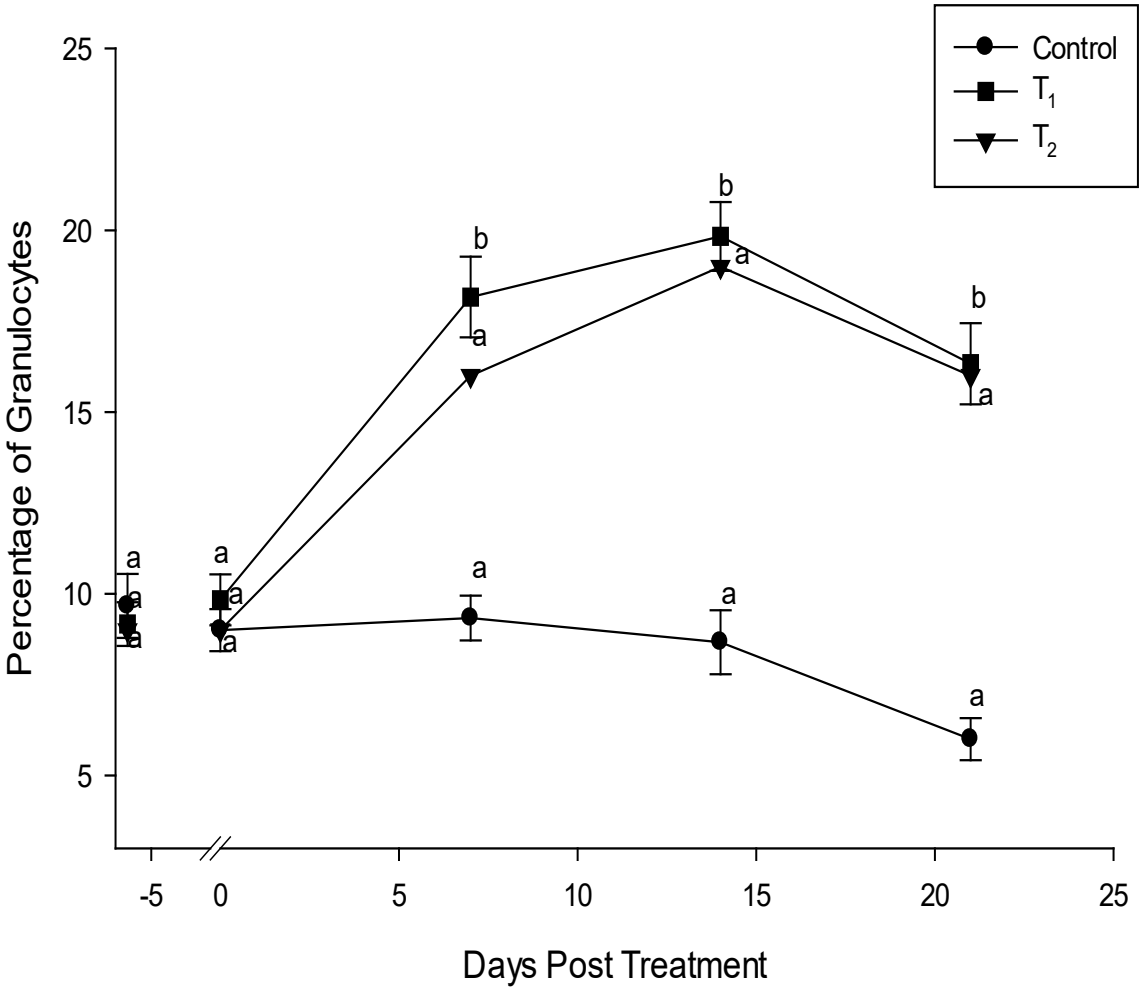
Figure 3.1.17 Effect of CUD administered through water additive on Percentage of granulocytes in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



Feed additive route

The Figure 3.1.18 illustrates a significant increasing (P<0.05) percentage of granulocytes found in treatment group when compared to untreated control group, in feed additive administration of CUD. It's increasing from 0^{th} day to 14^{th} day in the experiment. In T_1 group significant ($P < 0.05$) difference was observed.

Figure 3.1.18 Effect of CUD administered through feed additive on Percentage of granulocytes in Oreochromis mossasmbicus; each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual posttreatment days shown with different alphabets representing significant difference (P<0.05)



Monocytes
Water additive route

The Figure 3.1.19 illustrates no significance was observe. An apparent decrease (P<0.05) in the number of monocytes was observed in the treatment groups as shown in the graph.

Figure 3.1.19 Effect of CUD administered through water additive on Percentage of monocytes in Oreochromis mossasmbicus

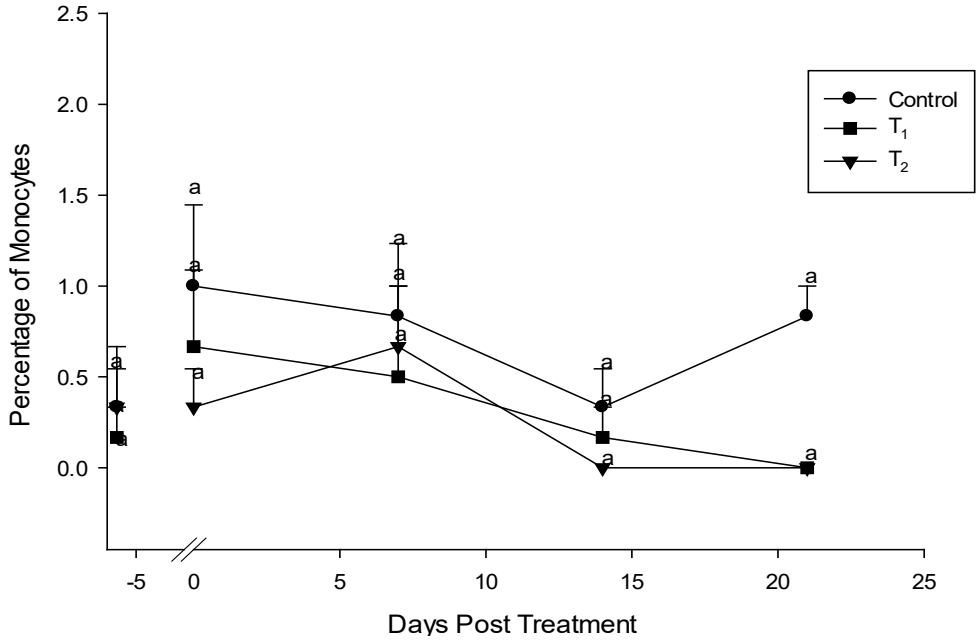
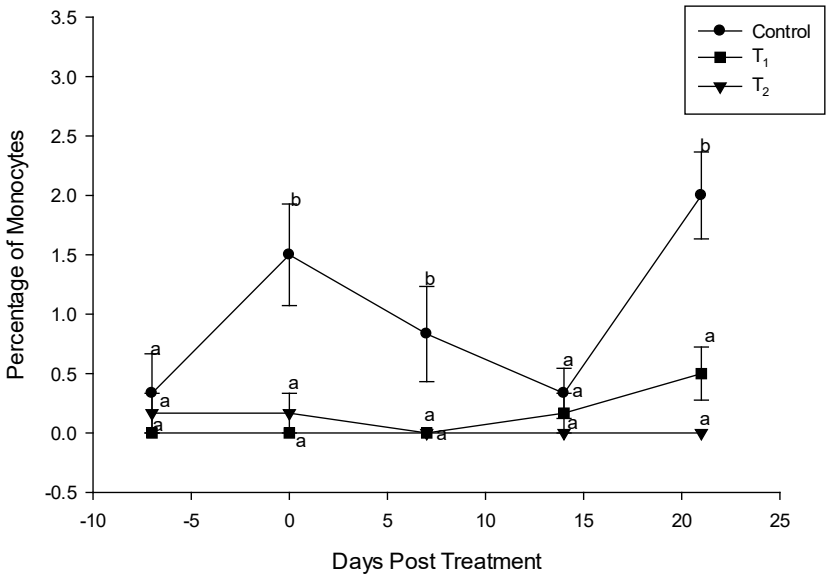


Figure 3.1.20 Effect of CUD administered through feed additive on Percentage of monocytes in *Oreochromis mossasmbicus*



Each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

Feed additive route

The Figure 3.1.20 illustrates no significance was found between the groups. An apparent decrease ($P<0.05$) in the number of monocytes was observed in the treatment groups as shown in the graph.

f. Neutrophil activity

Water additive route

The Figure 3.1.21 illustrates a significant increasing ($P < 0.001$) neutrophil activity when compared to that of untreated control group, in direct administration of *Bos indicus* urine treated group which is T_1. The T_2 didn't show any moderate changes or negative change. In Control group the neutrophil activity suddenly decreases after the immunization (Heat killed *A.hydrophila* injection (0^{th}day)) and from 7^{th} day of immunizaton the neutrophil activity occur in the same level till the end of experiment. However, for control T_2 the 7^{th} day of immunization neutrophil activity shows no modulation till the end of experiment ($P>0.05$).

Feed additive route

The Figure 3.1.22 illustrates a significant increase ($P< 0.05$) in neutrophil activity when compared to that of untreated control group, in feed supplement administration of *Bos indicus* and *Bos taurus* urine treated group which is T_1 & T_2. In the control group the neutrophil activity suddenly decreased after the immunization period. In the 7^{th} day of immunization the neutrophil activity shows increased and peak value obtain from 14^{th} day ($P<0.00$). For T_1group, the peak day of response occurred on the 14^{th} day of immunization. There was a significant augmentation of neutrophil activity ($P<0.001$) by *Bos indicus* urine on the 14^{th} day with 0.161 activity / OD at 620 nm.

Figure 3.1.21 Effect of CUD administered through water additive on neutrophil activity in Oreochromis mossasmbicus

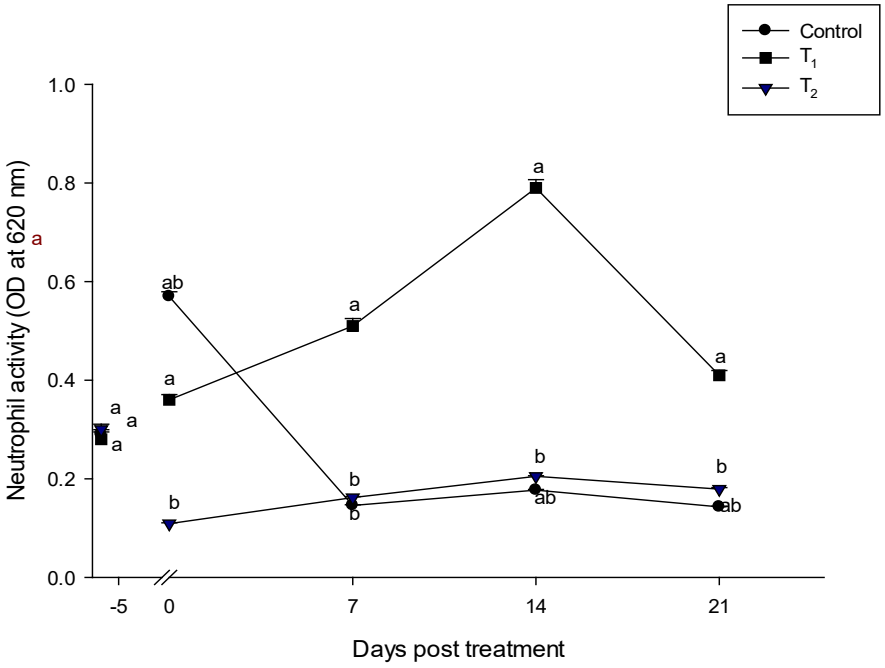
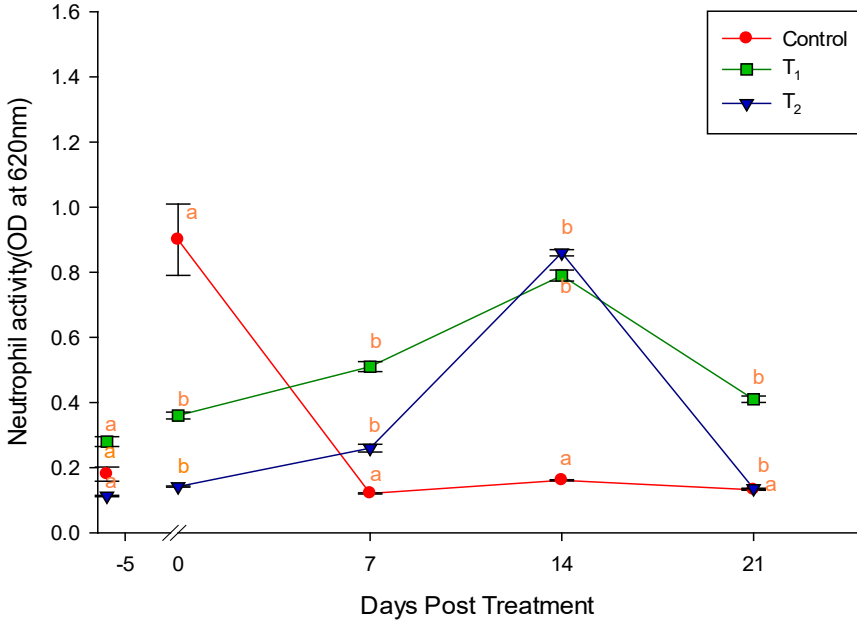


Figure 3.1.22 Effect of CUD administered through feed additive on neutrophil activity in *Oreochromis mossasmbicus*



Each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

g. Serum lysozyme activity

Water additive route

As shown in figure 3.1.23, the serum lysozyme activity measured in unit ml^{-1}, was higher in both the CUD treated groups than the untreated control group on all the days of after immunization. The difference was significant ($P<0.001$) throughout the study period. The maximum activity was observed on day 14 in the $T_1$and T_2 groups (1115.6 units ml^{-1}, $P<0.001$; 1085.1 units ml^{-1}, $P<0.001$). Among the treatment group and the days of experiment, the T_1 treated group showed highest activity on 14th day post treatment.

Feed additive route

The lysozyme activity was found significantly higher ($p<0.001$) in the treatment groups compared with control. The maximum activity was observed on day 14th in the T_1 and T_2 groups (1001.3 units ml^{-1}, $P<0.001$; 997.6 units ml^{-1}, $P<0.001$) at the same time the very least activity (430.6 units ml^{-1} $P>0.05$) was found in control group (fig 3.1.24).

Figure 3.1.23 Effect of CUD administered through water additive on serum lysozyme activity in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

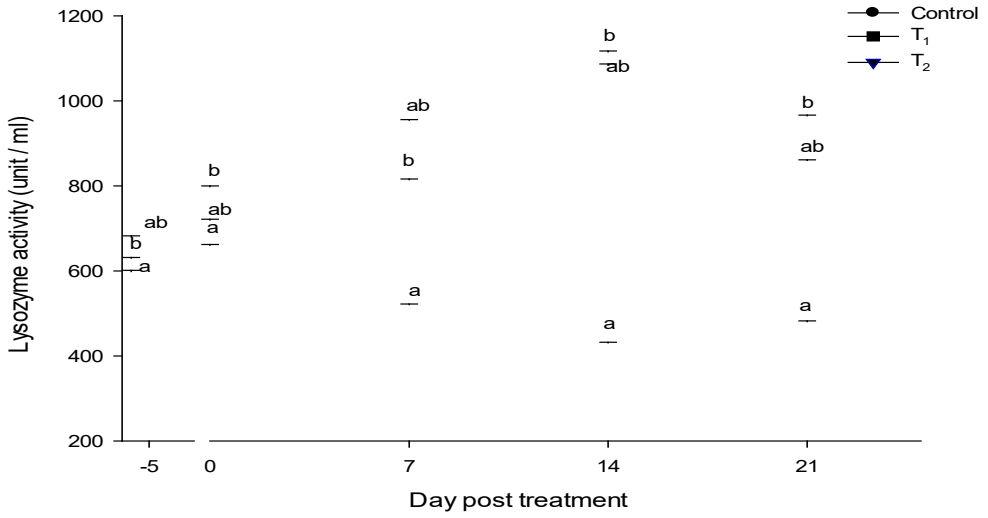
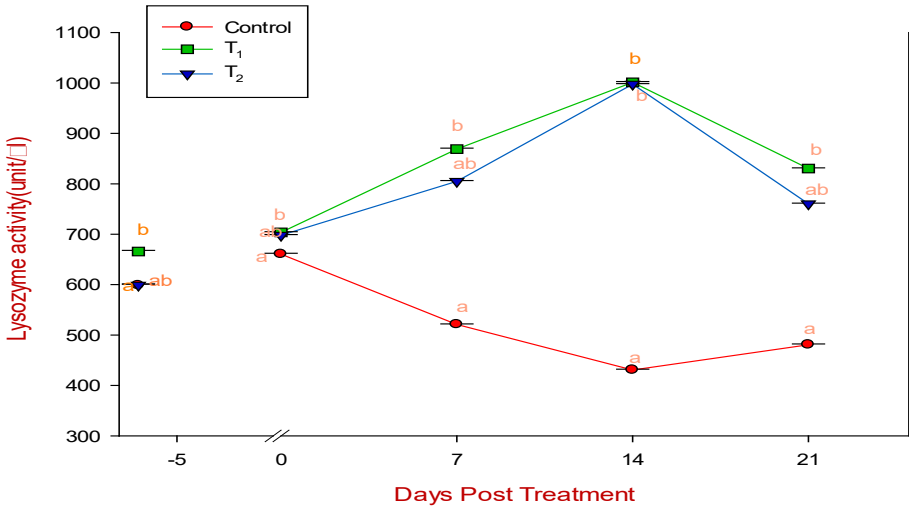


Figure 3.1.24 Effect of CUD administered through feed additive on serum lysozyme activity in *Oreochromis mossasmbicus ;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



h. Serum myeloperoxidase activity

Water additive route

Effect of CUD on the serum myeloperoxidase activity is depicted in figure 3.1.25. The treatment groups significantly (P<0.001) elevated the activity on the 7th day of experiment. The maximum activity was observed on 7thday in T_1 group (P<0.001) (i.e) *Bos indicus* urine treated group (0.86 activity /OD at 450nm) and minimum by control (0.27 activity /OD at 450nm).

Feed additive route

Effect of CUD as feed supplement on the serum myeloperoxidase activity is depicted in figure 3.1.26. The treatment group significantly (P<0.05) elevated the activity on the 7th day of experiment. The maximum activity was observed on 7th day in T_1 group (0.78 activity /OD at 450nm; P<0.001). The minimum activity by control group (0.27

activity/OD at 450nm). For both T_1 and T_2 the maximum activity was exhibited on the 7th day of immunization. The control group showed no significant fluctuation over the days.

Figure 3.1.25 Effect of CUD administered through water additive on serum myeloperoxidase activity in *Oreochromis mossasmbicus*

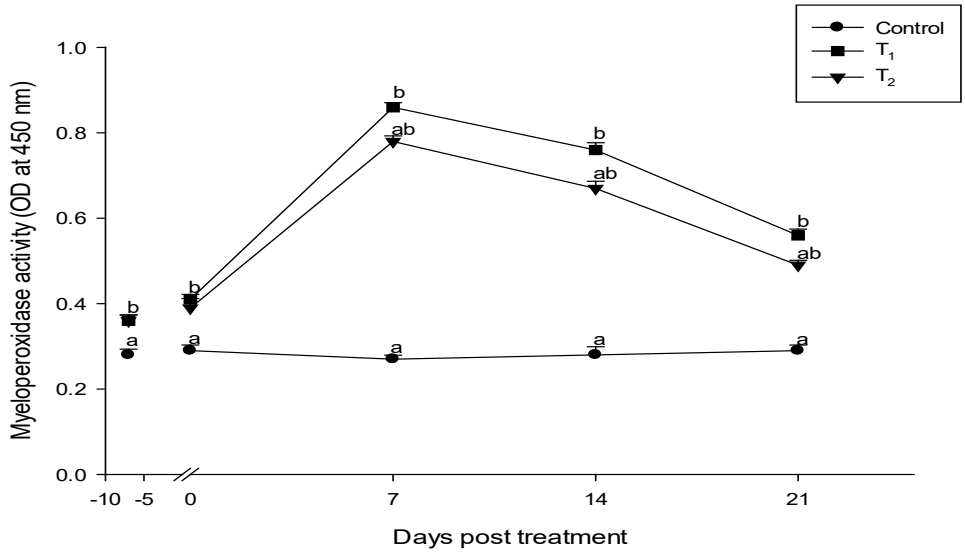
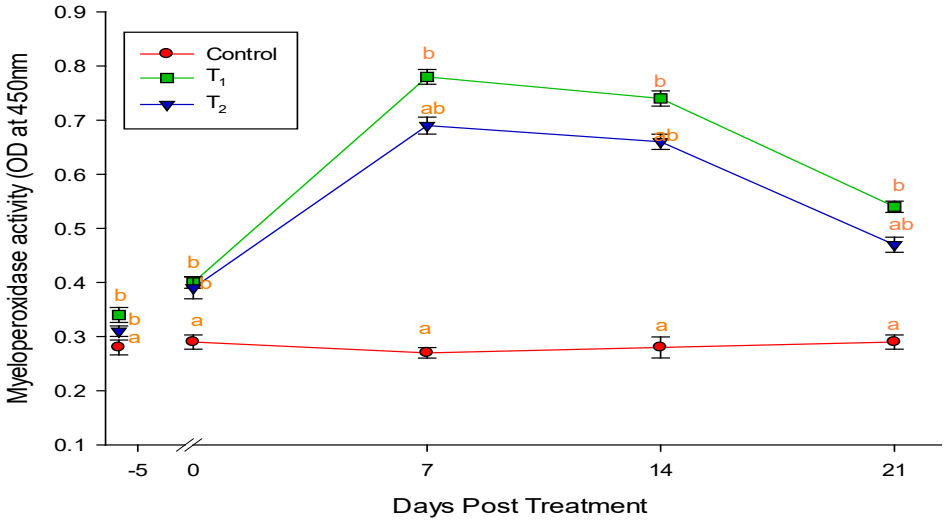


Figure 3.1.26 Effect of CUD administered through feed additive on serum myeloperoxidase activity in *Oreochromis mossasmbicus*



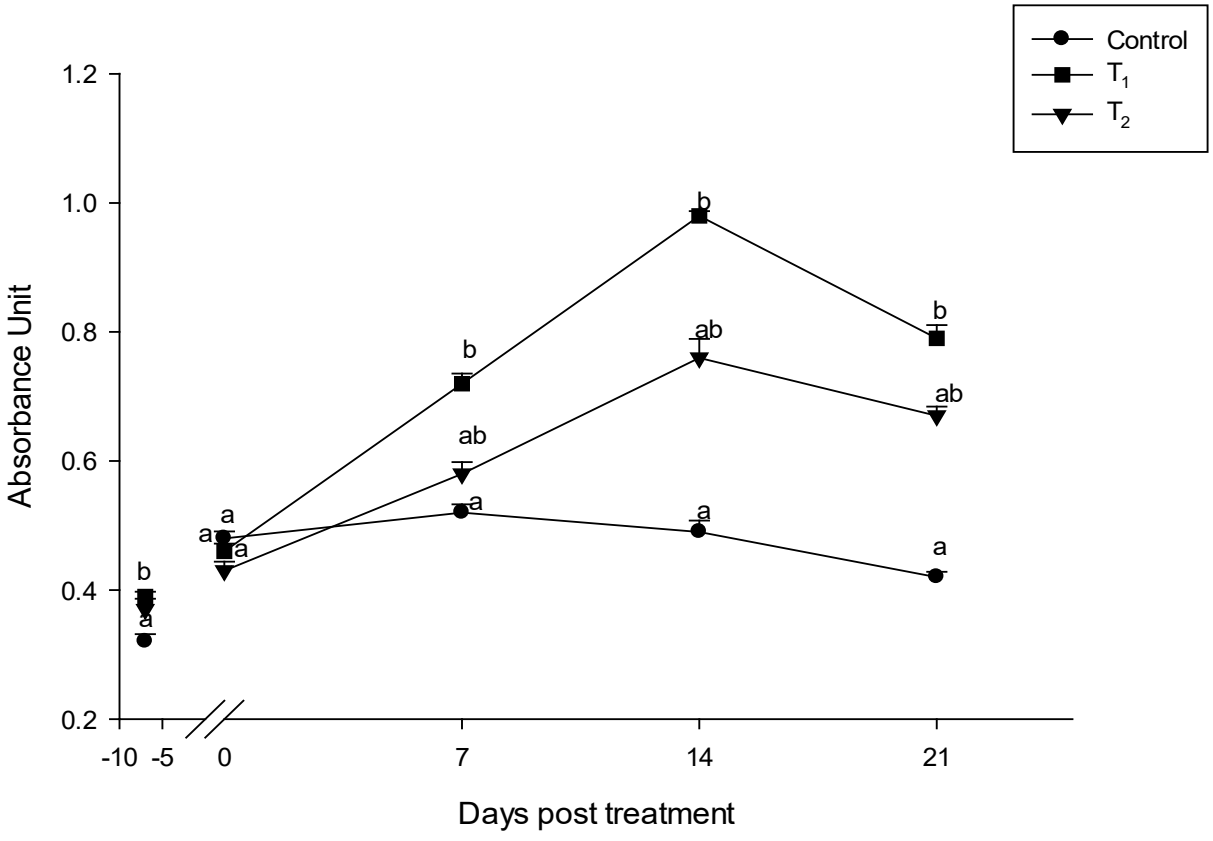
Each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

i. Serum Bactericidal activity

Water additive route

The effect of direct administration of cow urine distillate (CUD) on Serum Bactericidal activity of *Oreochromis mossambicus* was illustrated in fig 3.1.27 which shows the variation between *Bos indicus* and *Bos taurus* when compared with the control group. The Serum Bactericidal activity was significantly (P<0.001) higher in both breed CUD treated groups while compared to untreated control group throughout the study period. The maximum activity was observed on 14th day in T_1 and T_2 group (P<0.001).

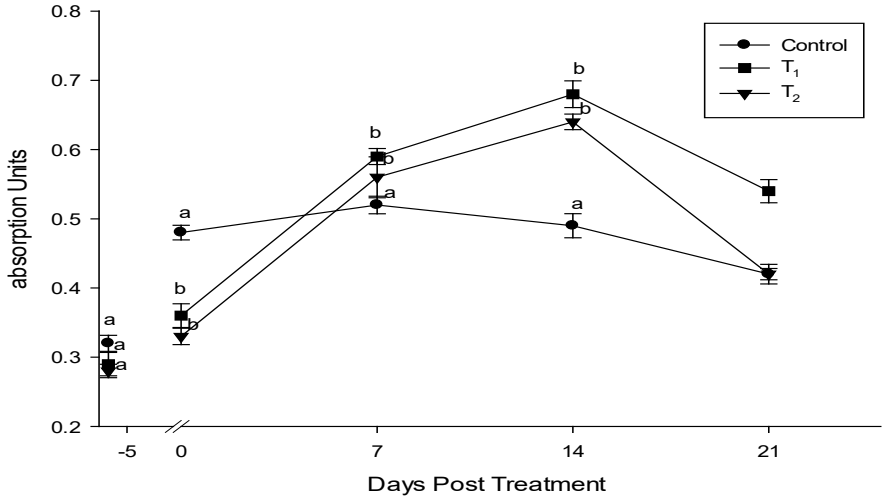
Figure 3.1.27 Effect of CUD administered through water additive on serum Bactericidal activity in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



Feed additive route

The effect of Feed supplementation administration of cow urine distillate (CUD) on Serum Bactericidal activity of *Oreochromis mossambicus* was illustrated in fig 3.1.28 which shows. The similar effects as in water additive route with the maximum activity observed on 14th day in T_1 and T_2 group ($P<0.001$).

Figure 3.1.28 Effect of CUD administered through feed additive on serum Bactericidal activity in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



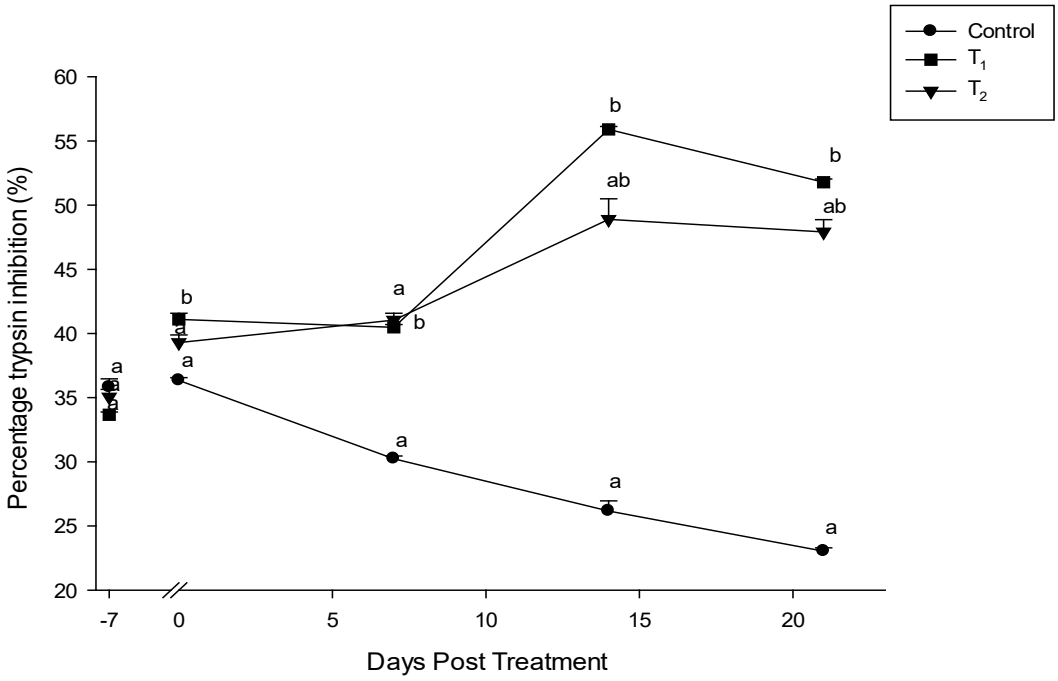
Antiprotease activity

Water additive route

The Figure 3.1.29 illustrates a significant increase ($P < 0.001$) in antiprotease activity found in treatment groups when compared to untreated control group, through water additive administration of *Bos indicus* & *Bos taurus* urine treated group which is T_1 & T_2 respectively. It is measured in terms of Percentage trypsin inhibition (%). It is significantly ($P < 0.001$) higher on the 14thday in treatment groups with T_1 showing maximum activity (55.9 %) and control showing the minimum activity (26.16%). The fig

clearly shows a negative trend in control and positive trend in treatment groups from the 7th day of immunization to 14th day.

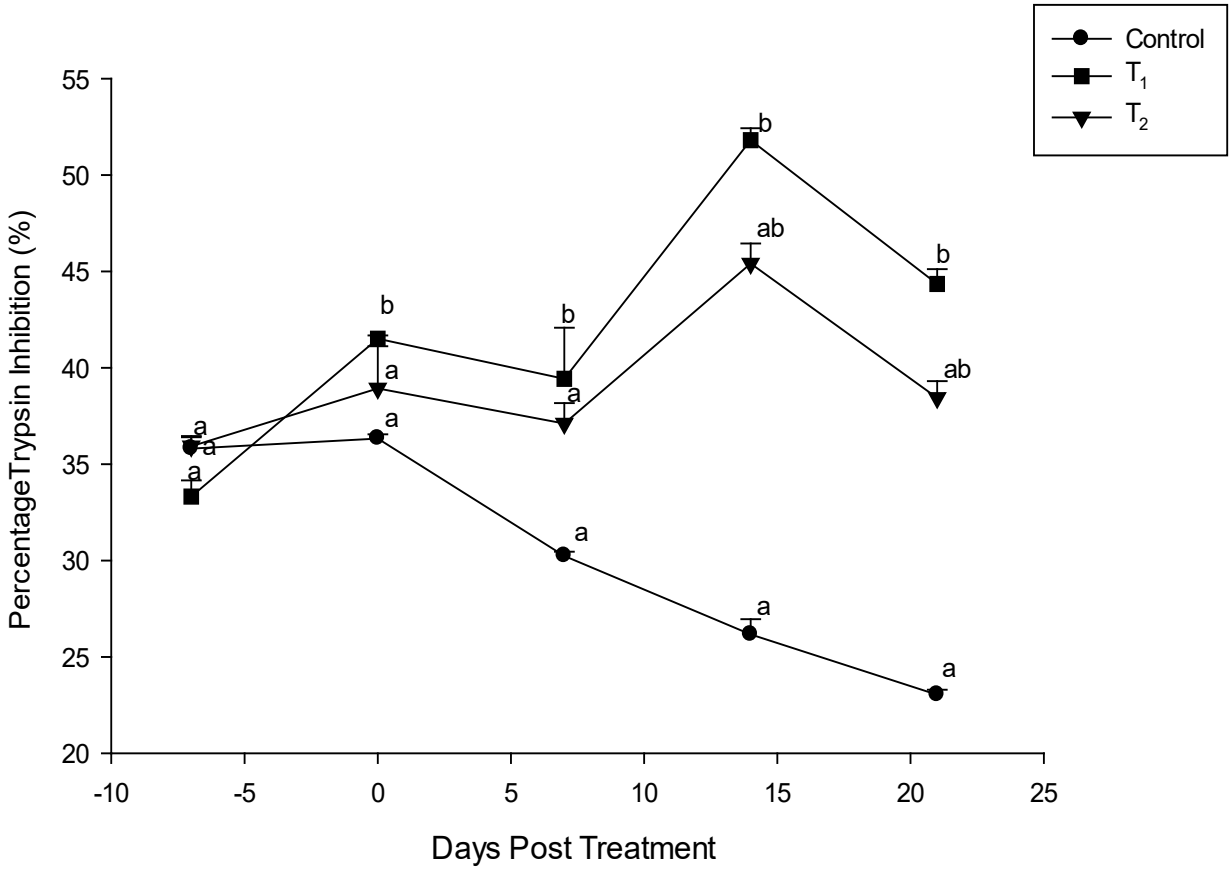
Figure 3.1.29 Effect of CUD administered through water additive on serum antiprotease activity in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



Feed additive route

The Figure 3.1.30 illustrates a significant increase (P < 0.001) in antiprotease activity found in treatment groups when compared to untreated control group, through water additive administration of *Bos indicus* & *Bos taurus* urine treated group which is T_1 & T_2 respectively. It is measured in terms of Percentage trypsin inhibition (%). It is significantly (P < 0.001) higher on the 14thday in treatment groups with T_1 showing maximum activity (51.81 %) and control showing the minimum activity (26.16%). The fig clearly shows a negative trend in control and positive trend in treatment groups from the 7th day of immunization to 14th day and peak day of response occurred.

Figure 3.1.30 Effect of CUD administered through feed additive on serum antiprotease activity in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



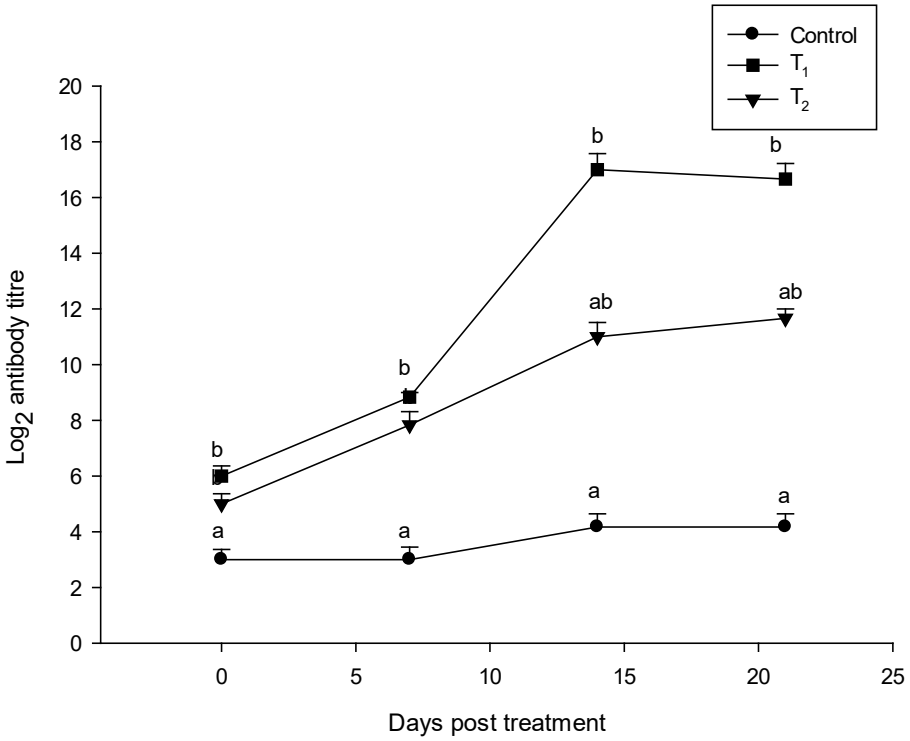
II. Specific immune parameters

Antibody Titration

Water additive route

Fig 3.1.31 illustrates the effect of direct administration of cow urine distillate (CUD) on Serum Bacterial titer of *Oreochromis mossambicus* between *Bos indicus* and *Bos taurus* with the comparison of control group. In treatment groups it evident is that there was significant (P<0.01) increase when compared to that of untreated control group. From

Figure 3.1.31 Effect of CUD administered through water additive on serum Antibody titer in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)

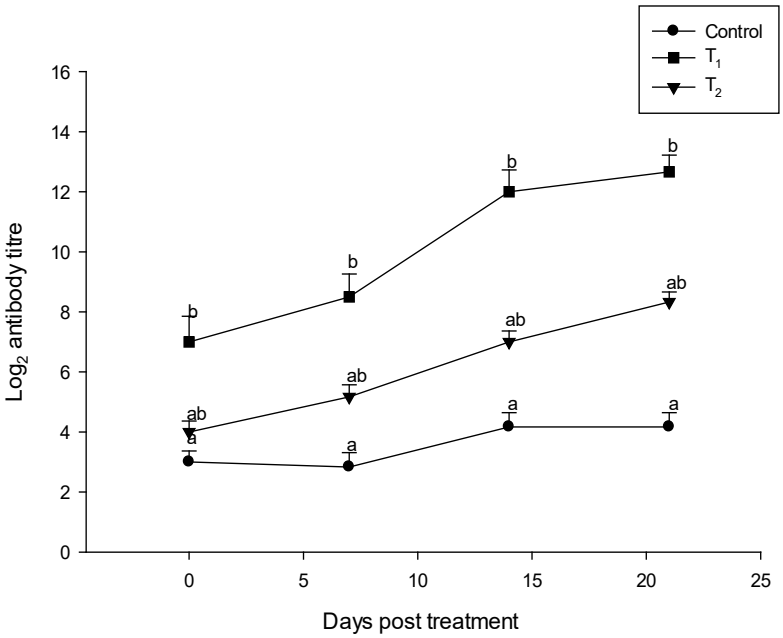


the graph, it is clear that, all the three were having almost same value during the initial days, and in treatment groups the antibody level increased continuously over the days. In both treatment groups the 14th day showed significantly higher values. Among the treatment group and the days of experiment, the T_1 treated group showed highest activity on 14thday post treatment (17.2; P<0.01) while the control group showed the minimum activity (4.16; P<0.01).

Feed additive route

Fig 3.1.32 depicts the effect of feed supplement administration of cow urine distillate (CUD) on Serum Bactericidal agglutination of *Oreochromis mossambicus* between *Bos indicus* and *Bos taurus* when compared to control group. In treatment groups it is evident

Figure 3.1.32 Effect of CUD administered through feed additive on serum anti body titre in *Oreochromis mossasmbicus;* each point represents mean ± SE of 6 fish; a posteriori Holm-Sidak comparison of control and treated groups on individual post treatment days shown with different alphabets representing significant difference (P<0.05)



that there was significant (P<0.01) increase compared to that of untreated control group over the period of experiment. From the graph, it is clear that, all the three were having almost same value during the initial days, and the treatment group increasing continuously with peak day response on the 21st day. A shift in peak day from 14th to 21st day was observed in all groups. In the treatment groups the 21st day have significantly highest value was occur. Among the treatment groups and the days of experiment, the T_1 treated group showed highest activity on day 21post immunization.

III. Internal Morphological observation

Morphological investigation of the lymphoid organs like structure, shape and colour of both route and different group were recorded. No notable difference was observed in the colour, shape and structural pattern of the lymphoid organs of control and treatment groups through water and as feed additive routes.

Organosomatic index

Hepatosomatic index, Splenosomatic index, Thymosomatic index and Mucosomatic index

Water additive route

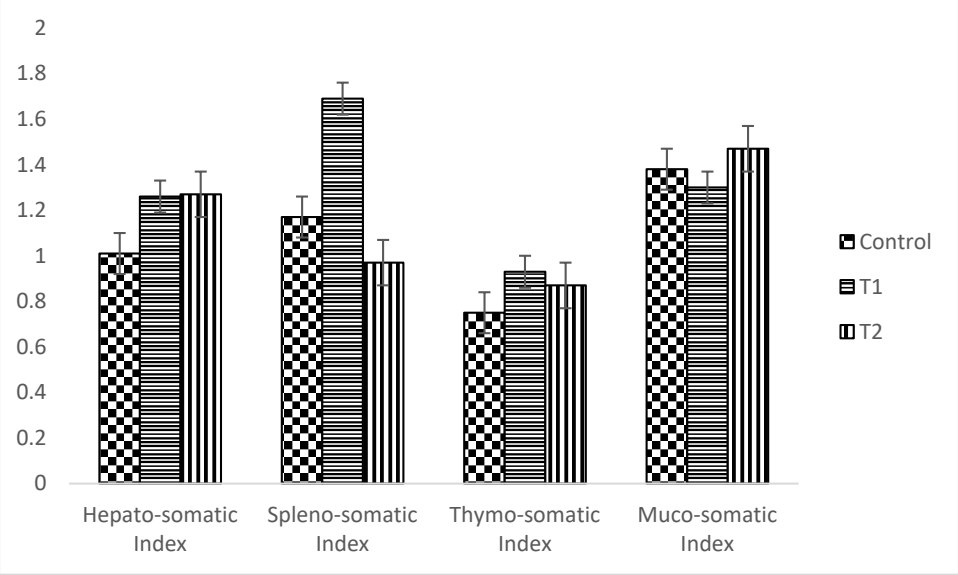
The highest value of **Hepato-somatic index** was recorded in the T_2 as 1.27±0.05 and least value was recorded in the control as 1.01±0.09.

The highest value of **Spleno-somatic index** was recorded in the T_1 of as 1.69±0.08 and least value was recorded in the control as 1.17±0.06.

The highest value of **Thymo-somatic index** was recorded in the T_1 as 0.93±0.05 and least value was recorded in the control as 0.75 ±0.08.

The highest value of **Muco-somatic index** was recorded in the T_2 as 1.47±0.01 and least value was recorded in the T_1 as 1.30±0.04 (Fig. 3.1.33).

Figure 3.1.33 Effect of CUD administered through water additive on Organosomatic index in *Oreochromis mossasmbicus*



Feed additive route

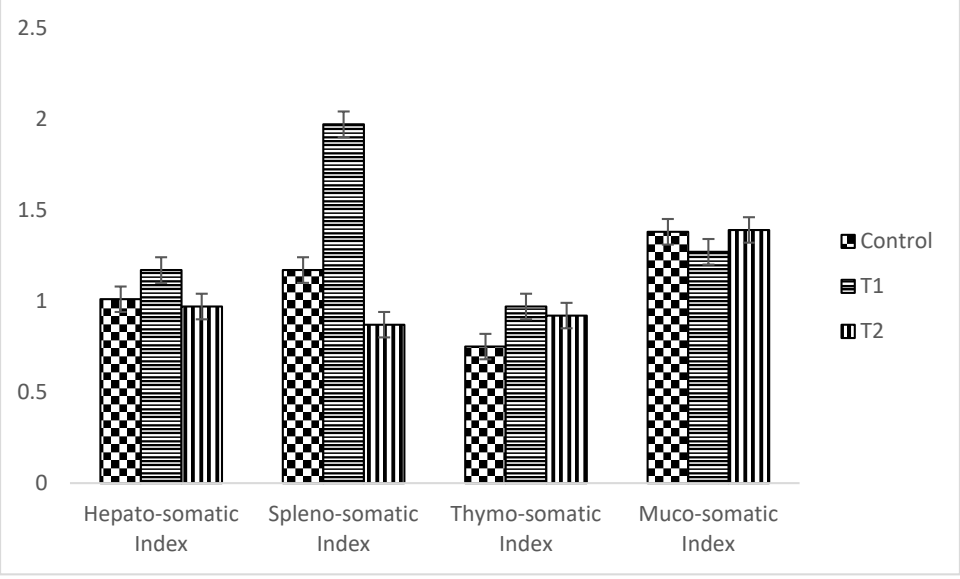
The maximum **Hepato-somatic index** was recorded in the T_2 as 1.17±0.05 and least value was recorded in the control as 1.01±0.09.

The highest value of **Spleno-somatic index** was recorded in the T_1 as 1.97±0.03 and least value was recorded in the control as 1.17±0.06.

The highest value of **Thymo-somatic index** was recorded in the T_1 as 0.97±0.04 and least value was recorded in the control as 0.75 ±0.08.

The highest value of **Muco-somatic index** was recorded in the T_2 as 1.39±0.07 and least value was recorded in the T_1 as 1.27±0.03 (Fig. 3.1.34).

Figure 3.1.34 Effect of CUD administered through feed additive on Organosomatic index in *Oreochromis mossasmbicus*

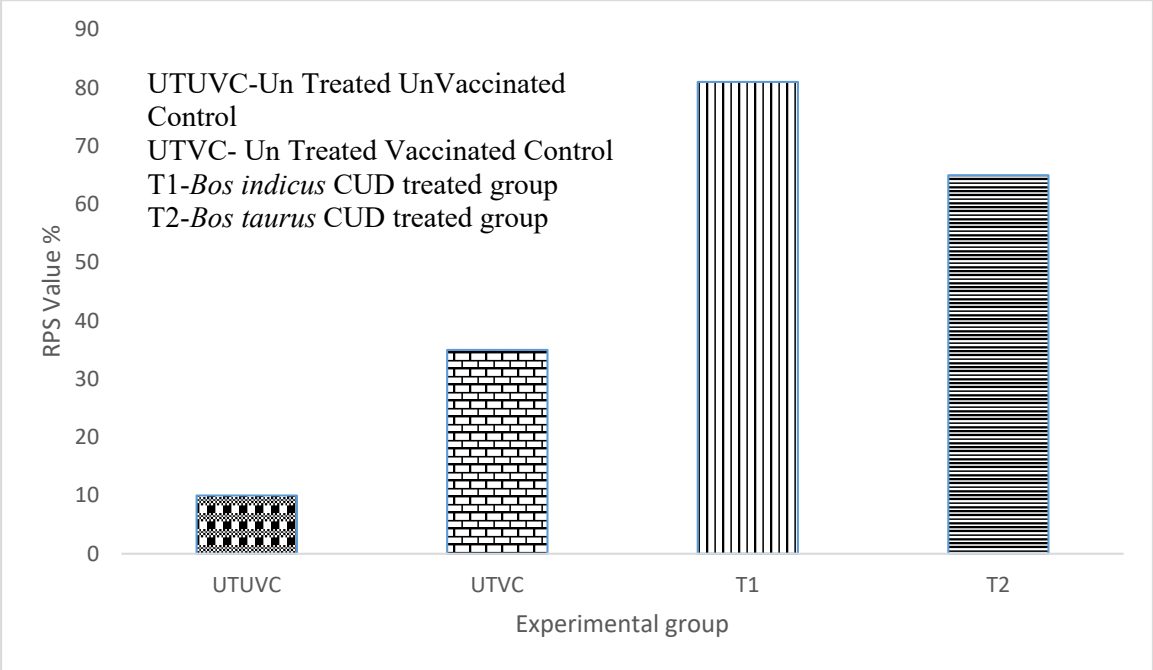


IV. Disease resistance

Water additive route

Figure 3.1.35 illustrates a significant reduction (P >0.001) in mortality when compared to that of untreated, unvaccinated control group, on administration of *Bos indicus* & *Bos taurus* urine treated group which is T_1 & T_2. RPS value reached up to 80% in T_1. In T_2 significant reduction in mortality was observed with untreated, unvaccinated Control and Untreated, vaccinated Control. Both control group did not provide significant protection, which is reflected on the high mortality observed.

Figure 3.1.35 Effect of CUD administered through water additive on RPS values in *Oreochromis mossasmbicus*

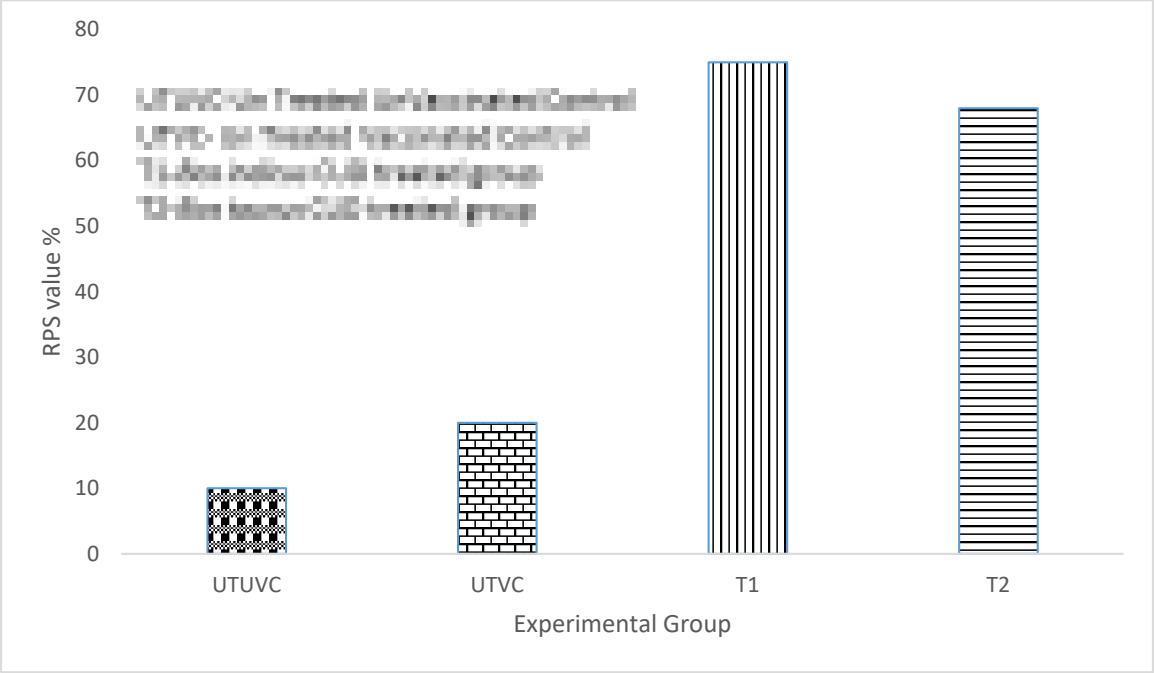


Feed additive route

Figure 3.1.36 illustrates a significant reduction (P < 0.001) in mortality when compared to that of untreated control group, on administration of *Bos indicus* & *Bos taurus* urine feed supplement treated group which is T_1 & T_2. RPS value reached up to 75% in T_1. In T_2 significant reduction in mortality was observed with untreated, unvaccinated control

and untreated, vaccinated control. Both control group did not provide significant protection, which is reflected on the high mortality observed.

Figure 3.1.36 Effect of CUD administered through feed additive on RPS values in *Oreochromis mossasmbicus*



Chapter II - 3.2 Study of Growth and Food utilization Parameters influenced by *Bos indicus* and *Bos taurus* Urine Distillate

The study of growth and Food utilization responses in *Oreochro*mis *mossambicus* fingerlings were compared for the influence of cow urine distillate of two different breeds *Bos indicus* and *Bos taurus*. The Cow urine distillate was administered through two different routes – water additive (v/v) and Feed additive at 0.1% concentration (w/v), which was found as the optimal (fig 2.1, fig 2.2 & fig 2.3).The result of growth parameters,

conditional factors, internal morphological analysis, food utilization parameters and survival parameters were recorded.

3.2.1 Effect of cow urine distillate administered through different routes on Growth parameters in *Oreochromis mossambicus*

i. Water additive

The results revealed that on the every 10th day of experimental period, the growth parameters were significantly higher in both experimental groups (T_1 & T_2) when compared to the control. The influences of the cow urine distillate on the fish body length on every 10^{th} day were presented in Table 3.2.1. 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 on every 10^{th} day were presented in Table 3.2.2.

10^{th} day

The weight gain was found higher in T_2 (0.122 gm) and least growth of 0.002 gm was recorded in Control. In T_1 0.073gm of growth (weight gain) was recorded. The highest growth rate of 0.00792 mg/day was recorded in T_2, when compared with control followed by T_1. The maximum specific growth rate was recorded in T_2 that was noted as 0.7628 % and minimum value observing the control. The maximum average daily growth of 0.0122 g/day was observed in T_2 and least average daily growth of 0.0002 g/day was observed in control. The Percentage of increase in body weight was highest in T_2 group (7.927%) and in T_1 it was 5.324% and 0.013% on control (Table 3.2.2, Fig 3.2.1).

20th day

The expose of cow urine distillate on the growth parameters on 20th day is presented in Table 2.2. An augmented growth (weight gain) was recorded in T_1 and T_2 with 0.0315 and 0.0338gm as well as a higher growth rate of 0.0011487g/day and 0.00109g/day in T_1 and T_2 respectively when compared with control. The maximum average daily growth of 0.00169 g/day was observed in T_2 and least average daily growth of 0.0006 g/day was observed in control, as well as maximum specific growth rate was recorded as 0.11357 (%) at T_1 and minimum specific growth rate was recorded as in control (Table 3.2.2).The percentage increase in body weight in T_1 and T_2 groups are 2.297 % and 2.196% respectively with in significantly higher when compared to control 0.417% (Table 3.2.2 & fig 3.2.1).

30th day

The influence of cow urine on the growth parameters on 30th day is presented in Table 3.2.2. A stimulated growth (weight gain) of 0.069g in T_2 and 0.036g in T_1 was recorded, when compared to control (0.026g). The highest growth rate of 0.0014 mg/day was recorded in T_2, when compared with 0.0005 mg/day of control. The maximum average daily growth of 0.008 g/day was observed in T_2 and least average daily growth of 0.00085 g/day was observed in control. The maximum specific growth rate of 0.1461 (%) in T_2 and minimum specific growth rate of 0.06 (%) in control was recorded. When compared to the control with 1.75 %, of percentage increase in body weight, it was fairly higher in T_2 with 4.48 % and in T_1 it was 2.62% the growth rate and the percentage increase body weight were shown the figure 3.2.1 & Table 3.2.2.

Figure 3.2.1 Effect of CUD administered through water additive on PIBW in *Oreochromis mossasmbicus*

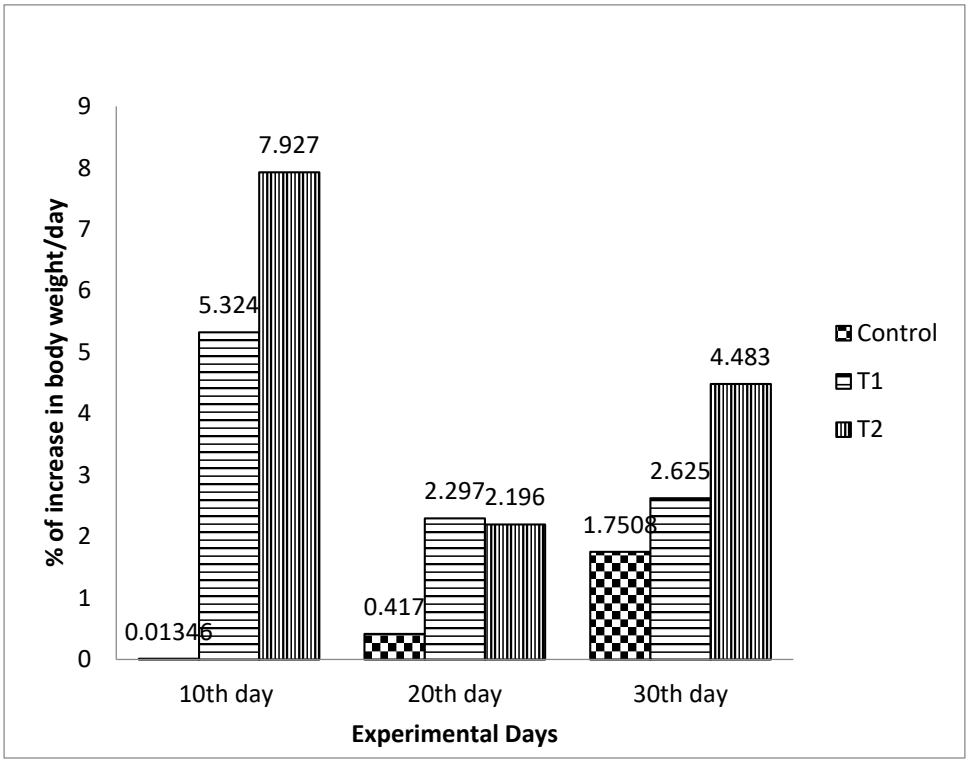


Table 3.2.1 Effect of cow urine distillate on Length of *Oreochromis mossambicus* as water additive

In day	Control	T1 (*Bos indicus*)	T2 (*Bos taurus*)
0^{th} day	4.71 ±0.108	4.78 ± 0.077	4.71 ±0.161
10^{th} day	4.74 ±0.132	4.81 ±0.071	4.7 ±0.089
20^{th} day	4.81 ±0.060	4.97±0.079	4.84 ±0.081
30^{th} day	4.93 ±0.088	5.2 ±0.070	4.9 ± 0.086

Table 3.2.2 Effect of Cow Urine Distillate on Growth parameters *of Oreochromis mossambicus* fingerlings as water additive

	10th day			20th day			30th day		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
G (g)	0.002	0.073	0.122	0.0062	0.0315	0.0338	0.026	0.036	0.069
GR(mg/D)	0.0013	0.00532	0.00792	0.0008	0.001148	0.00109	0.0005836	0.000875	0.0014
ADG	0.0002	0.0073	0.0122	0.00062	0.001575	0.00169	0.000853	0.0012	0.0089
SGR%	0.01345	0.5187	0.7628	0.02083	0.1135	0.1086	0.06	0.08639	0.1461
PIBW%	0.01346	5.324	7.927	0.417	2.297	2.196	1.7508	2.625	4.483

ii. Feed additive route

The growth response of *Oreochromis mossambicus* in terms of total body weight and total body length (Table 3.2.3), growth (weight gain), growth rate, specific growth rate, average growth rate and percentage increase in body weight were increased on 10, 20 and 30th days, under the influence of *Bos indicus* CUD and *Bos taurus* CUD through feed additive (table 3.2.4 and fig 3.2.2).

10th day

The 10th day growth response of *Oreochromis mossambicus* in terms of increase in body weight, growth rate, specific growth rate (SGR) are presented in Table 3.2.4 and the percentage increase body weight was shown in figure 3.2.2.The maximum growth rate was found in T_2 (0.0029 mg/day), which was treated with Cow urine distillate and least growth rate, was found in control (0.00058 mg/day). The maximum average daily growth of 0.0024g/day was noted in T_2 and minimum average daily growth of 0.00085 g/day was noted in control. Highest specific growth rate of 0.288% was noted in T_2 and least specific growth rate of 0.06% was noted in control. The highest percentage increase in body weight of 2.92% was recorded in T_2 and least percentage increase in body weight of 0.013% was recorded in Control.

20th day

The growth parameters indices growth, growth rate, average daily growth rate, specific growth rate and survival rate where presented in Table 3.2.4. The growth was found higher in T_1 0.0541g which had suspended CUD. Least growth of 0.006 g was recorded in control. The maximum growth rate of 0.00189 g/day was recorded in T_2 and minimum growth rate of 0.0004 was recorded in control followed by 0.0008 mg/day in T_2.

Least average daily growth rate of 0.00062 mg/day was also recorded in control and highest average daily growth rate of 0.0270 mg/day was recorded in T_2 as well as the maximum specific growth rate was noted in same groups. Figure 3.2.2 shows that the highest percentage increase in body weight was exhibited as 3.788% in T_2 and minimum in control of 0.417%. The 20th day growth parameters were shown Table 3.2.4.

30th day

The growth parameter indices like growth (weight gain), growth rate, average daily growth rate, specific growth rate and survival rate were recorded on the 30th day and tabulated in table 3.2.4. The all growth parameters significantly higher in treatment groups T_1 and T_2 when compared to control. The growth were found to be in fish treated with *Bos indicus* CUD (T_1) was found to be lesser (0.072g) than T_2 (0.079g) recorded. Least growth of 0.026 g was recorded in control. The maximum growth rate of 0.001842 g/day was recorded in T_2 and least growth rate of 0.0005g/day was recorded in control followed by 0.001808 mg/day in T_1. Least average daily growth rate of 0.00085 mg/day was also recorded in control and highest average daily growth rate of 0.026 g/day was recorded in T_2 and in T_1 it was 0.00036g/day. The maximum specific growth rate was noted in T_2 (0.1461%) and in T_1 it was observed as (0.086%). Highest percentage increase in body weight of 5.425% was found in T_1, while in T_2 5.528% was recorded 1.750% were noted in control (Table 3.2.4 & figure 3.2.2).

Figure 3.2.2 Effect of CUD administered through feed additive on PIBW in *Oreochromis mossasmbicus*

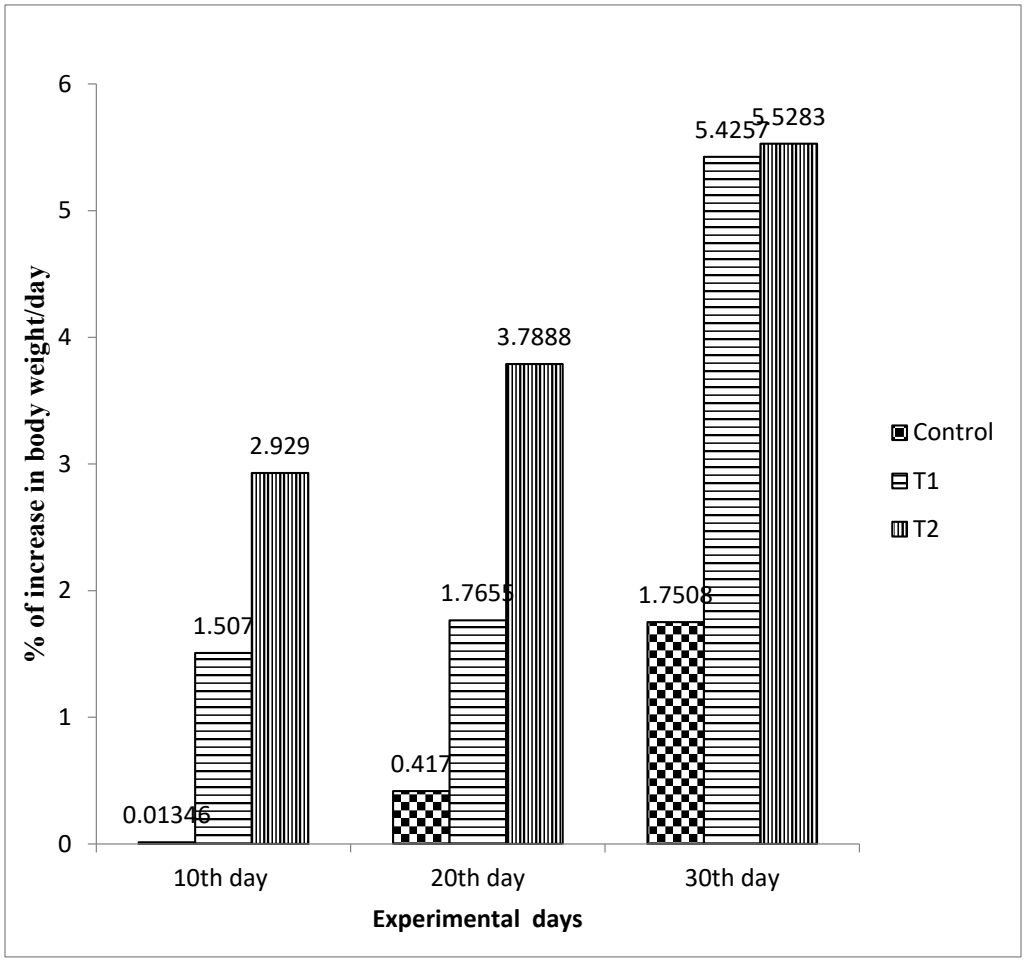


Table 3.2.3 Effect of cow urine distillate on Length of *Oreochromis mossambicus* as feed additive

In day	Control	T1(*Bos indicus*)	T2 (*Bos taurus*)
0^{th} day	4.71 ± 0.108	4.82 ± 0.128	5.01 ± 0.246
10^{th} day	4.74 ± 0.132	5.08 ± 0.067	5 ± 0.108
20^{th} day	4.76 ± 0.082	5.17 ± 0.096	5.03 ± 0.064
30^{th} day	4.93 ± 0.088	5.38 ± 0.139	5.2 ± 0.051

Table 3.2.4 Effect of Cow Urine Distillate on Growth parameters *of Oreochromis mossambicus* fingerlings as feed additive

	10^{th} day			20^{th} day			30^{th} day		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
G (g)	0.002	0.02	0.04	0.0062	0.0234	0.0541	0.026	0.072	0.079
GR(mg/D)	0.00058	0.001507	0.002929	0.0004	0.00088	0.00189	0.000583	0.001808	0.001842
ADG (g)	0.0002	0.002	0.004	0.00062	0.00117	0.00270	0.00085	0.00036	0.00263
SGR%	0.01345	0.149	0.288	0.02083	0.0875	0.185	0.06	0.17	0.18
PIBW%	0.01346	1.507	2.929	0.417	1.7655	3.7888	1.7508	5.4257	5.5283

3.2.2 Effect of cow urine distillate administered through different routes on Conditional factor (K Factor) in *Oreochromis mossambicus*

In the present study, the condition factor of *Oreochromis mossambicus* administered with CUD through different routes. When CUD was administered as water additive, the highest "k" factor was calculated in T_1 group (1.58) and the lowest was in T_2 (1.07) and control has 1.26. When the CUD was given as feed additive, the highest "k" factor was recorded in T_1 group with 1.26 k value and the lowest k value was observed in control (0.939). (Table 3.2.5 & Figure 3.2.3).

Figure 3.2.3 Effect of CUD administered through different routes on Condition factor in *Oreochromis mossasmbicus*

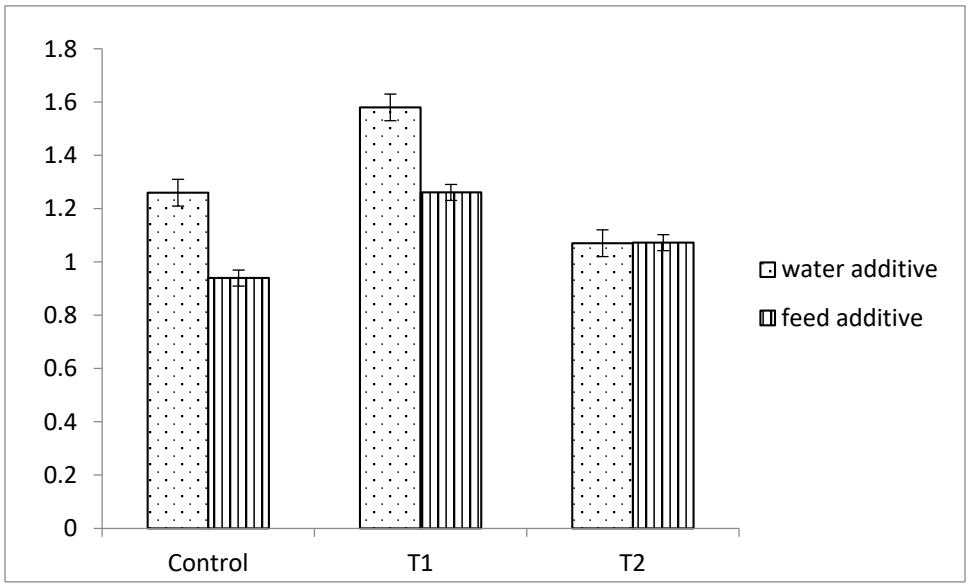


Table 3.2.5 Effect of cow urine distillate administered through different routes on Conditional factor (K Factor) in *Oreochromis mossambicus*

Parameters	Water additive	Feed additive
Control	1.26	0.9397
T_1	1.58	1.2610
T_2	1.07	1.0724

3.2.3 Effect of cow urine distillate administered through different routes on internal morphological analysis in *Oreochromis mossambicus*

i. Water additive route

Relative Length of Gut, Somato-Gastric Index and Hepato-Somatic index

Relative Length of Gut defined as the ratio of the length of intestine of fish to standard body length. Somato-gastric index defined as the ratio of the total body length to total intestine length and the hepato-somatic index as an index of weight of liver to the weight of body was studied in the present investigation. The maximum value of RLG was calculated in T_1 (2.761) and minimum was in control (2.321). The maximum value of Somato Gastric Index in T_1 group (0.376) and minimum was observed in control (0.315). The highest value of hepato-somatic index of 0.985 in T_1 and the lowest of 0.853 in control was observed. The RLG, SGI and HIS of *O. mossambicus* was estimated on 30th day of experiment and was tabulated as Table 3.2.6 and presented in figure 3.2.4.

Figure 3.2.4 Effect of CUD administered through water additive on internal morphology in *Oreochromis mossasmbicus*

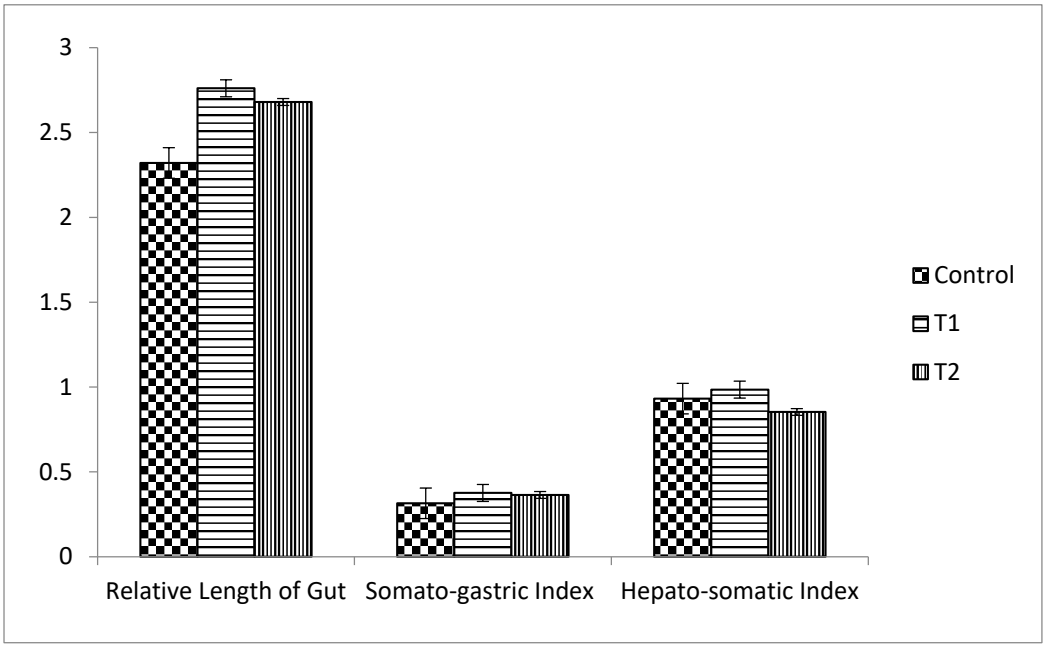


Table 3.2.6 Effect of cow urine distillate administered as water additive on internal morphological analysis in *Oreochromis mossambicus*

Parameters	Relative Length of Gut	Somato-gastric index	Hepato-somatic Index
Control	2.321 ± 0.105	0.315± 0.045	0.932 ±0.076
T_1	2.761± 0.084	0.376±0.017	0.985±0.062
T_2	2.680±0.129	0.364± 0.011	0.853±0.077

ii. Feed additive route

Relative Length of Gut, Somato-gastric Index and Hepato-somatic index

The maximum value of RLG was calculated in T_1 (3.319) and minimum was in control (2.321). The maximum value of Somato Gastric Index in T_1 group (0.475) and minimum was observed in control (0.315). The highest value of hepato-somatic index of

1.043 in T_1 and the lowest of 0.675 in T_2 was observed. The RLG, SGI and HIS of *O. mossambicus* was estimated on the 30th day feed supplement route average value of relative length of gut, somato-gastric index and hepato-somatic index tabulated as Table 3.2.7 and Figure 3.2.5.

Figure 3.2.5 Effect of CUD administered through feed additive on internal morphology in *Oreochromis mossasmbicus*

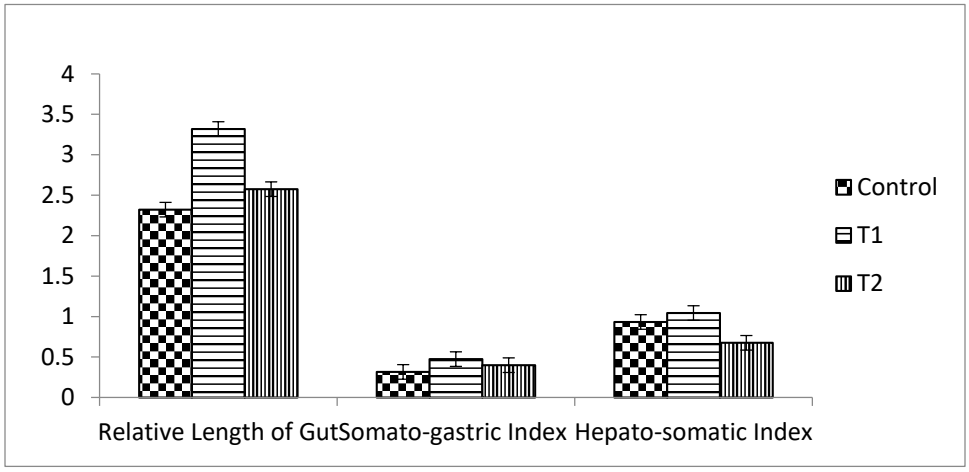


Table 3.2.7 Effect of cow urine distillate administered as feed additive on internal morphological analysis in *Oreochromis mossambicus*

Parameters	Relative Length of Gut	Somato-gastric index	Hepato-somatic Index
Control	2.321 ± 0.105	0.315±0.045	0.932 ±0.076
T_1	3.319± 0.510	0.475±0.0188	1.043±0.0337
T_2	2.575± 0.090	0.399±0.0145	0.675±0.0326

3.2.4 Effect of cow urine distillate administered through different routes on Food utilization Parameters in *Oreochromis mossambicus*

i. Water additive

The food utilization responses of *Oreochromis mossambicus* fingerlings under different breeds of cow urine distillate treatments was studied by administering through water additive. The food utilization responses of *Oreochromis mossambicus* in terms of Feeding rate, Feed absorbed, Absorption Rate, Percentage of Feeding Rate, Percentage of Absorption Rate, Absorption Efficiency, Gross conversion efficiency and Net conversion efficiency were observed on 10^{th}, 20^{th} and 30^{th} day of experimental period.

10^{th} day

Table 3.2.8 shows the 10^{th} day food utilization parameters influenced by CUD. The food utilization parameters were higher in experimental fishes treated with Cow Urine Distillate (CUD), when compared to the control. The maximum feeding rate was record as 0.029 mg/day in T_1, 0.02723mg/day in T_2, and 0.0208mg/day in control. The highest values of food absorbed, absorption rate, absorption efficiency, Percentage of Feeding Rate, Percentage of Absorption Rate were record in T_1 *Bos indicus* CUD treated group while compared to other groups. The values of feeding rate and food absorption was shown in figure 3.2.6. The maximum Gross conversion efficiency of 20.91% and Net Conservation efficiency of 32.36% were noted in T_2. The minimum results were recorded in control (Table 3.2.8).

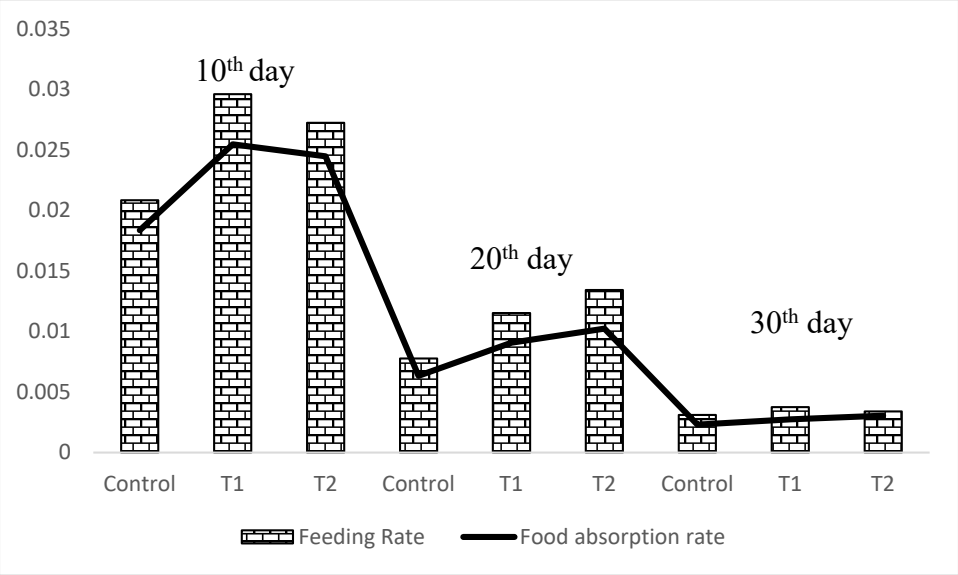
20th day

Various food utilization parameters like Feeding rate, Food absorbed, Absorption rate were higher in treatment group T_1 and T_2 when compared to the control. The effect on feeding rate and food absorption rate was shown the Figure 3.2.6 *Bos taurus* CUD treated group (T_2) shows highest absorption efficiency (81.68 mg/day), the Percentage of Feeding Rate (1.342%), Percentage of Absorption Rate (1.023%), Gross conversion efficiency (10.304 %) as compared to the other group. Net Conservation efficiency (12.649%) were higher in T_1 which was shown in Table 3.2.8. The minimum performance was exhibited by the control groups which is lesser than CUD treated groups (Table 3.2.8)

30th day

Bos indicus CUD (T_1) through direct administration in water had maximum Feeding rate (0.00375mg/day), Food Absorption (0.00305mg/day), Food Absorption rate (0.00305mg/day), and Absorption efficiency (8 9.59mg/day), the Percentage of Feeding Rate (14.12%/day), Percentage of Absorption Rate (0.30%/day) were are all noted to be highest in *Bos taurus* CUD treated group (T_2) as compared to the other group. The Gross conversion efficiency of T_1 is 53.33 %, T_2 is 43.82 % and it is a 16.29% in control. The maximum Net Conservation efficiency of 81.00% was recorded in T_1, and 21.37% minimum values were recorded in control.

Figure 3.2.6 Effect of cow urine distillate administered through water additive route on Feeding and Food absorption rate in *Oreochromis mossambicus*



ii. Feed additive route

The food utilization responses of *Oreochromis mossambicus* fingerlings under different breeds of cow urine distillate treatments were studied by giving CUD as feed additive on 10th, 20th and 30th day of experimental period.

10th day

Table 3.2.9 shows the effect of CUD as feed supplement on 10th day food utilization parameters of *Oreochromis mossambicus*. The maximum feeding rate (0.0283 mg/day), food absorbed (0.1887mg/day), food absorption rate (0.0250mg/day), absorption efficiency (88.17mg/day), percentage of feeding rate (2.83%), percentage of food absorption rate (2.504%) was noted in T_1, when compared to other treated group and control. The gross conversion efficiency of 14.136%, Net Conservation efficiency of 17.748% were record in T_2. The effect of CUD on feeding and food absorption rate was shown in Figure 3.2.7.

Table 3.2.8 Effect of cow urine distillate administered as water additive on Food utilization Parameters in *Oreochromis mossambicus*

	10^{th} day			20^{th} day			30^{th} day		
	C	T1	T2	C	T1	T2	C	T1	T2
FR (mg/day)	0.020843	0.029602	0.02723	0.007764	0.011516	0.013429	0.0031	0.00375	0.0034
FA (mg/day)	0.1236	0.159	0.1857	0.085411	0.13239	0.13306	0.1044	0.113	0.1412
FAR (mg/day)	0.018364	0.025459	0.02447	0.006342	0.009075	0.010239	0.0023	0.002747	0.00305
AE (mg/day)	88.10202	89.27885	76.44231	76.24778	78.80826	81.68533	75.542	73.186	89.59
PFR (%)	2.084	2.972	2.760	0.776	1.156	1.342	10.44	11.3	14.12
PAR (%)	1.836	2.545	2.447	0.634	0.907	1.023	0.23	0.27	0.30
GCE (%)	06.236	19.288	26.645	8.1774	9.9689	10.304	16.129	53.33	43.82
NCE (%)	07.079	20.911	32.366	10.724	12.649	12.614	21.739	81.00	48.85

20th day

Maximum effect on various food utilization parameters like feeding rate (0.0098mg/g/day), food absorbed (0.11354 mg/day), absorption rate (0.0075 mg/g/day), absorption efficiency (81.68mg/day), percentage of feeding rate (0.980%), percentage of food absorption rate (0.753) was recorded in T_1 which was treated with *Bos indicus* cow urine distillate when compared to control. however T_2 exhibits higher effects on the gross conversion efficiency (25.38%) and Net Conservation efficiency (34.94%). All the parameters studied were found to be least in control (Table 3.2.9). The effect of CUD on feeding and food absorption rate was shown in Figure 3.2.7.

30th day

The influence of various breeds of cow urine distillate on the food utilization parameters on the 30th day are presented in (Table 3.2.9). Fish fed with CUD (T_1 &T_2) supplemented feed given groups had maximum feeding rate (0.028 & 0.02 mg/day), absorption rate (0.078 & 0.073 mg/day), food absorption rate (0.0025 & 0.001 mg/day), absorption efficiency (83.84% & 76.54), grass conversion efficiency (81.818% & 50.00%) when compared to other treated groups. The minimum result was marked in the control group. Net Conservation Efficiency highest value recorded in T_2 and T_1 (100% & 94.26%) respectively. The results for the influence of cow urine administered through feed supplementation on food utilization parameters on the 30th day for selected parameters were graphically represented in Figure 3.2.7.

Table 3. 2.7 Effect of cow urine distillate administered through feed additive route on Feeding and Food absorption rate in *Oreochromis mossambicus*

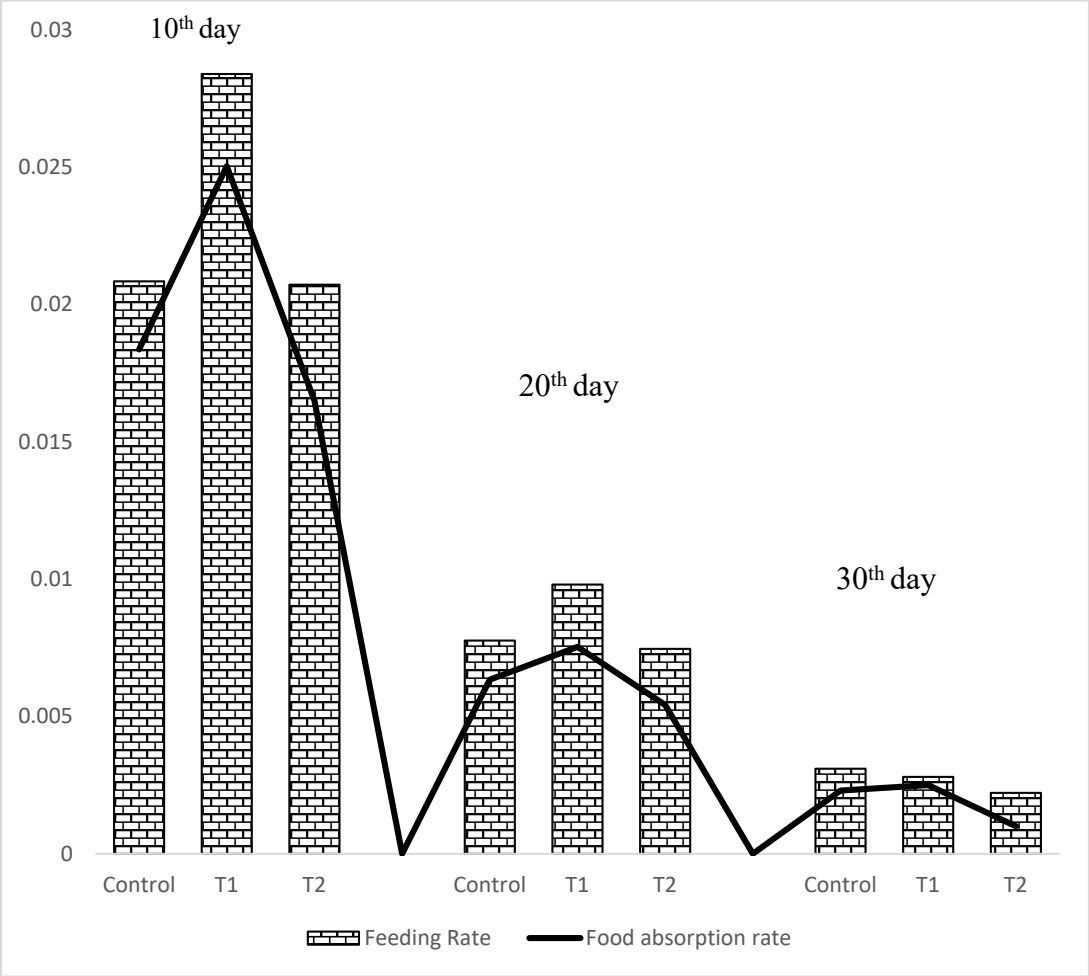


Table 3.2.9 Effect of cow urine distillate administered as feed additive on Food utilization Parameters in *Oreochromis mossambicus*

	10^{th} day			20^{th} day			30thn day		
	C	T1	T2	C	T1	T2	C	T1	T2
FR (mg/day)	0.020843	0.028398	0.020721	0.007764	0.009806	0.007462	0.0031	0.0028	0.00222
FA (mg/day)	0.1236	0.1887	0.11549	0.085411	0.11354	0.075875	0.1044	0.0789	0.0731
FAR (mg/day)	0.018364	0.02504	0.016504	0.006342	0.007533	0.005421	0.0023	0.0025	0.001
AE (mg/day)	88.10202	88.17757	79.64828	76.24778	81.68533	76.82522	75.542	83.846	76.544
PFR (%)	2.084	2.839	2.072	0.776	0.980	0.746	10.44	2.839	2.072
PAR (%)	1.836	2.504	1.650	0.634	0.753	0.542	0.23	2.504	1.650
GCE (%)	06.236	5.3071	14.136	8.1774	10.304	25.388	16.129	81.818	50.00
NCE (%)	07.079	6.0186	17.748	10.724	12.614	34.943	21.739	94.264	100.00

3.3.5 Effect of cow urine distillate administered through different routes on Gastro-Somatic Index (GSI) in *Oreochromis mossambicus*

The gastro-somatic index refers to feeding intensity. The gastro-somatic index shows a relation of weight of stomach to the body weight of the fish. The gastro-somatic index of *O. mossambicus* was estimated on 30th day of experiment. In the present study, the gastro-somatic index of *Oreochromis mossambicus* administered with CUD through different routes. When CUD was administered as water additive, the highest GSI calculated in T_1 group (30.133) and the lowest was in control (19.808). When the CUD was given feed additive, the highest GSI in T_1 (28.554) and lowest in control (19.808) its show Table 3.2.10 and Figure 3.2.8.

Figure 3.2.8 Effect of CUD administered through different routes on Gastro-somatic index in *Oreochromis mossasmbicus*

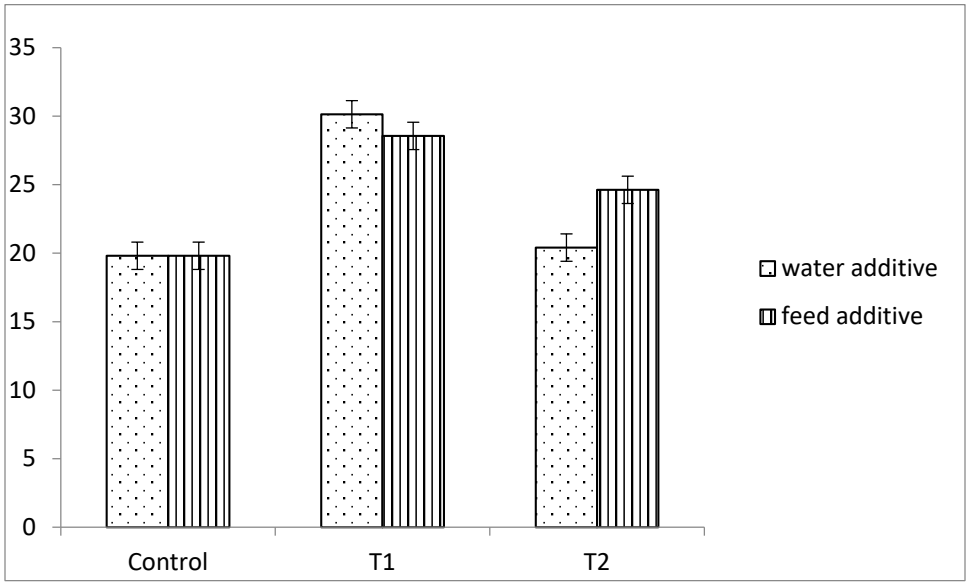


Table 3.2.10 Effect of cow urine distillate administered through different routes on Gastro somatic index in *Oreochromis mossambicus*

Parameters	Water additive	Feed additive
Control	28.5542 ± 5.742	19.808 ± 0.605
T_1	30.133 ± 3.264	28.554 ± 0.510
T_2	20.407 ± 1.947	24.619 ± 0.685

3.3.6 Feed proximate analysis

The components used in control feed and experimental feeds were given in Table 2.2 and proximate analysis of the control and experimental feeds. Table 3.2.11 & 3.2.12. figure 3.2.9, 3.2.10 & 3.2.11 shows the proximate values for the control and experimental feeds. Supplemented with the *Bos indicus* CUD (T_1) and *Bos taurus* CUD (T_2) a comparision table of the same data (Table 3.2.12) clearly shows that T_2 has maximum crude fat of 7.5% and minimum in T_1 (7.1%) while T_1 recorded maximum crude fiber (12.1%) and T_2 had minimum a value (11.6%). Carbohydrate and protein content shows only minor differences.

Table 3.2.11 Physical properties of experimental feed

	Pellet stability (%)	Moisture (%)	Dry matter (%)	Ash content (%)		
				Total Ash	Water soluble	Water insoluble
Control	98.96	1.3	98.7	7.5	2.3	5.2
T_1	98.65	3.5	96.5	6.0	1.8	4.2
T_2	97.97	4.1	95.9	7.0	2.1	4.9

Table 3.2.12 Biochemical composition of experimental feed

	Carbohydrate (%)	Protein (%)	Crude Fat (%)	Crude fiber (%)
Control	40.6	40.2	7.3	11.9
T_1	40.5	40.3	7.1	12.1
T_2	40.7	40.2	7.5	11.6

Figure 3.2.9 Proximate analysis of control feed

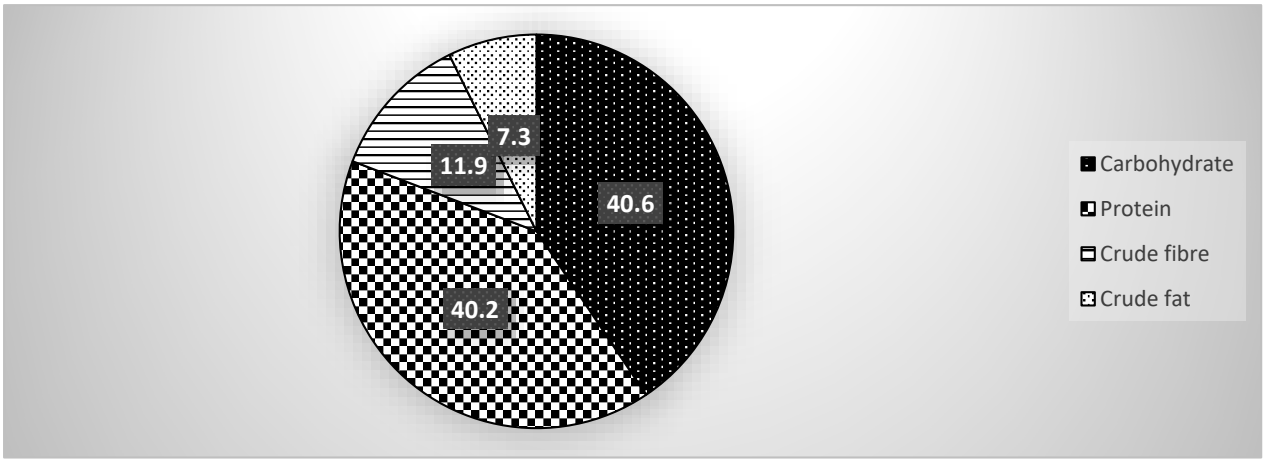


Figure 3.2.10 Proximate analysis of T_1 Feed

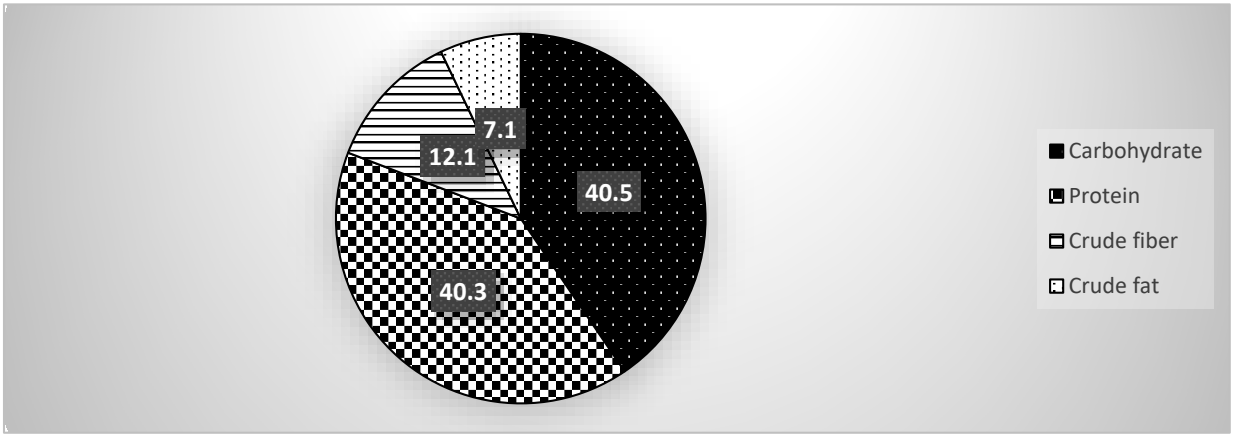
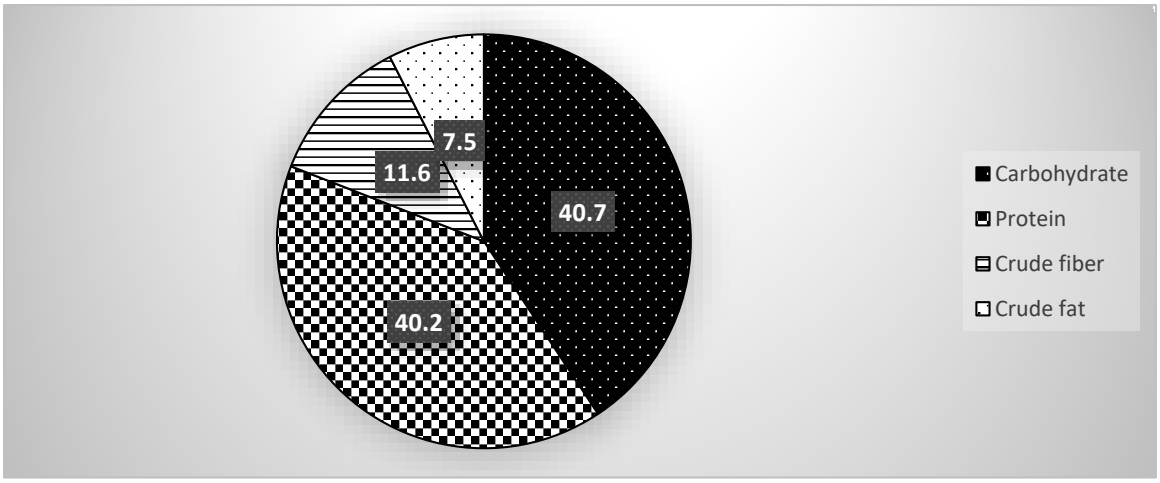


Figure 3.2.11 Proximate analysis of T_2 Feed

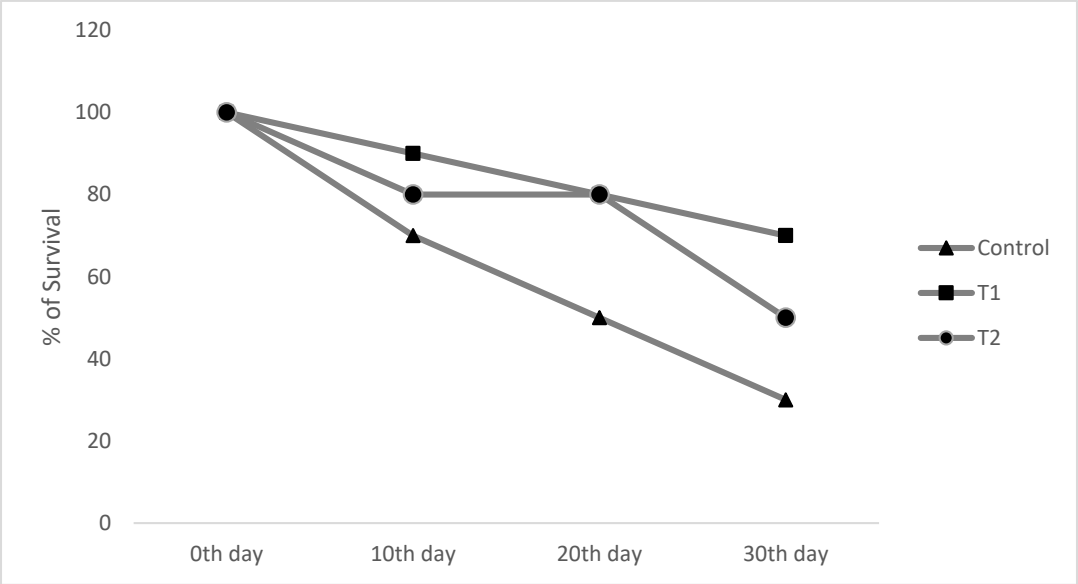


3.2.7 Effect of cow urine distillate administered through different routes on survival Parameters in *Oreochromis mossambicus*

I. Water additive

Fig 3.2.12 indicates that CUD of *Bos indicus* conferred the maximum survival rate in *Oreochromis mossambicus* fingerlings with 80% on the 30th day. The control group shows 30% survival rate on the 30th day.

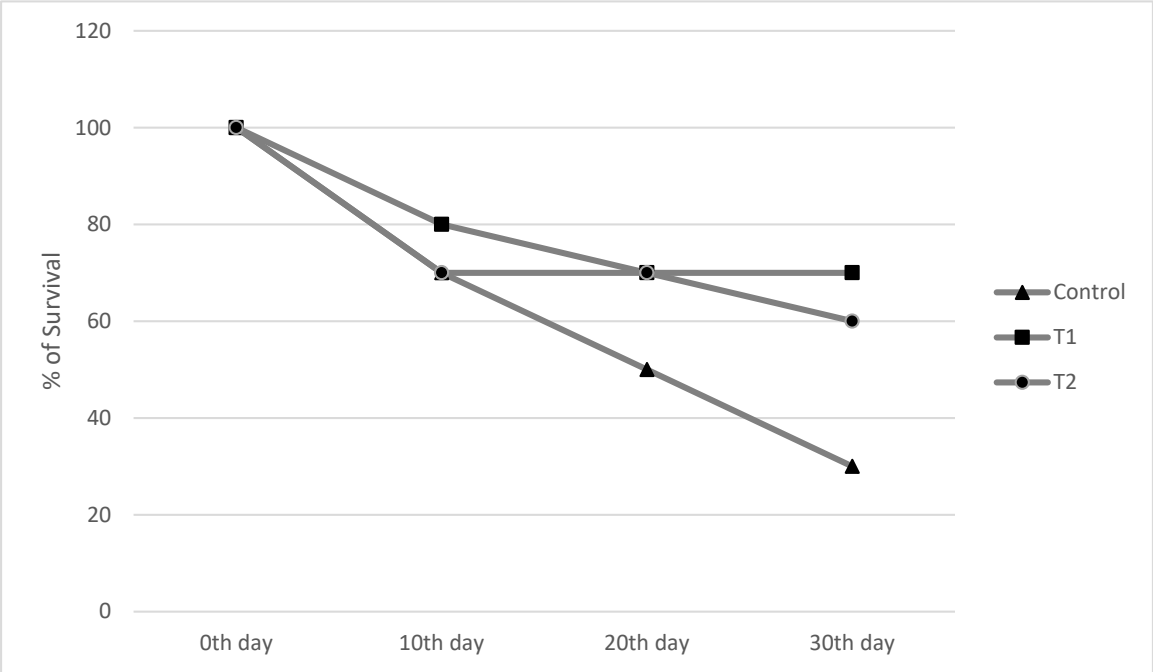
Figure 3.2.12 Effect of CUD administered through water additive on survival rate in *Oreochromis mossasmbicus*



II. Feed additive

Fig 3.2.13 indicates that CUD of *Bos indicus* provided the maximum survival rate in *Oreochromis mossambicus* fingerlings with 70% on the 30th day. The control group shows 30% survival rate on the 30th day.

Figure 3.2.13 Effect of CUD administered through feed additive on survival rate in *Oreochromis mossasmbicus*



CHAPTER III - 3 .3 STUDY OF BIO CHEMICAL PARAMETERS INFLUENCED BY *BOS INDICUS* AND *BOS TAURUS* URINE DISTILLATE

The Bio chemical responses of *Oreochromis mossambicus* to different breeds of cow urine distillate treatments (*Bos indicus* & *Bos taurus*) were studied by administering through two different routes.

3.3.1 Effect of *Bos indicus* and *Bos taurus* urine Distillate on muscle and liver Biochemistry was administered as through different route

The muscle and liver Biochemistry status of *Oreochromis mossambicus* after seven days exposure to two breeds of CUD as water additive and feed additive was recorded.

I. Muscle

Water additive route

Muscle protein

Protein content of *Oreochromis mossambicus* was estimated on wet weight basis. Maximum protein content of 33.726 ± 1.415 mg/g was recorded in T_1 which was treated with *Bos indicus* CUD. Minimum protein content of 24.9 ± 0.371 mg/g was recorded in control.

Muscle carbohydrate

The composition of carbohydrate in *Oreochromis mossambicus* was estimated on wet weight basis maximum carbohydrate content of muscle 13.011 ± 0.290 mg/g was recorded in Control. The minimum carbohydrate content of muscle 12.518 ± 0.427 mg/g was recorded in T_2 (Fig.3.3.1,Table 3.3.1).

Muscle lipid

The result shows that lipid content of *Oreochromis mossambcus* was Table 3.3.1. Maximum lipid content of muscle 24.743 ± 1.184 mg/g was recorded in T_2. Minimum lipid content of muscle 6.153 ± 0.592 mg/g was recorded in control (Fig. 3.3.1, Table 3.3.1).

Feed additive route

Muscle protein

Protein content of *Oreochromis mossambicus* was estimated on wet weight basis. Fig 3.3.2 and Table 3.3.2 shows that maximum protein content of 28.604 ± 3.389 mg/g was recorded in T_1 which was treated with *Bos indicus* CUD. Minimum protein content of 24.9 ± 0.371 mg/g was recorded in control.

Muscle carbohydrate

The composition of carbohydrate in *Oreochromis mossambicus* was estimated on wet weight basis Maximum carbohydrate content of muscle 13.011 ± 0.290 mg/g was recorded in control. The minimum carbohydrate content of muscle 8.314 ± 2.085 mg/g was recorded in T_2 which was treated with *Bos indicus* CUD (Fig. 3.3.2, Table 3.3.2).

Muscle lipid

The result shows that lipid content of *Oreochromis mossambicus* was Table 3.3.2. Maximum lipid content of muscle 35.879 ± 2.135 mg/g was recorded in T2. Minimum lipid content of muscle 6.153 ± 0.592 mg/g was recorded in Control (Fig. 3.3.2, Table 3.3.2).

II. Liver

Water additive route

Liver protein

Fig. 3.3.1 and Table 3.3.1 shows that there was maximum protein content in liver 15.341 ± 0.338 mg/g of T1 which had been treated with CUD of *Bos indicus* breed. Minimum protein content of liver 14.396 ± 0.409 mg/g was recorded in control.

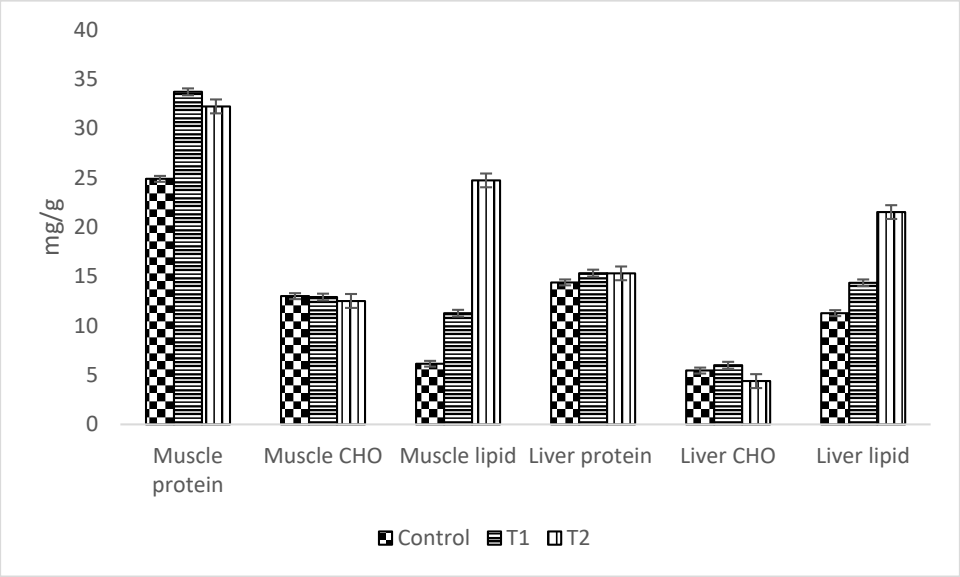
Liver carbohydrate

Maximum carbohydrate content in liver 6.003± 0.122 mg/g was recorded in T_1. The minimum carbohydrate content of 4.393 ± 0.153 mg/g was recorded in T_2 (Fig.3.3.1, Table 3.3.1) which was treated with *Bos taurus* CUD.

Liver lipid

Maximum lipid content in liver was found as 21.528 ± 1.184 mg/g in T_2 and minimum 11.280 ± 1.180 recorded as control (Fig. 3.3.1, Table 3.3.1 and Table 3.3.1).

Figure 3.3.1 Effect of CUD administered as water additive on Muscle and Liver Bio chemistry in *Oreochromis mossasmbicus*



Feed additive route

Liver protein

Maximum protein content of liver 18.111 ± 1.014 mg/g was recorded in T_1. Minimum protein content of liver 14.396 ± 0.409 mg/g was recorded in control (Fig. 3.3.2 and Table 3.3.2).

Table 3.3.1 Effect of Cow urine distillate on Muscle and Liver Biochemistry in *Oreochromis mosssambicus* through water additive

	Muscle protein	Muscle CHO	Muscle lipid	Liver protein	Liver CHO	Liver lipid
Control	24.9 ± 0.371	13.011 ± 0.290	6.153 ± 0.592	14.396 ± 0.409	5.470 ± 0.045	11.280 ± 1.180
T1	33.726 ± 1.415	12.916 ±0.662	11.281 ± 1.184	15.341 ± 0.338	6.003± 0.122	14.358 ± 1.184
T2	32.245 ± 0.750	12.518 ± 0.427	24.743 ± 1.184	15.324 ± 0.055	4.393 ± 0.153	21.528 ± 1.184

Table 3.3.2 Effect of Cow urine distillate on Muscle and Liver Biochemistry in *Oreochromis mosssambicus* through feed additive

	Muscle protein	Muscle CHO	Muscle lipid	Liver protein	Liver CHO	Liver lipid
Control	24.900 ± 0.371	13.011 ± 0.290	6.153 ± 0.592	14.396 ± 0.409	5.470 ± 0.045	11.280 ± 1.80
T1	28.604 ± 3.389	8.445 ± 2.275	30.768 ± 0.591	18.111 ± 1.014	6.098 ± 0.317	22.905 ± 2.920
T2	27.541 ± 2.558	8.314 ± 2.085	35.879 ± 2.135	14.912 ± 1.353	5.188 ± 0.548	24.615 ± 2.644

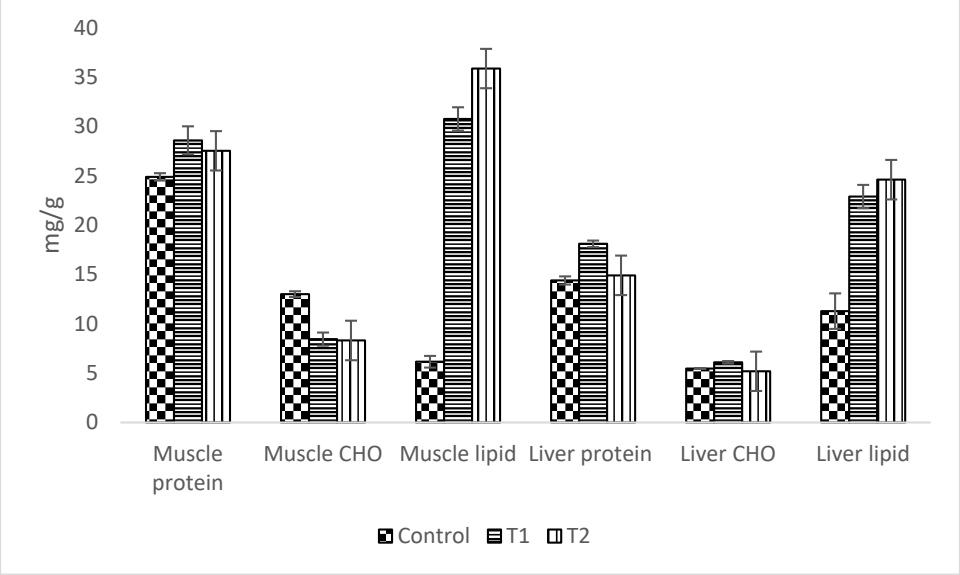
Liver carbohydrate

Maximum carbohydrate content in liver 6.098 ± 0.317 mg/g was recorded in T1. The minimum carbohydrate content 5.188 ± 0.548 mg/g was recorded in T2 (Fig. 3.3.2, Table 3.3.2).

Liver lipid

Maximum lipid content in liver of 24.615 ± 2.644 mg/g was recorded in T2 and minimum lipid content of 11.280 ± 1.80 mg/g was recorded in control (Fig. 3.3.2, Table 3.3.2and Table 3.3.2).

Figure 3.3.2 Effect of CUD administered as feed additive on Liver and Muscle Bio chemistry in *Oreochromis mossasmbicus*

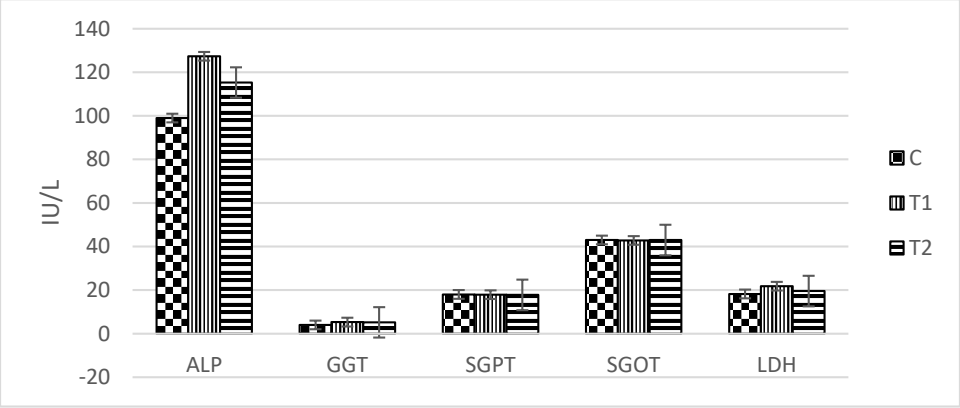


3.3.2 Effect of CUD administered through different routes on Liver function parameter in *Oreochromis mossasmbicus*

Water additive

From the fig 3.3.3 it is clear the value of ALP of control was 99 IU/L, while it was 127.33 IU/L in T_1 and decreased as 115.3 IU/L in T_2. The value of GGT in control was 4 IU/L, it was 5.3 IU/L in T_1 and decreased as 5.2 IU/L in T_2. The value of SGPT's control was 18 IU/L, it was 17.9 IU/L in T_1 and it was decreased as 17.8 IU/L in T_2.The value of SGOT's control was 43 IU/L, it was 42.8 IU/L in T1 and increased as 43 IU/L in T_2.The value of LDH's control was 18.21 IU/L, it was 21.77 IU/L in T_1 and decreased as 19.57 IU/L in T_2.

Figure 3.3.3 Effect of CUD administered as water additive on Liver function parameters in *Oreochromis mossasmbicus*

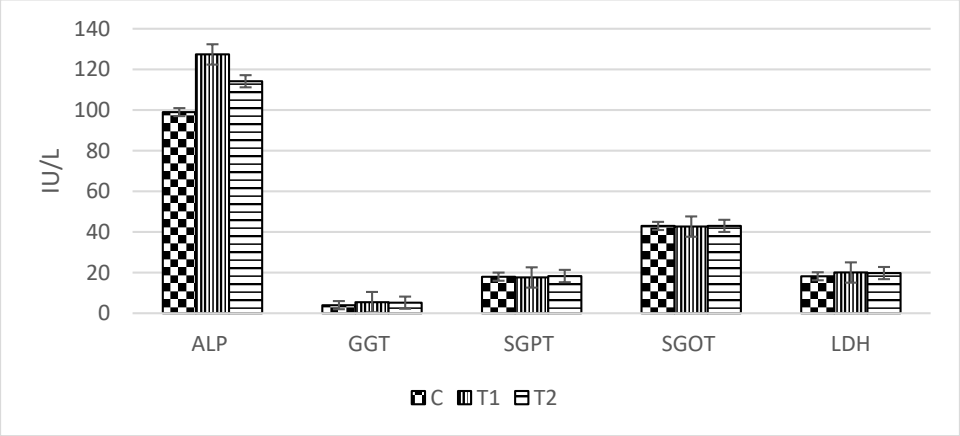


Feed additive

From the fig 3.3.4 it is clear the value of ALP of control was 99 IU/L, it was 127.4 IU/L in T_1 and decreased as 114.2 IU/L in T_2, the value of GGT's control was 4 IU/L, it was 5.48 IU/L in T_1 and decreased as 5.23 IU/L in T_2. The value of SGPT's control was

18 IU/L, it was 17.66 IU/L in T_1 and it was increased as 18.33 IU/L in T_2. The value of SGOT's control was 43 IU/L, it was 42.7 IU/L in T_1 and increased as 43 IU/L in T_2. The value of LDH's control was 18.21 IU/L, it was 20.10 IU/L in T_1 and decreased as 19.77 IU/L in T_2.

Figure 3.3.4 Effect of CUD administered as feed additive on Liver function parameters in *Oreochromis mossasmbicus*

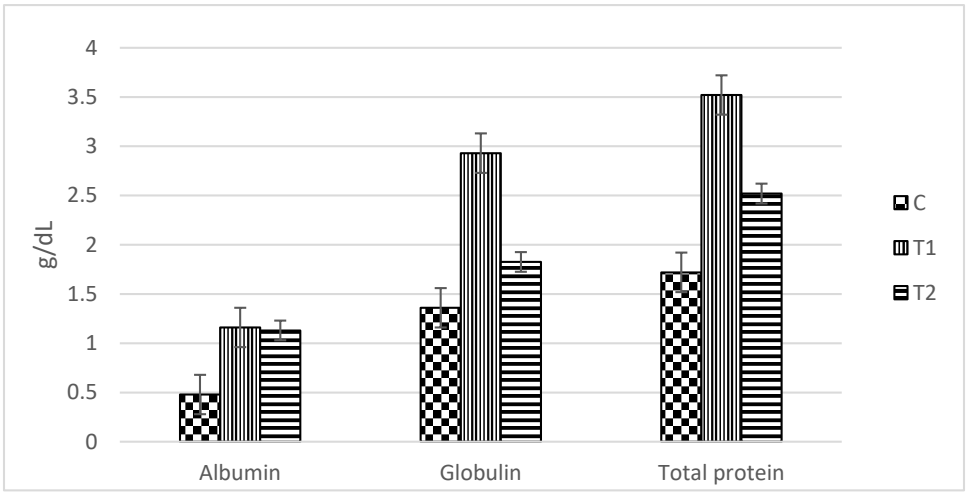


3.3.3 Effect of CUD administered through different routes on serum proteins in *Oreochromis mossasmbicus*

Water additive

The value of Albumin of control was 0.48 g/dL, it was 1.16 g/dL in T_1 and decreased as 1.13 g/dL in T_2. The value of Globulin's control was 1.36 g/dL, it was 2.93 g/dL in T_1 and decreased as 1.826 g/dL in T_2. The value of Total protein's control was 1.72 g/dL, it was 3.52 g/dL in T_1 and decreased as 2.52 g/dL in T_2 (fig 3.3.5).

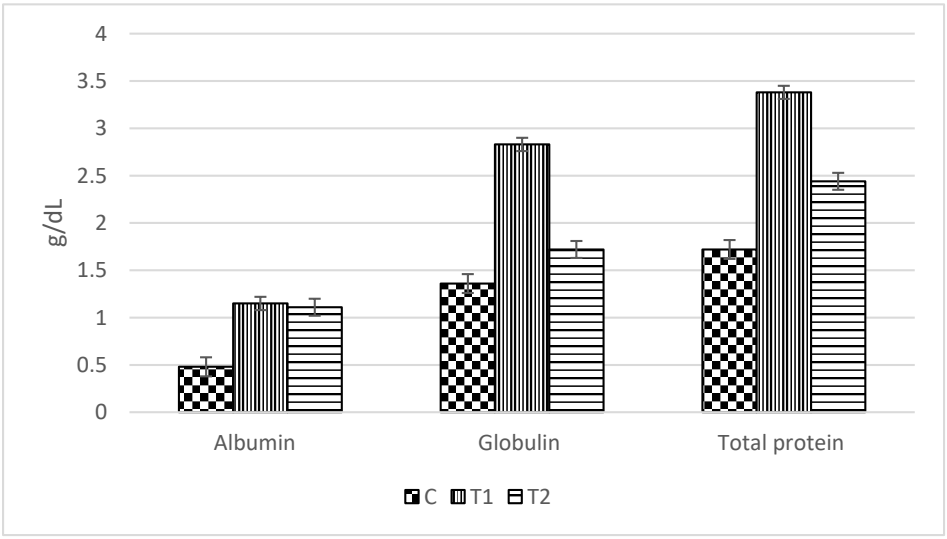
Figure 3.3.5 Effect of CUD administered as water additive on serum proteins parameter in *Oreochromis mossasmbicus*



Feed additive

The value of Albumin of control was 0.48 g/dL, it was 1.15 g/dL in T_1 and decreased as 1.11 g/dL in T_2. The value of Globulin's control was 1.36 g/dL, it was 2.83 g/dL in T_1 and decreased as 1.72 g/dL in T_2. The value of Total protein's control was 1.72 g/dL, it was 3.38 g/dL in T_1 and decreased as 2.44 g/dL in T_2 (fig 3.3.6).

Figure 3.3.6 Effect of CUD administered as feed additive on serum proteins parameters in *Oreochromis mossasmbicus*

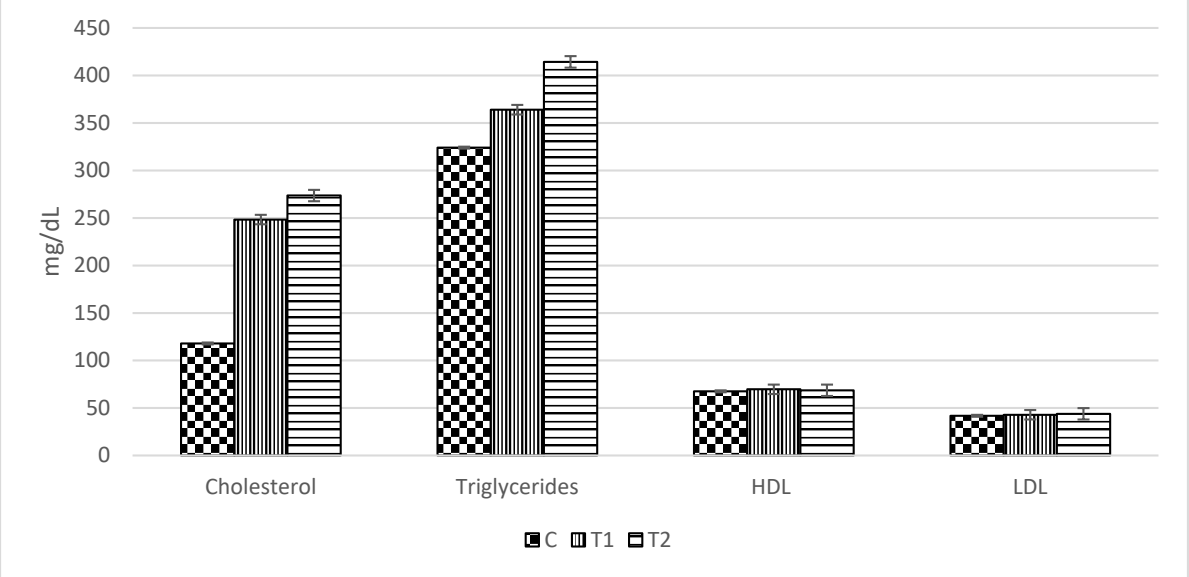


3.3.4 Effect of CUD administered through different routes on the lipid profile in *Oreochromis mossasmbicus*

Water additive

The fig 3.3.7 shows value of Cholesterol of control was 118 mg/dL, it was 248.33 mg/dL in T_1 and increased as 273.66 mg/dL in T_2. The value of Triglycerides's control was 324 mg/dL, it was 364 mg/dL in T_1 and increased as 414.33 mg/dL in T_2. The value of HDL's control was 67.63 mg/dL, it was 69.72 mg/dL in T_1 and decreased as 68.62 mg/dL in T_2. The value of LDL's control was 41.766 mg/dL, it was 42.85 mg/dL in T_1 and increased as 43.85 mg/dL in T_2.

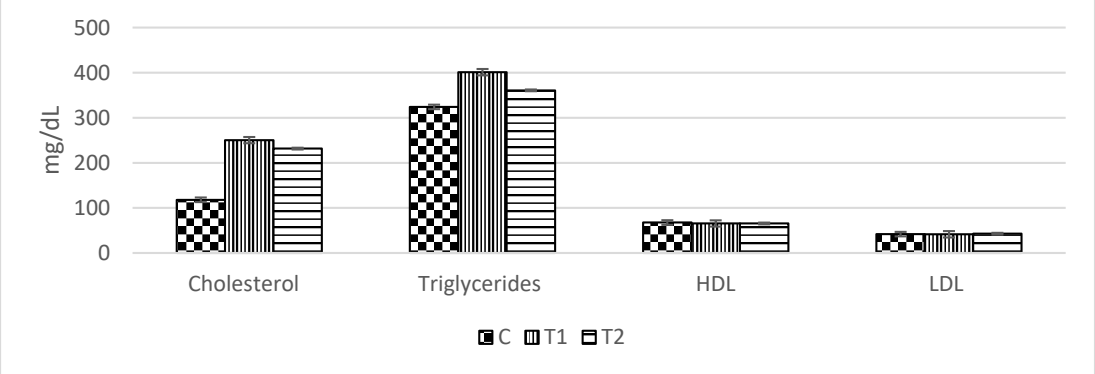
Figure 3.3.7 Effect of CUD administered as water additive on the lipid profile parameters in *Oreochromis mossasmbicus*



Feed additive

The fig 3.3.8 shows value of Cholesterol of control was 118 mg/dL, it was 250.33 mg/dL in T_1 and decreased as 231.66 mg/dL in T_2. The value of Triglycerides's control was 324 mg/dL, it was 401 mg/dL in T_1 and decreased as 360.66 mg/dL in T_2. The value of HDL's control was 67.63 mg/dL, it was 65.59 mg/dL in T_1 and increased as 65.62 mg/dL in T_2. The value of LDL's control was 41.766 mg/dL, it was 41.48 mg/dL in T_1 and increased as 42.85 mg/dL in T_2.

Figure 3.3.8 Effect of CUD administered as feed additive on the lipid profile parameters in *Oreochromis mossasmbicus*



3.3.5 Effect of CUD administered through different routes on the serum Creatinine and Bilirubin in *Oreochromis mossasmbicus*

Water additive

The fig 3.3.9 and 3.3.10 illustrates value of Creatinine of control was 0.99 mg/dL, it was 0.98 mg/dL in T_1 and decreased as 0.97 mg/dL in T_2. The value of Bilirubin control was 0.01 mg/dL, it was 0.01 mg/dL in T_1 and increased as 0.02 mg/dL in T_2.

Figure 3.3.9 Effect of CUD administered as water additive on Creatinine of *Oreochromis mossasmbicus*

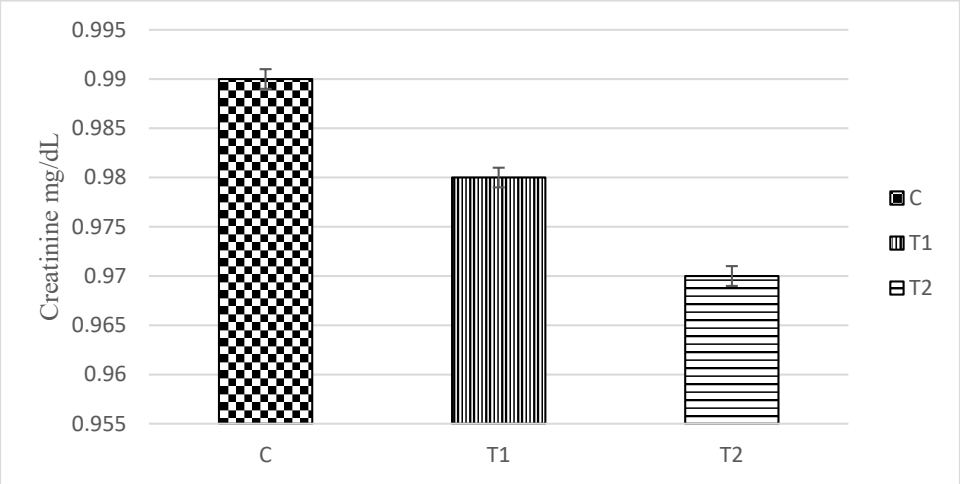
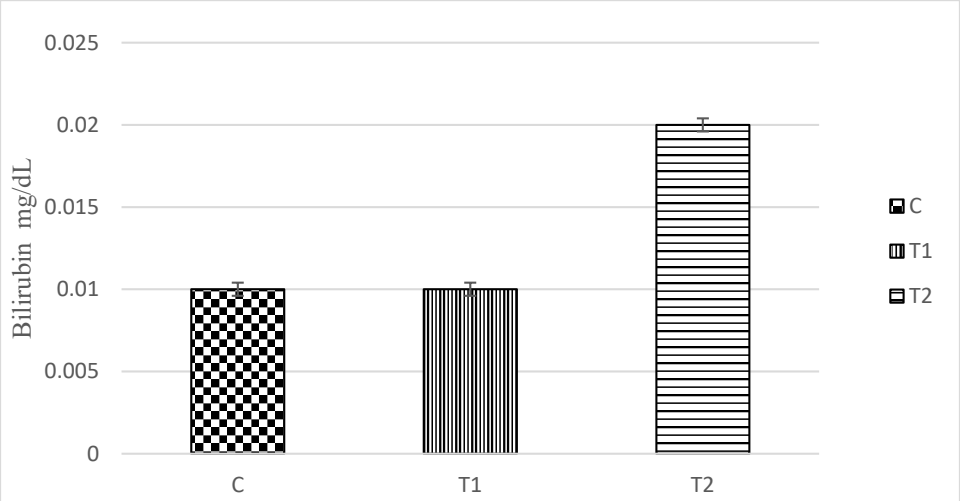


Figure 3.3.10 Effect of CUD administered as water additive on Bilirubin of *Oreochromis mossasmbicus*



Feed additive

The fig 3.3.11 and 3.3.12 illustrates value of Creatinine of control was 0.99 mg/dL, it was 0.98 mg/dL in T_1 and decreased as 0.97 mg/dL in T_2. The value of Bilirubin control was 0.01 mg/dL, it was 0.01 mg/dL in T_1 and increased as 0.013 mg/dL in T_2.

Figure 3.3.11 Effect of CUD administered as feed additive on serum Creatinine of *Oreochromis mossasmbicus*

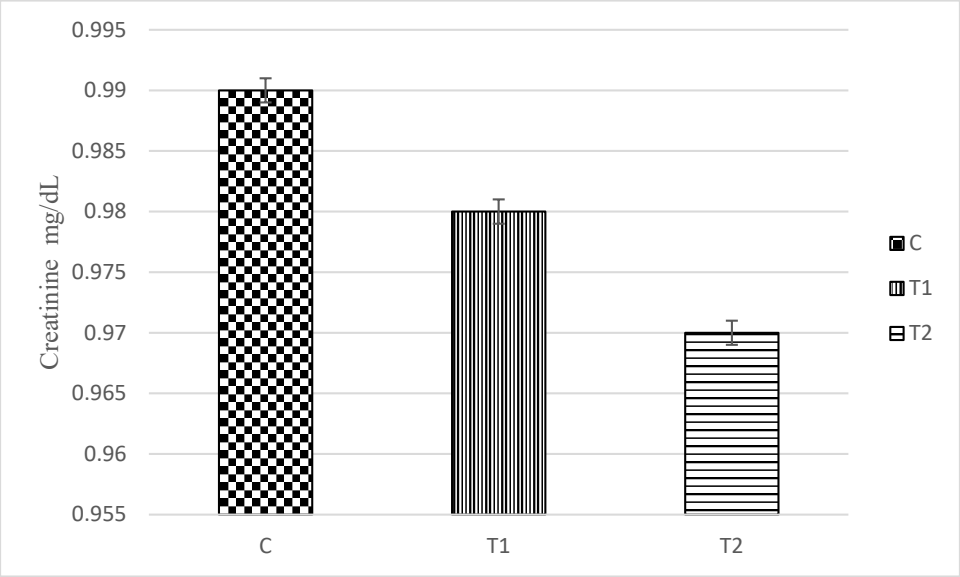
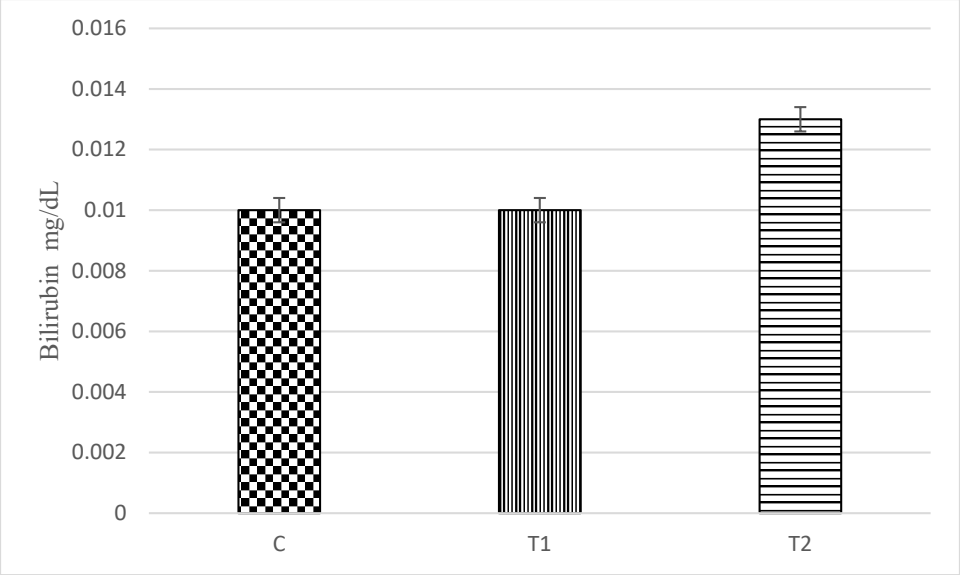


Figure 3.3.12 Effect of CUD administered as feed additive on serum Bilirubin of *Oreochromis mossasmbicus*



CHAPTER IV - 3.4 EFFECT OF *BOS INDICUS* URINE DISTILLATE IN FIELD

Field trials are normally applied when a final prototype is available, or a complete research is to be evaluated. Because of the relative time and expense of running a field trial

it is not common to use them in the early stages of research development, but rather to use them for evaluation purposes. The study of immune parameter, growth responses and survival rate in *Oreochro*mis *mossambicus* were compared for the *Bos indicus* urine distillate and untreated control group in different ponds. The Cow urine distillate was administered through water additive route at 0.1% concentration (v/v), which was used for laboratory studies was applied in field study. The result of immune parameter, growth parameters, survival rate and water quality parameters were evaluated.

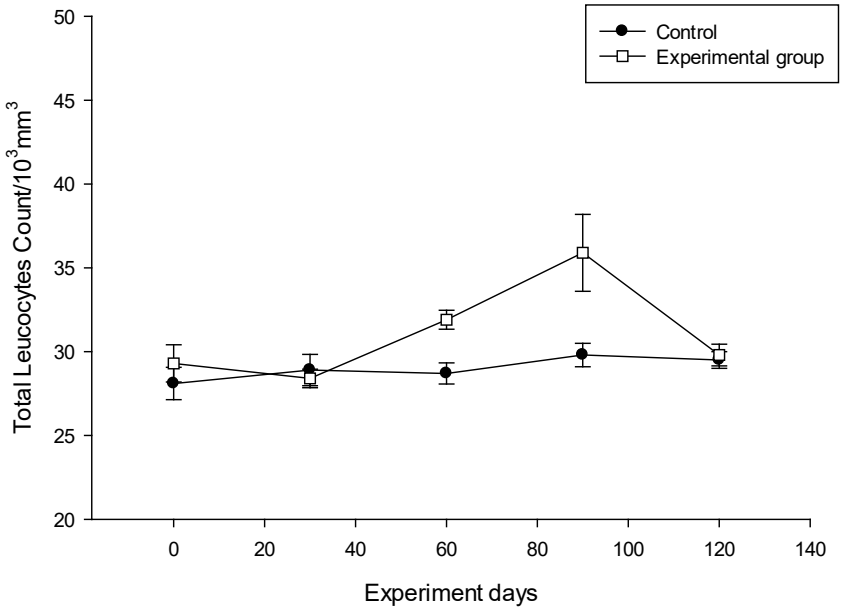
3.4.1 Immunological parameters

The various immunological parameters which were specific and non-specific were studied during present investigation. The results are as follows:

Total Leucocyte Count (TLC)

The fig 3.4.1 represents the difference between control and experimental group in effect of CUD administered through water additive on total leucocytes in *Oreochromis*

Figure 3.4.1 Effect of CUD administered through water additive on Total leucocytes in *Oreochromis mossasmbicus*

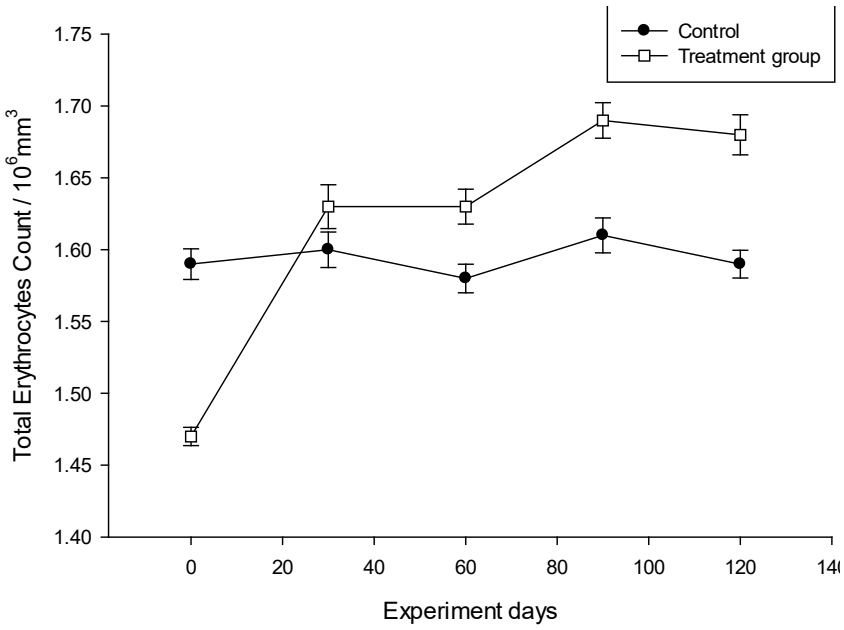


mossasmbicus. From the graph it is clear that, the control group variation is very less over the period of study whereas the experimental variation was high. The experimental group is almost high than control group over the period of time except it was only less around 30th day. By looking at the overall data it can be stated that, the experimental group value is fluctuating highly notably around 90th day as a peak response while control group values are equivalent over the period of study.

Total Erythrocyte Count (TEC)

The fig 3.4.2 presents effect of CUD administered as water additive on Total Erythrocyte Count (TEC) in *Oreochromis mossasmbicus*, from the graph it is clear that, the variation among control group over the study period is less whereas the variation between experimental group is high. The value of experiment group was very low on 0th day of study and it has been increased more than control group around 30th day and continuously increased till the final day of the study.

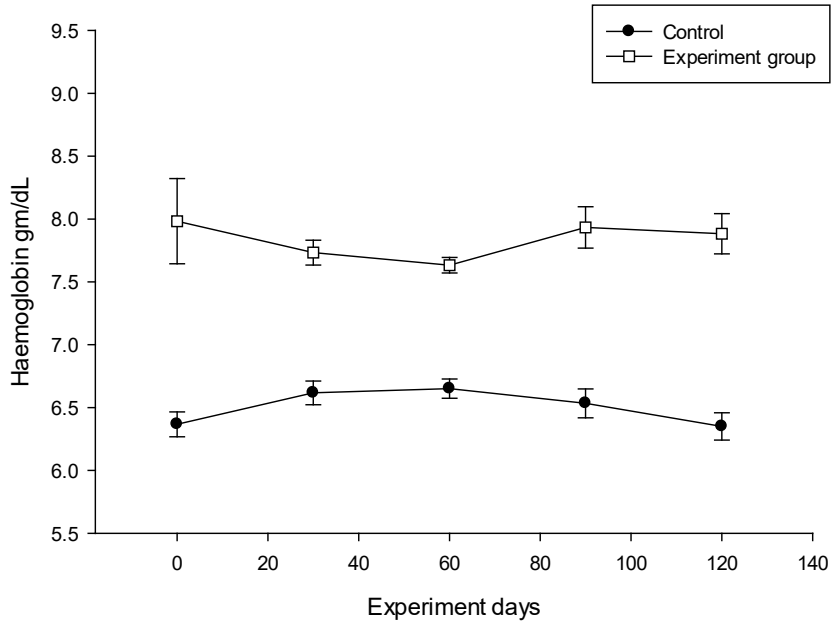
Figure 3.4.2 Effect of CUD administered through water additive on Total Erythrocyte Count (TEC) in *Oreochromis mossasmbicus*



Haemoglobin (Hb)

The fig 3.4.3 illustrates the effect of CUD administered through water additive on Heamoglobin in *Oreochromis mossasmbicus*. From the graph it can be identified that, the variation of control and experimental group continues from the 0^{th} day to final day of study. Another notable thing is that, experimental groups values are increasing when the control groups values are decreasing, which depicts the disproportional relationship between two groups.

Figure 3.4.3 Effect of CUD administered through water additive on Heamoglobin in *Oreochromis mossasmbicus*

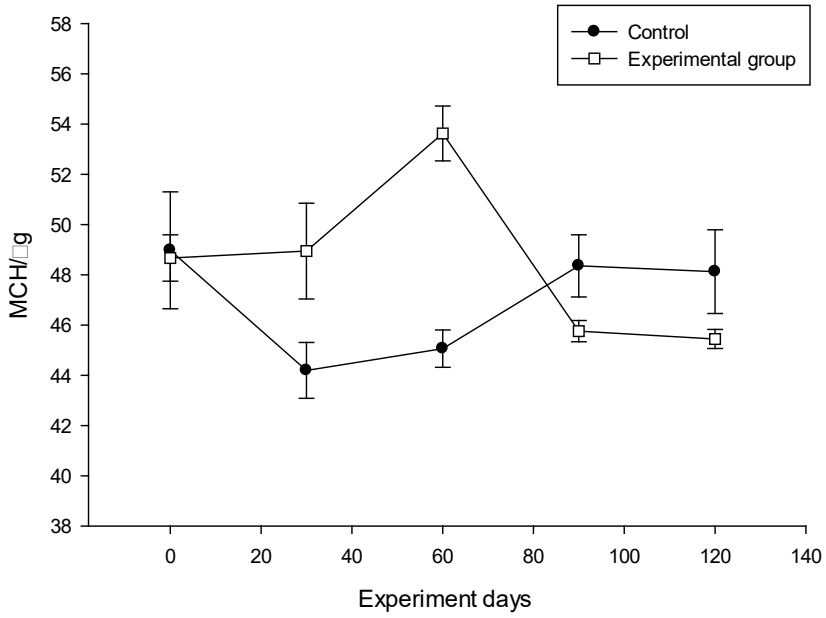


Mean corpuscular haemoglobin (MCH)

The fig 3.4.4 depicts the effect of CUD administered through water additive on Mean Corpuscular Haemoglobin in *Oreochromis mossasmbicus*. By looking at the graph it is clear that, the values of control and experimental group are same on the first day but it has varied over the time period, notably the control group value was decreased from the first day to around 85^{th} day and continued with less value than first day. The value variation

of experimental group illustrates that, it was increased between first day to around 80th day and it was continues with less value till the last day. Another interested thing is that, the graph illustrates the disproportional relationship between the two groups as when one group value increases the other one decreases.

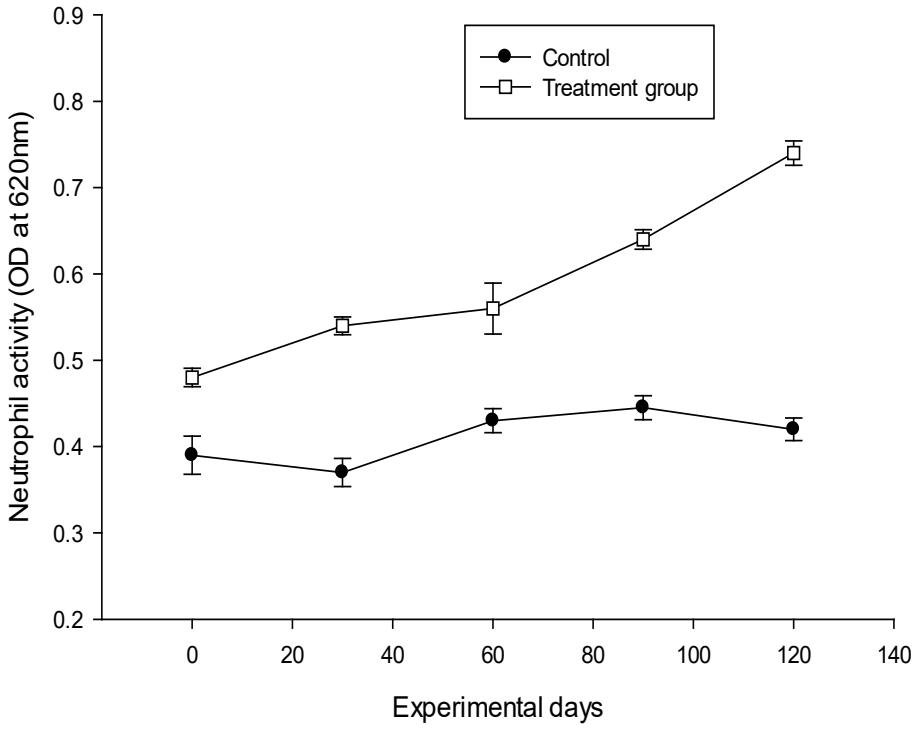
Figure 3.4.4 Effect of CUD administered through water additive on Mean Corpuscular Haemoglobin in *Oreochromis mossasmbicus*



Neutrophil activity

The fig 3.4.5 represents the effect of CUD administered through water additive on neutrophil activity in *Oreochromis mossasmbicus*. From the graph it is visible that, the value between control and experimental group was different even on first day as experimental group value was higher than control group. The experimental group value has increased continuously over the period of study, whereas control group had fluctuation. The variation of two groups on the first day was less and it has increased high in the end day of the study.

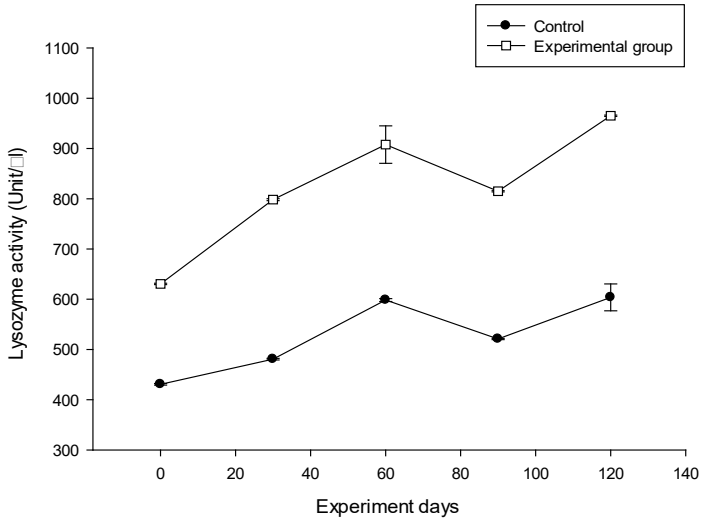
3.4.5 Effect of CUD administered through water additive on neutrophil activity in *Oreochromis mossasmbicus*



Lysozyme activity

The fig 3.4.6 represents the effect of CUD administered through water additive on serum lysozyme activity in *Oreochromis mossasmbicus*. From the graph it is clear that, the values between control and experimental group is varied in the entire study period, as experimental group was having higher value while control group was having lesser value. The graph also illustrates that, the increasing trend of both values are proportional to one another. Both the values were increased continuously from the day first and decreased around the 90th day, and having increasing trend till the final day of the study.

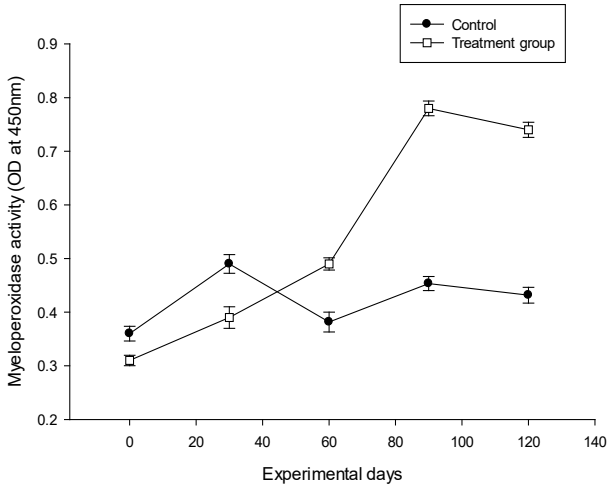
Figure 3.4.6 Effect of CUD administered through water additive on serum lysozyme activity in *Oreochromis mossasmbicus*



Myeloperoxidase activity

The fig 3.4.7 shows the effect of CUD administered through water additive on serum myeloperoxidase activity in *Oreochromis mossasmbicus*. By looking at the graph it

Figure 3.4.7 Effect of CUD administered through water additive on serum myeloperoxidase activity in *Oreochromis mossasmbicus*



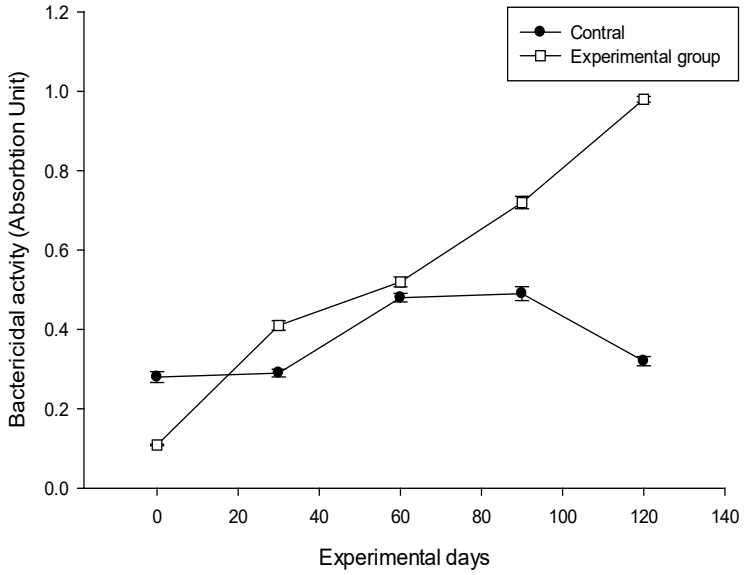
is clear that, during the beginning of the study the value of experimental group was lesser

than the control group but around 40th day the value of experimental group was increased than control group and it was continued till the final day of the study. The notable thing is that, the sudden in increase of experimental group was happened around 60th day when control group value was decreased and maintained almost same values till the end of study.

Bactericidal activity

The fig 3.4.8 illustrates effect of CUD administered through water additive on serum Bactericidal activity in *Oreochromis mossasmbicus*. From the graph it is visible that, the experimental group which was having less value than control group in the beginning

Figure 3.4.8 Effect of CUD administered through water additive on serum Bactericidal activity in *Oreochromis mossasmbicus*

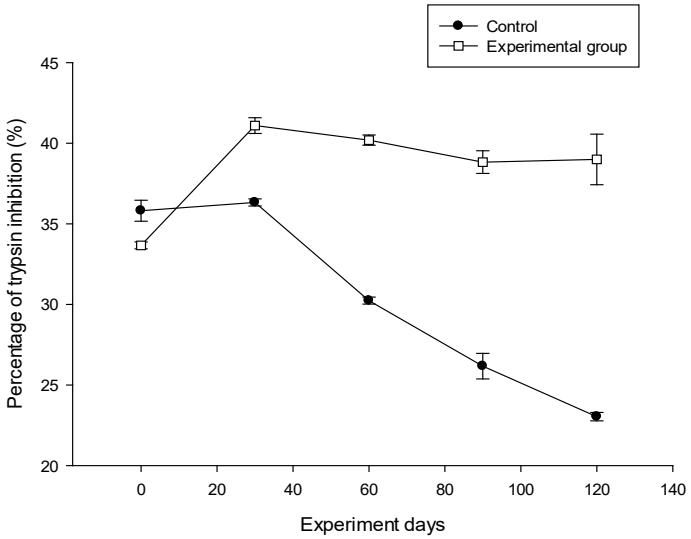


of the study was increased higher than control group around 30th day and continued till the final day of study. The control group which was having higher value than experimental group was decreased around 30th day and increased around 60th day, again decreased around end of the study.

Serum antiprotease activity

The fig 3.4.9 illustrates the effect of CUD administered through water additive on serum antiprotease activity in *Oreochromis mossasmbicus*. From the graph it is visible that, in the beginning, the experimental group value was less than control group and experimental group value was increased than control group around 0^{th} day (i.e 7 days after CUD treatment) and it was increased continuously. The value of control group over the study period showed the decreasing trend. Notably, the difference between two groups were less in the beginning days and it was increased highly during end of the study period.

3.4.9 Effect of CUD administered through water additive on serum antiprotease activity in *Oreochromis mossasmbicus*

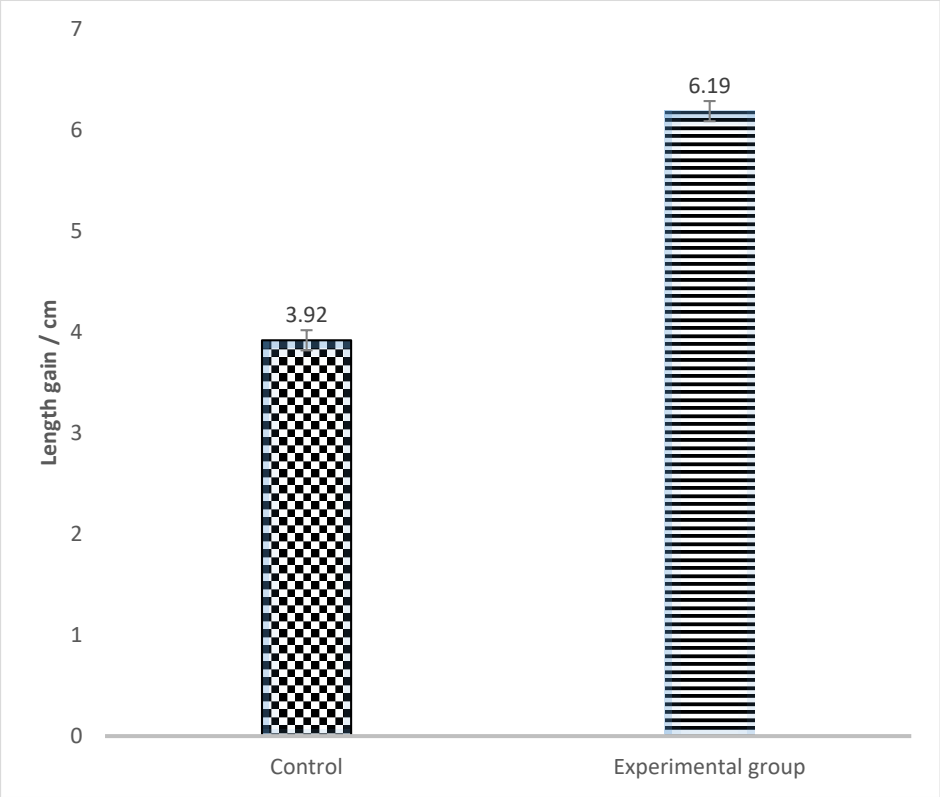


3.4.2 Growth parameters

The study of growth responses in *Oreochro*mis *mossambicus* were compared for the cow urine distillate treated group and untreated control group in different ponds. The weight gain was found highest in T group (290.42 gm) when compared to untreated control (193.22 gm). The highest growth rate of 604.58 mg/day was recorded in T group, when compared with

control (387 mg/day). The maximum specific growth rate was recorded in T group that was noted as 0. 0.84 (%) and minimum value observing the control (0.58).

Figure 3.4.10 Effect of CUD administered through water additive on Total body length in *Oreochromis mossasmbicus*



The maximum average daily growth of 2.42 g/day was observed in T group on the 120th day and least average daily growth of 1.61 g/day was observed in control. The Percentage of increase in body weight also highest in experimental group (116.25) and 80.39 on control (Table 3.4.1). Fig 3.4.10 revealed that length of control and experimental fish. The highest length found in the experimental group (6.19 cm).

Table : 3.4.1 Effect of *Bos indicus* CUD on the growth Parameters of *Oreochromis mossambicus* in field Trials

Parameter	Control pond	Experimental pond
Initial Length(cm)	23.51	23.61
Final Length(cm)	27.43	29.8
Length Gain (cm)	3.92	6.19
Initial weight (W_1)	240.35	249.81
Final weight (W_2)	433.57	580.23
Weight gain (g)	193.22	290.42
Growth rate(mg/day)	387.00	604.58
Average Daily Growth (g/day)	1.61	2.42
Specific growth rate (%)	0.58	0.84
Percentage of increase in body (%)	80.39	116.25

3.4.3 Survival Test

The highest survival was observed in Experimental group when compared to untreated control group. It was 95% survival in experimental group and 85% in control.

3.4.4 Water Quality parameters

Measurements of water quality parameters like (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 Control and experimental pond at throughout the study period (Table 3.4.2 & 3.4.3). In the control pond temperature was noticed in acceptable range at 27.±0.08 to 29.98±0.40°C. The pH values were observed in

Table : 3.4.2 Water quality Parameters of Control pond in field during trial period

Parameters	15th day	30th day	45th day	60th day	75th day	90th day	105th day	120th day
Temp (˚C)	28.15± 0.40	27.02±0.12	29.9±0.08	29.8± 0.34	29.98±0.08	29.01±0.08	27.0±0.08	28.08±0.03
pH	7.17±0.13	7.16±0.17	7.64±0.36	7.20±0.07	7.95±0.27	7.13±0.16	7.02±0.115	7.87±0.12
Salinity	0.21±0.013	0.22±0.019	0.21±0.01	0.20±0.005	0.24±0.017	0.21±0.04	0.21±0.011	0.21±0.013
DO	5.14±0.04	5.13±0.033	5.13±0.02	5.122±0.017	5.13±0.03	5.12±0.03	5.23±0.108	5.14±0.06
Ammonia	0.05±0.006	0.05±0.004	0.05±0.01	0.03±0.006	0.05±0.01	0.04±0.013	0.05±0.010	0.05±0.01
Conductivity	5.24±0.078	5.22±0.15	5.12±0.01	5.13±0.04	5.40±0.14	4.61±0.57	4.38±0.45	3.81±0.22
TDS	2.41±0.42	2.09±0.03	2.17±0.14	2.10±0.04	2.09±0.03	2.24±0.12	2.41±0.18	2.29±0.15
Turbidity	6.12±0.59	5.20±0.051	5.28±0.18	5.38±0.19	5.88±0.79	4.38±0.14	3.88±0.29	4.32±0.73

Table : 3.4.3 Water quality Parameters of Experimental pond in field during trial period

Parameters	15th day	30th day	45th day	60th day	75th day	90th day	105th day	120th day
Tempe ˚C)	27.15± 0.40	29.02±0.12	29.9±0.08	29.6± 0.24	28.98±0.08	27.04±0.07	26.0±0.08	26.09±0.03
pH	6.97±0.16	6.96±0.27	7.24±0.46	7.40±0.09	7.95±0.27	7.53±0.12	7.52±0.21	7.97±0.42
Salinity	0.24±0.023	0.26±0.029	0.24±0.01	0.22±0.003	0.26±0.019	0.24±0.04	0.23±0.012	0.21±0.013
DO	5.94±0.04	5.63±0.033	5.73±0.02	6.122±0.017	5.63±0.03	5.17±0.03	5.3±0.108	5.1±0.06
Ammonia	0.06±0.009	0.05±0.007	0.04±0.03	0.04±0.005	0.06±0.02	0.04±0.023	0.05±0.020	0.04±0.91
Conductivity	5.54±0.078	5.32±0.15	5.42±0.01	6.13±0.24	6.40±0.17	6.61±0.67	6.38±0.55	5.81±0.32
TDS	2.71±0.92	2.43±0.08	2.98±0.16	2.18±0.05	2.19±0.33	2.43±0.42	2.47±0.78	2.49±0.65
Turbidity	7.12±0.58	6.21±0.651	6.28±0.98	6.58±0.29	5.98±0.93	6.38±0.24	6.81±0.92	7.32±0.73

Control pond during the study periods were 7.95±0.27 as maximum on 75th day and 7.02±0.11 as minimum on 120th day. During the present study salinity ranged between 0.20±0.005 mg/l to 0.24±0.017 mg/l for C groups. The lowest salinity was observed on 60th day and highest salinity was observed on 75th day. The DO levels measured in the pond showed maximum levels on 105th day (5.23±0.18 mg/l), and minimum on 60th day (5.12±0.01 mg/l). The ammonia levels showed at maximum on 15th day (0.005 mg/L) and minimum level on 60th day (0.039±0.006 mg/L). Conductivity was found to be varying between 3.81±0.011 µS to 5.24±0.07 µS. The maximum was observed on 15th day and minimum was observed on 120th day. Total dissolved solids ranged from 2.09±0.03 mg/l to 2.41±0.42 mg/l. Turbidity ranged between 3.88±0.2 NTU to 6.12±0.59 NTU. Minimum turbidity was noted on 105th day and maximum turbidity was noted on 15th day. In experimental pond higher value of turbidity was noticed when compared to control pond.

CHAPTER V - 3.5 COMPARATIVE STUDY OF PHYSICAL, CHEMICAL AND MICROSCOPIC CHARACTERISTICS OF COW URINE

Physical, chemical and microscopic characteristics of fresh urine and distillate (FCU & CUD) of two different breeds (*Bos indicus* & *Bos taurus*) was studied.

3.5.1 Physical characteristics

Colour

The FCU of *Bos indicus* was looking dark amber while that of *Bos taurus* was looking amber. However both the CUD's studied were coloreless.

Plate 3.5.1 Colour of Fresh Cow Urine and Cow Urine Distillate

Odour and Taste

Odour and taste were determined by using normal sensational observation of the sample. FCU of both breeds had aromatic odour and CUD of both breed had ammoniac odour. The results suggest the presence of ammonia ions in CUD and FCU, which gives urine its typical odour.

pH

pH of FCU of *Bos indicus* and *Bos taurus* was recorded as 7.9 and 8.9 respectively while that of CUD of both breeds 8.1 and 8.66 (Table 3.5.1). It is not getting much more difference from FCU and CUD.

Transparency, Turbidity and Specific Gravity

Plate 3.5.2 and 3.5.3 shows that CUD is more transparent and less turbid when compared to FCU. Plate 3.5.2 indicates that among FCU, the *Bos indicus* is more turbid than *Bos taurus*. There is slight difference in the specific gravity of FCU and CUD of *Bos indicus* and *Bos taurus.* FCU of *Bos indicus* SG is 1.026 and that of *Bos taurus* is 1.032. While the SG of CUD in *Bos indicus* is 0.992 and that of *Bos taurus* is 1.000 (Table 3.5.1).

Plate 3.5.2 Transparency, Turbidity and Specific Gravity of both breed FCU

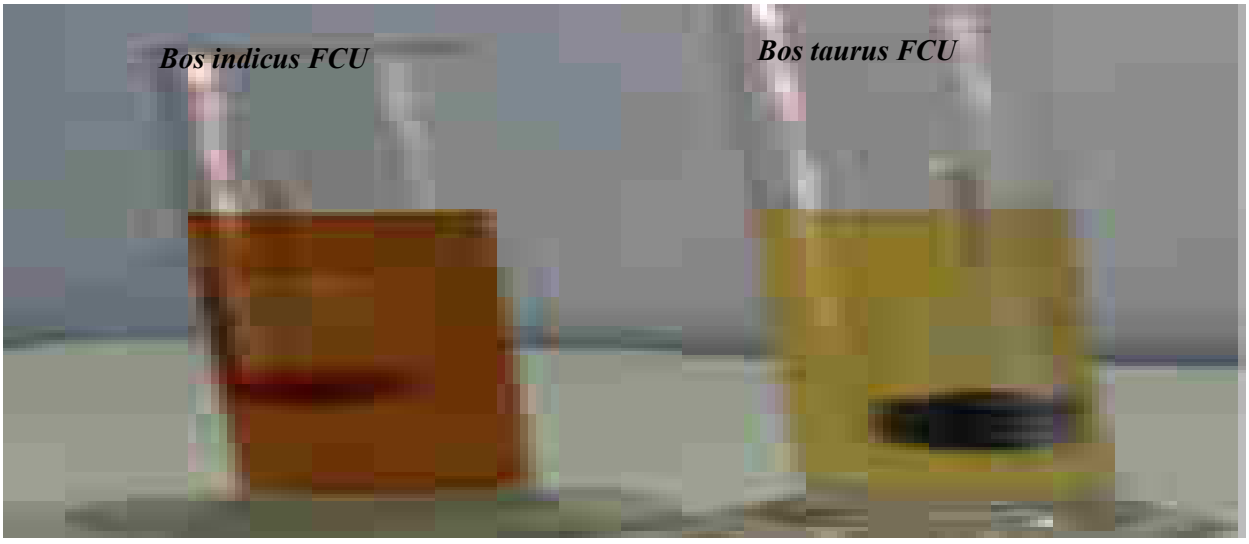


Plate 3.5.3 Transparency, Turbidity and Specific Gravity of both breed CUD

Weight

The weight of FCU of *Bos indicus* is 1.063g and 1.013g in *Bos taurus*. Weight of CUD is 0.99g, 1.03g in *Bos indicus* and *Bos taurus* respectively.

Conductivity

The conductivity measurement is related to the concentration of electrolytes in the urine. The conductivity of FCU is 29.8mS in *Bos indicus* and 39.7mS *in Bos taurus* conductivity of CUD is 16.2mS and 23.3mS in *Bos indicus* and *Bos taurus* respectively.

Salinity

Salinity is measure of all the salts dissolved in urine. The salinity of FCU is 16.8 ppt in *Bos indicus* and 20.5 ppt *in Bos taurus.* Conductivity of CUD is 29.4 ppt and 36.2 ppt in *Bos indicus* and *Bos taurus* respectively.

Total dissolved solids (TDS)

TDS indicates the Total Dissolved Solids (TDS) of a solution, i.e. the concentration of dissolved solid particles. The TDS of FCU is 19.4 ppt in *Bos indicus* and 23.22 ppt *in Bos taurus*. TDS of CUD is 32.9 ppt in *Bos indicus* and 48.1 ppt in *Bos taurus* respectively.

Table 3.5.1 Physical characteristics of Fresh Cow Urine (FCU) and Cow Urine Distillate (CUD)

Parameter	Fresh Cow Urine		Cow Urine Distillate	
	Bos indicus	***Bos taurus***	***Bos indicus***	***Bos Taurus***
Colour	Dark Amber	Amber	Colorless	Colorless
Odur	Aromatic	Aromatic	Ammoniac	Ammoniac
Taste	Tangy	Tangy	Pungent	Pungent
pH	7.9	8.9	8.92	8.66
Transparency	Clear	Clear	Very Clear	Very Clear
Turbidity	Clear	Clear	Very Clear	Very Clear
Specific gravity	1.026	1.032	0.992	1.000
Weight (g/ml)	1.063	1.013	0.99	1.03
Temperature (˚C)	30	29	26	27
Conductivity (mS)	29.8	39.7	16.2	23.7
Salinity (ppm)	16.8	20.5	29.4	36.2
TDS (ppm)	19.4	23.2	32.9	48.1
Moisture (%)	96.11	97.99	96.23	98.64
Organic content (%)	1.22	1.57	1.75	0.05
Ash content (%)	2.67	0.53	3.2	1.31

Moisture, Organic content and Ash content

The Moisture content was found to be high in all the samples. The moisture content of the sample is more than 96% in all the sample. FCU has 96.11% moisture in *Bos indicus* and 97.99 % *in Bos taurus*. And the moisture content of CUD is 96.23% in *Bos indicus* and 98.64% *Bos taurus* respectively. The amount of total ash, water soluble ash, Water soluble and insoluble matters are less than 5%. The ash content of FCU is 2.67% in *Bos indicus* and 0.53% *in Bos taurus* ash content of CUD is 3.2% *Bos indicus* and 1.31% *Bos taurus* respectively. Inorganic matters of FUC is 1.22%, 1.57%, *Bos indicus* and *Bos taurus* & CUD is 1.75%, and 0.05% respectively.

3.5.2 Chemical characteristics

Albumin, Glucose, Ketone Bodies, Bilirubin, Bile salt, Bile pigment and Blood contents are negative in both bred and both form (FCU & CUD). Other than this urea, uric acid, calcium, total phenol, creatinine were highly present in *Bos taurus* FCU and lesser in *Bos indicus* FCU. Protein was present only in FCU and not detected in CUD. Urea, uric acid, calcium, total phenol, creatinine are minimum quantity detected in CUD when compared to FCU. Ammonia content is highly present in CUD compare to FCU. (Table 3.5.2).

Table 3.5.2 Chemical characteristics of Fresh Cow Urine (FCU) and Cow Urine Distillate (CUD)

Qualitative estimation				
Parameter	**Fresh Cow Urine**		**Cow Urine Distillate**	
	Bos indicus	***Bos taurus***	***Bos indicus***	***Bos taurus***
Glucose	Nil	Nil	Nil	Nil
Albumin	Nil	Nil	Nil	Nil
Ketone Bodies	Nil	Nil	Nil	Nil
Bilirubin	Nil	Nil	Nil	Nil
Bile salt	Nil	Nil	Nil	Nil
Bile pigment	Nil	Nil	Nil	Nil
Presence of Blood	Nil	Nil	Nil	Nil
Protein	Positive	Positive	Nil	Nil
Calcium	Positive	Positive	Positive	Positive
Creatinine	Positive	Positive	Positive	Positive
Total phenolic content	Positive	Positive	Positive	Positive
Quantitative estimation				
Protein (mg)	0.05	0.08	-	-
Total phenolic content (mg/ml)	4.84	2.76	0.024	0.015
Creatinine (mmol/kg W)	0.89	1.19	0.62	0.98
Calcium(mEq)	0.057	0.019	0.035	0.002
Ammonia(mg)	0.042	0.053	0.089	0.098
Urea (mg/ml)	5.126	6.23	0.404	2.0
Uric acid(g/lit)	1.343	1.53	0.34	1.2

3.5.3 Microscopic studies

Both FCU & CUD samples of both cow breeds were found to be negative for microorganism, casts, crystals, blood cells, epithelial cells, bacteria, fungi and protozoa. The results were recorded in Table 3.5.3.

Table 3.5.3 Microscopic characteristics of Fresh Cow Urine (FCU) and Cow Urine Distillate (CUD)

Parameter	Fresh Cow Urine		Cow Urine Distillate	
	Bos indicus	***Bos taurus***	***Bos indicus***	***Bos taurus***
Crystals	Nil	Nil	Nil	Nil
RBC	Nil	Nil	Nil	Nil
WBC	Nil	Nil	Nil	Nil
Epithelial cells	Nil	Nil	Nil	Nil
Bacteria	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Fungus	Nil	Nil	Nil	Nil
Protozoa	Nil	Nil	Nil	Nil

CHAPTER VI- 3.6. EFFECT OF *BOS INDICUS* AND *BOS TAURUS* URINE DISTILLATE ON WATER QUALITY

I. Comparison of physico-chemical characteristics of tank water in under the influence of Cow urine distillate exposure as water additive route

Temperature

There was no significant fluctuations observed in treated groups. But the untreated control tank showed fluctuation from 28.15 to 27.02 and then to 29.9˚C. But the treated groups had a range of 29.01 to 29.98˚C in T1 and 29.02 to 29.9 in T2.

pH

When compared with control there is no alteration in pH due to the addition of CUD of both breeds. In control it increased from 7.1 to 7.64, while in T1 it is reduced from 7.2 to 7.13 and in T2 it is increased from 6.9 to 7.4.

Salinity

The salinity in control was maintained in 0.21. In T_1 a slight increase was observed (from 0.20 to 0.21mg/l). In T_2 it is decreased from 0.26 to 0.22mg/l.

Dissolved oxygen

There is no much differences in the dissolved oxygen content of control and T_1 which in T_2 while in T_2 slightly from 5.63 to 6.122 mg / l.

Ammonia

The ammonia level remained stable in control. In T_2 it increased initially from 0.03 to 0.05 mg/l on 7th day but was maintained at 0.04 mg/l till 28th day. In T_2 ammonia level decreased from 0.05 to 0.04 mg/l.

Conductivity

Control showed no variations in conductivity which is treatment T_1 it is decreased from 5.13 to 4.61 µs and in T_2 it is increased from 5.32 to 6.13 µs.

TDS

There was no variation observed in TDS with a range of 2.09 to 2.41 mg/l in control; 2.09 to 2.24 in T_1 and 2.18 to 2.98 mg/l in T_2.

Turbidity

The turbidity was decreased from 6.12 to 5.28 NTU in control and 5.38 to 4.38 in T_1.But it is increased from 6.21 to 6.58 NTU in T_2.

3.6.1 Physico-chemical characteristics of water samples during the study period (Water additive)

	Control			T_1			T_2		
	0^{th} day	**7^{th} day**	**28^{th} day**	**0^{th} day**	**7^{th} day**	**28^{th} day**	**0^{th} day**	**7^{th} day**	**28^{th} day**
Temp (˚C)	28.15± 0.4	27.02±0.12	29.9±0.08	29.8± 0.34	29.98±0.08	29.01±0.08	29.02±0.12	29.9±0.08	29.6± 0.24
pH	7.17±0.13	7.16±0.17	7.64±0.36	7.20±0.07	7.95±0.27	7.13±0.16	6.96±0.27	7.24±0.46	7.40±0.09
Salinity (mg/l)	0.21±0.013	0.22±0.019	0.21±0.01	0.20±0.005	0.24±0.017	0.21±0.04	0.26±0.029	0.24±0.01	0.22±0.003
DO (mg/l)	5.14±0.04	5.13±0.033	5.13±0.02	5.122±0.017	5.13±0.03	5.12±0.03	5.63±0.033	5.73±0.02	6.122±0.01
Ammonia(mg/l)	0.05±0.006	0.05±0.004	0.05±0.01	0.03±0.006	0.05±0.01	0.04±0.013	0.05±0.007	0.04±0.03	0.04±0.005
Conductivity (µS)	5.24±0.078	5.22±0.15	5.12±0.01	5.13±0.04	5.40±0.14	4.61±0.57	5.32±0.15	5.42±0.01	6.13±0.24
TDS (mg/l)	2.41±0.42	2.09±0.03	2.17±0.14	2.10±0.04	2.09±0.03	2.24±0.12	2.43±0.08	2.98±0.16	2.18±0.05
Turbidity(NTU)	6.12±0.59	5.20±0.051	5.28±0.18	5.38±0.19	5.88±0.79	4.38±0.14	6.21±0.651	6.28±0.98	6.58±0.29

II. Comparison of physico-chemical characteristics of tank water in under the influence of Cow urine distillate exposure as feed supplement

Temperature

Temperature increased from 27.15 to 29.02° C in control. But it is decreased in T_1 from 29.9 TO 28.9^O C and from 27.04 to 26.09 in T_2.

pH

PH is stable in control but in treatment tanks it was increased from 7.64 to 7.95 in T_1 and from 7.53 to 7.97 in T_2.

Salinity

Slight increase in salinity was observed in control (0.24 to 0.26 mg/l) and T_1 (0.21 to 0.26 mg/l).But it was decreased from 0.42 to 0.21 mg/l in T_2.

Dissolve Oxygen

Dissolve oxygen decrease from 5.94 to 5.63 mg/l in control. But it was increased from 5.13 to 6.12 on 7th day and 5.63 on 28th day in T_1. In T_2 there was no modulation observed.

Ammonia

No alteration was observed in T_2 while it was increased from 0.05 to 0.06 mg/l in T_1. In control a slight decrease of 0.01 mg/l was observed (0.66 to 0.05 mg/l).

3.6.2 Physico-chemical characteristics of water samples during the study period (Feed supplement)

Parameters	Control			T_1			T_2		
	0^{th} day	7^{th} day	28^{th} day	0^{th} day	7^{th} day	28^{th} day	0^{th} day	7^{th} day	28^{th} day
Temp (˚C)	27.15± 0.4	29.9±0.08	29.02±0.12	29.9±0.08	29.6± 0.24	28.98±0.08	27.04±0.07	26.0±0.08	26.09±0.03
pH	6.97±0.16	7.24±0.46	6.96±0.27	7.64±0.36	7.40±0.09	7.95±0.27	7.53±0.12	7.52±0.21	7.97±0.42
Salinity (mg/l)	0.24±0.023	0.24±0.01	0.26±0.029	0.21±0.01	0.22±0.003	0.26±0.019	0.24±0.04	0.23±0.012	0.21±0.013
DO (mg/l)	5.94±0.04	5.73±0.02	5.63±0.033	5.13±0.02	6.122±0.017	5.63±0.03	5.17±0.03	5.3±0.108	5.1±0.06
Ammonia(mg/l)	0.06±0.009	0.04±0.03	0.05±0.007	0.05±0.01	0.04±0.005	0.06±0.02	0.04±0.023	0.05±0.020	0.04±0.91
Conductivity (µS)	5.54±0.078	5.42±0.01	5.32±0.15	5.12±0.01	6.13±0.24	6.40±0.17	6.61±0.67	6.38±0.55	5.81±0.32
TDS (mg/l)	2.71±0.92	2.98±0.16	2.43±0.08	2.17±0.14	2.18±0.05	2.19±0.33	2.43±0.42	2.47±0.78	2.49±0.65
Turbidity(NTU)	7.12±0.58	6.28±0.98	6.21±0.651	6.58±0.29	6.58±0.29	5.98±0.93	6.38±0.24	6.81±0.92	7.32±0.73

Conductivity

Control and T_2 showed decrease in conductivity in control it was from 5.54 to 5.32 µs and in T_2 it was from 6.61 to 5.81 µs. But T_1 has increased conductivity from 5.12 to 6.40 µs.

Total Dissolved Salt

Total dissolved salt was decreased in control from 2.71 to 2.43 mg/l. But in T_1 it gradually increases from 2.17 to 2.19 mg/l and in T_2 also it was increased from 2.43 to 2.49 mg/l.

Turbidity

Turbidity was decreased in both control and T_1 .But t_2 it was increased from 6.38 to 7.32 mg/l.

Fish had great significance in the lifetime of humanity, existence and usual significant source of protein and provided that certain other useful products as well as economic sustenance to many nations. The gradual corrosion of commercial fish stocks due to over-exploitation and modification of the habitation is one intention why the science - fish biology came into existence (Royce, 1972).Certain common considerations apply to nearly all research investigations on fishes, whether conducted in the field or a laboratory scenery. This division introduces concepts and procedures that can be adapted to the situation and circumstances for each investigator.

Fish has now been considered as the model organism for conducting different experimental studies, including those of Immunological, pharmacological and toxicological research, fish has also been used as a new model organism to create the experimental. The possibilities for the application of investigation and discoveries to both humanoid and ecological fitness problems makes fish species attractive and valued alternative models in the immunogenetics, oncology and toxicity research. The tilapia (*Oreochromis mossambicus*), a tropical freshwater fish, can be kept easily and cheaply in large numbers in the laboratory, where it breeds all year around. Therefore, the *Oreochromis mossambicus* has been recognized as a suitable model for experimental studies. The following sections provide a summary regarding background information on both these species regarding natural distribution, habitat, reproduction, external anatomy, function and histological structure as described for teleost species.

Cows, similar to other bovins, are ruminants. They have a unique gastrointestinal system that allows them to digest cellulose and other otherwise indigestible plant materials with the aid of symbiotic microorganisms living in their rumen, or first stomach. Cows eat mainly grasses and seeds. Like all mammals, cows yield milk to nourish their young. Cows are very protecting and caring for their calves.

Cow urine is considered to be the effective animal product used in the general health improvement (Khanuja *et al.,* 2005). It contains various volatile and non - volatile compounds which has significant antimicirobial activity (Shaw *et al.,* 2007). In ayurvedic system cow urine is widely used in combination with drugs and pharmaceuticals. Cow urine contains few essential component such as estrogen, nitrogen, phosphorus, pheromone, potassium, chloride, calcium, and urinary proteins (Biddl, *et al.,* 2007; Yan, *et al.,* 2007; Bravo, *et al.,* 2003).

In recent years, lot of interest has been generated among scientific community to develop or scientifically validate the Indigenous Technical Knowledge (ITK) as an alternate therapeutic or preventive approach using cow urine or its products. Several scientists from different laboratories of the Indian subcontinent like CSIR, AIIMS, GB Pant University, IVRI and certain NGOs are also working on different medicinal properties of cow urine. In fact there are several medicinal preparations available with NGOs, who are also marketing 'cowpathy' drugs under FDA license. Several students of Masters and Doctoral degree programmes are working on the medicinal properties of cow urine and other products of cows' urine (Chauhan and Singhal, 2006). Very recently Indian Scientists at the Junagadh Agricultural University (JAU), who analysed of urine samples of 400 Gir cows (*Bos indicus*), found traces of gold ranging from three mg to 10 mg from one litre

urine, *The Times of India* (http://indianexpress.com/article/cities/ahmedabad/gold-cow-urine-junagadh-agricultural-university-test-result-2882431/) reported.

For administration of any immunostimulants in aquaculture different routes are followed. Among them, water additive (Timmermans, 1987; Zhou *et al.,* 2009a; Merrifield *et al.,* 2009; Gupta *et al.,* 2014), and feed supplement (Sakai 1999, Boonyaratpalin *et al.,* 1995, Burrells *et al.,* 2001, Burrells *et al.,* 2001, Low *et al.,* 2003, Volman *et al.,* 2008, Soosean *et al.,* 2010, Harikrishnan *et al.,* 2011) are widely in practical.

Immunological parameters

In Indian scenario, Aquaculture has become a means for earning livelihood for the economically distressed farmers in India due to its promising results in productivity and national economy. Aquatic organisms rearing are currently the fastest growing industry in our national livestock sector which is benefiting us from production and advantages in prices along with provision of proteinacous food. In India, fish forming is recognized as an important cottage as well as fast growing large commercial aquaculture industry. Sustained economically viable fishery production demands the stringent control of various infectious diseases affecting the fish which incur huge economic losses to the fish farmers. To overcome this and for the maintenance of healthy flocks for commercial purposes, it is needed to augment the immune system in the susceptible fishes. Livestock and fish population are affected by many infectious diseases which cause immunosuppression leading to failure of vaccination against these diseases. In spite of timely vaccination by established methods, failure and breakdown of immunity has become common. Fish farming is always prone to a heavy risk of increased disease incidences leading to high mortality even after scheduled mass vaccination programmes are implemented. Some

pesticides and chemicals may lead to immunosuppression in the fish. To overcome these immunosuppressive conditions, modulation of micro-environment of the immune system seems to be essential. This can be achieved by immunomodulators or immunostimulating compounds. So we are trying Cow Urine Distillate as working immunomodulators or immunostimulators.

In the present study, two different breed of cow urine distillate treatment were studied by administrating through two different routes. Among them Water additive route had significant stimulatory effect on the specific and non-specific immune parameters. However, both breed CUD when supplemented in diet stimulate immune system to a lesser extent and CUD of both breeds in both routes conferred disease resistance with less mortality when compared to that of untreated control (fig 3.1.35 and 3.1.36). Similar results have been reported by Mala and Venkatalakshmi, 2015; Total leukocyte count (TLC) was increased in fish exposed with feed supplemented with CUD compared with fish fed with feed without CUD. The results from the present experiment also revealed an increase in TLC in fish administered with CUD compared with the control. This indicated the increased immune response in the fish administered with CUD, probably due to it's the immuno-stimulatory effects. Similar report was observed by Logambal and Venkatalakshmi, 2000 and Dinakaran Michael *et al.,* 2001; in *Occimum sanctum* leaf water extract and Ascorbic acid respectively. Similar report was observed by various animal models when the CUD was used. The cow urine distillate (CUD) was found to have immunomodulatory effect in mice as it enhances both T-and B-cell proliferation and also increased the level of IgG (Chauhan *et al.,* 2001). Recently, the cow urine has also been granted U.S. patents (No. 6896907 & 6410059) for its synergistic properties with

antibiotics, antifungal and anti-cancer drugs as bio-enhancer. It has provided the base for further research on immunomodulatory properties of indigenous cow urine. It has also been reported that CUD enhances B and T lymphocyte blastogenesis and increased IgG antibody titer in avian species (Kumar *et al.,* 2004 and Garg *et al.,* 2005). Immunomodulatory effect of cow urine distillate on humoral and cell mediated immune response against NDV vaccination in broiler chicks was observed when administered orally (Subha Ganguly, 2013). Immunomodulatory effect of cow urine or its distillate has been reported by many workers (Ganguly *et al.,* 2011, Ambwani *et al.,* 2005 and Garg *et al.,* 2005) and therefore this has made the base for present research. Awadhiya *et al.,* 1981; Srikumar *et al.,* 2006; Kumari, 2007 and Rakhi, 2004; showed increased cell mediated immune (CMI) response in CUD treated groups. The findings were also in accordance with those of (Chauhan *et al.,* 2004; Ambwani 2005 and Garg *et al.,* 2005) worked on lymphocytes blastogenic activity with respective mitogens using lymphocyte proliferation assay. The antigenotoxic and antioxidant properties of cow urine distillate and redistillate were reported *invitro* by Krishnamoorthi *et al.,* 2004. Copper has the power to destroy diseases and act as an antidote. Cytokines and amino acids might play a role in immune enhancement. *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 and Singh, 2001). Keeping in view all the above facts, the present investigation was planned to study the immunomodulatory effect of cow urine distillate in aquatic filed.

Lymphocytes are one of the most important protective factors of the fish against the microbial agents. The T cell, while stimulating, secrete cytokines, including interleukin 4 which leads to the reinforcement of the growth of the precursor cells of the hematopoietic

cells (Panigrahi *et al.,* 2008). In the present study, *O. mossambicus* blood cells were characterized microscopically and hematological indices were analyzed. It is well known that blood sampling, laboratory techniques, seasonal variations, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation could affect hematological data (Van Vuren and Hattingh, 1978).

In previous works, there was significant increase in total leucocyte count (TLC) and lymphocyte count in cow urine distillate given immunostimulation in rohu as compared to control group. Similar (Mala and Venkatalakshmi, 2015; Padmapriya and Venkatalakshmi, 2014; Priya and Venkatalakshmi, 2016) in fish on administration of cow urine and its distillate (0.1% v/v) concentration for a week on 21 days study.

Lymphocytes are numerically predominant white blood cells in fish (Swarnlata 1995; Kumar, *et a.l,* 1999). Lymphocytes of fish have been regarded as immunocompetent. Thus, they are responsible for the production of antibodies (Ellis *et. al.,* 1978). It is widely accepted that fishes, like most other vertebrates, have a common leucocytes pattern consisting of granulocytes, monocytes, lymphocytes and thrombocytes.

Haematological techniques are gaining importance for environmental monitoring and assessment of physiological condition of fishes (Shah and Altindag, 2004). Haematological picture has frequently been utilized for the detection of pathophysiological changes under different stressful conditions (Nussey *et al.,* 1995). It can then help to improve fish cultivation by facilitating early detection of prevailing stress and or diseases that could affect their production performance (Rehulka *et al.,* 2004 and Tavares-Dias and Barcellos, 2005). Fish blood is being studied increasingly in experimental research and environmental monitoring as a possible indicator of physiological and pathological

changes in fishery management and disease investigations (Mulcahy, 1975). Alteration in physiological and biochemical parameters of experimental fish has recently emerged as an important tool for water quality assessment in the field of environmental research. This is because blood in the gill has direct contact with the water medium and any unfavourable change in the water could be reflected in the circulatory system. These studies could be used to indicate the health status of fish as well as water quality. Blood chemistry has long been a helpful diagnostic tool in pathological, toxicological and general clinical tests. Fish blood parameters are suitable biomarkers for evaluating the potential risk of chemicals (Roche and Boge, 1996). Fish live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components. Blood being the medium of intercellular and intracellular transport, which comes in direct contact with various organs and tissues of the body, the physiological state of an animal at a particular time is reflected in its blood.

Haematological parameters can provide satisfactory information on the physiological response of fish to environmental stressors for two major reasons, namely, the close association of the circulatory system with the external environment and the ease of availability of fish blood (Houston, 1997; Cazenave *et al.,* 2005). The use of primary haematological indices such as blood haemoglobin (Hb), packed cell volume (PCV) and red blood cell count (RBC count) in assessing CUD of two different breeds were considered. Changes in the erythrocyte count or in haemoglobin values following chronic stress are useful indicators of blood volume changes (haemodilution or haemoconcentration) that have occurred. Observed haematological values such as Total erythrocyte count (TEC), Haemoglobin (Hb), Haematocrit (Hct) finds application in

assessing the functional status and even oxygen carrying capacity of blood stream (Shah and Altindag, 2004). Mean corpuscular volume (MCV) (size/ state of RBCs), Mean corpuscular haemoglobin (MCH) (average Hb content of single RBC) and Mean corpuscular haemoglobin concentration (MCHC) (average Hb concentration in 100ml of haematocrit are the calculated values of blood parameters (TEC, Hb and Hct) which fluctuate under varying eco-physiological and biological conditions and prove to be an asset in diagnosing the structural and functional status of body/ organs exposed to toxicants. Such studies with reference to fish acquires an added significance in that just by having a look at these values, it becomes easy for fish farmer to tell upon not only its health status but even the quality of fish.

Internal morphological observation of the lymphoid organs - spleen, head kidney, thymus and MALT were observed. Similar investigation was done by Cossarini-Dunier *et al.,* (1987) to study the immunosupressive effect of lindane on lymphoid organs and on either antibody production or on spleen weight. Dunier *et al.,* (1994) investigated the lindane in rainbow trout (*Oncorhynchus mykiss*) to cause a strong depressing effect on the chemiluminiscent response of head kidney macrophages in trout exposed to lindane via injection. Das and Mukherjee (2000a) reported the effect of hexachlorocyclohexane pesticide on the liver and kidney of *Labeo rohita.* and observed swelling of the hepatocytes with diffuse necrosis and marked swelling of blood vessels in the liver tissue. Tubules of the kidney tissue were reported by them to have distended tubular cells of posterior kidney showing marked necrotic changes. Sakr *et al.,* (2001) studied the effect of or ganophosphorus insecticide (Hostathion) on the liver of the catfish *Clarias gariepinus*. The insecticide caused changes in the liver which included cytoplasmic vacuolization of the

hepatocytes, damage of blood sinusoids, blood vessel congestion and inflammatory leucocytic infilterations. Vinodhini and Narayanan, 2009; studied the alterations in selected organs of *Cyprinus carpio* after exposure to heavy metals. The liver of control fish was found by them to exhibit a normal structural pattern compared to expose to heavy metals which exhibited vacuolation, presence of haemosiderin and fibrosis. Similarly observed Romano *et al.,* (1999) studied the structure of developing thymus of the marine teleost, *Diplodus puntazzo*. Lam *et al.,* (2002) described the Morphologic transformation of the thymus in developing zebrafish. The transformation of the developing zebrafish thymus from 1 week post fertilization (1 wpf) to 15 week postfertilization (15 wpf) was described. Bodammer *et al.,* (1990) examined the Spleen and head kidney of striped bass *Morona saxatilis.*

Organosomatic index of the spleno-somatic index (SSI), Hepto somatic index, thymosomatic and mucosomatic index were observed. The significant difference was recorded between control and treatment groups. The weight of the spleen was expressed as the percentage of total body weight. Alterations in this index could indicate an abnormal condition in the spleen such as necrosis or swelling due to infection (Goede and Barton, 1990). Spleen size is considered a useful diagnostic factor because the spleen is a hematopoietic organ (Anderson, 1990) and dysfunction could have effects at the whole-organism level. Seasonal changes also affect the SSI (Sanatan Singh *et al.,* 2015; White and Fletcher, 1985). Liver activity was increased with feeding intensity of the fish. The hepatosomatic index and activity of digestion is related with each other. Finally, as with the HSI, factors that cause a disproportionate change in body weight will affect the SSI. Nonspecific stressors (e.g., hypoxia) can result in altered spleen morphology. Studies on

six species of teleost fish found that transient hypoxic conditions or severe exercise caused the spleen to contract fully and then decrease in size and hemoglobin content (Yamamoto and Itazawa, 1985; Yamamoto, 1988). Hence the increase SSI proves that CUD relieved the fish from stress and best owed the fish with good health.

Growth and food utlization

The effect of CUD in *Bos indicus* and *Bos taurus* on the growth parameters were measured for every 10 days and it was recorded in table 3.2.2 and table 3.2.4. Its shows that the water additive route is more effective than feed additive, which could be noted from the fact that the maximum growth parameters were recorded in water additive route. It was shown in figure 3.2.1 and 3.2.2 i.e Percentage of Increase in Body Weight (PIBW). Growth is the appropriate index which reveals the role of any growth promoter in the medium. Growth rate, determines the quality of the product and total annual yield (Ebanasar, 1995). Garg *et al.,* (2005) was evaluated the effect of distilled cow urine on the nutrient utilization by the white leghorn layers. The results showed that there was increase in feed intake, decreased feed conversion ratio and feed efficiency ratio. Digestibility of dry matter, crude protein, ether extract, crude fiber and organic matter increased significantly in the cow urine treated group. The findings of present study have practical importance in maximizing the growth and survival of fingerlings by 0.1% CUD treatment. The present study demonstrated that the CUD is efficient in *Oreochromis mossambicus* fingerlings for better growth performance at 0.1% concentration treatment when compared to untreated control group. Cow urine is well known for 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 *Labio rohita* (Sattanathan and Venkatalakshmi, 2015) and in *C. mrigala* (Padmapriya and Venkatalakshmi, 2014). In the present study the results confirm that the Cow Urine Distillate is capable of promoting growth and food utilization of cultured fishes as in the present experimental model of *Oreochromis mossambicus* fingerlings compared to control. 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 (Table 3.5.2) and hence it may be the reason for the promotion of growth.

Utilized food is digested and assimilated for maintenance and growth of any organism. Apart from providing energy, they play vital role in the growth of aquatic fauna. Analysis of food utilization and growth of aquatic fauna is the thrust area at present. (Joycy Jay Manoharam, 1980; Marimuthu Sakthivel and Pandi Baskaran, 1995; Swain *et al.,* 1996

and Shalendra Singh *et al.,* 1998; Anupriya, 2007). Feeding and growth studies are available giving special reference to growth and quality of food. (Jobling, 1993; Sadhana and Neelakandan, 1996; Parameswaran and Murugesan, 1975; Joycy Jay Manoharam, 1982; Baskaran *et al.,* 1990; Elangumaran *et al.,* 1992; Ebanasar and Jayaprakas, 1994 and 1996).

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 highest survival was noted in CUD exposed groups as compared to the control. Similar results were observed by Priya and Venkatalakshmi, (2016) in *rohita* by using fresh Gir cow urine. This shows that the fish were under stress free environment and also acted as good stress reliever. Ration level is an important factor governing the growth of fish (Brett, 1979; Chiu *et al.,* 1987). The relationship between growth rates and ration in fish is very important, because feed accounts for 50% of the cost of the intensive fish culture (Gunther *et al.,* 1992). Growth rate and ration interact to determine FCR and are used to estimate the daily ration for a particular fish stock. Similar to all animals, fish will lose weight when their nutrient intake rate falls below that required for daily maintenance. As food availability increases, the quantity consumed by the fish will also increase, giving a linear increase in specific growth rate (SGR%) up to the point maximum voluntary food

intake. Growth rate is linearly correlated to food intake (Peres and Oliva-Teles, 2005). If fish are fed above their appetite, the extra food will be wasted and an artificially high FCR will result. So high FCRs can result from both over and under feeding does not necessarily result in higher growth. Beyond a certain level, over feeding has no effect on growth, and result in a poor growth (De Silva and Anderson, 1995) and will also cause water pollution for aquaculture (Store-bakken and Austerng, 1987).

As literature reveals, the present study also confirms the potential of cow urine distillate in promoting the health, which was expressed as good survival rate, increased growth rate and feeding rate in *Oreochromis mossambicus* fingerlings. This has direct applicability in the field of aquaculture.

The T_2 group showed very high level growth rate, food absorption rate, Gross conversion efficiency and Net conversion efficiency but low "k" value, survival rate and length when compared to the T_1 group *Bos indicus* CUD treated fishes.

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 protein and lipid contents (Weatherley and Gill, 1983). The obtained data are in agreement with Fletcher and White, 1976; and White and Fletcher, 1985; found fluctuation in k values of fish and attributed these changes in k factors to the feeding rate and to lipid mobilization. So *Bos indicus* CUD treated fishes are having highest living capacity to survival rate and protein content helped in increasing body length when compare to T_2 and control.

However in this 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 and economically safe 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 method of increasing productivity. Hence it can be concluded that *Bos indicus* CUD can fulfill the needs of aquatic farmers to increase fish production, interms of quality and quantity at low cost.

Feed Formulation and proximate analysis

Fishes are the efficiently animal sources of protein for human beings. These animals convert feed protein into body protein more significantly than any other organisms. Hence, the complete success of the fish farming keeping depends upon the feed given to them. Fish meal is used as an animal protein source in the artificial feeds of these animals. As the availability of the quality fishmeal has become scarce, an alternate source has been tried by some scientists. The feed should be made established on the exact knowledge of their nutritious supplies so that the optimal growth can be reached at a given time. The balanced nourishment to be given to these organisms should contain nutrients such as lipid, carbohydrate, protein, vitamins and minerals to meet basal energy requirements and also to ensure healthy growth. Of all the components of the formulated feed, protein plays a

major portion in the feed. It is also a costly component. The part of the protein in the feed should be neither more nor less than the optimum required for the organisms.

In the current study, the feed was prepared in the form of pellets for the fish. Many researchers (Nakanuira and Kasahara, 1956 and Tabachek, 1988) have detected that pelleted form of a feedstuff is more suitable for fish as it supports the fish to get al.,l the crucial nutrients and as it is convenient to store. Since the moisture content in the dry pellet is small, the chance for the formation of mould minimized, and insect pests also can be banned easily. Hence, in the present study, the fish feed was prepared in the form of long and thin pellets.

The feed should contain not only all the nutrients required for normal growth in the right proportion. Fishes are the major animal protein sources for human consumption and their feed conversion efficiencies are higher than those of other organisms. The ingredients used in fish feeds are locally available and quite inexpensive. Groundnut oil cake is commonly used in animal feed as a source of vegetable protein and it is available throughout the year. The amino acid profile of soy powder shows that it is rich in arginine and the limiting amino acids are tryptophan, methionine and lysine. However, in the compounded feed, the lack of certain amino acids is taken care of by adding certain ingredients which are rich in those limiting amino acids. In formulated fish feed, the wheat powder is a help as energy source besides rice bran. Kim *et al.,* (1984), Belal *et al.,* (1995) and Desilva and Gunasekera (1989) have used wheat powder in the feed for fish not only as an energy source but also as a ring binder. The other binding ingredients like gum arabic and align are physiologically active and hence they are considered to have growth

inhibiting effects (Belal *et al.,* 1995). So wheat powder was used in the present study as a binding compound and as an energy source.

The energy source need of fish is much less compared to that of warm-blooded animals. Fish need energy mainly to maintain position and for movement. Due to ammonia excretion, the use of energy source is extremely minimized. However lacking of extra energy outcomes in reduced fish growth rate (length and weight). Fish mainly eat to satisfy their energy needs and excess energy reduces the consumption as high energy feed fulfill the energy requirement in less quantity. Peanut oil cake is usually used a fish feed (Raj, 1989 and Daniel and Sahayaraj, 1990) as a vegetable source of protein and fishmeal as an animal source of protein.

The dietary protein requirement of fish differs from fish to fish. Several experiments have been earned out by some scientists to optimize the protein requirement for different species/classes of fish. It has been studied that the dietary protein necessity of *Salmo gairdneri* was 45 percent (Higuera *et al.,.*1988), in *Cyprinus carpio* was 40 percent (Kim and OIU 1985), in *rohu* and *catla* were 5 percent (Jayaram and Sherry, 1980 and Mohanty *et al.,* 1990), in *Oreochromis nilolicus* was 34 - 36 percent (Desilva and Gunasekera, 1989). Ogino and Saito 1970 reported that the optimum utilization of protein by carp was gained while fed on diets having 35 to 40 percent of protein. For the present study, the protein level for the compounded feed for *Oreochromis mossambicus* was fixed at 40 percent level. Feed is given to fish in pellets form, since providing the feedstuff for fishes in the form of pellets is the general practice method. Dry pellets are simple to prepare, store, transport, handle and distribute. They can also be easily protected from fungi and insects. Some workers used pelleted diet for fish culture (Raj, 1994). In pelleted feeds

required protein percentage can easily be calculated (Ali, 1980). Locally available ingredients can easily be incorporated into the feed to decrease the cost of the feed. Conventional and non-conventional compounds like natural weeds and grasses (Raj, 1984)., hide meat flash powder (Raj and Kandasamy1991), natural wild seeds (Daniel and Raj, 1992), natural wild leaves (Raj, 1994).Taking this in view the ingredients used for the pellet preparation in the present study were selected. To maximize the feed utilization among the rearing organisms, their feeding behavior should be taken into account. In aquaculture operation, the size and the shape of the feed pellets play a role in eliciting responses from animals which capture them. The physical attributes of the pellets namely texture, length, color, density, flavor, etc. not only affect the capability of the fish to capture but also stimulate the fish to eat them. The shape and size of the pellets are probably to be significant in every phase of the feeding sequence by manipulating their detectability, attractiveness and ease of capture (Stradmeyer *et al.,* 1988).

The texture and hardness of the pellets also play a major role in encouraging the fish to eat formulated feed. Soft pellets are readily established by the fish regardless of their length (Knights, 1985 and Mearns, 1990). In the current study, the length of the pellets used for the ranged from 2 to 5 cm. There was no modification in the width and permanency of the pellets. The stability of the feed pellets depends on the ingredient composition, nature of the ingredients, their processing method, moisture content, etc. Higher fat content affects the gelatinization and reduces the pelleting stability.Winfree and Stickney reported in 1984 that vegetal proteins rise the stability. In the current study, the stability of the feed pellets ranged from 97 to 98 percent. The variance may be due to the increase in the percentage of the length of pellets. Raj and Kutty (1979) have detected a feed stability of 96.9, 93.0 and

95.8 percent in dry feed pellets of l mm diameter incorporating 60 percent *(Albizzia lebbeck* and *Enterolobium saman* seed kernel powder respectively. Venugopal and Kesavanath (1984) studied a feed stability of 92.8. 91.9 and 87.7 in the pellets incorporated with fishmeal, colocassia leaf and fish silage. They also noted that increase in moisture, in turn, altered the stability of the feed pellets. In the present study feed supplemented with CUD showed 98.65 and 97.97% stability which indicates that CUD could be a good feed additive practically.

However, the success of rearing fishes dependeds upon the feed given. Some experiments have been carried out by various researchers to optimize the percentage of protein required for fish (Mohanty *et al.,* 1990 and Ogino and Saito, 1970).

Low-cost components like soya bean meal, copra cake, corn flavor, rice bran, napier and carpet grass meals were verified for their digestibility in grass carp (Law, 1986). Jayaram and Sherty (1980) studied the effect of three pelleted feeds incorporating silk worm pupae, peanut oil cake and fish meal as sources of protein on *rohu*, *catla* and *carp*. Silkworm pupae and fishmeal diets showed conversion ratios of 2.5 and 2.6 for carp. The digestibility and amino acid availability of soya bean, poultry meat meal blend based diets for *Oreochromis niloticus (L)* fingerlings were tested by (Sadiku and Jauncey,1995). Both diameter and length of pellets affected the feeding time because Salmons take a longer time to capture small pellets. Though they initially caught longer pellets, finally they were rejected and only the smaller pellets were ingested.

Biochemical analysis of Liver and muscle

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).

Essential fatty acid deficient diets also may result in growth retardation and physiological symptoms (Takeuchi and Watanabe, 1977), Warmwater fishes are able to utilize much higher levels of carbohydrate compared to cold water fishes. However, if adequate carbohydrate in the diet, other nutrients such as protein and lipid may be catabolized for energy, to provide metabolic intermediates for the synthesis of other biological compounds (Gaber, 2000). The concentration of hexokinase, a key enzyme in glucose utilization in animals, is relatively low in fishes (Walton and Cowey,1982). Excess dietary carbohydrate also may lead to fat deposition by stimulating the activities of lipogenic enzymes (Likimani and Wilson, 1982).

Biochemical studies of fish tissue are of considerable interest for their specificity in relation to the food values of the fish and for the evaluation of their physiological needs at different periods of life. It is also necessary to have the data on the composition of fish in order to make the best use of it as food and also to develop the technology of processing

fish products. Generally changes in chemical composition of body have been known to reflect storage or depletion of energy reserves. The values of body composition in fishes vary considerably within and between species, with fish size, sexual condition, feeding, time of the year and activity (Weatherly and gill, 1987). Food composition, environment and genetic trait are also known to influence chemical composition of fish (Oni *et al.,* 1983).

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 (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).

Carbohydrates are considered to be the first among the organic nutrients to be depleted and degraded in response to stress conditions imposed on animals. Carbohydrates are important, since these provide the energy for the animal required for performing different processes (Lehninger, 2004; Harper, 2003). Alteration in carbohydrate metabolism is prone to have deleterious effect on the survival of the animal (Srinivasa murthy, 1983; Radhaiah, 1988; Rama Murthy, 1988; Veeraiah, 2002 and Madhavi, 2005). Impairment of carbohydrate metabolism is one of the outstanding biochemical lesions caused by the action of toxic compounds (Matias 1983; Srinivasa murthy, 1983; Radhaiah, 1988; Rama Murthy, 1988).

Alterations in biochemical components like protein, carbohydrate and lipid as response to environmental stress are authenticated by many investigators; Ramakrishna and Sivakumar (1993) in *Oreochromis mossambicus*, Malla Reddy and Bashamohideen (1995) in *Cyprinus carpio* , Singh *et al.,* (1996) in *Heteropneustes fossilis*, Tilak *et al.,* (2001) in *Labeo rohita* , Kumar and Saradhamani (2004) in *Cirrhinus mrigal*, Saraswathi (2004) in *Labeo rohita*, Arockia Rita and John Mitton (2006) in *Oreochromis mossambicus* and Prabhakara Rao and Radhakrishnaiah (2006) in *Cyprinus carpio.*

Understanding of the protein components of cell becomes necessary in the light of the radical changes taking place in protein profiles during pesticide intoxication. Both the protein degradation and synthesis are sensitive over a wide range of conditions and show changes to a variety of physical and chemical modulators. The physiological and biochemical alterations observed in an animal under any physiological stress can be correlated with the structural and functional changes of cellular proteins. Proteins occupy

a unique position in the metabolism of cell because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways (Lehninger, 2004; Harper, 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, etc.

Bio chemical analysis of *Oreochromis mossambicus* treated with *Bos taurus* (T_2) showed high level fat content and low level protein when compared to the control and *Bos indicus* CUD treated group (T_1). Excessive lipid levels in fish diets may reduce fish growth and produce fatty fish (Garling and Wilson, 1977).

Serum Bio Chemistry

The serum biochemical profile can reflect the health status, nutrition status and the adaptability to environment of physiological or pathological changes of fish affected by external factors (Shi *et al.,* 2012). Some researchers held that the change of fish serum biochemical indices might be caused by stress, or by some change of amino acids in diet (Lin *et al.,* 2015) Alkaline phosphatase (ALP) catalyses the hydrolysis of p-nitrophenyl phosphate at pH 10.4, liberating p-nitrophenol and phosphate. The rate of p-Nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alkaline phosphatase presence. γ-Glutamyl Transferase (GGT) is an enzyme found mainly in serum from hepatic origin, though the highest levels are in the kidneys. GGT catalyzes the transfer of amino group between L γ-Glutamyl 3-carboxy-4 nitroanilide and Glycyl glycine to form L γ-Glutamyl glycyl glycine and 5-amino 2-nitrobenzoate. The rate of formation of 5-amino-2 nitrobenzoate is measured as an increase in absorbance which is proportional to the GGT activity. SGPT is found in a variety of tissues but is mainly found

in the liver. Increased levels are found hepatic diseases. Slight elevation of the enzymes is also seen in myocardial infarction. SGPT (ALAT) catalyzes the transfer of amino group between L Alanine and α Keto glularate to form Pyruvate and Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT (ALAT) activity. SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood. Elevated levels are found in acute renal diseases, primary muscle diseases. SGOT (ASAT) catalyzes the transfer of amino group between L-Aspartate and α Ketoglutarate to form Oxaloacetate and Glutamate. The Oxaloacetate formed reacts with NADH in the presence of Malate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT (ASAT) activity. LDH is found in many body tissues particularly heart, liver, skeletal muscle and kidney. LDH is found in the form of isoenzymes based on their electrophoretic mobility with each isoenzyme being primarily from different organs. Lactate dehydrogenase catalyzes the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the LDH activity.

In all groups liver functional enzymes noted no significant difference. The treatment groups showed nearly control value and it clearly illustrates CUD treatment is not harmful at 0.1% concentration. Both water and feed additive route have same status (fig 3.3.3 and fig 3.3.4). Padmapriya (2016) and Priya (2016) reported similar results in *C. mrigala* and *rohita* respectively by using fresh Gir cow urine.

Albumin consists of approximately 60% of the total proteins in the body, the other major part being globulin. It is synthesized in the liver and maintains the osmotic pressure in blood. Albumin also helps in the transportation of drugs, hormones and enzymes. Decreased levels are seen in liver diseases. Malnutrition, Kidney disorders, increased fluid loss during extensive burns and decreased absorption in gastro-intestinal diseases (Doumas and Watson 1971). Proteins are constituents of muscle, enzymes, hormones and several other key functional and structural entities in the body. They are involved in the maintenance of the normal distribution of water between blood and the tissues. Consisting mainly of albumin and globulin the fractions vary independently and widely of diseases. Decreased levels are found mainly in malnutrition, impaired synthesis, protein losses as in hemorrhage or excessive protein catabolism (Gornall, *et al.,* 1949, Doumas. 1975). The serum protein parameters are albumin, globulin and total protein values significantly differ from control in both routes. The maximum protein values were recorded in T_1 group and the minimum in control group (fig 3.3.5 and 3.3.6).

Measurement of serum cholesterol levels can serve as an indicator of liver function, biliary function, intestinal absorption, propensity toward coronary artery disease, thyroid function and adrenal disease. (Roeschlau et al., 1914; Richmond 1973) Cholesterol levels are important in the diagnosis and classification of hyper lipo-proteinemias. Stress age, gender hormonal balance and pregnancy affect normal cholesterol levels (Report of the National Cholesterol Educational Program, 1988). The highest cholesterol profile observed in T_2 group in both route and minimum value recorded in control (fig 3.3.7 and 3.3.8).

Sulfanilic acid reacts with sodium nitrite to form diazotized Sulfanilic acid. In the presence of accelerator (cetrimide), conjugated and unconjugated bilirubin reacts with

diazotized Sulfanilic acid to form azobilirubin. In the absence of accelerator only conjugated bilirubin reacts (Peariman FC and Lee RTY 1974; Zoppi *et al.,* 1976). Serum bilirubin and creatinine in all groups were noted in the same range (Fig 3.3.9 and 3.3.10).

Field Trials

In 2008, a total of 1,336,340 experimental animals were used for field studies. Among them 1,332,422 were fishes, 2,655 were mammals and 1,253 were birds. NARA (National Aquatic Resource Research and Development Agency) in 2008 reported that 70% of the animals used for field trials are fishes. Nearly all of these fish were part of vaccine trials (https://norecopa.no/media/6299/baeverfjord.pdf). In the present study also fishes were used for testing the efficiency of CUD as water additives.The results obtained in this study confirm previous results on the use of growth promotors in *Oreochromis mossambicus* in the lab and in field trials. This study shows the potential of this type of water additives as a growth promoter in the pond grow out of tilapia in the absence of major disease threats. Further work is underway to investigate the impact on survival in production conditions where disease pressure is affecting fish health and farm productivity.

(i) Immune parameters

lmmunostimulants have long played a major role in disease control in aquaculture practices in the world (Austin and Austin, 2007; Austin *et al.,* 1995). Sakai, (1999) has extensively reviewed the role of immunostimulants in control of fish disease. Substances like bacterial components, chemical agents, extracts of marine animal or plant extracts have been tried as immunostimulants to protect the fish against several diseases caused by the pathogens. (Kajita *et al.,* 1990; Raa *et al.,* 1992; Mulero *et al.,* 1998; Esteban *et al.,* 2005; Logambal *et al.,* 2000; Esteban *et al.,* 2001; Miles *et al.,* 2001; Ortuno *et al.,* 2001; Villamil

et al., 2003; Dautremepuits *et al.,* 2004). lmmunostimulants are generally used to enhance the non-specific system of fish. In the present study all the immunological parameters were increased in treatment pond when compared to control group.

(ii) Growth promotion and Survival rate

The use of functional feed additives to promote growth and/or disease resistance has been demonstrated with different aquaculture species in controlled laboratory studies. However, demonstrating the commercial cost efficiency of these strategies requires field evaluations under the specific challenges encountered in production. During the current field trial, conditions were favorable and there were no disease outbreaks during the growout, resulting in an excellent survival at harvest (control pond averaged 85% and treatment pond 95%). Despite the excellent productivity in the treatment group, CUD promoted a significant improvement of survival. The economic analysis showed that the water additive resulted in increased revenues for the farmer and a return on investment.

(iii) Water quality in pond

A knowledge of the biotic and abiotic factors affecting the cultivable species of fish is a pre-requisite for their successful culture. Environmental conditions influence the culture performance of fish and their survival and growth. Factors controlling the quality of water which determines to a great extent the success or failure of culture operations are extremely varied, maintenance of optimum water quality is essential for the optimum survival and growth of fish. The physico chemical parameter found no significantly difference with treatment pond and control pond. But some parameter like pH, temperature and turbidity were slightly changed in treatment pond (fig 3.4.2 and fig 3.4.3). Hence it is

proved that cow urine even though an excretory product with high urea content, could be used as an pond fertilizer without doubting for its influence on water quality.

Physico chemical characteristics of Cow urine

Many products obtained from cow are useful to humanity either in medicines, agriculture or religious purpose. Panchgavya (Five essential products of cow viz., urine, milk, ghee, curd and dung) is having therapeutic and spiritual significance in India. All these five (Panchgavya) having therapeutic properties and are used either single or in combination with herbal or minerals against many diseases (Jarald *et al.,* 2008). An exhaustive reference of cow's urine having curative properties in skin diseases, especially leprosy, is referred in Charak Samhita.Use of cow's urine has been suggested for bath, anointing and intake. Feeding of cow urine increased the feed in take in white leghorn layers (Garg *et al.,* 2005). The medicinal values of cow urine are due to the presence of many minerals, vitamins and other trace elements. Hence in this chapter deals with the physicochemical and microscopic characterization of cow urine and their distillate of selected cow breeds was done.

For animals, the essential primary or macro elements are Ca, N, P, S, K, Na, C1, Mg, and S (together with C, H and O) (Thornton 1983),while Cu, Co, I, Fe, Mn, Mo, Se and Zn are classified as essential microelements (Underwood, 1966).

Webb *et al.* (1968a, 1968b) attributed the disease in domestic animals to mineralization and stated that regional geochemical reconnaissance surveys illustrate their potential value not only in prospecting for minerals but also in investigating the influence of nutritional imbalance on plants, animals and man.

Modern works on animal nutrition (Underwwood, 1971; Mc Donald *et al.,* 1981: Maynard *et al.,* 1979; Georgievskii *et al.,* 1981; Bondi, 1987) exhaustively discussed the significance of trace element interaction in animal products.

The ayurvedic texts (Kantavallabha Charyulu, 1952; Venkata Sastry, 1959; Jolly, 1977; Ambikadutta Shastri, 1979; Pardhnsaradhi sarma 1979; Priyavrat Sharma, 1981; Pandey, 1986) they point out that the urine of cow, goat, buffalo, sheep, horse, donkey, camel prescribed for treatment of various physiological disorder of men.

Urinalysis is information on urine production and urine components (chemicals, cells, casts, crystals). It is very useful tool to evaluate healthy and diseased/sick individuals. By this, valuable information can be obtained particularly about the urinary system and in general about the other organs/systems of the body in minutes. In diseases not yet diagnosed, urinalysis might be helpful for diagnosis of the pooled sample obtain to the experiment test. The urinalysis test end report with regards to the properties of the cow urine used for the study. The report revealed that the cow was healthy, has no sign of abnormality as at the time the urine was collected. A complete urinalysis, therefore, includes determining physical properties, verifying the urine's chemical properties and microscopic examination. Hence the Fresh Cow Urine (FCU) and Cow Urine Distilled (CUD) were analyzed for physico chemical characteristics.

Physical characteristics

Urine is formed to keep the composition of the extracellular fluids constant, and generally most substances that are present in extracellular fluid are also present in urine (Reece, 2005). Urine contains mainly water, minerals, urine cast and other waste products

of the body and the composition of urine varies according to the species, breed, season, physiological status, quality and quantity of water consumed (Siener and Hesse, 2002).

Colour

As show the result in Table 3.5.1 & plate 3.5.1 the depth of the colour depends on the urine concentration. The usual colour of urine is yellow to light amber in cattle and it depends primarily on the concentration of urochromes. Urine colour may be light to dark yellow and pale pink in bovines suffering from urolithiasis (Sharma *et al.,*2006). Yellow to amber normal color it may be due to the presence of urochrome and urobilin. Normal urine colour varies from almost clear to dark yellow as a result of urobilin pigment. In a study conducted on calves suffering from obstructive urolithiasis, urine was found to be of different colours and different appearances than normal. The variation in the colour of urine of the affected animals on day zero probably could be due to the difference in the concentration of urine, accumulation of sediments and haemorrhage. Dirty yellow coloured urine might be due to the presence of sedulous materials in the urinary bladder. Brownish urine is indicative of mixing of blood in the urine, which could be due to haematuria or nephritis reddish colouration of urine is indicative of haematuria, which could be caused by injury by calculi or inadvertent haemorrhage while performing surgery (Kannan, K.V.A. and Lawrence 2010).

Odour and Taste

Odour and taste are determined by using normal sensational observation of the sample. FCU of both breeds had aromatic odour and CUD of both breed have ammoniac odour. Ammonia ions present in CUD and FCU, give urine its typical odour. A foul or feculent odour may signify urinary tract infection. Sweet or fruity smelling urine may be

present in diabetes, ketosis, or maple syrup urine disease. Phenylketonuria gives off a musty odour, isovaleric academia a sweaty foot odour, while hypermethioninaemia gives off a rancid or fishy odour (Fogazzi *et al.,* 2008a). The taste of FCU is tangy in both breeds and taste of CUD in both breeds was Pungently. An interesting observation is that there are people, not less in number, consume 20 to 50 ml at morning FCU (gomutra) every day. Taste and odour are, thus, not obstacles in consumption and utility of FCU.

Transparency and Turbidity

Transparency and Turbidity are clear in both breed of FCU (plate 3.5.2). Freshly voided urine from the healthy animals is usually clear, except in horses where it is thick and cloudy owing to the presence of calcium carbonate crystals and mucus (Fenton *et al.,* 2009). Cloudy urine may not necessarily indicate pathology, as many samples become cloudy upon standing (Kalim *et al.,* 2011). Interestingly, in bovine obstructive urolithiasis urine may still be transparent and clear (Braun 2006 and Sharma, *et al.,* 2006). Cloudy or turbid urine is most commonly caused by urinary tract infections but may be due to contamination from vaginal secretions, faecal material, gross haematuria, crystals, or lipids as in chyluria (Fogazzi *et al.,* 2008a). The turbidity can be assessed visually or more accurately using spectrophotometry by comparing the sample turbidity with that of a set of standards (Parrah *et al.,* 2011).

pH

Measurement of urine pH can be made using urinalysis strips, narrow range pH meter and portable pH meter. Of these, portable pH meter is highly accurate for the measurement of the urinary pH (Nappert and Naylor 2001). The water content of the urine is within the normal range and the color of the urine and the pH show that the concentration

is of significant value in the acidic region. Urine pH depends upon the type of diet. If the animal is on a vegetable diet, power of hydrogen ion of urine of herbivorously animals will be alkaline, and carnivorously animals urine power of hydrogen ion will be acidic. pH of urine is a measurement of the kidney's ability to conserve hydrogen ions. Thus it provides a rough but useful estimate of the body's acid-base status. However, urine pH does not necessarily reflect the body's pH, as it is highly influenced by diet, recent feeding, bacterial infection, storage time, metabolic and respiratory alkalosis, and urinary retention. The urinary pH in normal cattle is usually on the alkaline side and may range from 7.4 to 8.4 (Mavangira *et al.,* 2012). Diet can have both immediate and short-term effects on urine pH. High protein diets, such as those consumed by carnivores produce neutral to acidic urine. Herbivores tend to produce alkaline urine. Any animal may produce alkaline urine immediately after eating due to buffering that occurs in response to gastric acids. The urine alkaline nature is frequently linked to urinary tract infections. The bacteria break down urea and form ammonia contributing towards the alkalinity of urine. The renal tubular disease and obstruction may also create alkaline urine. Acidic urine is commonly observed in animals with diabetes mellitus, especially if the animal is ketoacidosis (Darling *et al.,* 2009). An exact measurement of urine pH is critical for clinical decision making especially in urolithiasis cases (Gazi *et al.,* 2012). In bovine obstructive urolithiasis, urine is usually alkaline (Sharma *et al.,*2006). However acidic urine is also not an uncommon finding. The release of ammonia owing to the breakdown of urea in the retained urine renders it alkaline (Parrah *et al.,* 2011). Urine pH plays a major role in the formation of uroliths. Struvite and calcium apatite uroliths are mostly found in urine with alkaline pH (Fenton, T.R. *et al.,* 2009 and Singh 2005), while cystine stones are formed at the acidic pH. However, pH is

variable in the formation of urate, silicate and calcium oxalate stones (Lulich and Osborne 1992).

Specific Gravity

SG is the concentration of dissolved solutes (substances in a solution) in the urine, and it redirects the ability of the kidneys to concentrate the urine (conserve water). The SG of a solution thus depends on the number and the molecular weight (size) of particles in the solution. It is a valuable to test, as the loss of concentrating ability of the kidneys is among the first signs of renal tubular disease. Urine with an SG outside the range i.e. > (1.020-1.040) suggests alteration by the renal tubules. Specific Gravity of Urine can be recorded either using urinometer method. The latter have one calibration scale for dogs and large animal urine samples, which is similar to human urine samples and a separate scale for cat urine samples (Wisnewski *et al.,* 2004). Specific Gravity is a valuable test for evaluating kidneys. The low Urine Specific Gravity could be caused by osmotic dieresis, loss of medullary tonicity (medullary washout), resistance, and deficiency in the antidiuretic hormone. Osmotic diuresis occurs in diabetes mellitus, Fanconi syndrome and primary renal glucosuria, where an excess amount of glucose in the glomerular filtrate prevents water being reabsorbed in the distal tubules. Loss of hypertonicity in the renal medulla may result from hypoadrenocorticism (loss of sodium), liver disease, prolonged or vigorous fluid therapy. Resistance to Anti diuretic hormone (ADH) termed nephrogenic diabetes insipidous, occurs commonly secondary to many conditions including hypercalcemia, chronic liver diseases, pyometra, hyperadreno-cotism and hypokalaemia. Specific gravity in health varies with the state of hydration and fluid intake. The specific gravity range of normal cattle urine is 1.025-1.045 with an average of 1.035 (Kannan and

Lawrence 2010) and in obstructive urolithiasis it ranges in cattle from 1.008 to 1.025 (Braun 2006). The amount of urine excreted daily varies with the diet, work, external temperature, water consumption, season and other factors. The specific gravity of urine varies with the relative proportion of dissolved matter and water. The greater the volume, lower will be the specific gravity. Cattle produce on an average of 17-45 ml/kg body weight/day of urine with mean specific gravity of 1.030-1.045 (Reece, 2005). The both breed urine SG is normal. In *Bos indicus* it is 1.026 and for *Bos taurus* it is 1.032.

Weight

The weight of FCU in *Bos indicus* and *in Bos taurus* is higher when compared to CUD of *Bos indicus* and *Bos taurus* respectively. The weight of cow urine depends on the soluble particles in the urine. Thus the higher weight of FCU than CUD indicates that FCU has more particles as compared to the CUD.

Conductivity

The conductivity measurement is related to the concentration of electrolytes in the urine. In contrast to osmolality and specific gravity, uncharged particles (e.g. glucose, urea) do not contribute to conductivity. The conductivity can be used as a substitute for osmolality (reference range: 8–32 mS/cm). There is a good agreement between conductivity, urine density, and osmolality (Hofmann *et al.,* 1995). The Conductivity correlates better with osmolality than the creatinine concentration and therefore strength serves as a better indicator of hydration status (Hofmann *et al.,* 1995; Hannemann-Pohl and Kampf, 1999). The estimate the ability of urine to conduct an electrical current. A determination of the urine conductivity which is a non-linear function of the electrolyte concentration in the urine.

Salinity

Salinity is the dissolved salt or saltiness content of the given sample. Salinity is a major factor in determining many aspects of the chemistry of biological processes inside it and is a thermodynamic form variable that, along with temperature and pressure, governs physical characteristics like the density and heat capacity. Salinity is the measure of all the salts dissolved in water. Salinity is the measuring saltiness or dissolved salt content of water. Salinity is a major factor in determining many aspects of the chemistry of natural waters and biological (organic) practices within it and is a thermodynamic state variable that, along with pressure and temperature, governs physical characteristics like the density and heat capacity of the water. CUD contains higher salinity than FCU.

Total dissolved solids (TDS)

TDS, which stands for Total Dissolved Solids, refers to the amount of organic and inorganic dissolved substances that may be found in urine, such as minerals, metals and salts. A TDS Meter indicates the Total Dissolved Solids (TDS) of a solution, i.e. the concentration of dissolved solid particles. Total Dissolved ionized solids, such as minerals and salts, increase the EC (electrical conductivity) of a solution. For it is a volume measure of ionized solids, electrical conductivity (EC) can be used to estimate TDS. The TDS is a determined of the combined content of all organic and inorganic materials contained in the liquid in molecular, ionized or micro-granular suspended form. The operational description is that the solids necessity the minor sufficient to survive filtration via a filter with 2µm (nominal size, or smaller) pores.

Chemical characteristic

Chemical analysis of urine can be used to identify the composition of the calculi (Neuman *et al.,* 1994). Kumar, 2001 studied various biochemical constituents in the urine of cow, buffalo and goat and arrived at a conclusion that, the biochemical constituent composition varies between the species. Parihar *et al.,.* 2004 measured the concentration of some minerals by atomic absorption spectrophotometry in the urine of Sahiwal, Crossbred and Non-descript (Desi breed) cattle. The result showed non-descript cattle have maximum concentration of zinc, potassium and calcium when compared to Sahiwal and crossbred cattle.

A healthy animal excretes little to no glucose in its urine. Glucose is freely filtered and then reabsorbed in the proximal tubules, preserving it to be utilized as an energy source. The presence of urine glucose is called glucosuria. Pathologic glucosuria is associated with diabetes mellitus, acute renal failure, and urinary obstruction in cats and milk fever in cattle (Roy 2008). In the present finding glucose was found negative in both breeds FCU & CUD.

Normally, the urine should not contain noticeable concentration of Albumin and the same was recorded in the present study.

Normally urine does not contain proteins (Radostitis, O.M. 2008 and Grover, P.K. and Resnick, M. 1995). The presence of protein in urine is called proteinuria. The reference range is negative to trace in most animals. Increased protein level in the urine might be due to acute nephritis or inflammatory exudation resulting from pyelitis, urethritis, cystitis and urolithiais. Here FCU has protein in *Bos indicus* (0.05mg) and in *Bos taurus* (0.08mg). The protein was absent in CUD of both breeds.

Creatinine is an unwanted product formed in muscle from a high-energy storage compound, creatinine phosphate. Creatine phosphate can be stored in muscle at approximately four times the concentration of adenosine triphosphate. In muscles, it spontaneously undergoes degradation to form a cyclic anhydride-creatinine. Urinary creatinine excretion was correlated with body weight in cattle by silva *et al.,* 2012 and with estrogen in prepartum and postpartum cows by Erb *et al.,* 1977. Hence it fluctuates with body weight and age. The high creatinine level in *Bos taurus* indicates its high body mass.

The CUD and FCU of both breeds did not contain a noticeable concentration of ketones to give a positive reading. Ketones including acetone, acetoacetate, and beta-hydroxybutyrate are generated in states of insulin deficiency and starvation. The presence of ketones in urine called ketonuria. Their reference range is negative to trace. The absence of ketone bodies in FCU and CUD of both breeds indicate that the cows are healthy and not under starvation.

Similar to other urine chemicals, bilirubin, bile salt and bile pigment should not be present and its only negative in both breeds FCU & CUD. If large amounts are present, the condition is referred to as bilirubinuria. There is a low renal threshold for bilirubin; hence even small increases in plasma bilirubin can lead to bilirubinuria. This bilirubinuria can be detected prior to hyperbilirubi-naemia. Bile salt lowers the surface tension of urine when sulphur is added to the surface of the abnormal urine. It sinks into the bottom of the tube in normal urine (Chew and Schenck (2009) and Jawalekar *et al.,* 2010).

In the present investigations it was were negative for blood in both breeds FCU and CUD. The Presence of blood in Urine may be related with hematurea or hemoglobinurea.Cow's urine was found to contain various inorganics, including silver,

traces of gold, Na-K in ratio of 4:1 (36%: 9% in dried urine), apart from about 3% urea (Apte, 2002). Urease hydrolyses the Urea into NH_3 (ammonia) and CO_2 (carbon dioxide). The ammonia created a response with alkaline hypochlorite (NaClO) and sodium salicylate in the occurrence of sodium nitroprusside as a coupling agent. The similar findings was observed by Bhadauria, in 2002. It was showed that Cow urine contents were 95% water, 2.5% urea and 2.5% minerals, salts, hormones, and enzymes. It contains, calcium, phosphorus, carbonic acid, potash, nitrogen, ammonia, manganese, urea, uric acid, amino acids, enzymes, etc. hence it is proved that the findings of present study is in line with the previous studies and also that the source of cow urine distillate was from healthy cows.

Microscopic characteristic

Many authors Identified of casts, cells, and crystals relies on the experience of the observer. Not surprisingly there is significant variation in the interpretation of urinary sediment findings (Wald *et al.,* 2009). The microscopic examination of urine is of great clinical importance. For example, there is no consensus among nephrologists as to how many granular or renal tubular cell casts are required to support a clinical diagnosis of acute tubular necrosis (ATN) (Chawla *et al.,* 2008). Inter-observer variability among nephrologists of common urinary findings has been reported. Ten nephrologists in a single center agreed on the presence of dysmorphic RBCs only 31% of the time (Wald et al., 2009). However, nephrologists may perform better than hospital-based technologists in the identification of some characteristics including renal tubular epithelial (RTE) cells, granular casts, RTE casts, and dysmorphic RBCs (Tsai *et al.,* 2005). The microscopic examination of urine is of a large clinical importance. The necessary structures to identify include crystals, erythrocytes, leukocytes, casts and bacteria (Gyory *et al.,* 1984).

The Cow urine was examined under the microscope for the presence of red and white blood cells, epithelial cells, crystals, casts, bacteria, fungus and protozoa. The cow urine may have cells that initiated in the blood, the kidney, or the junior urinary tract. Microscopic examination of urinary deposit can provide valuable clues regarding diseases and disorders involving. The presence of microorganisms or yeast and white blood cells helps to differentiate a urinary tract area transmission and a fouled urine sample. White blood cells (WBC) are not seen if the sample has been fouled.

Phase contrast microscopy may have advantages for cellular identification and is recommended in some guidelines (European Confederation of Laboratory Medicine, 2000; Kouri *et al.,* 2000). However, many nephrologists use bright field microscopy and this may be the only available option (Fogazzi and Grignani, 1998).

In different breeds of cow and the various forms (FCU and CUD), the colour of urine was found to be yellow, appearance was clear. The physical characteristics are not much more different detected. The urine was negative for the presence of ketone bodies, sugar, bile salts, bile pigments, casts, crystals and micro-organisms. There were no much significant differences in the breeds studied regarding physical, microscopic properties of the both cow urine and both forms. However, the chemical characteristics significantly vary between CUD and FCU. These characteristic analysis could be further used for characterization of active ingredients in the Fresh Cow Urine and Cow Urine Distillate.

Water Quality Parameter

The results showed that water quality was maintained well within normal range by CUDs of both breeds being an nitrogenous excretory product, cow urine is expected to increase the ammonia level and give stress to fish. And also any animal waste is expected

for microbial contamination and thereby decreasing dissolved oxygen and increasing BOD. However the results of the present investigation blows out the assumptions and proves that cow urine does not alter the water quality and instead, an increase in dissolved oxygen was observed. In this point of view feed additive was found to be better than water additive route in maintaining the water quality.

However for practical application in field administration of CUD through water would be much easier, non-stressful, less labor-intensive and also allows large scale administration. It is also cheap and effective. The feed additive method also has practical feasibility and cost effectiveness in increasing productivity. Hence it could be concluded that *Bos indicus* CUD can fulfil the needs of aquatic farmers to increase fish production in terms of quality and quality in an economical and eco-friendly manner.

- Knowing the importance of route of administration, the present work was designed. The Cow urine distillate was administered through two different routes – water additive (v/v) and Feed additive at 0.1% concentration (w/v), which was found as the optimal.
- In the present study, two different breed of cow urine distillate treatment were studied by administrating through two different routes. 1. Water additive route – Dissolving CUD directly in fish environment 2. Feed additive – mixing CUD in feed during preparation.
- The results revealed that is all the parameters studies, the CUD of *Bos indicus* showed better effects significantly than that of *Bos taurus*.
- The water additive route was established as better route of administration of CUD for growth and survival enhancement also to get disease protection.
- 0.1% concentration was found to be the optimal concentration for CUD of both breeds based on the results of haemotological studies done.
- For all non-specific defense mechanism the peak day response was observed to be on 14^{th} day
- Maximum disease resistance was bestowed by CUD of *Bos indicus* against *A.hydrophila* infection. Hence it could be suggested that for CUD of *Bos indicus* is an better alternative to chemical therapeutics at 0.1% concentration as water additive.
- The results were reassured by extending the work from lab to land (i.e) field trails and similar results were obtained.
- The water quality was measured frequently to check whether cow urine being a nitrogenous waste product has caused any alternatives. But it does not cause any changes and all the parameters remained stable.

- The physico-chemical analysis of CUD and its distillate showed negligible differences except in protein levels in both breeds, but there is are notable differences between the characteristics of FCU and CUD.
- Hence it could be conclude that this study shows the potential of this type of water additives as a growth promoter in the pond growout of tilapia in the absence of major disease threats. Further work is underway to investigate the impact on survival in production conditions where disease pressure is affecting fish health and farm productivity.

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APPENDIX

Two Way Analysis of Variance

Source of Variation	DF	SS	MS	F	P
Treatment Days	4	820.429	205.107	83.736	<0.001
Treatment Groups	2	3552.828	1776.414	725.225	<0.001
Treatment DayxTreatment Group	8	2796.851	349.606	142.728	<0.001
Residual	75	183.710	2.449		
Total	89	7353.818	82.627		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Treatment Days depends on what level of Treatment Groups is present. There is a statistically significant interaction between Treatment Days and Treatment Groups. (P = <0.001)

Power of performed test with alpha = 0.0500: for Treatment Days : 1.000

Power of performed test with alpha = 0.0500: for Treatment Groups : 1.000

Power of performed test with alpha = 0.0500: for Treatment Day x Treatment Group : 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor: Treatment Groups

Comparison	Diff of Means	t	P	P<0.050
T1 vs. Control	14.270	35.313	<0.001	Yes
T2 vs. Control	12.127	30.009	<0.001	Yes
T1 vs. T2	2.143	5.304	<0.001	Yes

Comparisons for factor: Treatment Groups within -7

Comparison	Diff of Means	t	P	P<0.05
Control vs. T1	2.150	2.379	0.058	No
T2 vs. T1	1.383	1.531	0.243	No

Control vs. T2	0.767	0.848	0.399	No

Comparisons for factor: Treatment Groups within 0

Comparison	**Diff of Means**	**t**	**P**	**P<0.05**
T1 vs. Control	4.767	5.275	<0.001	Yes
T2 vs. Control	2.967	3.283	0.003	Yes
T1 vs. T2	1.800	1.992	0.050	No

Comparisons for factor: Treatment Groups within 7

Comparison	**Diff of Means**	**t**	**P**	**P<0.05**
T1 vs. Control	29.733	32.905	<0.001	Yes
T2 vs. Control	22.733	25.159	<0.001	Yes
T1 vs. T2	7.000	7.747	<0.001	Yes

So, All Pairwise Multiple Comparison Procedures (Holm-Sidak method) of control and treated shown with different alphabets representing significant difference (P<0.05)

Comparisons for factor: Treatment Groups within -7

Comparison	**Diff of Means**	**different alphabets**
Control vs. T1	2.150	a
Control vs. T2	0.767	a
T2 vs. T1	1.383	a

Comparisons for factor: Treatment Groups within 0

Comparison	**Diff of Means**	**different alphabets**
Control vs. T1	4.767	a
Control vs. T2	2.967	b
T2 vs. T1	1.800	b

Comparisons for factor: Treatment Groups within 7

Comparison	**Diff of Means**	**different alphabets**
Control vs. T1	29.733	a
Control vs. T2	22.733	b
T2 vs. T1	7.000	ab

BEST: International Journal of Humanities, Arts, Medicine and Sciences (BEST: IJHAMS)
ISSN (P): 2348-0521, ISSN (E): 2454-4728
Vol. 4, Issue 3, Mar 2016, 121-132

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

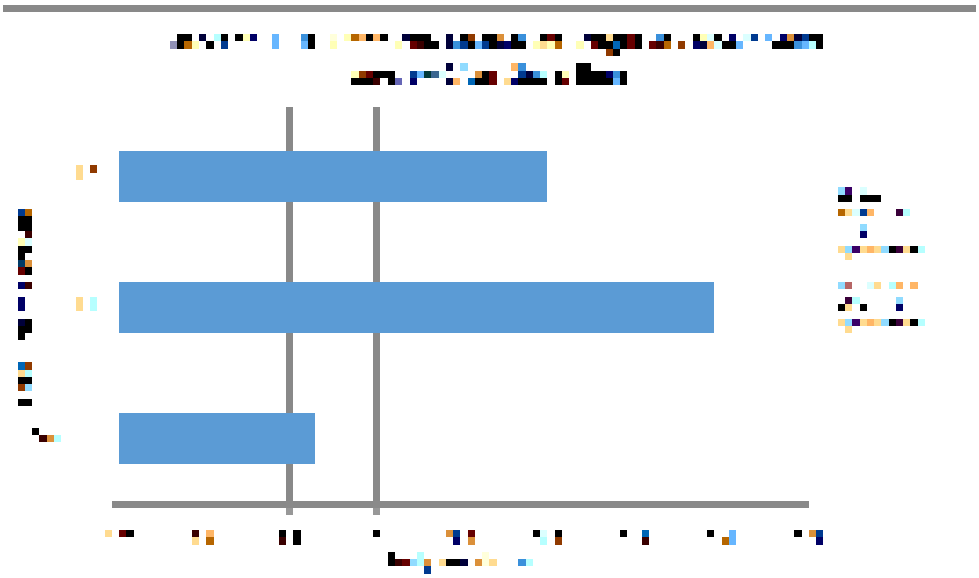

Figure 2

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

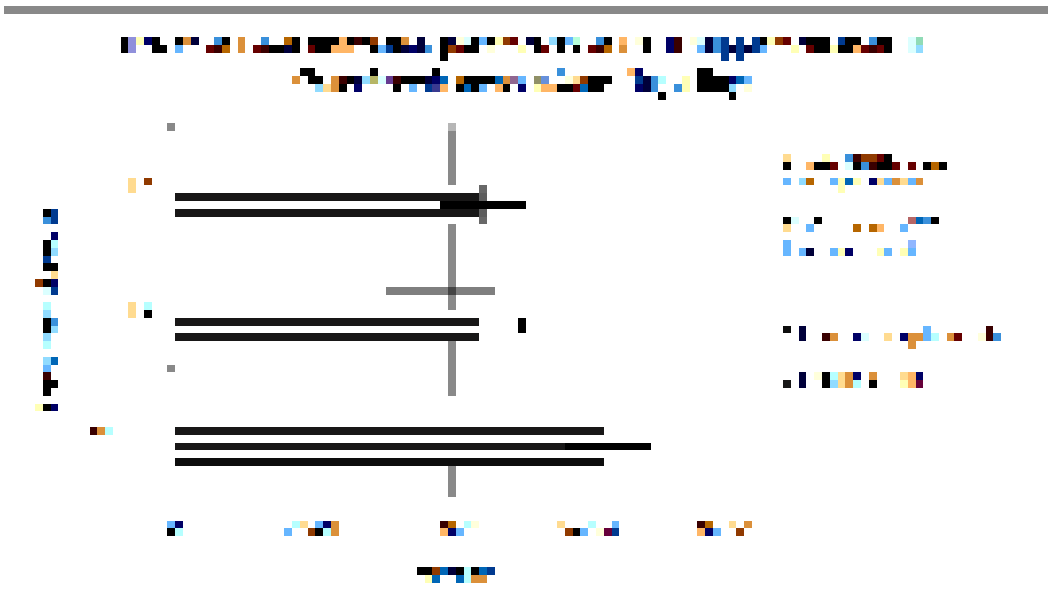

Figure 3

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

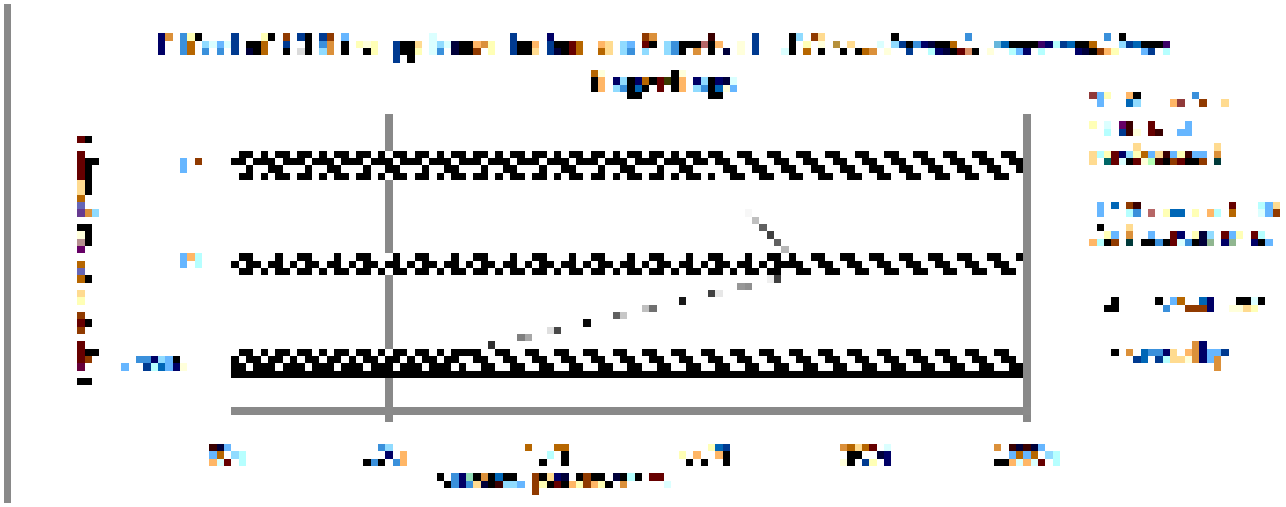

Figure 4

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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RESEARCH

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Publication History
Received: 08 March 2015
Accepted: 24 March 2015
Published: 1 April 2015

Citation
Durga B, Mala G, Venkatlakshmi S. Optimization of Concentration of *Bos indicus* Cow Urine Distillate (CUD) in *Oreochromis mossambicus* (Peter) for Immunostimulatory activity. *Drug Discovery*, 2015, 10(24), 76-83

Optimization of Concentration of *Bos indicus* Cow Urine Distillate (CUD) in *Oreochromis mossambicus* (Peter) for Immunostimulatory activity

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Abstract

Cows were regarded as wealth and were the backbone of the economy of ancient Indians. The sacred Indian cow, *Bos indicus,* is believed to be a "mobile hospital" for the treatment of many diseases. Wars were fought for acquiring cows. Cattle were one of the most frequently used animals described in Vedas. Cows were regarded as mother ("*Gau-mata*") and referred to as *Aghnya.* Atharvaveda provides interesting information about ailments of animals, herbal medicines, and cure of diseases. Urine was also considered as an antidote to poisons (Sushrut Samhita). From the ancient period, cow's urine has been used as a medicine. In Veda, cow's urine was compared to the nectar. The present study is aimed at investigating the immunostimulatory effect of cow urine distillate of *Bos indicus* (Gir breed) in *Oreochromis mossambicus* (Peter). Since cow urine is employed for curing many human ailments and also its immunomodulatory potential was well reported in literature it was thought worthy to find its optimal concentration for its application in aquaculture. Aquaculture is an ecofriendly, socially sound and economically viable innovative technology to manage water resources on low capital input basis. However there is a continuous threatening for the industry due to fish pathogens. The results revealed that 0.001% of cow urine distillate (CUD) is the optimal concentration for its effect on neutrophil activity.

Key words: CUD, *Bos Indicus*, *Oreochromis mossambicus,* Neutrophil activity, Immunomodulation, Aquaculture.

Introduction

Cow urine therapy and all traditional practices from Indian systems of medicine have a strong scientific base. Traditional systems in medicines, whether from Ayurveda or Siddha or the use of cow urine distillate as immunomodulator are based on classical texts , systems, practices and products handed down over generations going back to Charaka, Sushrutha, Vagabhatta, the Ashtangahridaya and the Samhitas. Cow urine has been described in 'Sushrutha Samhita' and 'Ashtanga Sangraha' to be the most effective substance/secretion of animal origin with innumerable therapeutic values. In Ayurveda cow urine is suggested for improving general health (Khanuja 2007). According to a recent online report of 'Love4Cow Trust' by researchers at Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow have identified a fraction of cow urine distillate as a bioenhancer of commonly used antibiotics and anti-cancer drugs. It increases life span and purifies blood from all sorts of impurities.

Cow urine is entirely sterile after secretion and has antiseptic effect. It acts like a disinfectant and prophylactic agent. Most of the medicines are made by distilling urine and collecting Vapours termed as ark (distillate). It is proved as a universal curer of blood disorders, leucorrhoea and even leprosy. This therapy has been reported to be beneficial for dreaded diseases like Cancer, AIDS and diabetes (*Chauan R.S* 2004). Literature shows that cow urine has enormous enhancing properties on biological systems. Cow urine one of the ingredients in panchagavya is believed to have therapeutic value. In India cow urine is used by majority of rural population as Folklore remedy in almost all the states. Urine therapy was not only used in India, but for several Centuries in many parts of the Globe. However, cow urine has not been examined for their effect on disease resistance & new immunostimulant promotion in aquaculture. Aquatic ecosystems are well utilized for aquaculture in recent decades. The use of unutilized resources and modern technology hiked aquaculture production. However, the number of outbreaks of bacterial diseases in cultured fish has also increased and it causes decline in aquaculture production. Fish health is closely associated with characteristics of aquatic environment.

Bos indicus

Bos indicus are the hardiest of high yielders in the world. The *Bos indicus* are 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 1150 – 1600 kg of milk lactation. The *Bos indicus* are famous milk cattle breed of India for their tolerance to stress conditions and resistance to various tropical diseases.

Oreochromis mossambicus

Oreochromis mossambicus often used as a good experimental model and is extensively used in genetic and physiological studies in relation to pollution, stress, or growth promotors (Baskaran *et al,* 1989; Pratap *et al,* 1989) , Tilapia is selected as the experimental model because of its economical importance worldwide and also Tilapia eat a wide range of natural food organisms; tolerate poor water quality and easily spawned. Tilapia is a good fish for warm water aquaculture. Consumers like tilapia for their firm flesh and mild flavor. Worldwide harvest of farmed tilapia has now surpassed 800,000 metric tons and of the most widely farmed fresh water fish in the world, tilapias are second only to carps. (Popma and Masseri, 1999).

METHODOLOGY

Animal maintenance:

Oreochromis mossambicus a common fresh water cichlid fish was used for the study. Fish procured from local fish form were stocked in large fiber tanks. The experiments were carried out in plastic tubs of 70 lit capacities. Fish of both sexes weighing 10-25gm were used in the study. Water was changed frequently to avoid stress due to ammonia accumulation. The animals were fed *ad libitum* with a balanced fish diet prepared in our laboratory.

Cow urine collection

Cow Urine was collected from Gir breed in selected six healthy cows. These cows were selected after obtaining certificate from veterinary doctor they are disease free. Cow Urine was collected sterile container in the early morning from each cows, and then total three litters (collected from six cows) were pooled together for the distillation. The cows in the Gosala (cow farm) at Govindapuram , in Kumbakonam have been considered for the sample collection.

CUD Preparation

The collected urine samples were distillated at 50^0C - 60^0 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.

Aeromonas hydrophila

It is a gram negative, facultative rod shaped bacteria belonging to the family Vibrionaceae. It causes hemorrhagic septicemia in warm water fishes like channel catfish, tilapia (Amin *et al*., 1985; Leung *et al*., 1995). It may become an opportunistic pathogen (Grizzle and Kirya, 1993) infecting fish under stressful conditions or in concern with other pathogens (Noga *et al*., 1991). *Aeromonas hydrophila* is a causative agent for one of the major fish diseases in India. It was cultured in Tryptic soy broth (Himedia, India).

Preparation of heat killed whole cell vaccine

Single colony of *A. hydrophila* from the agar plate was inoculated in the tryptic soy broth. After 24 hrs, the bacterial cells in the broth were subjected to 60°C for one hour in a water bath. The sterility was checked by inoculating a sample on nutrient agar plates. The heat killed bacterial culture was centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded and the pellet was re-suspended in sterile PBS .Then the bacterial number was calculated by measuring optical density (OD) in a spectrophotometer and also confirmed by plate count method. The final bacterial concentration was adjusted to $1x10^8$ cfu/ml by serial dilution (Rajesh Kumar *et.ai.,2008).* The experimental fishes were challenged intraperitoneally with the bacterial suspension of 0.2ml ($1x10^8$ cfu ml^{-1}).

Exposure to CUD

After acclimatization, three groups of fish were treated with cow urine distillate in different concentrations for seven days (0.1%.0.01%,0.001% concentration). A control group was kept separately. On the seventh day immunization was done with 10^8 of cells of heat killed *A.hydrophila.* The neutrophil activity was assessed on the 4^{th}, 8^{th},$12^{th.}$ and 16^{th} days of post immunization.

Serial bleeding

The fish were bled serially using one ml tuberculin syringe (Glass van) with 26-gauge needle from the common cardinal vein situated just below the gills, at regular intervals of seven days after immunization (Michael *et al.,* 1994). The blood drawn was collected in heparinzed heamatological tubes. Great care were taken to avoid foaming when drawing the blood into micropipette as this readily resulted in hemolysis.(P. Sivanatarajan and T. Sivaramakrishnan, *2013*).

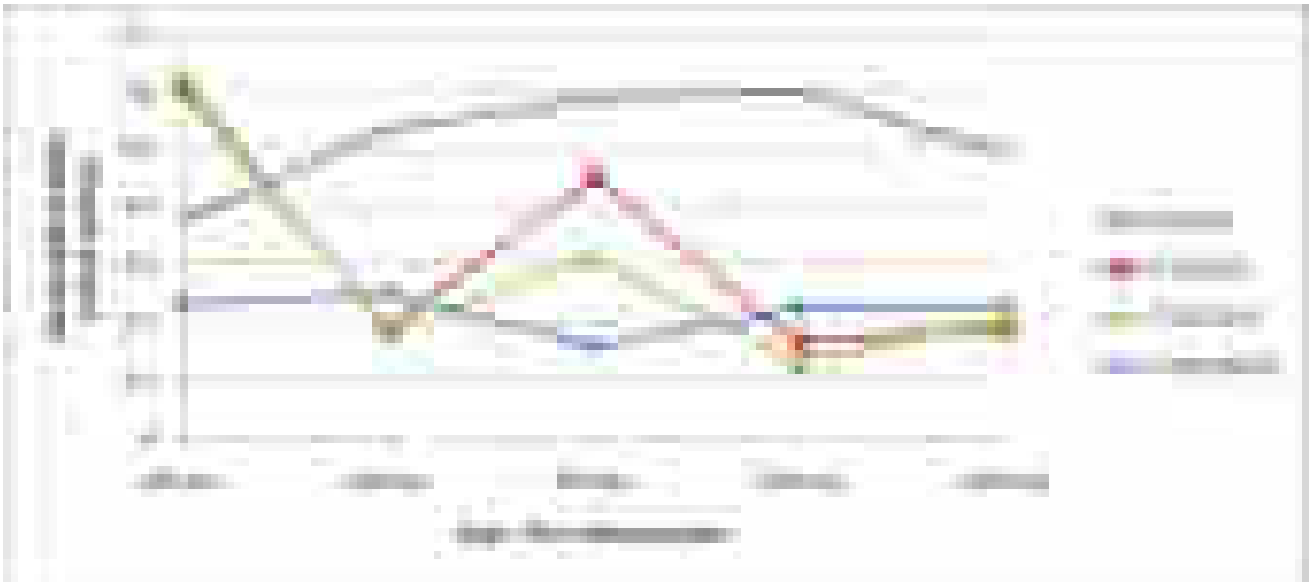
Neutrophil activity

The NBT assay was assessed by following modified Stasiack and Bauman method. 50µl samples of blood were taken from the experimental fish with a 1ml Tuberculin heparinized syringe. Collected blood was transfer into the flat bottom ELISA plate. The plate was incubate at 37ºC in water bath for 1 hour (to facilitate cell adherence).The incubating plate was washed 3 times in PBS (100µl) to remove non-adherence cells. Add 100µl of 0.2% NBT as coloring agent and Incubate for 1 hr at 37º C. After incubation fix with 100% methanol (100µl) for 2-3 min after 3min discard. Wash with 70% methanol (100µl).After washing plates was dry. Add 120 µl of 2N KOH and 140 µl of DMSO-mix properly. Take absorbance at 620 nm in ELISA reader. The NBT assay was done on 4^{th}, 8^{th}, 12^{th} and 16^{th} days post stimulation with cow urine and after immunization.

Result and Discussion

The neutrophil activity (OD at 620 nm) of experimental groups in different concentration of Gir breed Cow Urine Distillate (CUD) is compared to control. On the whole the minimum value occurred in T_2 (0.01%) on the 12^{th} day of post immunization and the maximum value occurred in T_3 (0.001%) on the same day. The high OD value in T_3 indicates the high level of neutrophil activity. Compared to control the effect of T_3 on the neutrophil activity is better. The control group shows no modulation in the neutrophil activity. T_1 group & T_2 group is affected by immunization of *A.hydrophila* . Hence a drop in neutrophil activity occurred. Since on the 12^{th} day of post immunization, peak value of 0.601 is occurred in T_3 group (0.001%) it is considered as the optimum concentration for immunostimulation of neutrophil activity.

Effect of different concentration of Gir CUD on the neutrophil activity of *Oreochromis mossambicus*



It has been already reported that 0.1% concentration as the optimal dose for CUD treatment in literature for growth enhancement (Padmapriya and Venkatalakshmi, 2014) (Sattanathan and Venkatalakshmi., 2015). This different in the optimal concentration for the same product on difference effects like growth and neutrophil activity could be attributed to the fact that for any biological activity modulators need higher does for gross effects. Whereas very minimal dose is required for a fine effect like neutrophil activity enhancement. Similar report was observed by Venkatalakshmi & Michael (2001) and (Dinakaran Michael *et,al.,*1998) in *Ocimum sanctum* leaf water extract and Ascorbic acid respectively. It has been shown that higher concentration caused immunosuppression and still higher concentration as toxic. But lesser doses were proved to be immunostimulative. Hence the observation of the present is in line with the previous reports.

The requirement of cow urine distillate for giving disease protection is very less (0.001%). This gives a scope for cost effective, eco friendly, Organic way of Aquaculture management practices.

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