Observation of heavy metals accumulation and growth performance of Koi, *Anabas testudineus*(Bloch, 1792) by using different market feeds

A thesis submitted to the Department of Fisheries, University of Dhaka

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Submitted By

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Declaration

I hereby declare that the dissertation entitled **"Observation of heavy metals accumulation and growth performance of Koi, *Anabas testudineus*(Bloch, 1792) by using different market feeds"** submitted to the Department of Fisheries, University of Dhaka for the degree of Master of Science (MS) is based on self-investigation carried out under the supervisions of Dr. KanizFatema, Professor and Chairperson, Department of Fisheries, University of Dhaka, Dhaka- 1000, Bangladesh.

I also declare that this or any part of this work has not been submitted for any other degree anywhere. All sources of knowledge used have been duly acknowledged.

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Certification

We certify that the research work embodied in this thesis entitled **"Observation of heavy metals accumulation and growth performance of Koi, *Anabas testudineus*(Bloch, 1792) by using different market feeds"** submitted by Md. NazmusSakib, roll number: 806, session: 2015-16, registration number: 2011-112-769 has been carried out under our supervision.

This is further to certify that it is an original work and suitable for the partial fulfillment of the degree of Master of Science (MS) in Fisheries from the Department of Fisheries, University of Dhaka.

We wish every success in his life.

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Abstract

The study was conducted to determine the concentration of heavy metals in some commonly used commercial fish feeds. This study also observed the bioaccumulation of Cr, Cu, Cd, Pb and Ni in fish organs such as intestines, muscle and gills of Koi (*Anabustestudineous*)after culturing them for twelve weeks by using differentmarket fish feeds.

The present study showed that concentration (33.42 mg/kg) of Ni was found the highest in Madina feed among five heavy metals that exceeded the permissible limit set by FAO. In cultured Koi, the concentration of studied heavy metals was found higher than initial concentration in fish feeds and collected fingerling samples. Metal levels in cultured fish followed the ranking: Cu >Pb> Cr > Cd > Niand order of bioaccumulation in individual organs was intestines > gills > muscle. The highest concentration (368.67mg/kg) of Cu observed in intestines of Koi given Madina feed and the lowest concentration (14.03 mg/kg) was recorded in muscle of Koi receiving Quality feed. The highest concentration of Pb (138.28 mg/kg) observed in intestines of Koi givenMadina feed and the lowest concentration (0.00 mg/kg) was recorded in muscle of Koi receiving Quality feed.Bioaccumulation of Cr (3.20 mg/kg) was the highest in gills of fishes taken Mega feed and the lowest (0.00 mg/kg) in intestine of Koi cultured with different feeds.The concentration of Cu, Pb and Cr exceed the tolerable limit in fishes cultured with the experimental feeds. The concentrations of Cd,and Ni were below the permissible range approved by FAO.

As Koi fingerlings were cultured, the growth performance parameters like condition factor (K), average daily gain (ADG), specific growth rate (SGR) and feed conversion ratio (FCR) were also determined in different feeding levels. The highest condition factor was observed at 30th day period in Handmade feed (B3) as 3.13±0.20 and lowest was observed at 60th day period in Quality feed as 1.58±.096. The highest Average Daily Gain (ADG) during the study period was found to be0.11±0.001 at 30th day period in Handmade feed (B3) and the lowest value of ADG was recorded .03±.004at 90th day period in Mega feed. The highest specific growth rate was 4.68±.39 found in Madina feed in 60th day of culture and lowest was determined in 0.80±.01 during 60th day in Handmade feed (B3). The highest FCR value 3.74±0.38 was observed in T1 during 30th

day period and lowest FCR value (1.29±0.13) was observed in Quality feed during 90^{th} day period.

Considering the present study, Koi cultured with these experimental feeds is not safe for human consumption. Because higher concentrationthan allowable limitof these heavy metalsmight cause harmful effect on human body after consuming those fishes and cancreate cancer and other heavy metal-related diseases on human body.

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List of Abbreviations and Symbols

AAS	Atomic Absorption Spectrometer
BBS	Bangladesh Bureau of Statistics
BCSIR	Bangladesh Council of Scientific and Industrial Research
Cd	Cadmium
Cu	Copper
Cr	Chromium
DoF	Department of Fisheries
et al.	et alia(L), and others
EU	European Union
FAO	Food and Agriculture Organization
Fe	Iron
FRSS	Fisheries Resources Survey System
GDP	Gross Domestic Product
GNP	Gross National Product
GOB	Government of the People’s Republic of Bangladesh
Hg	Mercury
HI	Hazard index
Kg	Kilogram
g	Gram
Pb	Lead
mg/Kg	Milligram per kilogram

Na	Sodium
Ni	Nickel
ppm	Parts per million
SD	Standard Deviation
SPSS	Scientific Package for Social Sciences
TF	Transfer Factor
TR	Target cancer risk
USEPA	US Environmental Protection Agency
WHO	World Health Organization

Chapter 1

Introduction

1.1 Background

Bioaccumulation is the accumulation of a contaminant into an organism or a biological community, resulting either from direct uptake from the water or from ingestion. It is used to describe both the dynamic accumulation process and the final accumulation of a contaminant in an organism. In the dynamic sense, bioaccumulation describes any accumulation of chemicals in biota resulting uptake, storage and sequestration of contaminants (Braunbecket al.1998). Bioaccumulation measurements refer to studies or methods monitoring the uptake and retention of pollutants like metals or biocides by organisms such as fish (Roux, 1991; Nusseyet al, 2000). The accumulation of metals in an organism's body can take place, if the rate of uptake by the organism exceeds the rate of elimination (Oronsaye, 1987; Oguzie, 2003). The bioaccumulation of heavy metals in living organisms and biomagnifications describes the processes and path- ways of pollutants from one trophic level to another. Various species of fishes are mostly used as bio-indicators of heavy metals contamination.Bioaccumulation in fish can be predicted by models (Svobodovaet al.2004). The acidic conditions of aquatic environment might cause free divalent ions of many heavy metals to be absorbed by fish gills (Part *et al.* 1985). The concentrations of heavy metalsin the organs of fish showed that the aquatic environment is polluted (Farkaset al.2000).

Pollution of the aquatic environment by inorganic chemicals has been considered a major threat to the aquatic organisms including fishes, mollusks, crustaceans etc. The agricultural drainage water containing pesticides, fertilizers, effluents of industrial activities and runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions and heavy metals (ECDG, 2002). In the aquatic food chain, fishes are one of the most important organisms which are very sensitive to heavy metals contamination. Most of the freshwater fishes are confined to specific microhabitat within inter connected river/stream system. If such system becomes contaminated by heavy metals, fish species either shift to less polluted segment of river/stream system or die off which ultimately disturb the food chains (Rashed, 2001).

Pollutants enter fish through a number of routes: via skin, gills, oral consumption of water, food and non-food particles. On absorption, pollutants are transported in the blood stream to either a storage point (i.e bone) or to the liver for transformation and/or storage (Nussey, et al, 2006). If transferred in the Liver, pollutants are either stored there or excreted in bile or passed back to the blood for possible excretion via gills or kidneys or stored in fat (Heath, 1991).

1.2 Heavy metals

1.2.1 Definition of heavy metals

The term heavy metals is a general collective term which applies to group of metals and metalloids with atomic density greater than 4gm/cm^3 or 5 times or more greater than water (Duruibeet al. 2007), they are also known as trace elements because they occur in minute concentrations in biological systems. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu), iron (Fe), and the platinum group elements.

1.2.2Classification of heavy metals

Heavy metals are generally of two types, (i) essential heavy metalsand (ii) Non-essential heavy metals**.** Both types of heavy metals may become toxic to plants and animals.

(i) Essential heavy metals

Essential heavy metals are vanadium (V), manganese (Mn), iron (Fe), cobalt(Co), copper(Cu), zinc(Zn), selenium (Se), strontium (Sr) and molybdenum (Mo).

(ii) Non-essential heavy metals

Non-essential heavy metals are chromium (Cr), aluminum (Al), Arsenic (As), Barium (Ba), Bismuth (Bi), Cadmium(Cd), Lead (Pb),Mercury (Hg),Nickel (Ni),Uranium (U),and Tin (Sn).

1.2.3 Sources of heavy metal

Heavy metals occur as natural constituents of the earth crust, and are persistent environmental contaminants since they cannot be degraded or destroyed. They enter the body system through food, air, and water and bio-accumulate over a period of time (Lenntech, 2004; UNEP/GPA, 2004). In rocks, they exist as their ores in different chemical forms, from which they are recovered as minerals. Heavy metal ores include sulfides, such as iron, arsenic, lead, lead-zinc, cobalt, gold, silver and nickel sulfides; oxides such as aluminum, manganese, gold, selenium and antimony. Some exist and can be recovered as both sulfide and oxide ores such as iron, copper and cobalt. Ore minerals tend to occur in families whereby metals that exist naturally as sulfides would mostly occur together, likewise for oxides. Therefore, sulphides of lead, cadmium, arsenic and mercury would naturally be found occurring together with sulphides of iron (pyrite,

FeS_2) and copper (chalcopyrite, $CuFeS_2$) as minors, which are obtained as by-products of various hydrometallurgical processes or as part of exhaust fumes in pyro-metallurgical and other processes that follow after mining to recover them. During mining processes, some metals are left behind as tailings scattered in open and partially covered pits; some are transported through wind and flood, creating various environmental problems (Habashi, 1992).

Heavy metals are emitted both in elemental and compound (organic and inorganic) forms. Anthropogenic sources of emission are the various industrial point sources including former and present mining sites, foundries and smelters, combustion by-products and traffics (UNEP / GPA, 2004). Cadmium is released as a by- product of zinc (and occasionally lead) refining; lead is emitted during its mining and smelting activities, from automobile exhausts (by combustion of petroleum fuels treated with tetraethyl lead antiknock) and from old lead paints; mercury is emitted by the degassing of the earth's crust. Generally, metals are emitted during their mining and processing activities (Lenntech, 2004).

Environmental pollution by heavy metals is very prominent in areas of mining and old mine sites and pollution reduces with increasing distance away from mining sites (Peplow, 1999). These metals are leached out and in sloppy areas, are carried by acid water downstream or run-off to the sea. Through mining activities, water bodies are most emphatically polluted (Garbarino*et al*. 1995; INECAR, 2000). The potential for contamination is increased when mining exposes metal-bearing ores rather than natural exposure of ore bodies through erosion (Garbarino et al. 1995), and when mined ores are dumped on the earth surfaces in manual dressing processes. Through rivers and streams, the metals are transported as either dissolved species in water or as an integral part of suspended sediments, (dissolved species in water have the greatest potential of causing the most deleterious effects). They may then be stored in river bed sediments or seep into the underground water thereby contaminating water from underground sources, particularly wells; and the extent of contamination will depend on the nearness of the well to the mining site. Wells located near mining sites have been reported to contain heavy metals at levels that exceed drinking water criteria (Garbarino et al. 1995; Peplow, 1999).

Table 1The tolerance limits of some heavy metals are shown in following table: United State Environment Protection Agency (USEPA) maximum contamination levels for heavy metal concentration in air, soil and water.

Heavy metal	Max conc. in air (mg/m^3)	Max conc. in sludge (ppm or mg/kg)	Max conc. in drinking water(mg/l)	Max conc. in H_2O supporting aquatic life(mg/l or ppm)
Cd	0.1-0.2	85	0.005	0.08δ
Pb	-	420	0.01*(0.0)	0.0058δ
Zn	1,5*	7500	5.00	0.0766δ
Hg	-	<1	0.002	0.05
Ag	5	-	0.0	0.1
As	-	-	0.01	-

(Value in bracket is the desirable limit; WHO ; 1adapted from U.S. – OSHA; 2 EPA, July 1992; USEPA, 1987; Georgia Code, 1993; Florida Code, 1993; Washington Code, 1992; Texas Code, 1991; North Carolina, 1991; *1 for chloride fume, 5 for oxide fume; -- no guideline available).

Table 2 Guideline in drinking water by the World Health Organization (WHO) and National Agency for Food and Drugs Administration and Control (NAFDC), Nigeria.

Heavy metal	Max. acceptable conc. (WHO) Mg/l	Max. acceptable conc. (NAFDC) Mg/l
Zn	5	5
Arsenic	0.01	0.0
Magnesium	50	30

Calcium	50	50
Cadmium	0.003	0.0
Lead	0.01	0.0
Silver	0.001	0.0
Mercury		0.0

1.2.4Heavy metal accumulation

In natural aquatic ecosystems, metals occur in low concentrations. As they cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals and thus, causing heavy metal pollution in water bodies (Abdel-Bakiet al. 2011). Metals entering the aquatic ecosystem can be deposited in aquatic organisms through the effects of bioconcentration, bioaccumulation via the food chain process and become toxic when accumulation reaches a substantially high level (Huang, 2003). In fish, which is often at the higher level of the aquatic food chain, substantial amounts of metals may accumulate in their soft and hard tissues (Mansour and Sidky, 2002).

Metal residues problems in the fish tissues are serious, as reflected by the high metal concentrations recorded in the water and sediments (Romeo et al. 1999; Wong*et*et al. 2001). The gills are directly in contact with water. Therefore, the concentration of metals in gills reflects their concentration in water where the fish live, whereas the concentrations in liver represent storage of metals in the water (Romeo et al. 1999)

The commercial and edible species have been widely investigated in order to check for those hazardous to human health (Begümet al. 2005). Heavy metals such as copper, iron, chromium and nickel are essential metals since they play an important role in biological systems, whereas cadmium and lead are non-essential metals, as they are toxic, even in trace amounts (Fernandeset al.2008). For the normal metabolism of the fish, the essential metals must be taken up from water, food or sediment (Canli and Kalay, 1998). These essential metals can also produce toxic effects when the metal intake is excessively elevated (Tüzen, 2003).

1.3Review of literature

1.3.1 For accumulation of heavy metals

Some of the important documented on accumulation of heavy metalsin the present study are as follows:

Alinaet al. (2012) intensively studied the level of heavy metals in marine ecosystem and the marine fish and shellfish from the Straits of Malacca and analyzedCd, As and Pb and Hg using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) and Flow Injection Mercury System (FIMS).

Noor et al.(2012)studieda number of heavy metals (Cd and Pb) in edible part of selected pelagic fish, demersal fish and shellfish that available in Malaysian local market. They found that a number of heavy metals in pelagic fish were the lowest while the highest in shellfish.

McFarlane and Franzin, (2011) examined the concentrations of Cd, Cu, and Hg in livers of northern pike (*Esoxlucius*) and white sucker (*Catostomuscommersoni*) from five lakes in the vicinity ofManitoba. Concentrations of Cd in livers increased with increasing age in both species. Concentrations of Cu and Hg increased with age only in pike livers.

Abdallah, (2008) studied the concentrations of five trace metals namely, Cd, Pb, Cu, Cr and Zn in muscles of some commercially fish species collected from two coastal areas of the Egyptian coast of the Mediterranean Sea west of Alexandria (El-Mex Bay and Eastern Harbour). For all trace element examined, in all fishes zinc was the highest (up to 57 µg/g) followed by Cr, Cu, Pb and Cd. The levels of Cr surpassed the maximum permissible concentration in most fish tissues, followed by Pb and Cd in some species. Cu and Zn concentrations were found to be below the maximum permissible levels proposed by Food and Agriculture Organization (FAO).

Saeed et al. (2008) reported that the concentrations of Fe, Zn, Cu, Mn, Cd and Pb in *O. niloticus*(Tilapia) fish tissues, water and sediments in northern Delta Lakes. They found that the edible part of *O. niloticus*from Lake Edku and Manzala contained the highest levels of Cd while fish from Manzala Lake contained the highest level of Pb. They reported that Nile tilapia caught from these two Lakes may pose health hazards to consumers.

Authman and Abbas, (2007) studied the accumulation and the distribution of Cu and Zn in *Tilapia zillii and M. cephalus* and suggested that the consumption of fishes of the lake could pose the health damage to the local populationwhose diet consist mainly of fish.This study also reported that the trend of accumulation of metals in fish organs was apparent in liver, gills and muscles, respectively. Moreover they reported that *T. zillii*seemed to be more contaminated with Zn and Cu than *M. Cephalus*. This study result was in disagreement with many previous findings which pointed that *Mugil*species seemed to be more accumulated with heavy metals than tilapia species.

Rashed, (2001)The author showed indicated that *T. niloticus*is one of the aquatic organisms affected by heavy metals. He showed that according to the Egyptian Organization for Standardization, concentrations of Cr, Cu, Fe, Mn, Ni, Co, Zn and Sr in the edible parts of the investigated fish were in the safety permissible level for human uses.

Al-Weher, (2008)investigatedthe levels of Cd, Cu and Zn in three fish species, *Oreochromisaureus, Cyprinuscarpio*and *Clariaslazera*, collected from the Northern Jordan Valley wereinvestigated. He found that levels of these heavy metals inmuscles of the three fish species were within acceptable limits by FAO standards, except for the Zn concentration in muscles of *Oreochromisaureus (*70.76±31.21 μg/gdrywt.) which were attributed to the increase of agricultural influx and some otheranthropogenic activity in that area.

Al- Bader, (2008)determined vanadium (V), Cd, Zn, arsenic (As), Ni, Pb and mercury (Hg) levels in most commonly available fish species in Saudi markets. Results showed that the concentrations of metals were below the maximum allowed limit by the Saudi and international legislations for fish human consumption permissible limit.

Khalifa et al. (2010) determined concentrations of Co, Cd, Pb, Fe and Cu in different tissues of six samples of fishes of the Mediterranean Sea.

Hassan et al. (2011)investigated distribution of seven trace metals namely Zn,Mn, Cd, Cu, Co, Ni, and Pb in mullet and other commercial fish species along the coast of Al-Hodiedah Red Sea coast. They found that the values of trace metals observed were within the acceptable Worldwide range. They recommended continuous monitoring program should be carried outon the Red sea coast of Yemen, and that the levels of heavy metals must remain within the prescribed Worldwide ratio.

MoletAl. (2010) investigated the concentrations of Zn, Cu, As, Cd, Hg and Pb in seven popular fish species of Ataturk Dam Lake, Euphrates, Turkey. They found that the fish from Ataturk Dam Lake are not heavily burdened with metals, but they should be controlled periodically to avoid excessive intake of trace metals by human, and monitoring the pollution of aquatic environment.

Yilmaz, (2009) compared the concentrations of Cd, Cu, Mn, Pb, and Zn in tissues of three economically important fish (*Anguilla anguilla*, *M. cephalus*and*O. niloticus*) inhabiting Koycegiz Lake-Mugla (Turkey), the highest concentration of trace metals in the tissues of *M.cephalus*in lake was attributed to the trophic characteristics of this species, that *M.cephalus*reflects the metal concentrations in surface and suspended particulate matter, showing high metal concentrations.The author considered *M. cephalus*as an adequate and most suitable species for use as biomonitors of trace metals pollution in the Koycegiz Lake. Consequently, he recommended the use of these species as biological indicators as a tool for future monitoring programs, to evaluate the evolution of heavy metal pollution in that area. Results indicated that concentrations of Pb and Zn for *O. niloticus*; Pb, Zn and Cd for *A. anguilla*; and Cd and Zn for *M. cephalus*were found higher than the Turkish Food Codex, European Units and WHO limits for human consumption in edible parts of the fish samples and posed a risk to human health.

1.3.2 For growth performances

Some of the important documented on growth performances to the present study are as follows:

Plavnik and Hurwitz, (1985) showed that there is an increasing body of evidence from work with domestic animals that the compensatory growth response may be influenced by several factors such as the severity of feed restriction imposed, dietary composition, age, sex, and state of sexual maturity of animals.

Larsson and Lewander, (1973) noted that many fish species are subjected to a natural starvation period during a part of year (and) *have* therefore developed an impressive ability to withstand a long period of starvation. Such period may extend to months and naturally, they cause more or less extensive reduce of body substances.

Jobling*et al.*(1993) examined the compensatory growth response of Arctic char, *Salvelinusalpinus*and noted that no significant differences in body weight between fish fed according to the different regimes were found during the period of food restriction was imposed.

Paul et al.(1995) showed that compensatory growth in Alaskan yellow fin Sole, *Pleuronectesasper,* following food deprivation. The result showed that *P. asper*had limited capacity for compensatory growth and when feed was scarce yellow fin sole allocated energy preferentially to growth in length instead of weight.

Mattilaet al.(2009) conducted an experiment on the effect of the length of repeated feed deprivation between single meals on compensatory growth of pikeperch (*Sander lucioperca*) which were reared for 58 days according to one of the following feeding regimes: control (fed once every day); 1 + 1 (fed every other day); 1 + 3 (one-day feeding followed by a three-day feed deprivation); and 1 + 6 (fed once a week). They reported that feeding of pikeperch every other day (1 + 1) did not significantly affect any other measured or calculated parameter than relative feed intake when compared to the controls which indicated full compensation. At lower feeding frequencies (1 + 3 and 1 + 6) pikeperch was capable of compensating only partially for the decreased number of feedings.

1.4Selection of elements and their toxicity

1.4.1 Basis of selection of elements

Elements such as Cd and Pb are non-essential metals and their toxic effect on human health is well known, while metals as, Cu,Ni and Zn are essential metals and the toxic effect of them on human health begins when they are present in high levels. These elements may be added to the ecosystem through human activities or from natural sources. Many studies assessed concentration of these heavy metals in fish species all over the world.

1.4.2 Selected elements and their toxicity

1.4.2.1 Cadmium (Cd)

Cadmium is a metal from group II B that has an atomic weight of 112.41with specific gravity of 8.65; the ionic form of cadmium (Cd^{2+}) is usually combined with ionic forms of oxygen (cadmium oxide, CdO), chlorine (cadmium chloride, $CdCl_2$), or sulfur (cadmium sulfate, $CdSO_4$). Cadmium is a natural element in the earth's crust. It is usually found as a mineral with other elements. All soils and rocks, including coal and mineral fertilizer, have some cadmium in them. In industry and consumer products, it is used for batteries (Ni-Cd batteries of mobile phones), pigments, metal coatings and plastics. It is also a constituent in many other things such as alloys. Cd enters air from mining, industry, and burning coal and household wastes. Its particles can travel long distance in air before falling to ground or water (Singh, 2005).

Toxicity symptoms induced by cadmium include gastrointestinal disorders, kidney failure and hypertension. It is also reported that, intoxication with Cd in pregnant women has been related to reduced pregnancy length and newborn weight and, recently, to disorders of the endocrine and/or immune system in children (Schoeters, 2006).

1.4.2.2 Lead (Pb)

Lead is a naturally occurring element; it is a member of Group 14 (IVA) of the periodic table, has an atomic weight of 207.2; with specific gravity of 11.34 and exists in three states: Pb (0), the metal; Pb (II); and Pb (IV). Lead is a blush-gray heavy metal and it is usually found combined with two or more other elements to form lead compounds (ATS, 2007). Lead is found in small amount in the earth's crust. It can be found in all parts of our environment. Most of it came from human activities, like mining, manufacturing and the burning of fossil fuels. The principal source of Pb in the marine environment appears to be the exhaust of vehicles run with leaded fuels that reach the sea water by a way of rain and wind blown dust (Castro et al. 1997). Lead is found in high concentration in muscles and organs of fish. Lead exposure has been mainly related to retardation of neurobehavioral development (Lidsky et al. 2003).The EU maximum residue limits permitted in fish is 0.3 µg/g for Pb, 0.1-0.3 µg/g for Cd (Herreros et al. 2008).

Moderate exposure to Pb and Cd can also significantly reduce human semen quality and is related to many diseases in adults and children (e.g., damage to DNA or impairment of the reproductive function) (Telisman et al. 2000).

1.4.2.3 Copper (Cu)

Copper belongs to group I-B of the periodic table, it has an atomic weight of 63.55 with a specific gravity of 8.96 with oxidation states of +2, +1. The important ores of Cu are Chalcocite ($CuFeS_2$), Cuprite (Cu_2O) and Malachite [$CuCO_3$. $Cu(OH)_2$]. Copper is widely used for wire production and in the electrical industry. Its main alloys are brass (with zinc) and bronze (with tin). Other applications are kitchenware, water delivery systems, and copper fertilizers (Bradi, 2005).

Copper is considered as an essential constituent of metalloenzymes of living organisms and is required for hemoglobin synthesis and in catalysis of metabolic reactions (Dural et al. 2007). It plays a crucial role in many biological enzyme systems that catalyze oxidation/ reduction reactions. However, if present at relatively high concentrations in the environment, toxicity to aquatic organisms may occur. Copper under ionic forms Cu^{2+}, Cu_2OH+ and $CuOH^+$ is toxic to fish (Moore, 1991).

High copper levels lead to an increase in the rate of free radical formation teratogenicity and chromosomal aberrations (Bhunya, 1987)

1.4.2.4 Nickel (Ni)

Nickel is a silvery-white, hard and malleable metal. It belongs to theiron-cobalt group (group VIII) of the periodic table, Ni has atomic weight of 58.71, with specific gravity of 8.9. It is very abundant element. It is found in all soils and is emitted from volcanoes. It normally occurs in oxidation states 0 and II. Nickel is used as analloy in the steel industry, electroplating, Ni/Cd batteries, arc-welding, rods, pigments or paints and ceramics, surgical and dental prosthesis, molds for ceramic and glass containers, computer components, and catalysts (Bradi, 2005). Ni is considered as an essential trace element. It acts as an activator of some enzyme systems but its toxicity at higher levels is more prominent. High levels of Ni can cause respiratory problems and it is carcinogenic (Sivaperumal et al. 2007).

1.4.2.5 Chromium (Cr)

Chromium has density of 7.2g/cm^3 and is the 21st most abundant element in Earth's crust with an average concentration of 100 ppm (Emsley, 2001). Chromium compounds are found in the environment, due to erosion of Cr -containing rocks, animals, plants, soil and can be a liquid, solid or gas. Cr can exist in valences of +3and +6 with oxidation state in Cr (III) being stable and give series of chromic compounds, like oxides (Cr_2O_3), chlorides ($CrCl_3$) and sulfates ($Cr_2(SO4)_3$) (Emsley, 2001; Gonzalez et al.2005). Cr is used in metal alloys such as stainless steel, protective coatings of metal (electroplating), magnetic tapes, and pigments for paints, cement, paper, rubber and its soluble form is used in wood preservatives as well as additive in water to prevent corrosion in industrial and other cooling system (Hingston, 2001; WHO, 2003). Hexavalent Cr is very toxic and mutagenic when inhaled and is a known human carcinogen. Breathing high levels of the element in this form can cause irritation to the lining of the nose and breathing problems such as asthma, cough, shortness of breath, or wheezingwhere long-term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation(Dayan and Paine, 2001).

Higher Cr mean levels than the recommended limit of 0.15 mg/kg have been recorded in fish from various rivers (FAO/WHO, 2003; Obasohan, 2008; Abdel-Bakiet al.2011).

1.5Rationale

Fish plays a significant role among the population in Bangladesh for supplying protein, essential vitamins, minerals and fatty acids. Fish accounts for about 70% of the animal protein uptake with annual fish consumption of about 14 kg per person (ADB 2005a). The average per capita fish consumption is lower than the world average of 16.1 kg a year (Hishamundaet al.2008). Nevertheless, the importance of aquaculture as a source of food has been well recognized in Bangladesh. The main production systems for freshwater aquaculture in Bangladesh are extensive and semi-intensive pond polyculture of Indian major carps and exotic carps which account for 80% of the total freshwater aquaculture production.

The Koi, (*Anabas testudineus*) naturally occurs in Bangladesh, India, Pakistan, Ceylon, Myanmar, Srilanka, Thailand, Cochin-China, Tongking, Southern China, Philippines, Polynesia and Malaysia (Sterba, 1983; Sen, 1985; Talwar and Jhingram, 1991). Once upon a time, climbing perch or Koi was very much abundant in almost all freshwater systems of Bangladesh (Mahmood, 2003). The availability of this fish is decreasing from natural system in the recent years. Ten years ago, Koi itself contributed 2.83% of the total pond catch of Bangladesh and gradually contribution declined to 0.85% (DoF, 1999). The reasons behind of severe decline of Koi fishes are ecological degradation, indiscriminate use of pesticides, destruction of habitats, obstruction of breeding migration and fishing pressure etc. Since the natural production of Koi is decreasing, fisheries biologists are thinking of its cultivation through intensive farming (DoF, 2002).

Very recent Koi fish has been introduced to our culture arena. It has a great demand in the market for its nutritive value and taste. Due to culture of Koi, brood and fry had been brought from Thailand with high price. Now it is being bred successfully in our country. Bangladesh Fisheries Research Institute (BFRI) developed the induced breeding and culture technology of exotic Koi. Many hatcheries have been established in our country with a view to producing Koi fry. Specially greater Mymensingh, Gazipur and Bogra are playing prominent role in this regard. The reasons behind the greater expansion of Thai Koi culture in Bangladesh are: a) the fish can tolerate our environment easily; b) It is cultivable under high stocking density; c) It is cultivable in deep or shallow water; d) they can survive in low oxygen level; e) It can be marketed at live condition; f) crop can be attained within 3-4 months; g) since it is nutritive and tasty so, it can be used as patient food. The present price of Thai Koi in the market is near about 150-160 Tk./kg. Thai Koi looks like our indigenous Koi but its body covers with gray color and small black spots. This fish is cultivable in ponds, tanks and cages successfully. It is tough to culture our indigenous Koi in pond providing supplementary feed to culture Thai Koi in pond successfully supplying artificial feed. The present study was under taken to evaluate performance level of different stocking densities.

Considering all those prospects of demand, Koi was selected to conduct this research study. Many researchers have worked on the bioaccumulation of the heavy metals in koi collected from various sources all over the worldand determined different heavy metal concentration accumulated from the environment the fish lived.Therefore, the present study is important to observe bio-accumulation of heavy metals and growth performance of Koi using different market feeds.

1.6Objectives

The objectives of this study are:

I. To determine theheavy metal concentration in different types of commercially available fish feeds.

II. To calculate heavy metals concentration in collected Koi fingerlings.

III. To observe the effect of different types of fish feeds on growth performance and survival rate of koi fingerlings.

IV. To assess the bioaccumulation of heavy metals from feeds to thefish body.

Chapter 2

Materials and Methods

2.1 Laboratory experiment

The experiment was carried out in two different laboratories of Bangladesh Council of Scientific and Industrial Research (BCSIR) in Dhaka, Bangladesh. The laboratory of Soil, Agronomy and Environment Section was used for heavy metal analysis and the laboratory of the Zoology section of BCSIR was usedfor biological analysis.

2.2 Period of the study

The experiment was carried out from August 2016 to January 2017.

2.3 Selection of sampling site

To collect fish and feed samples for this study, Mymensingh area has been selected because Mymensingh district is one of the renowned areas in Bangladesh for culturing different types of fishes and supplying various types of commercial fish feeds all over the country.

2.4 Selection of fish

To perform this research study koi fish was selected because of its faster growth, awide range of different water quality parameters tolerance and high survival rate in lab environments and they feed on different types of fish feeds. For this study koi (*Anabas testudineus*)was picked.

2.4.1 Classification

Table 3: The classification of *Anabas testudineus*

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Anabantidae

Genus: *Anabas*

Species: *Anabas testudineus*

Plate-1: Koi (*Anabas testudineus*)

2.5 Sample collection

2.5.1 Fish sample collection

Koi fingerlings were collected from M.O. Agro-Fisheries and Hatchery, Bagean, Trishal, Mymensingh. This hatchery is one of the largest and well-knownhatcheries in Mymensingh district for culturing healthy Koi fingerlings. These fingerlings were collected in a polythene bags with oxygenated water and transported to BCSIR laboratory, Dhaka for further analysis.

2.5.2 Feed sample collection

In this research, five types of fish feeds were used and four of them were commercial fish feeds which were collected from different hatcheries and fish feed shop in Mymensingh district and other one was made at Zoology Section, BCSIR, Dhaka.

2.6 Biological analysis

2.6.1 Experimental design

The experiment was conducted for three months from 16th August, 2016 to 17th November, 2017 in the glass aquaria of volume (30 × 14 × 6 inches) at Zoology Section ofBangladesh Council of Scientific and Industrial Research (BCSIR) in Dhaka, Bangladesh. The feeding trials were conducted on twelve aquaria and groups of 20 Koi fingerlings having similarbody length and weight was selected and randomly stocked to each aquarium with a duplicate for each experimental diet.The fingerlings in each aquarium were fed once in a day at a fixed feeding rate. Before stocking thefingerlings, each of the aquaria was cleaned up and prepared with all the facilities necessary to run the experiment efficiently. Each aquarium was filled with 60-liter clean tap water and water was changed once in a week. To supply adequate oxygen into the aquaria, aeration was done properly by setting up aerator in each aquarium.The feces and other dirt's in each aquarium were removed and dead fingerlings were counted before feeding. Different water quality parameters such as water temperature, pH, dissolved oxygen,and physical parameters such as length, standard length, width, weight of fingerlings were measured weekly. Five fingerlings were selected randomly from each aquarium to take the length and weight data. The weight (g) was taken on an analytical balance and the length (cm) and width (cm) were measured by a scale placing the fingerling on a transparent Petri dish.

2.6.2 Experimental feeds and their application

Five different types of experimental feeds were used in this research. Four of them were commercial feeds (Mega feed, Madina feed, Quality feed and Saudi Bangla feed). And another one was Handmade feed (B3). B3 was made by mixing corn grain, wheat, spirulina, shrimp grain, soybean, fat, vitamins and minerals. In first 30 days of the experiment feed was given at 10% of body weight, 5% of their body in next 30 days and 3% of body weight in 60 to 90 days of the experiment. Table 4 shows the name of different feeds and treatments used in the study.

Table 4: Different treatments used in Koi culture

Treatments	Name of the feeds
Treatment 1(T1)	Handmade feed (B_3)

Treatment 2 (T2)	Mega feed
Treatment 3 (T3)	Madina feed
Treatment 4 (T4)	Quality feed
Treatment 5 (T5)	Saudi Bangla feed

Plate-2: Experimental design for biological analysis of Koi

A. Mega feed

B. Madina feed

C. Quality feed

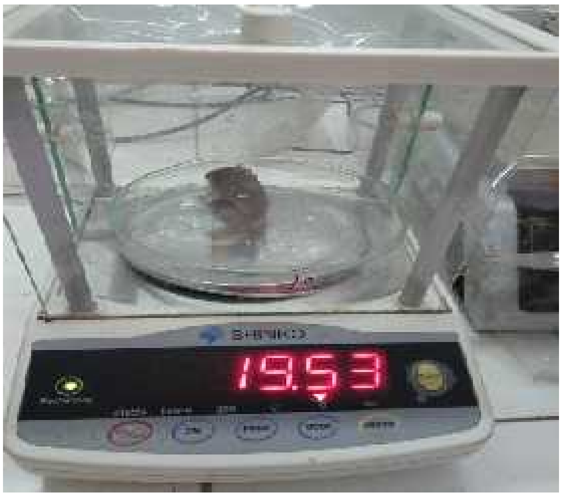
D. Saudi Bangla feed

E. B3 feed

F. Drying of Handmade feed in oven

G. Setting up of aquarium

H. Collected fish samples for measuring length and weight



I. Measuring of weight of koi

J. Measuring of length of koi

2.6.3. Analysis of experimental data

The following formula was used to determine the different growth parameters:

a)Length gain of fingerling (cm) = Average final length of fingerling – average initial length of fingerling

b)Weight gain of fingerling (g) = Average final weight of fingerling – average initial weight of fingerling

c)Percent gain in weight $= \frac{\text{Average final weight} - \text{average initial weight}}{\text{Average initial weight}} \times 100$

d) The survival rate $= \frac{\text{No.of fingerling alive}}{\text{Total number of stocked}} \times 100$

e)Feed Conversion Ratio (FCR)

The ratio of feed consumed by the fish and weight gain of the fish is called the FCR.Feed conversion ratio (FCR) was determined by the following formula as suggested byPayne (1987).

Food Conversion Ratio (FCR) $= \frac{\text{Feed consumed by the fish}}{\text{Weight gain of the fish (W2}-\text{W1)}}$

Where,

W2= Final weight, W1=Initial weight

f)Specific Growth Rate (SGR, %)

The percentage of the increase of body weight per day is called SGR. SGR (%) wascalculated by the following formula as suggested by Hopkins (1992)

Specific growth rate = $\frac{\ln W2-\ln W1}{T2-T1}\times 100$

Where,

$\ln W_2$ = Natural log of weight on time T

$\ln W_1$ = Natural log of initial weight

T_2= Final time, T_1= initial time

g)Average Daily Gain (ADG, g/day)

ADG is called the development of body weight per day. ADG was determined by the following formula as suggested by Jones (1967).

Average daily gain= $\frac{\text{Mean final weight gain}-\text{mean initial weight}}{\text{Time (T2}-\text{T1)}}$

Where

T_2= Final time, T_1= Initial time

2.6.4Water Quality Monitoring

Water quality was measured daily for providing adequate environment for cultured koi fingerlings. P^H meter was used to measure P^H, temperature was measured by digital thermometer and Do meter was used to determine dissolved oxygen (mg/L).

Water temperature (ºC)

Temperature is one of the most important factors that has a significant effect on growth performance of fish. The temperature of aquarium water was measured everyday by digital thermometer between 9.00-9.30 am and 4.00-4.30pm respectively.

Dissolved oxygen (DO)

The dissolved oxygen (DO) of aquarium water was recorded witha DO meter (HANNA, HI-9146, (USA) between 9.00-9.30 am and 4.00-4.30 p.m. respectively. Aerator was used to maintain proper dissolved oxygen concentration in the aquarium because lack of adequate DO in the water can cause fish mortality.

Hydrogen ion concentration (P^H)

P^H meter was used to determine P^H between 9.00-9.30 am and 4.00-4.30pm respectively. $P^H$was determined by P^Hmeter (HANNA, HI-8424, USA).

Plate-3: Frequently used laboratory instrument

A. P^{H} meter (HANNA, HI-8424, USA)

B. Digital mercury thermometer

C. DO meter (HANNA, HI-9146, USA)

D. Microwave Oven

2.7 Chemical analysis

2.7.1 Fish sample preparation for heavy metal analysis

To determine the initial concentration of heavy metals in the collected Koi fingerlings, pull sample was taken because fish samples were too small. The fish then meshed and 5g of meshed wet sample was taken into an oven and dried at $60^{\circ c}$ for 24 hours. After the sample was completely dried then it was grinded. 0.5g of dry sample was taken into a beaker and 10 ml of nitric acid was mixed with the sample. The mixer was then put into Fume chamber and digested at 120^{oC} until the solution becomes clear. Then the solution was diluted to 50 ml with deionized water and was filtered and collected into clean and sterilized plastic bottles for further analysis of heavy metals in the sample (Huq and Alam, 2005). The process of fish sample preparation is shown in the following diagram:

3-5 g of fish sample was kept in an oven at 60°C for 48 hours

⇩

Oven dried samples were homogenized using blender

⇩

1g of the dried samples were taken and 10ml of nitric acid was mixed with the samples

⇩

The mixer was digested at 80°C in Fume chamber

⇩

Samples were diluted to 50ml deionized water

⇩

The solution was filtered and collected into clean and sterilized bottles

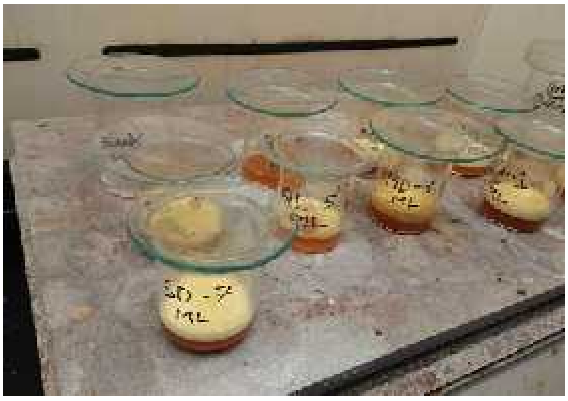
Plate-4: Sample preparation for heavy metal

A.Drying the sample in the oven

B. Blend the sample in the blander

C. Sample after blend

D. Packing the sample in zip bag



E.Mixing of nitric acid with samples

F. Digestion of samples in Fume chamber

G. Samples volume

H. Filtering of samples

I. Collecting samples in plastic bottles

J. Determination of heavy metals in Atomic Absorption Spectrophotometer (AAS)

After rearing those collected fingerlings for three months by feeding five types of different fish feeds, the fish was cut and after cutting gills, intestine and muscle were collected. To determine the heavy metal concentration in collected organs, sample preparing procedure was same as fish.

Plate-5: Collection of muscle, gills and liver from cultured koi

a. Capturing of fish for collected muscle, gills and liver

b. Cutting of fish

c. Collecting of muscle

d. Collected muscle in Petri dish

2.7.2 Feed sample preparation for heavy metal analysis

Feed samples do not need to be dried and sample preparation is same as fish sample preparation(Huq and Alam, 2005). Different steps of sample preparation are shown in the following flow chart:

1g of the feed samples was taken and 10 ml nitric acid was mixed with the samples

⇩

The mixture was kept in the Fume chamber and digested at 80℃ for 6 hours

⇩

The digested samples were diluted to 50 ml with deionized water

⇩

The solution was filtered and collected into clean and sterilized plastic bottles

2.7.3Determination of heavy metal

Fish and fish feed samples were analyzed at Soil, Agronomy and Environment Section; Biological Research Division, BCSIR, Dhaka. The heavy metals Cr, Pb, Cd, Ni, Cuwere analyzed by using Atomic Absorption Spectrometer (Model No: AA-7000, Shimadzu, Japan). The equipment was calibrated with chemical standard solutions prepared from commercially available chemicals and reagents used for the preparation.Deionized water was used throughout the study. Heavy metals concentrations were expressed as ppm.

Heavy metal was determined using following formula:

Concentration of heavy metals = (Reading – Blank reading) ×PDF×SDF

Where Primary Dilution Factor (PDF) $= \frac{\text{Volume}}{\text{Weight of sample}}$

Secondary Dilution Factor (SDF) = $\frac{\text{Secondary Volume}}{\text{Secondary Weight of sample}}$

2.8 Data analysis

The obtained data of water quality parameters, weight and length, specific growth rate,survival rate of the fingerling, and concentration of different heavy metals were analyzed by ANOVA followed by Turkey's HSD post hoc for multiple comparisons with the level of significance at $p < 0.05$ using statistical software SPSS (SPSS version 20). The obtained data was statistically tested to observe whether the influence of different treatments on the growth of fishes was significant or not.

Chapter 3

Results

3.1 Heavy metals determination in collected feeds

Various commercial feeds such as Mega feed, Madina feed, Quality feed and Saudi Bangla feedwere collected and one Handmade feed (B3) was made to determine different heavy metals. In collected fish feeds, the highest value of Nickel (33.42 mg/kg) was found in Madina feed and the lowest (1.72mg/kg) was recorded in Mega feed. The concentration of Lead (Pb) was found lowest among different heavy metals in collected fish feeds. The highest concentration of Pb was found (4.91 mg/kg) in Madina feed. The highest concentration (0.68 mg/kg) of Cd was recorded in Madina feed and the lowest (0.12mg/kg) was recorded in Mega feed. The highest concentration of Cu (18.25 mg/kg) was found in B3 feed and the lowest was in Madina feed. The highest concentration (0.58 mg/kg) of Cr was recorded in Madina feed and the lowest was recorded in Mega feed & B3 feed (Figure 1).

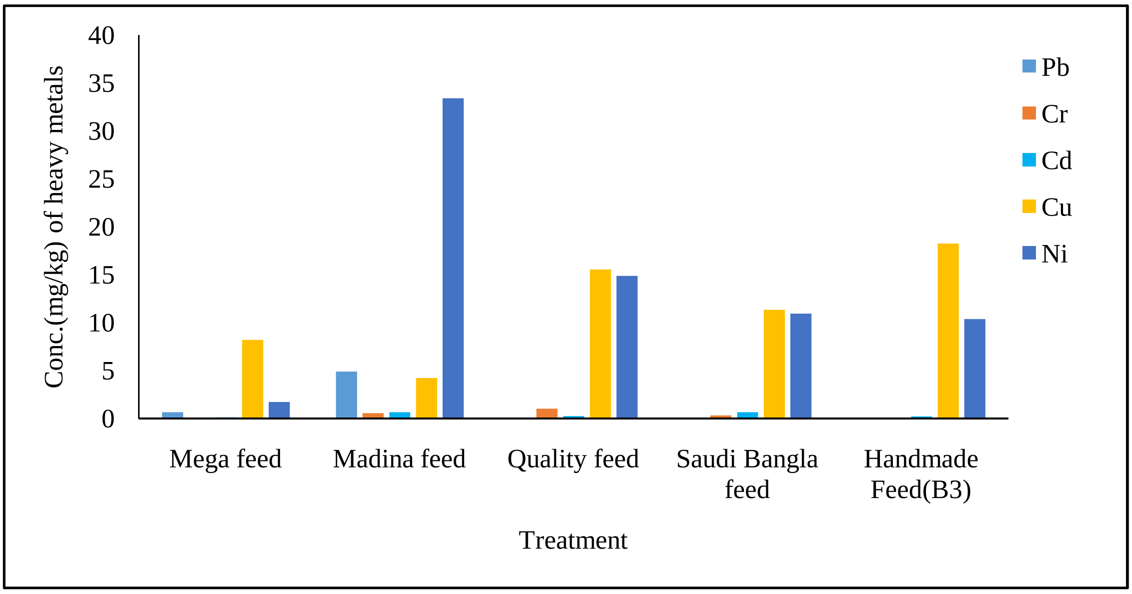


Figure1 Concentration of different heavy metals in collected sample of fish feeds

On the basis of the presence of heavy metals, Mega feed,Madina feed, Quality feed, Saudi Bangla feed, and Handmade feed (B3) were selected as experimental feeds for culturing of collected fingerling.

The concentrations of Cadmium (Cd), Copper (Cu), Lead (Pb),Chromium (Cr) and Nickel (Ni) in different experimental feeds are described below-

3.1.1 Concentration of Cadmium (Cd)

Chemical analysis for determination of Cadmium (Cd) of different experimental feeds showed that the lowest concentration (0.12 mg/kg) of Cd was found in Mega feed and the highest concentration(0.68 mg/kg) was found in Madina feed (figure2).

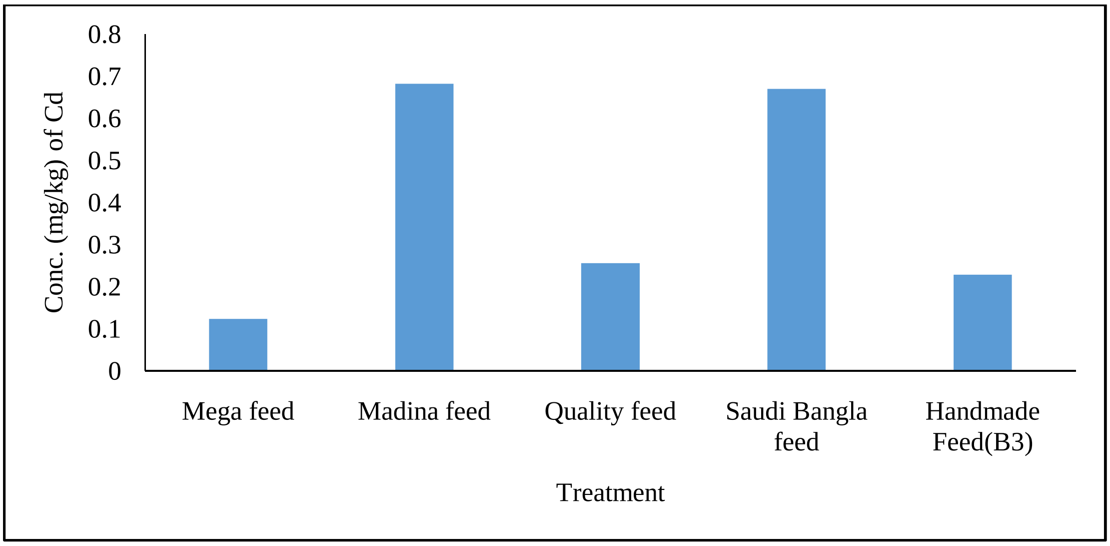


Figure2 Concentration of Cadmium in experimental feeds

3.1.2 Concentration of Copper (Cu)

The concentrations (18.25 mg/kg) of Cu was found in Handmade feed (B3) which was the highest among other feeds and the lowest concentration (4.24 mg/kg) was recorded in Madina feed (figure3).

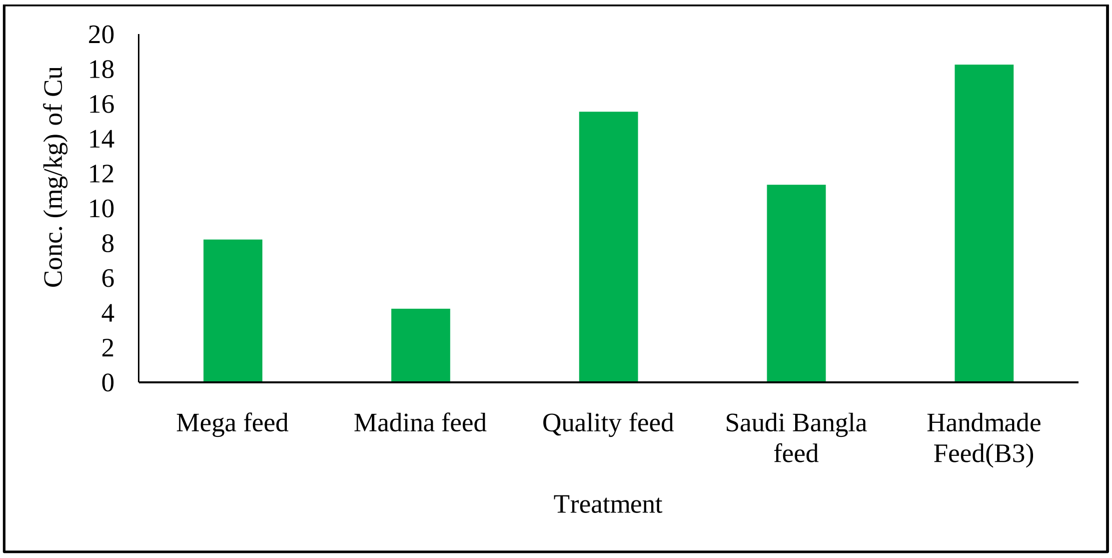


Figure3 Concentration of Copper in experiment feeds

3.1.3 Concentration of Lead (Pb)

Heavy metals of different experimental feeds showed that the lowest concentration of Pb was found in different feeds and the highest concentration (4.92 mg/kg) was found in Madina feed (figure4).

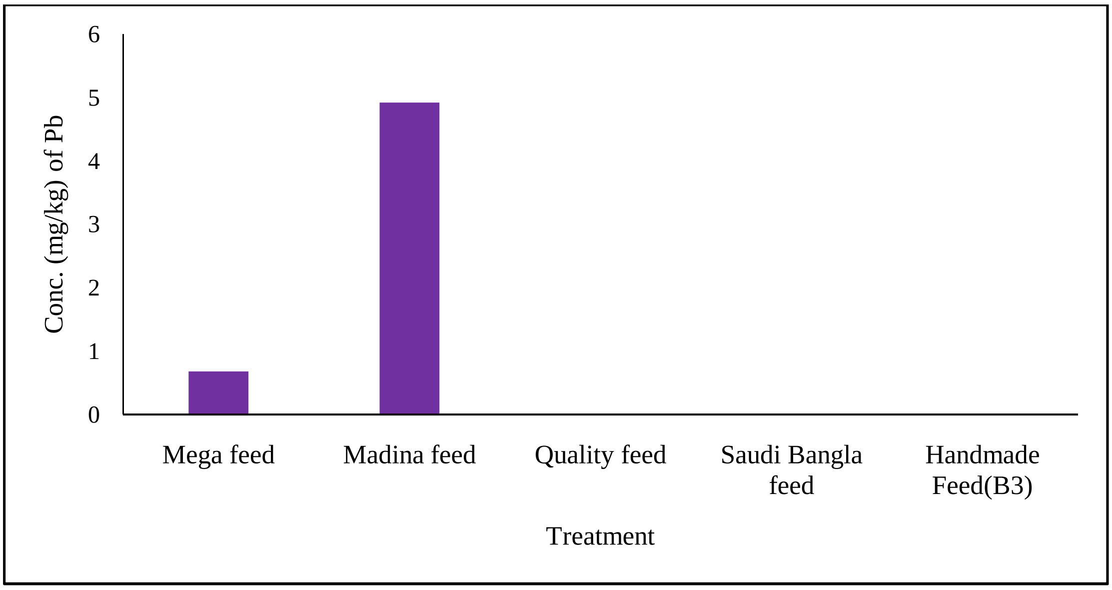


Figure4 Concentration of Lead in different treatments

3.1.4 Concentration of Chromium (Cr)

The concentration (1.04 mg/kg)of Cr was recorded in Quality feed which was highest among different experimental feeds. The lowest value (0.00 mg/kg) of Chromium was observed in Mega feed & Handmade feed (B3) (figure5).

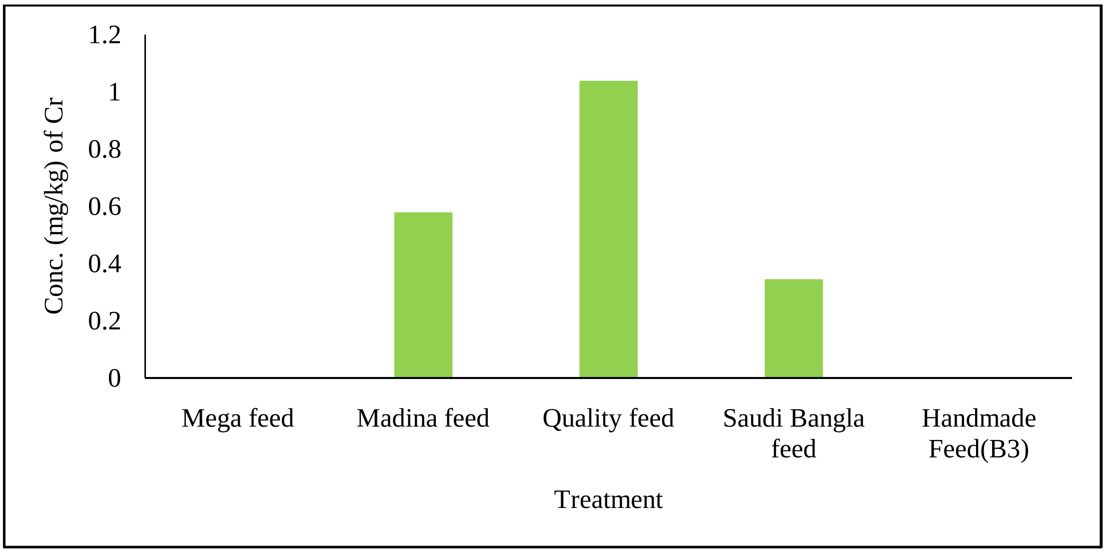


Figure 5 Concentration of Chromium in different experimental feeds

3.1.5 Concentration of Nickel (Ni)

The highest concentration (33.42 mg/kg) of Ni was found in Madina feed and the lowest (1.72 mg/kg) was observed in Mega feed (Figure 6).

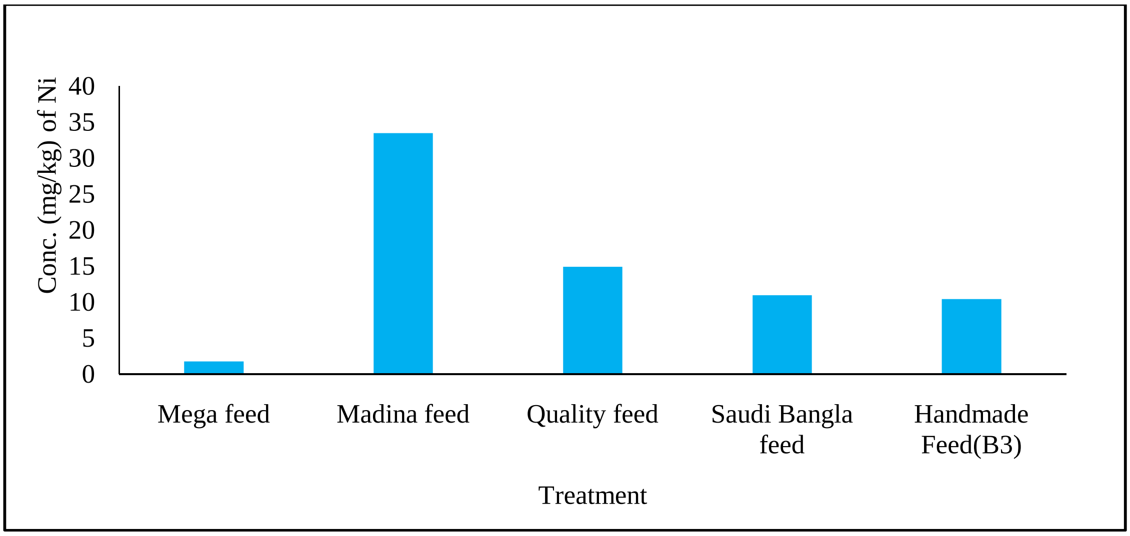


Figure 6 Concentration of Nickel in different experimental feeds

3.2 Choice of feed and culture of Koi

Different commercial feeds such as Mega feed,Madina feed, Quality feed, Saudi Bangla and Handmade feed (B3) were selected to culture Koi fingerlings for 12 weeks. Fingerlings were stocked in 10 aquariums with stocking density of 20 individuals per aquarium. Each treatment had a replication as each aquarium contained 60 liters of water.

3.3 Growth performance of Koi

Different growth parameters such as Condition Factor (K), Average Daily Gain (ADG), Specific Growth Rate (SGR) and Food Conversion Ratio (FCR) were determined in Koi at five different feeding frequencies.

3.3.1 Condition factor (K)

During 30th day of culture the highest condition factor of 3.13±0.20 was found in Handmade feed (B3) and the lowest of 1.81±0.01 was observed in Madina feed. The calculated condition factor revealed no significant difference between the treatments($p>0.05$) (Figure 7).

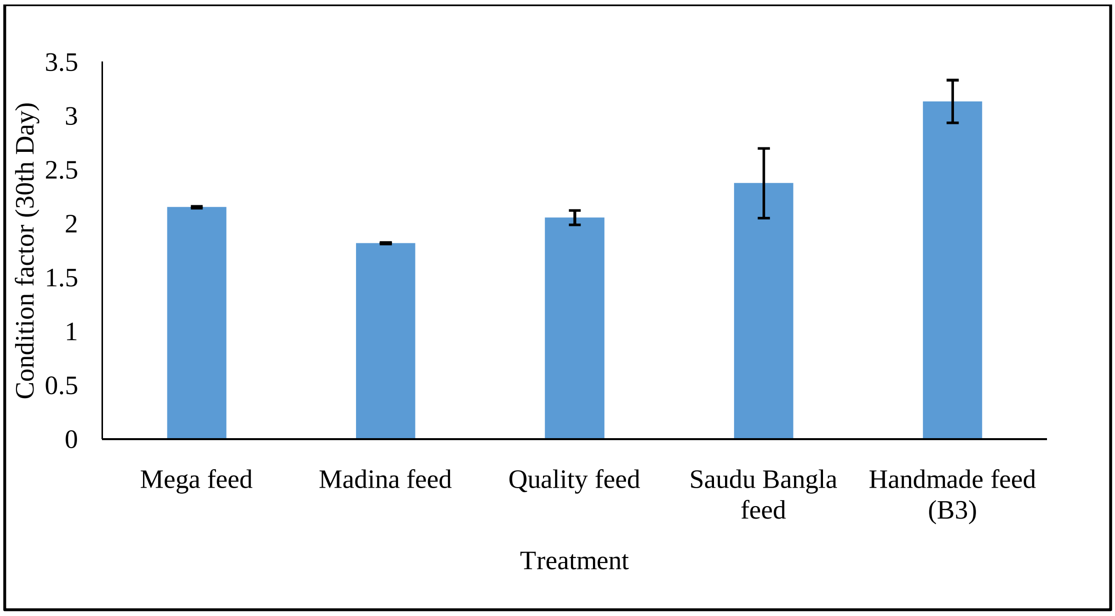


Figure 7 Comparative condition factor of Koi in 30th day of rearing period in different treatments (Mean ± SEM)

The value of condition factor was most prominent (2.61±0.19) in Saudi Bangla feed and the least (1.58±.096) in Quality feed during 60thday of the farming. There was significant differences ($p<0.05$) among different treatments (Figure 8).

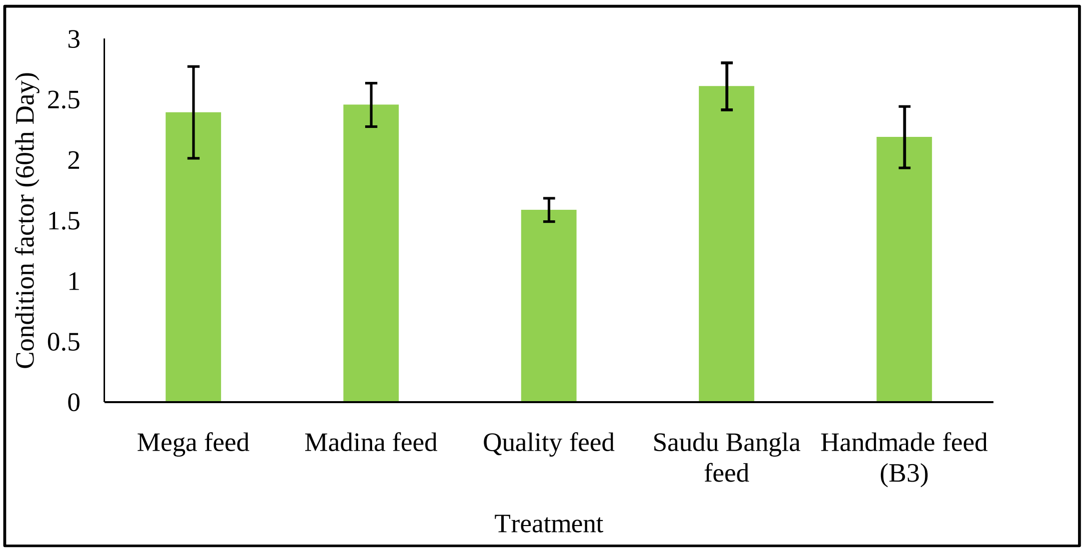


Figure 8 Comparative Condition factor of Koi in 60th day of rearing period in different treatments (Mean ± SEM)

During 90th day of farming the highest value (3.03±0.37) was recorded in Saudi Bangla feed and the lowest value (1.94±.06) was estimated in Quality feed (Figure 9). Statistical analysis showed that there is no significant difference among different treatments(p>0.05)(Figure 9).

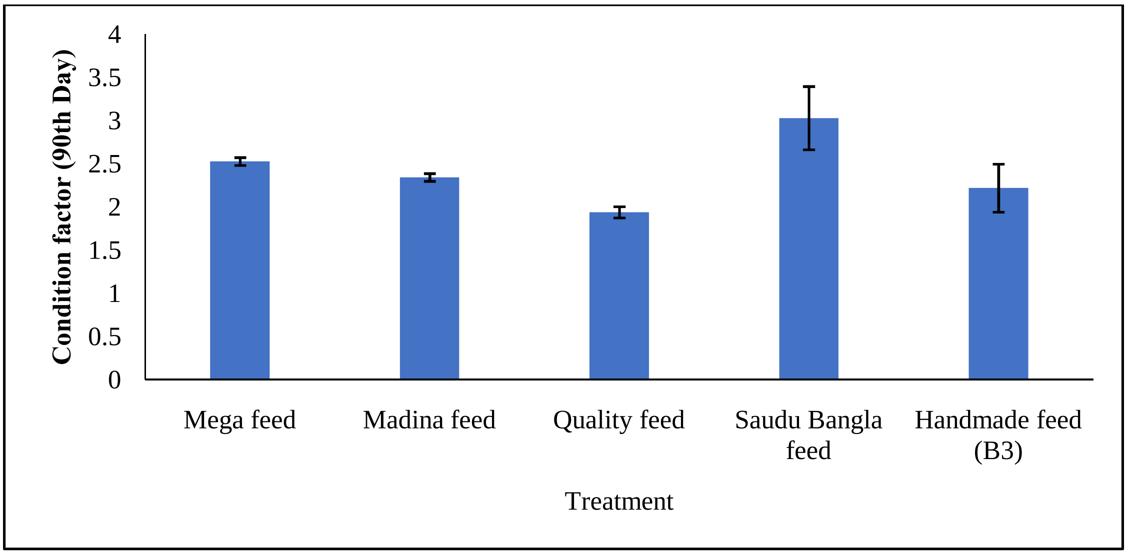


Figure 9 Comparative Condition factor of Koi in 90th day of rearing period in different treatments (Mean ± SEM)

3.3.2 Average Daily Gain (ADG)

The highest average daily gain was 0.11±0.001 found in Handmade feed (B3) and the lowest was 0.02±0.002, observed in Mega feed during 30th day period (Figure 10). The values of ADG among different treatments were not significantly different(p>0.05).

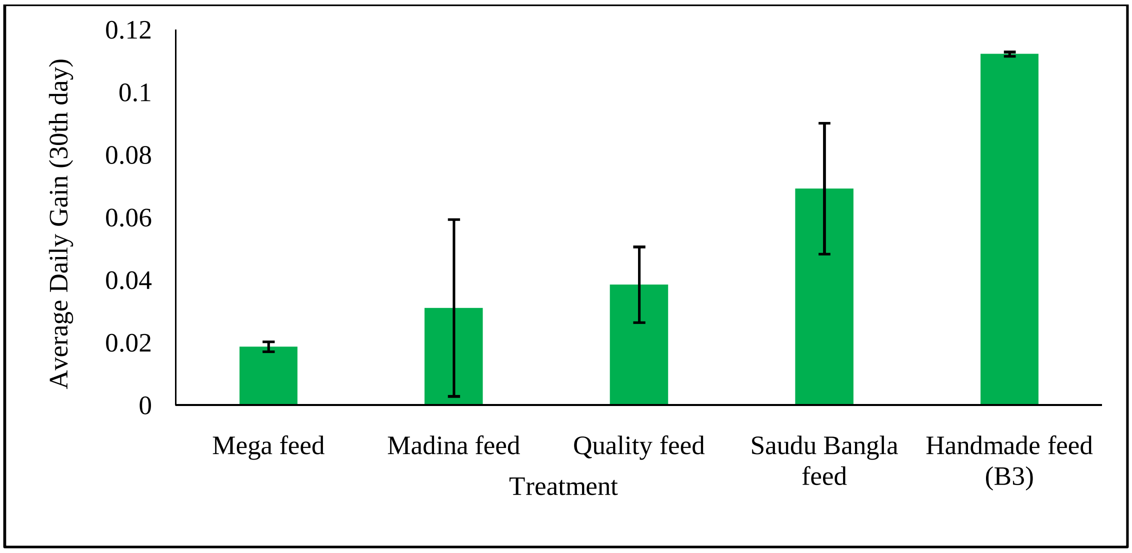


Figure 10 Comparative Average Daily Gain of Koi in 30th day of rearing period in different treatments (Mean ± SEM)

During 60th day period the highest (0.16±0.04) value of average daily gain (ADG) was calculated in Quality feed and the lowest (0.04±0.004) was calculated in Saudi Bangla(Figure 11). The value of ADGinMadina and quality feeds were significantly higher than other treatments.Statistical analysis showed that the values were significantly different($p<0.05$)among treatments.

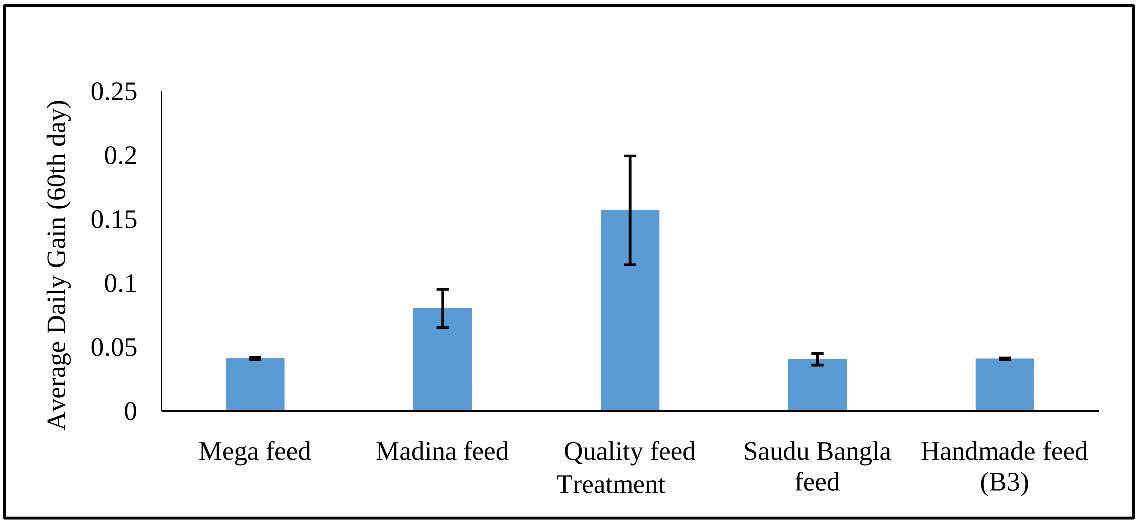


Figure 11 Comparative Average Daily Gain of Koi in 60th day of rearing period (Mean ± SEM)

The highest value of average daily gain was estimated 0.28±0.04 and the lowest value was 0.03±0.004 receiving Quality and Mega feed respectively (Figure 12). There was no significant variations ($p>0.05$) among treatments for average daily gain at 90th day period.

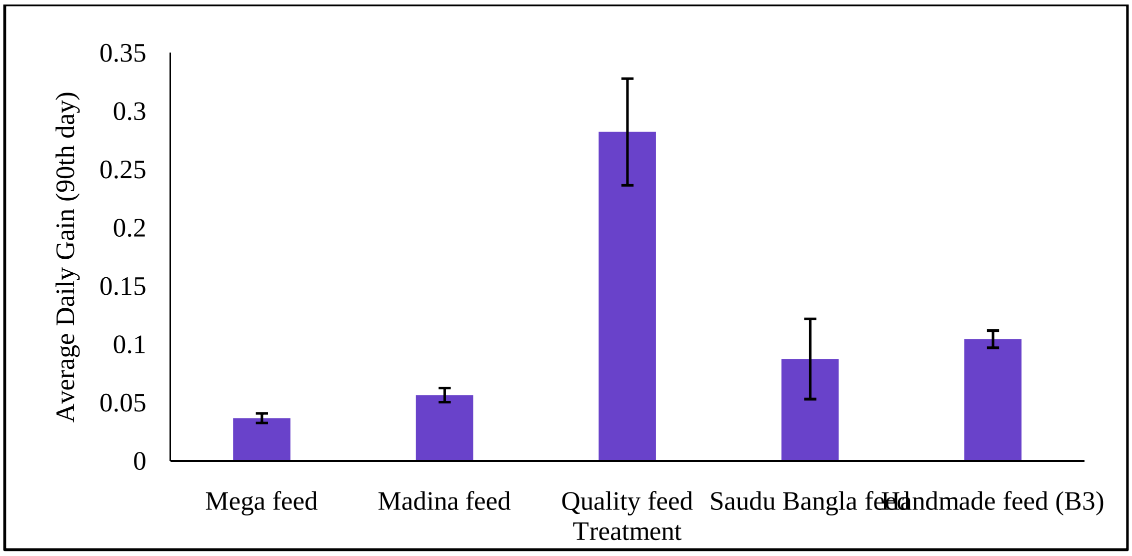


Figure 12 Comparative Average Daily Gain of Koi in 90th day of rearing period among different treatments (Mean ± SEM)

3.3.3 Specific growth rate (SGR)

The average specific growth rate ranged from4.59±.10 to 1.98±0.15and found in Handmade (B3) and Mega feed respectively (Figure 13) in 30th day of culture. There were no significant differences($p>0.05$) among different treatments.

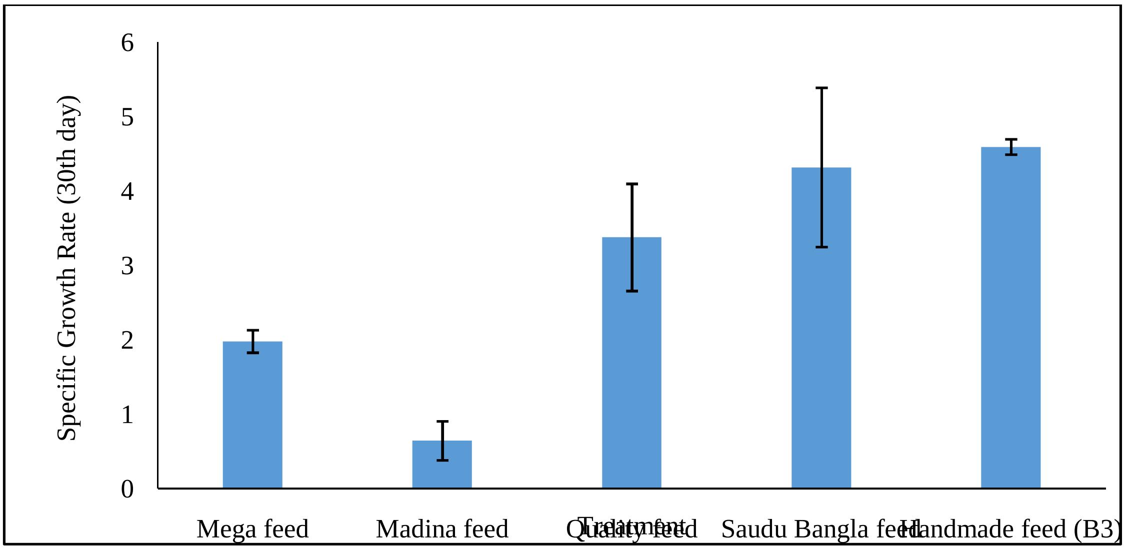


Figure 13 Comparative Specific Growth rate of Koi in 30th day of rearing period in different treatments (Mean ± SEM)

In 60th day period, the highest SGR was found 4.68±.39 in Madina feed and the lowest value was found 0.80±.01 inHandmade feed(B3) (Figure 14). The statistical analysis result showed that the values of SGR in 60th day were significantly varied ($p<0.05$)among different treatments.

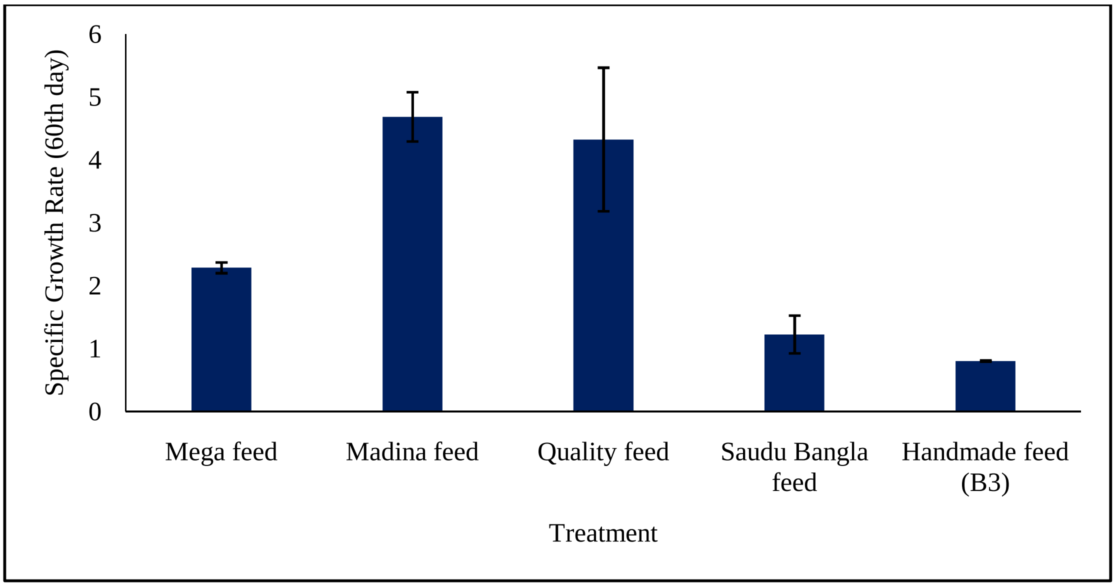


Figure 14 Comparative Specific Growth rate of Koi in 60th day of rearing period in different treatments (Mean ± SEM)

The specific growth rate varied from2.77±.031 to 1.22±.11 in the 90th day period and was found in Quality feed and Mega feed respectively (Figure 15). There were no significant differences (p>0.05)among treatments.

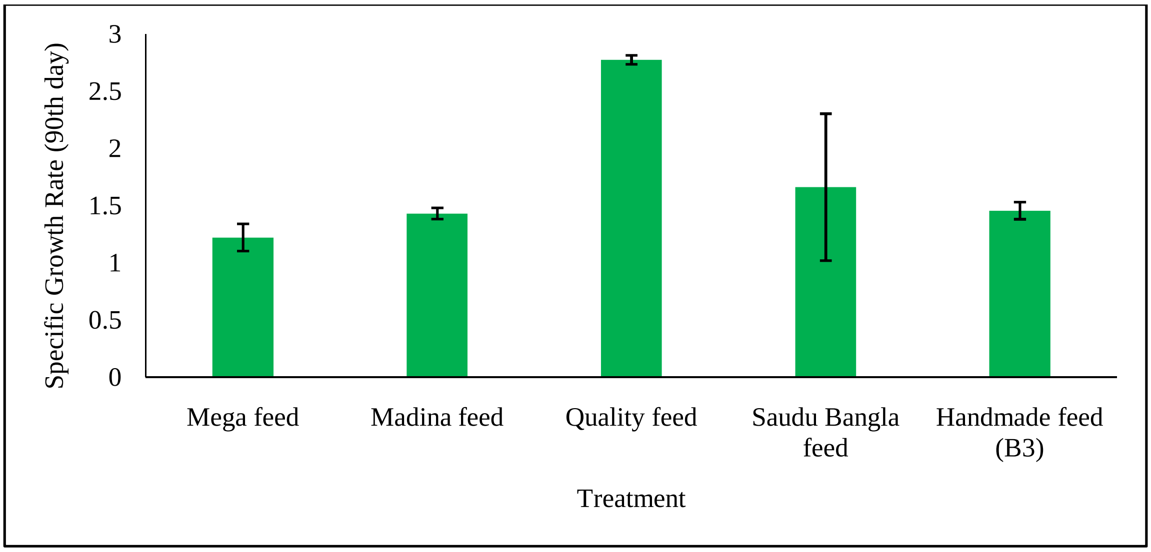


Figure 15 Comparative Specific Growth rate of Koi in 90th day of rearing period in different treatments (Mean ± SEM)

3.3.4 Feed conversion ratio (FCR)

The highest value of FCR was found in 3.74±0.38 and the lowest was found 1.46±0.14 in Mega and Handmade feed (B3) respectively during the 30th day of experiment (Figure 16). The mean value of FCR was not significantly different(p>0.05) among treatments.

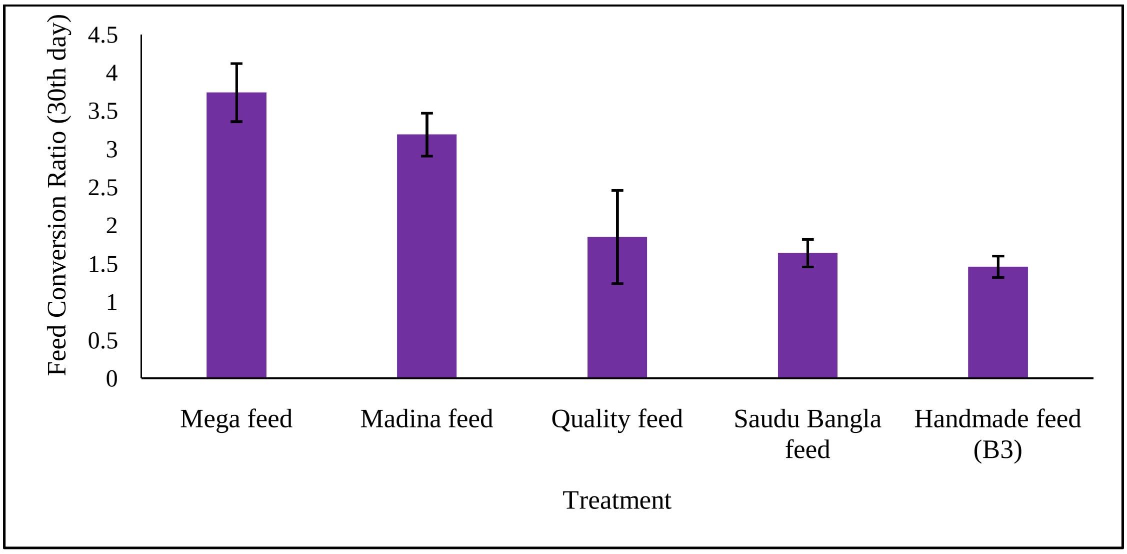


Figure 16 Comparative Feed Conversion Ratio of Koi in 30th day of rearing period in different treatments (Mean ± SEM)

The highest value of FCR was calculated 3.3±0.28 in Saudi Bangla and the lowest (1.35±0.24) in Madina feed during the 60th day period (Figure 17). Statistical analysis revealed that FCR values were not significantly different (p>0.05) among treatments.

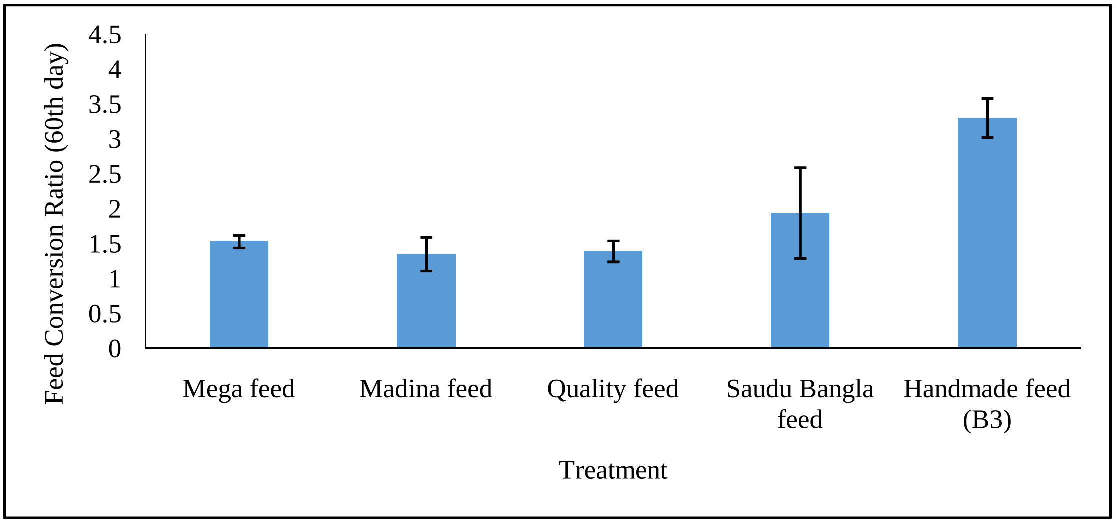


Figure 17 Comparative Feed Conversion Ratio of Koi in 60th day of rearing period in different treatments (Mean ± SEM)

The highest and lowest value of FCR ranged from2.18±0.34 to 1.29±0.11and found inMega and Quality feed respectively (Figure 18). There were no significant differences ($p>0.05$) among different treatments.

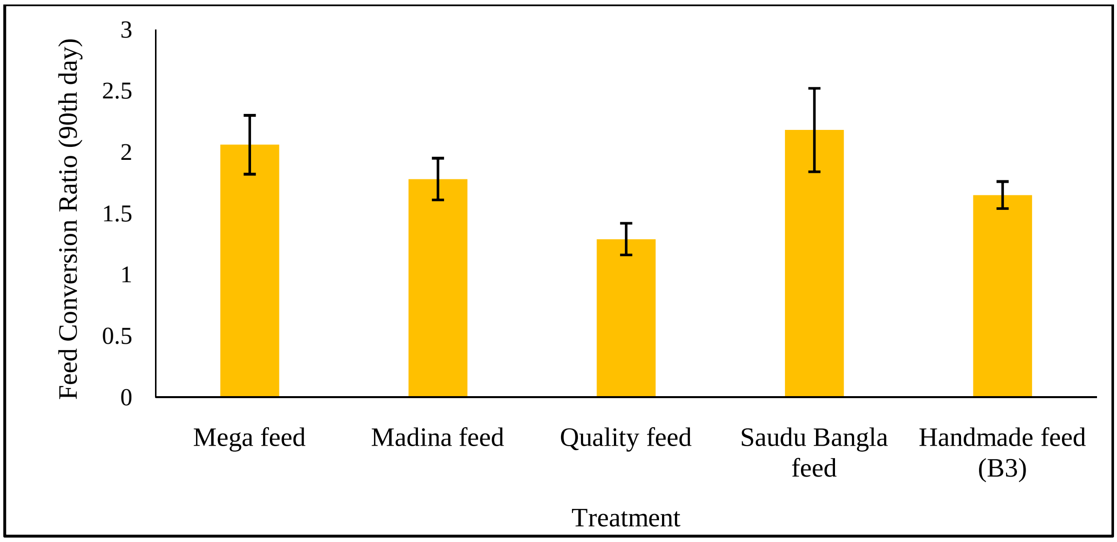


Figure 18 Comparative Food Conversion Ratio of Koi in 90th day of rearing period in different treatments (Mean ± SEM)

3.4 Heavy metal bioaccumulation

Initial concentration of different heavy metals in collected Koi fingerlings (pull sample) was determined by Atomic Absorption Spectrometer(AAS). Among different heavy metals in collected Koi fingerlings, the concentration of Cu was found to be the highest (2.99 mg/kg) followed by Cr and Cd. (Figure 19).

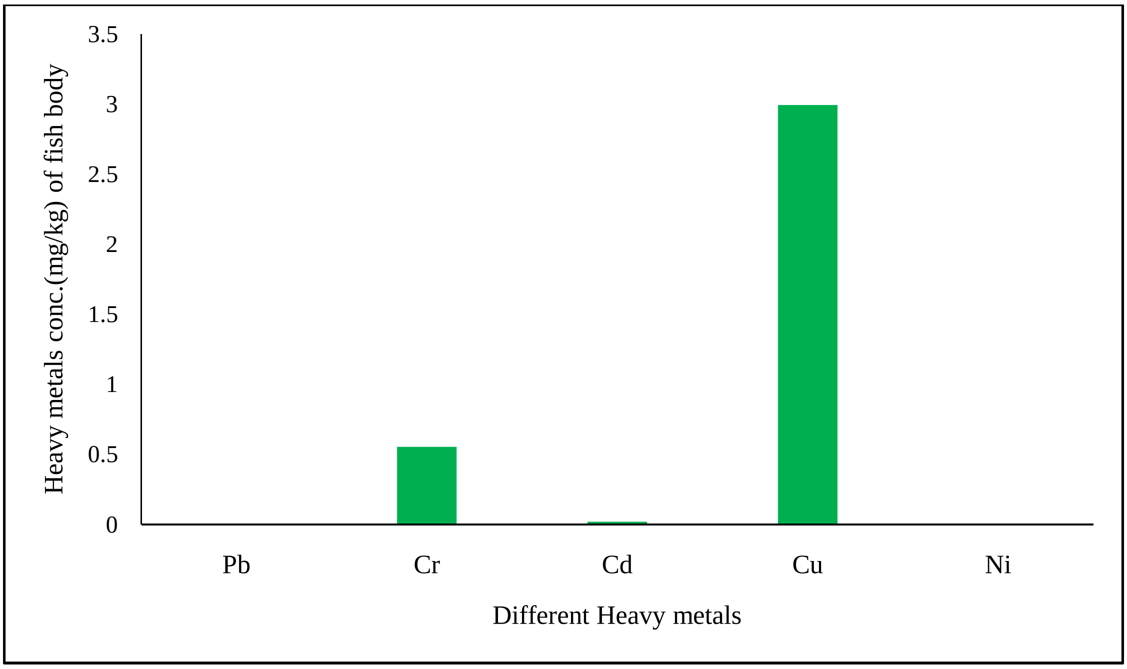


Figure 19 Concentration of different heavy metals on collected fingerlings of Koi

The highest concentration of Cu was found in cultured of Koi by feeding Mega feed followed by Pb, Cd, Cr, Ni (figure 20). Figure 20 showed that the concentration of heavy metals (Cu, Ni, Cd, Pb and Cr) were found highest in intestine followed by gills and muscle. The highest concentration of Cu was found to be 212.21 mg/kg in intestine, 85.14 mg/kg in gills and 52.10 mg/kg in muscle (figure 20).

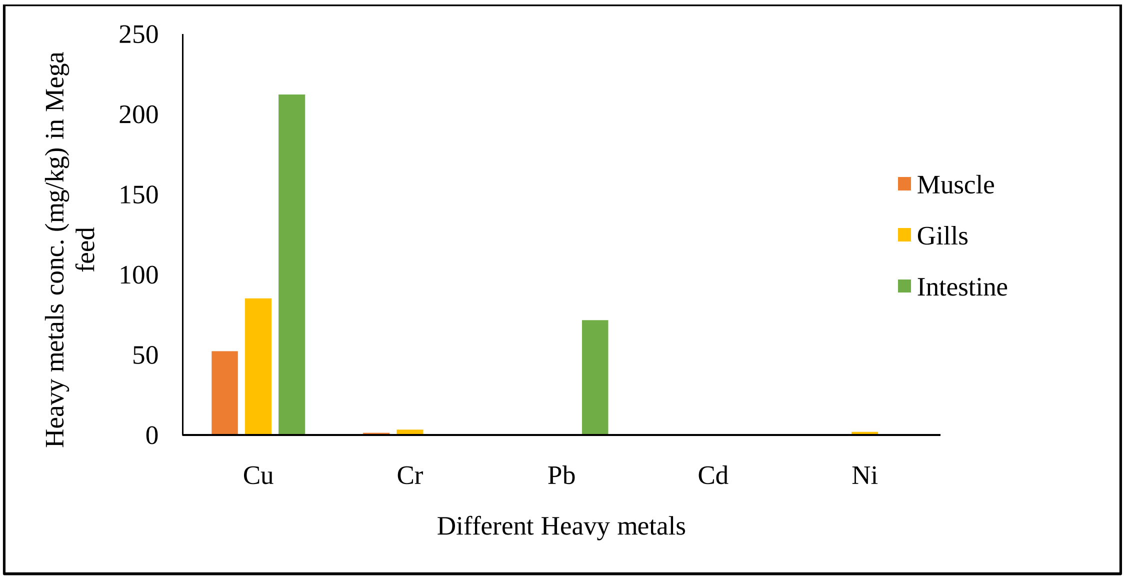


Figure 20 Concentration of heavy metals in intestine, gills and muscle of Koi receiving Mega feed

Figure 21 showed the concentration of different heavy metals in cultured Koi fingerlings receiving Madina feed. The concentration of Cu was found highest among different heavy metals followed by Pb, Cd, Cr and Ni. The highest concentration of heavy metals were foundin intestines and the lowest in muscle. The concentration of Cu was found 368.67 mg/kg in intestine, 65.72 mg/kg in gills and 55.42 mg/kg in muscle.

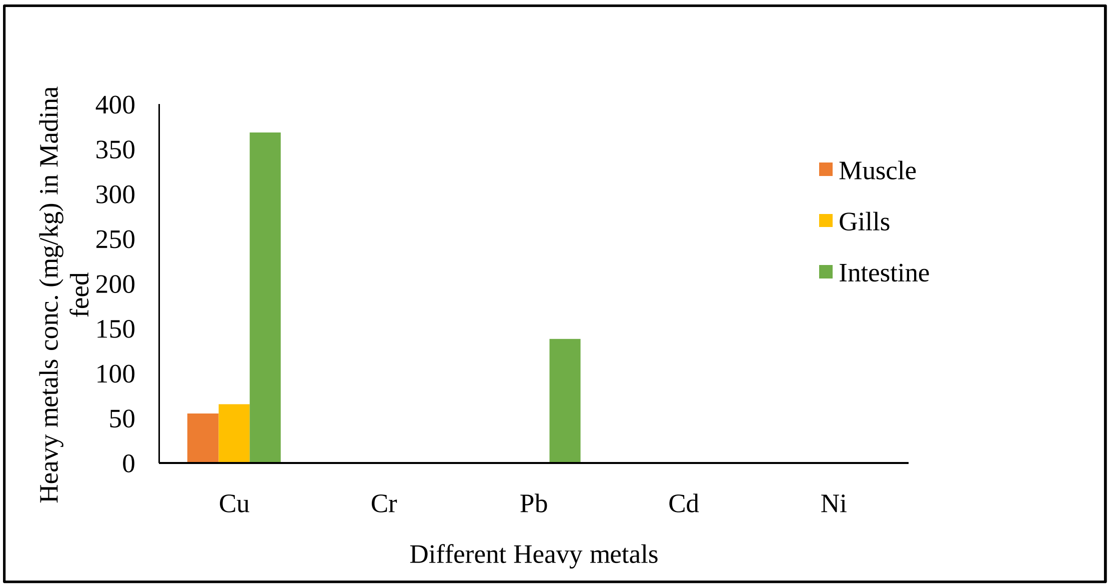


Figure 21 Concentration of different heavy metals in intestine, gills and muscle of Koi receiving Madina feed

Figure 22 found that the concentration of Cu was found to be the highest (43.35 mg/kg) in gills followed by intestine and muscle of cultured Koi receiving Quality feed. The concentration of Cd was found the lowest among different heavy metals.

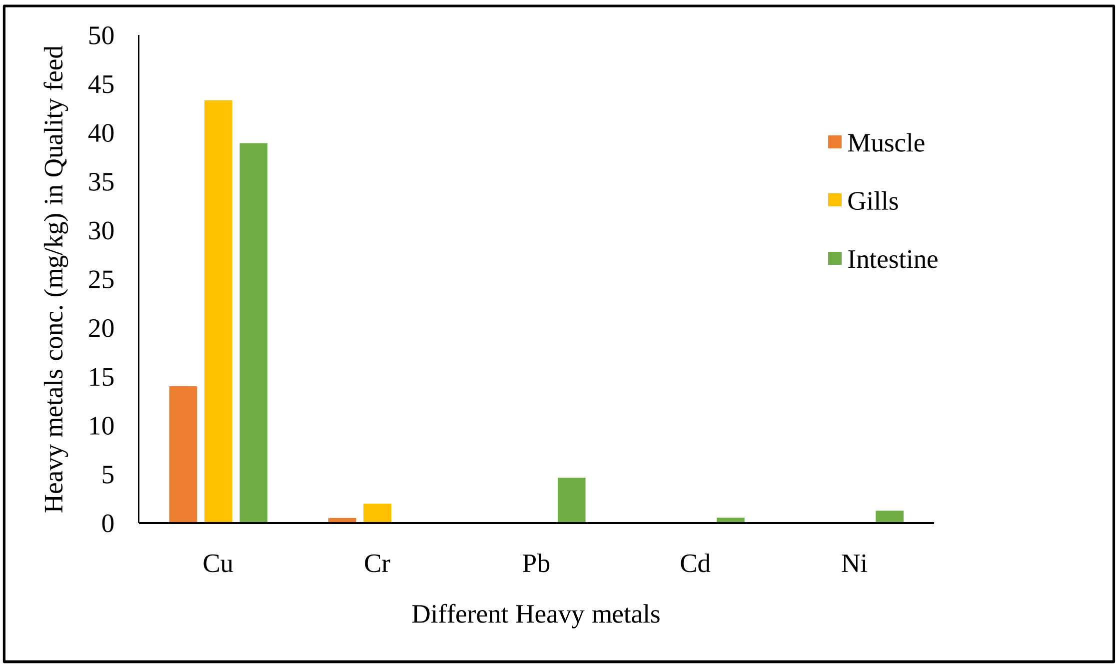


Figure 22 Concentration of different heavy metals in intestine, gills and muscle of Koi receiving Quality feed

Figure 23 showed that the concentration of Copper was found highest (319.13 mg/kg) in intestine followed by muscle (45.98mg/kg) and gills (32.64 mg/kg) of cultured Koi fingerlings in Saudi Bangla feed. The concentration of Ni was lower than other heavy metals. Ni was found in 0.11 mg/kg in muscle were observed in cultured fish receiving Saudi Bangla feed (figure 23).

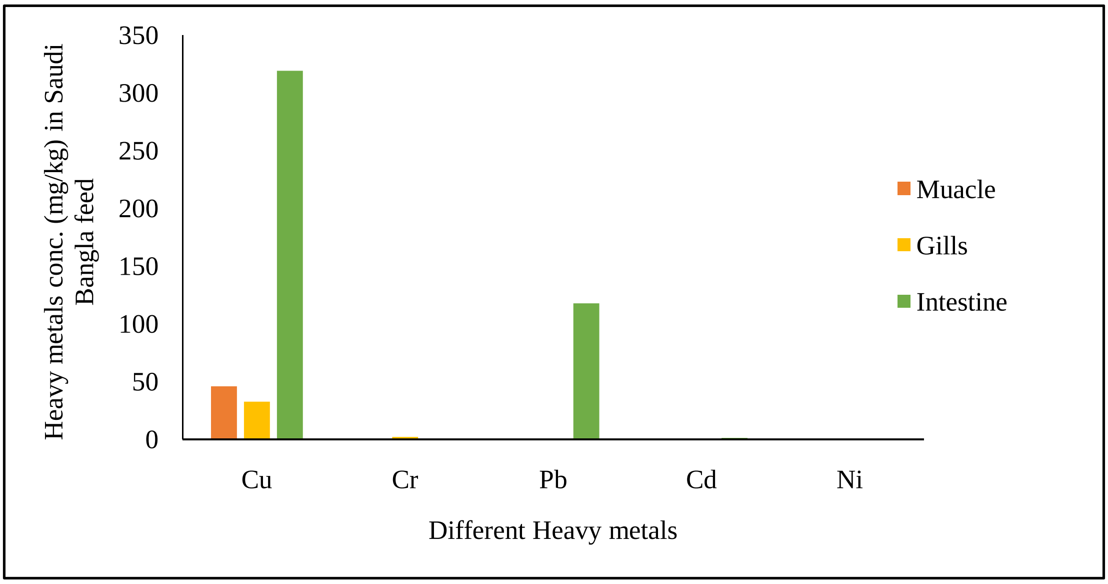


Figure 23 Concentration of different heavy metals in intestine, gills and muscle of Kio receiving Saudi Bangla feed

Figure 24showed that the concentration of Cu was found to be highest (289.89 mg/kg) in intestines followed by muscle and gills of cultured Koi receiving Handmade feed (B3). The concentration of Ni was found lowest among different heavy metals.

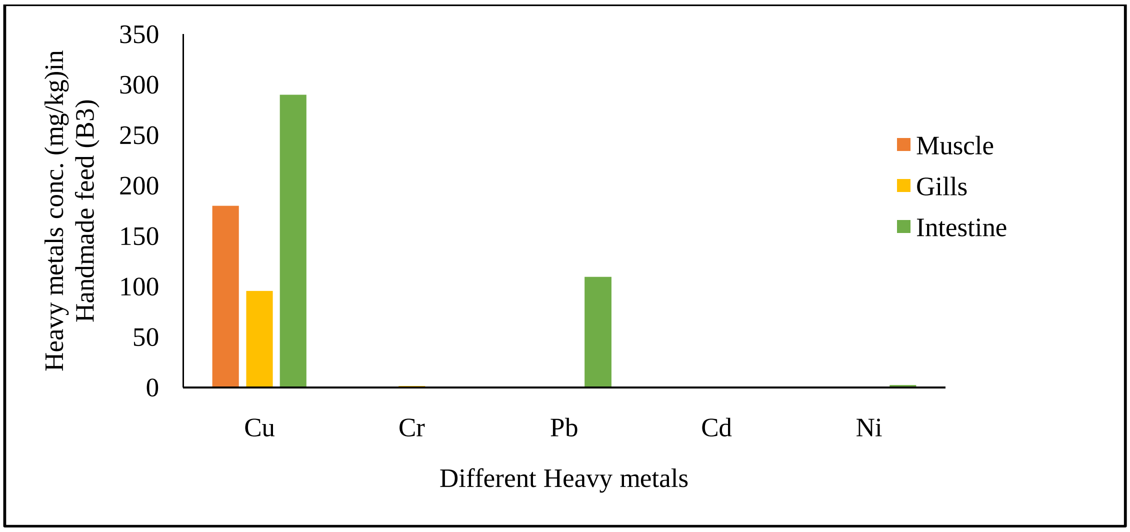


Figure 24 Concentration of different heavy metals in intestines, gills and muscle of Koi receiving Handmade feed (B3)

Figure 25 illustrated that the concentration of Cr in gills were found the highest and the lowest in Mega feed and Handmade feed (B3).Muscle concentration of Cr was found the highest and the lowest in Mega feed and Saudi Bangla feed. Intestine concentration of Cr was not found in those feed.

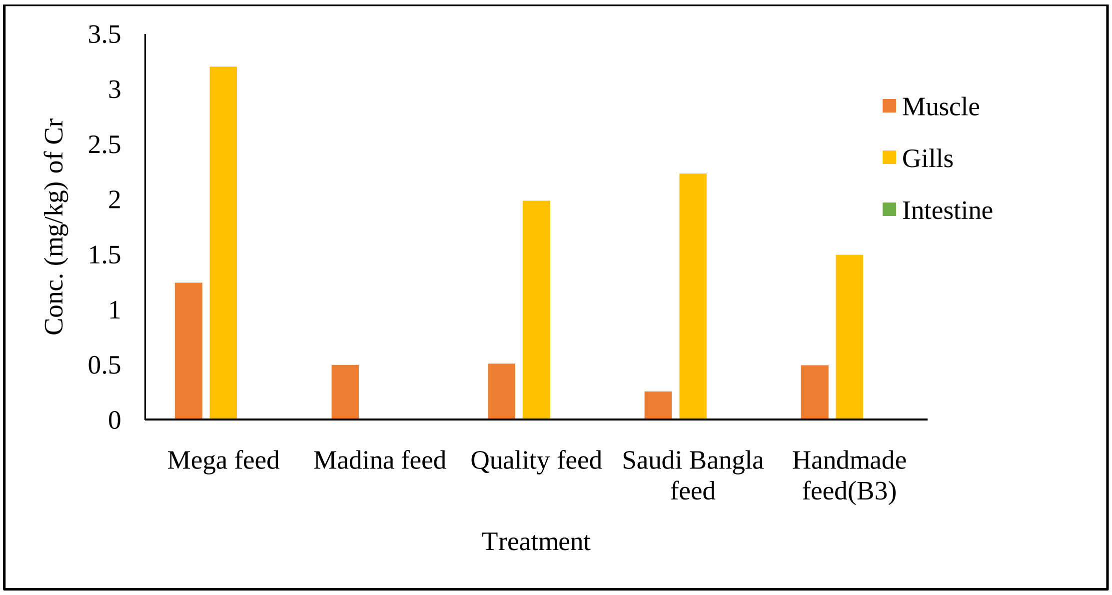


Figure 25Concentration of chromium in different organs of cultured Koi in five treatments

The concentration of Cu in intestines was found the highest in Madinafeed and the lowest in Quality feed. In muscle, the highest concentration.of Cu was observed of cultured fish receiving Handmade feed (B3) and the lowest in Koi taking Quality feed. The highest concentration of Cu in gills was found in fishes given B3 feed and lowest in Saudi Bangla feed (figure 26).

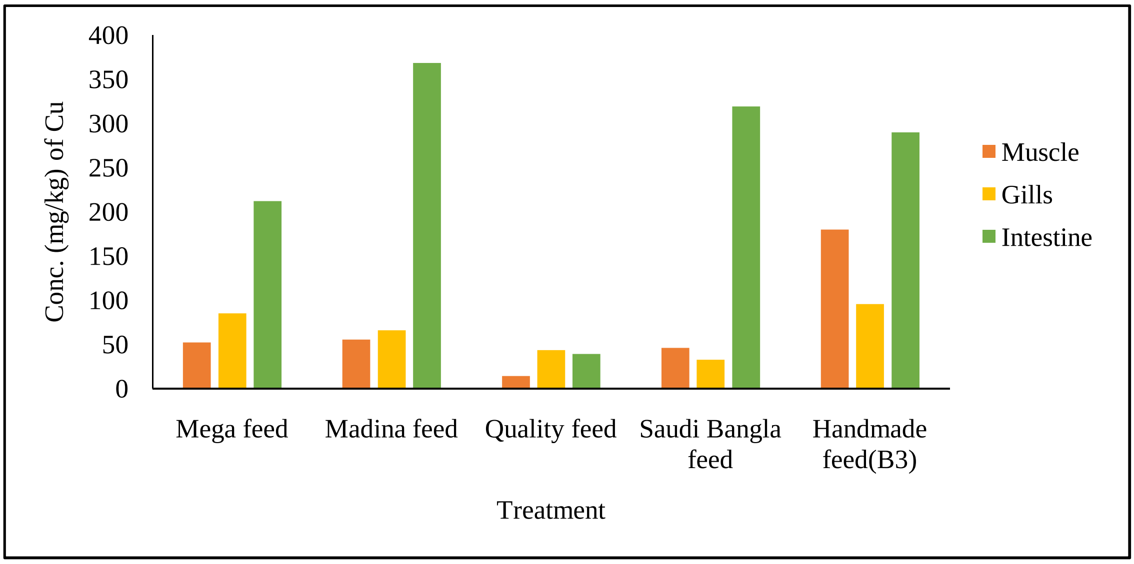


Figure 26Concentration of the Copper in different organs of cultured Koi in five treatments

Figure 27 showed that the concentration of Cadmium in intestines was found the highest in Saudi Bangla feed and the lowest in Mega feed. In muscle, the highest concentration of Cd was observed of cultured fish receiving Mega feed and the lowestin Koi taking Quality feed. The highest concentration of Cd in gills was found in fishes given B3 feed and the lowest in Saudi Bangla feed.

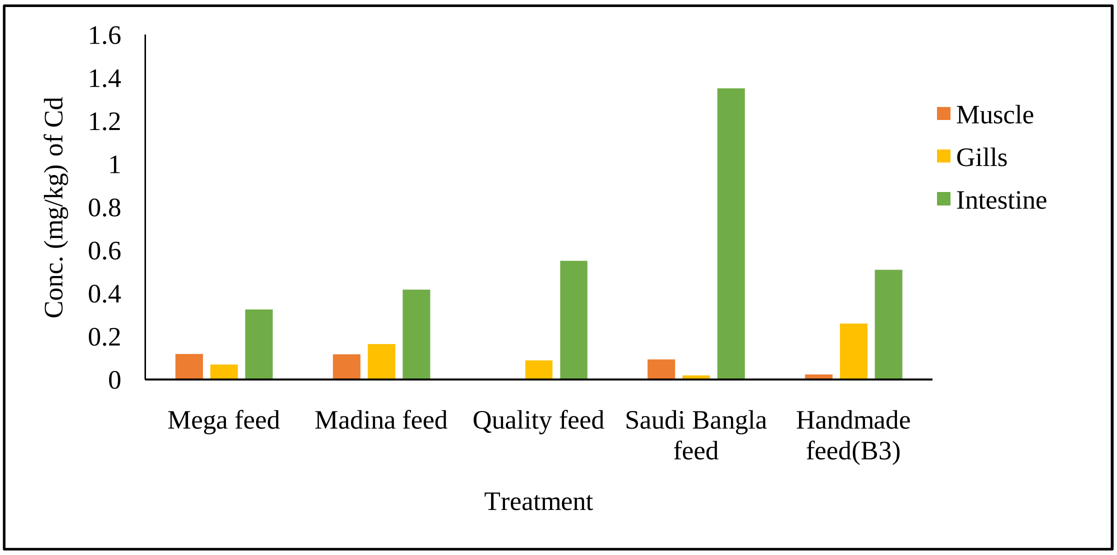


Figure 27Concentration of Cd in different organs of cultured Koi in five different treatments

The highest concentration of Lead in intestines found in Madina feed and lowest was found in Quality feed. The highest concentration of Pb in gills was observed in fishes taken Mega feed and lowest in fishes receiving Saudi Bangla feed (figure 28).

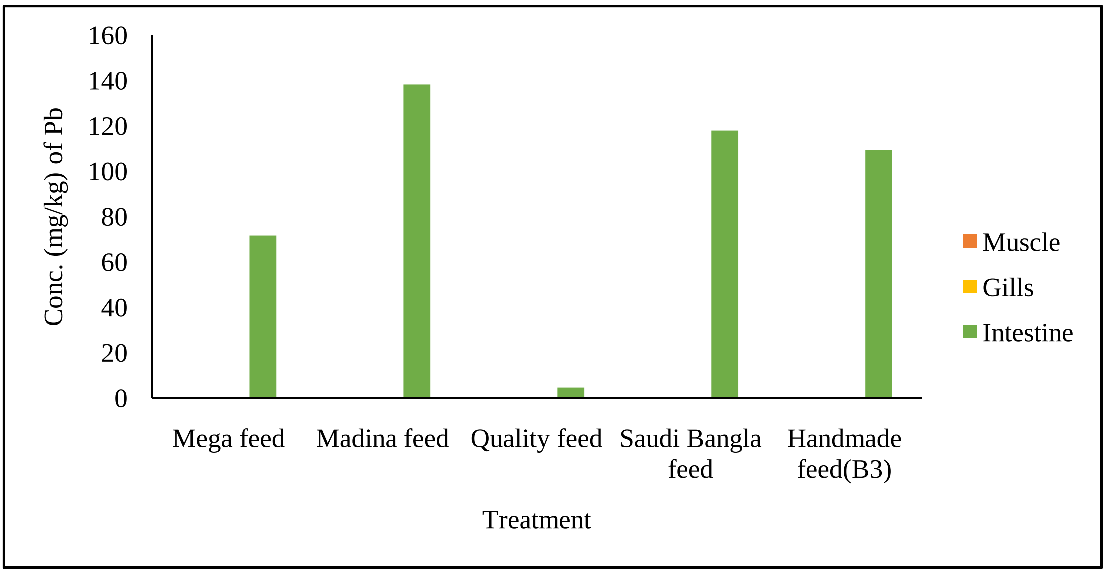


Figure 28 Concentration of Lead in different organs of cultured Koi under five treatments

The highest concentration of Nickel in intestine found in Handmade feed (B3) and the lowest was found in Madina& Saudi Bangla feeds. Ni concentration in gills was higher in Mega feed. The highest concentration of Ni in muscle was observed in fishes taken Handmade feed (B3) and the lowest in fishes receiving different feeds (Figure 29).

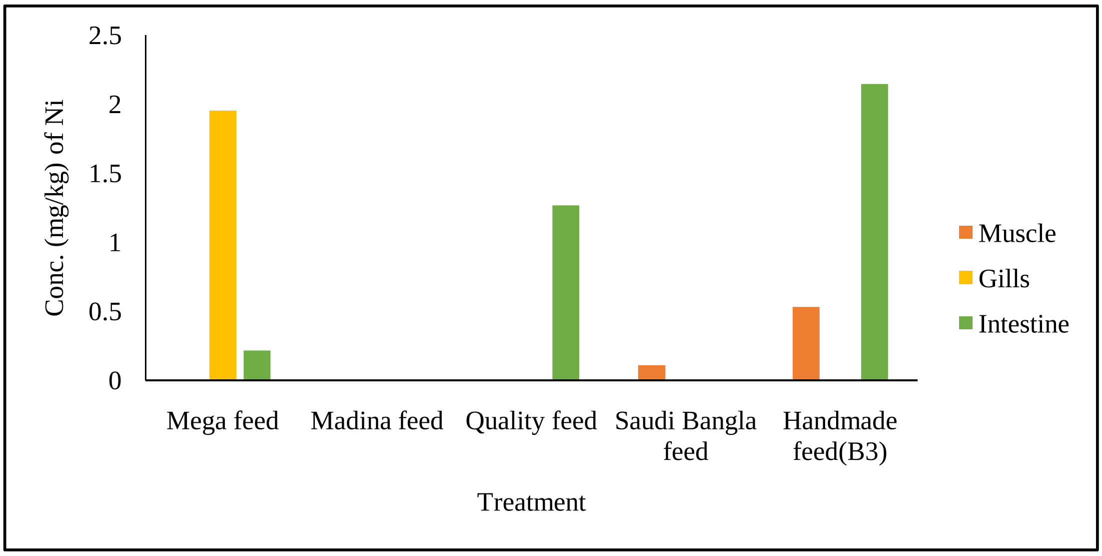


Figure 29 Concentration of Nickel in different organs of cultured Koi under five treatments

Figure 30 illustrated that the concentration of Cu was higher in cultured Koi receiving Mega feed than initial concentration. of Cu in feed and collected fish samples whereas concentration of Cr was low increase in final fish body. Concentration of Cd &Pb were also increased but Ni was decrease in cultured fish significantly.

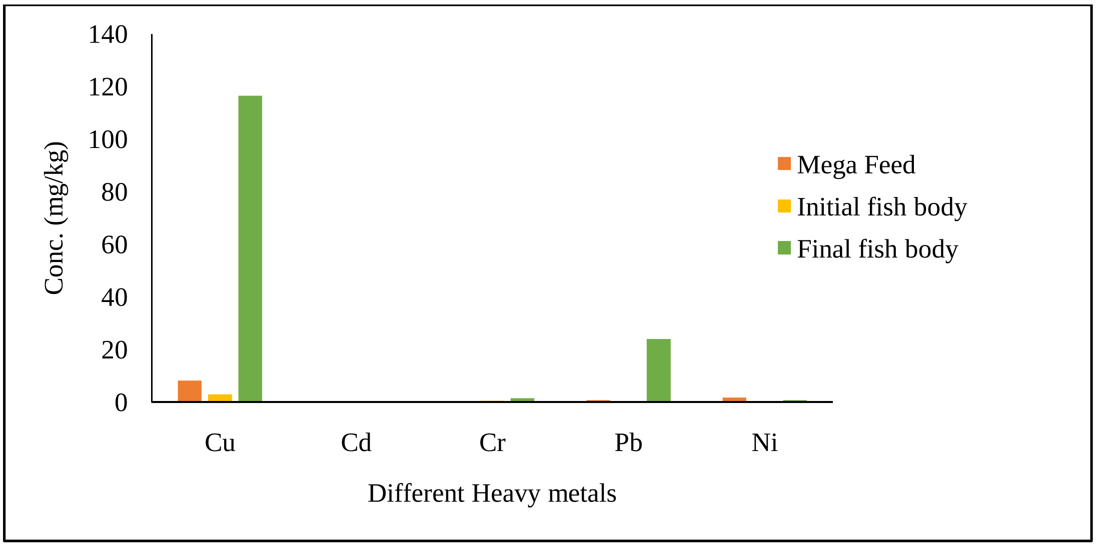


Figure 30 Comparative study of different heavy metals in Megafeed, collected fingerlings of Koi and cultured Koi given Mega feed

Concentration of Cuand Pb were increased noticeably in cultured Koi given Madina feed, in where conc. of Cr and Ni were decreased in cultured fish (figure 31).

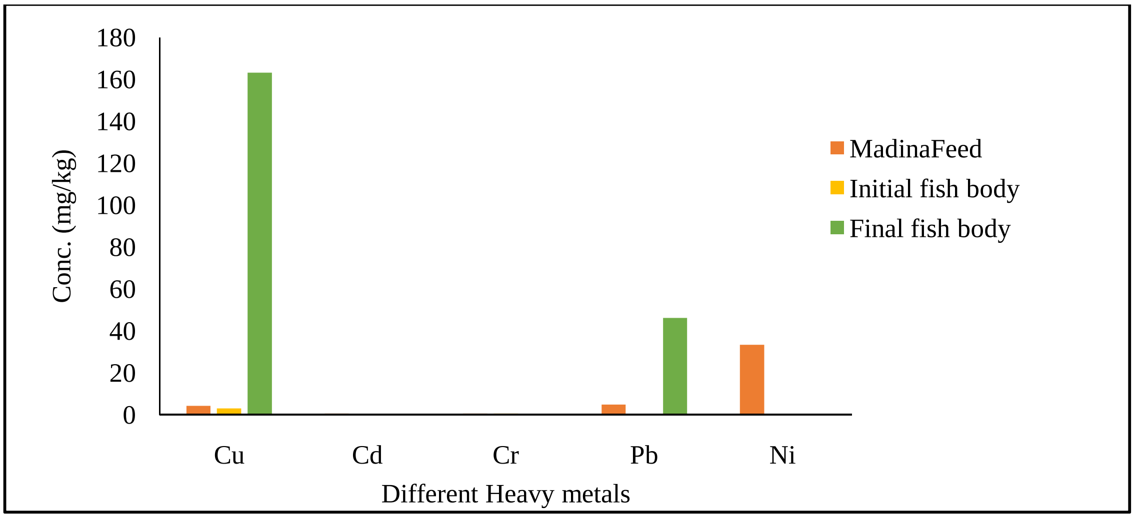


Figure 31 Comparative study of different heavy metals in Madina feed, collected fingerling of Koi body and cultured Koi given Madina feed

The concentrations of Cu, Pb, Cd were higher in cultured fish than collected feed and fingerlings of Koi receiving Quality feed. In this case the concentration of Cr, Ni were decreased in cultured fish (figure 32).

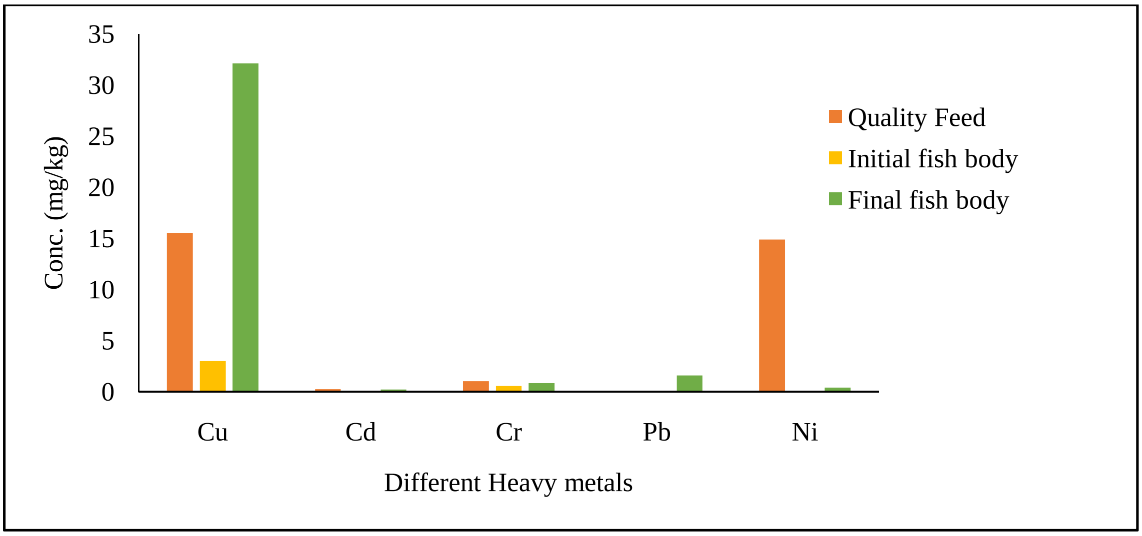


Figure 32 Comparative study of different heavy metals in Quality feed, collected fingerling of Koi and cultured Koi receiving Quality feed

Figure 33 indicates that the concentrations of Cu, Cd, Pb and Cr were higher in cultured fish given Saudi Bangla feed as conc. of Ni was in cultured Koi was decreased.

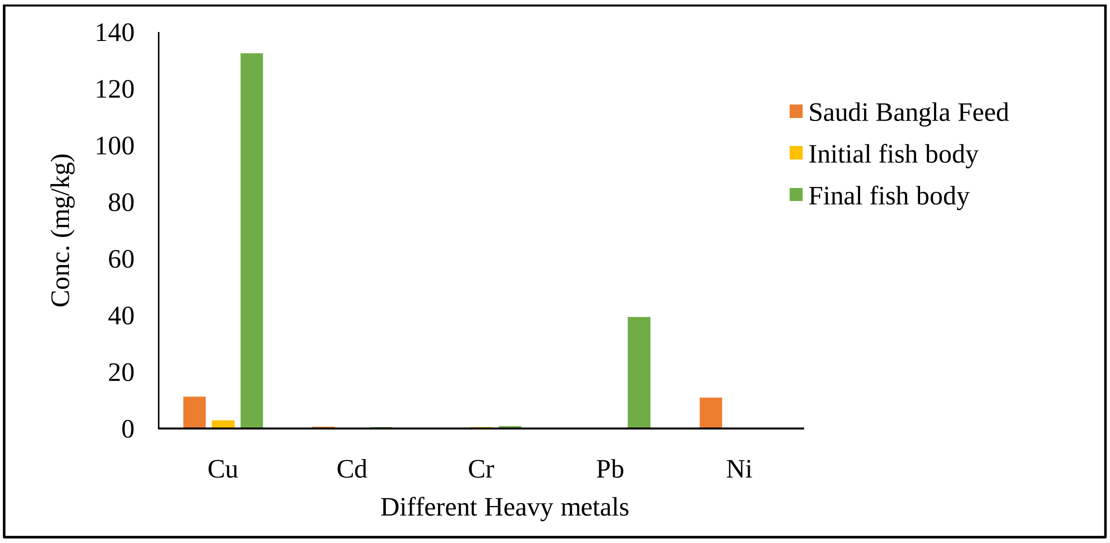


Figure 33 Comparative study of different heavy metals in Saudi Bangla feed collected fingerling of Koi and cultured Koi receiving Saudi Bangla feed

Cultured Koi given Handmade feed (B3) contained higher amount of Cu, Cr, Cd and Pb in their body than initial concentration of those metals in collected feeds and fingerlings. The concentration of Ni was decreased in cultured Koi(figure 34).

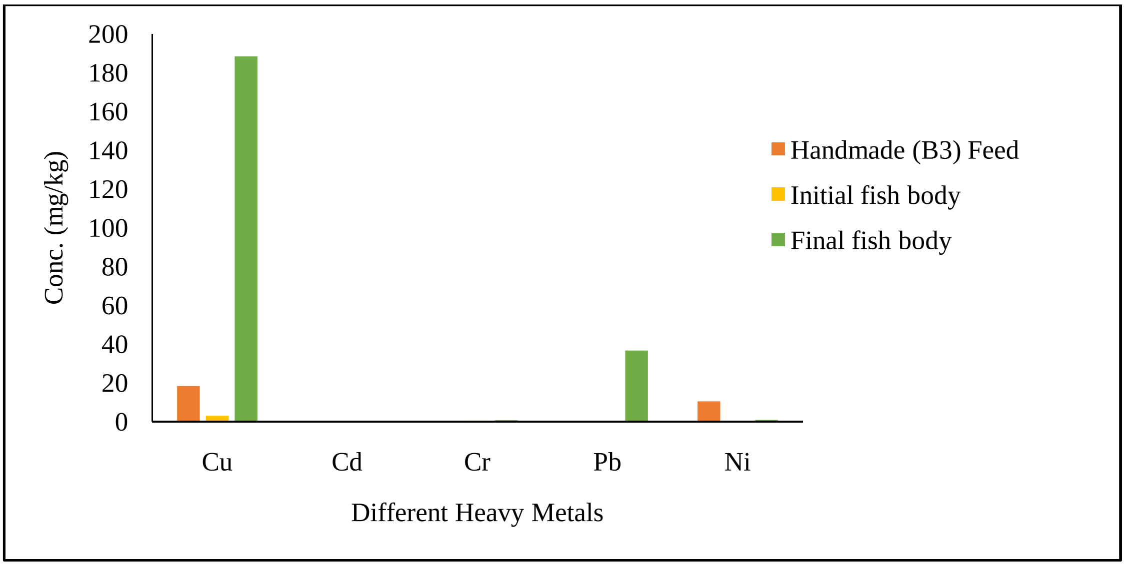


Figure 34Comparative study of different heavy metals in Handmade feed (B3), collected fingerlings of Koi and cultured Koi receiving Handmade feed (B3)

3.5 Water quality parameters

Different physicochemical parameters such as temperature (°C), dissolved oxygen (mg/l) and hydrogen ion concentration (pH) were recorded after each 30 days interval during the culture period of Koi.

Table 5The mean values (±SEM) of water quality parameters of different treatments

Parameters	Mega feed	Madina feed	Quality feed	Saudi Bangla feed	Handmade feed-B3	Level of significance (ANOVA)
Temperature	26.75±0.15	26.25±0.25	27.10±0.1	27.6±0.2	27.75±0.19	NS
Dissolve oxygen	5.16±1.67	6.06±0.08	6.41±0.02	6.37±0.09	5.8±0.1	NS

pH	8.01±0.1	8.08±0.01	8.12±.01	8.1±0.01	8.18±0.05	*

Table5 Water quality parameters (Mean±SEM) were recorded after 30 days interval from different treatments of experimental aquarium

NS: not significant (P>0.05), * Significant (P<0.05); Mean values with different superscript letters in each row indicate significantly difference (p<0.05).

Temperature

The water temperature of experimental ponds varied from 25.9 to 29.4°C during the study period. The highest average temperature (29.4°C) was recorded in Mega feed and lowest (25.9°C) was recorded in quality feed hence no significant difference (P>0.05) among the treatments. Average weekly variations of water temperature in pond under different treatments were shown in table-5.

Hydrogen ion concentration (pH)

The pH values of different treatments were found to be slightly alkaline ranging from 7.69 to 8.25. The highest average value of pH was recorded in Mega feed and lowest value was estimated Quality feed. There was significant difference in hydrogen ion concentration among different treatments when compared using ANOVA. The values of pHin Mega feed were significantly higher than other feeds.

Dissolve oxygen

The range of dissolved oxygen concentration in different treatments was 3.5 to 6.83 mg/l. The highest average value of DO was recorded in Mega feed and lowest value was recorded in Saudi Bangla feed. The values of DO in different treatments were not significant.

Chapter 4

Discussion

4.1 Heavy metals in collected feeds

The concentrations of different heavy metals were determined by using commercial fish feeds in Bangladesh. The result showed that there had been noticeable amount of different heavy metals in fish feeds. The concentrations of Cu and Pb were higher than other heavy metals. In Bangladesh, it has been accounted that the tannery solid wastes are changed to protein concentrate to be used as fish feed, poultry feed, and in production of organic fertilizers. It is a common phenomenon in the largest tanning area of the country for over the last several years, atHazaribagh tannery area at the southeast part of Dhaka city. One of the major concerns of these activities is the heavy metals, especially chromium, used in the tanning processes (Marufet al. 2007).

In the current regulations there areno maximum allowed concentrations of nickelfor feed ingredients and compound feed. Thelimits for the allowed nickel content indifferent human foods range between 0.1–8mg/kg (0.1 mg/kg in milk, 0.5 mg/kg in meatand meat products, 3 mg/kg in legumes, 5mg/kg in chocolate, 8 mg/kg in powderedcacao, 8 mg/kg in tea (Alexievaet al. 2007).This study showed that the highest concentration (33.42 mg/kg) of Ni was found in Madina feed and the lowest (1.72 mg/kg) was observed in Mega feed. The concentrations of Ni in Quality feed, Saudi Bangla feed and Handmade feed (B3) were 14.89 mg/kg, 10.94 mg/kg and 10.40 mg/kg respectively. The concentrations of Ni in all of the experimental feeds are above the acceptable limit except Mega feed.

According to FAO the acceptable limit of Cris 1.0 mg/kg. The concentration (1.04 mg/kg)of Cr was recorded in Quality feed which was highest among different experimental feeds. The lowest value (0.00 mg/kg) of Chromium was observed in Mega feed & Handmade feed (B3). The concentrations of Cr in Quality feed is above the acceptable limit.

The permissible cadmium concentration in feed ingredients of plant origin according tothe current regulations is 1 mg/kg and in those of animal origin 2 mg/kg (FAO 1983). The lowest concentration (0.12 mg/kg) of Cd was found in Mega feed and the highest concentration of 0.68 mg/kg was found in Madina feed. Therefore, this study found that the concentration of Cd is within acceptable limit.

The allowable limit of concentration of Copper as a food ingredient is 30 mg/kg (FAO 1983). This study showed that the concentrations (18.25 mg/kg) of Cu was found in Handmade feed (B3) which was the highest among other feeds and the lowest concentration (4.24 mg/kg) of copper was recorded in Madina feed. So, this study showed that the concentrations of Copper in different experimental feeds are in permitted limit.

The permitted lead content in feed ingredientsis 10 mg/kg according to World Health Organization (WHO, 1985). This study showed that the highestconcentration was recorded 4.92 mg/kg in Madina feed which is within the approved limit of WHO.

4.2 Growth performance of Koi

A number of feed industries have been established in Bangladesh and are producing fish feeds commercially. But these feeds are very costly and expected food conversion efficiency is not obtained. In this circumstance, proper utilization of these feeds can only improve the benefit level of feed by reducing feed costs that may be achieved by compensatory growth applying mixed feeding schedules. On the other hand, if the feeds are properly utilized, there is a less possibility of water quality deterioration, which ultimately will increase the production of fish.

Different growth performance parameters were measured under different feeding frequencies in koi. The growth parameters are condition factor, average daily gain, specificgrowth rate and food conversion ratio.

The highest condition factor was observed at 30^{th} day period in Handmade feed (B3)as 3.13±0.20and lowest was observed at 60th day period in Quality feed as 1.58±.096. In this study, the condition factor was increased in 30^{th} day period then decreased in 60^{th} day period. At the beginning of December 2016, the temperature was around 18 to 25ºC. It is well known that feeding frequency decreased in temperature below 20ºC. Therefore, the length and weight were decreased at 60^{th} day period. As condition factor depends on the weight and length, so the reason behind decreasing condition factor at 60^{th} day period was decreasing of water temperature. Rahmanet al. (1997) showed the survival and growth of catfish after giving supplementary feeds got the values of condition factor 0.81 to 0.87 %.

The results indicated that the highest Average Daily Gain (ADG) during the study period was found to be0.11±0.001 at 30th day period in Handmade feed (B3). The lowest value of ADG gain was recorded .03±.004at 90th day period in Mega feed. The statistical analysis indicated thatit is significant ($P<0.05$) difference among different treatments at 60th day period. The daily gain of Koi during the whole experimental period indicated that T5 (Handmade feed (B3)) and T4 (Saudi Bangla feed) groups showed significantly ($P<0.05$) higher values of daily gain as compared with groups fed T1 (Mega Feed) and T2 (Madina feed) respectively. The group fed on T2 (Madina feed) recorded the lowest value of daily gain. These results indicated that the average daily gain was decreased in 90th day period. Becauseaverage daily gain is decreased if temperature is decreased (Saber et al. 2004).Saadet al.2009 showed thattheaverage daily weight gain of tilapia increased graduallyreaching its maximum of 0.29, 0.41 and 0.38 g at the end ofthe experimental period for 20, 25 and 30°C, respectively. Itdecreased reaching 0.16 g at 15°C.

The highest and lowest average specific growth rate (SGR) was 4.59±.10 and 1.98±0.15 found in B3 and Mega feed respectively in 30th day of culture. In 60th day period, the highest SGR was found 4.68±.39 in Madina feed and the lowest value was found 0.80±.01 in Handmade feed (B3). And the values of the highest (2.77±.031) and the lowest (1.22±.11) specific growth ratein 90th day period were found in Quality feed and Mega feed respectively. Statistical analysis showed that the values of SGR in 60th day were significantly varied among different treatments. SGR progressively increase in feeding frequency and decreased with feed wastage. The significantly highest SGR in treatment T3 (Quality feed) during 30th day period might be due to the fact that fish have utilized efficiently the supplied feed. The SGR decreased in 30th and 90th day period, because the temperature was below 30ºC and for that reason food consumption rate became decreased. Hossain and Parween (1998) have recorded the highest SGR (1.8%) in *Heteropneustesfossilis* by supplemental diets which are similar to the present findings at 40th and 60th day of the study. Wing-Keonget al.(2000) reported SGR as 1.27%/day with 5% feeding BW/day in Bagrid catfish.

The highest FCR value 3.74±0.38was observed in T1 (Mega feed) during 30th day period and lowest FCR value (1.29±0.13)was observed in Quality feed during 90th day period. The lowest FCR value indicates that the experimental feeds were utilized properly.

Relatively better FCR value (1.6± .083) was detected by Marimuthuet al. (2010)in feeding catfish by feeding twice a day. Statistical analysis showed that there is not any significant difference among different treatments for FCR value.

4.3 Bioaccumulation of heavy metals

In cultured Koi, the concentration of studied of heavy metals were found higher than initial concentration in fish feeds and collected fingerling samples. So bioaccumulation has occurred in cultured fishes from experimental feeds and water used for culture. Heavy metal levels in cultured fish followed the ranking: Cu >Pb> Cr > Cd > Ni and as expected bioaccumulation was observed highest in intestines followed by gills and muscle. The highest concentration (368.67 mg/kg) of Cuobserved in intestineofKoi given Madina feed and the lowest concentration (14.03 mg/kg) was recorded in muscle of Koi receiving Quality feed. The highest concentration of Pb (138.28 mg/kg) observed in intestines of Koi givenMadina feed and the lowest concentration (0.00 mg/kg) was recorded in muscle of Koi receiving Quality feed.Bioaccumulation of Cr (3.20 mg/kg) was the highest in gills of fishes taken Mega feed and the lowest (0.00 mg/kg) in intestine of Koi cultured with different feeds.The concentration of Cu, Pb and Cr exceed the tolerable limit in fishes cultured with the experimental feeds. The concentrations of Cd,and Ni were below the permissible range approved by FAO.According to FAO the permissible limit of Cr is 1.00 mg/kg, Cu is 30 mg/kg and Pb is 10 mg/kg. The present study showed that concentration of Cr, Cu, Pb were significantly higher than the permissible limit in every organ studied.

Atli and Canli, (2011) indicated that kidney displayed the higher capacities on metal accumulation and metal-binding protein synthesis in O. niloticus. De Boecket al. (2003), Peyghanet al. (2003), and Soedariniet al. (2012) pointed out that liver is the key site for accumulating Cu and controlling homeostatic. Gill regulation is a time-dependent acclimation characterizing by both how quickly the acclimation is activated to prevent further effects and how long the acclimation stays in place (Diamond et al. 2006).As, Pb, Cd, Cr, Zn, and Cu in the muscle, gill, stomach, intestine, and liver of *Heteropneustesfossilis* caught at three different stations in the Buriganga River to assess the degree of heavy metal pollution. Significant differences in concentrations of analyzed elements were observed among different tissues, but not among the stations. The author showed that the liver appeared to be the main heavy metal storage tissue, whereas the muscle had the lowest levels of analyzed metals. The concentrations of metal in the muscles did not exceed the acceptable level as a source of food for human consumption.

4.4 Water quality parameters

Measurement of water quality parameters is pre-requisite for a maintaininga healthy aquatic environment and better production for aquatic organisms. This study found thatmeasured water quality parameters varied significantly.

Water temperature is one of the most importantfactors for aquatic organisms because it influences other physical and chemical factorsespecially dissolved oxygen content. With increase of water temperaturefood intake, metabolism and growth rate of fish increase. The temperature of the water available to aquarium fish depends upon geographic location, water supply and the design of the system. In the present study, water temperature in the aquarium water ranged from 25.9 to 29.4°C during the investigation period from October 2016 to December 2016 where the mean temperature was 27.02°C which was under the suitable range for growth. Boyd (1982) showed that the range of water temperature of 26.06 to 31.97°C is suitable for fish culture.

Among the dissolved gases, oxygen is most important and critical one in the naturalwater. Dissolved oxygen is required by all the aquatic organisms except anaerobicbacteria. Prolonged exposure to low concentrations of dissolved oxygen is harmful tofish. Continued exposure to low dissolved oxygen is also considered a precursor tobacterial infection in fish. The minimum concentration of dissolved oxygen tolerated byfish is obviously a function of the exposure time. A fish might survive in 0.5 mg/ldissolved oxygen concentration for a few hours but not for several days. In thepresent study, the concentrations of dissolved oxygen in the experimental aquarium werefluctuated and having the range from 3.5 to 6.83mg/l, which is more or less similar to thestudy of Zafar (1964); Banerjee, (1967);Azim et al. (1995) and Dewan, (1973). The mostsuitable range of dissolved oxygen in a water body for fish culture was suggested from5.0–8.0 mg/l (DoF, 1996). So, concentrations of dissolved oxygen were found quitesuitable for fish culture throughout the experimental period.

The pH of water has aprofound effect on the productivity of the water body. According toSwingle, (1967) the pH 6.5 to 9.0 is suitable for pond fish culture and pH more than 9.5 isunsuitable. Boyd, (1992) stated that the suitable range of pH is 6.5 to 8.5 for pond fishculture. DoF, (1996) reported that the suitable range of pH of a water body for fish culture should be 6.5 to 8.5. During the study period, pH values were slightly alkaline. Inthe present study, pH values inside aquariums varied from 7.69 to 8.25 which maintained thesuitable range. The mean values of pH were measured7.9±0.15, 7.6±0.12, 7.6±0.12, 7.42±.06, 7.4±.05 in T1 (Mega feed), T2 (Madina feed), T3 (Quality feed), T4 (Saudi Bangla feed) and T5 (Handmade feed (B3)) respectively.The measuredpH was found significantly different ($P<0.05$) among treatments.

Chapter 5

Conclusions and Recommendations

5.1Conclusions

The study was conducted to determine the levels of some physiochemical parameters, growth performance of Koi and bioaccumulation of heavy metals in Koi. From the above study, it can be concluded that the concentrations of heavy metals like lead, copper, cadmium, nickel, chromium in commonly used commercial feeds for Koi culture in Bangladesh that higher than the permissible limit set by FAO and WHO. The presence of the heavy metals in fish feeds could be observed due to uses of tannery wastes, industrial wastes to produce fish and poultry feeds. The analysis of different heavy metals in cultured koi fish confirms that there is ahigh chance of bioaccumulation of different heavy metals in thefish body by using commonly used commercial fish feeds in aqua firms. The determined concentration of Cu, Pb and Cr in thefinal fish body was so high. So this study may be concluded that fish consumers are exposed to health risks because most of the hatcheries in Bangladesh used those experimental feeds for koi culture. There is aneed for thedevelopment of fish consumption guidelines, so as to help fish consumers reduce exposure to toxic pollutants such as heavy metals. Local authorities, stakeholders and environmental authorities must act to reduce pollution of the lake and improve the health status of the fish in order to alleviate an impending public health disaster.

Fish gills and intestines should always be removed from fishes before cooking. Furthermore, fish intestines andgills should be removed from fishes before preserving them to avoid diffusion of the metals into surrounding tissues. The government should establish some laws regarding the production and raw materials used to produce fish and poultry feeds.

5.2Recommendations

The growing human population has increased the need for food supply. Because they are good protein sources, the demand for fish products has increased. Fishes fulfill 70% of the protein demand for thehuman population. So, fish culture is needed to fulfill the protein demand of growing people of our country. But if the concentration of different harmful heavy metals in fish feeds and water are in analarming rate, consuming those fish could cause aserious health risk, including cancer, lesions in skin and many other diseases. So, fish feed industries should stop using tannery wastes for producing fish feeds.

1) Only four commonly used commercial feeds were used in this study. There are lots of other fish feed companies in Bangladesh. Further study could be done by collecting several other local and commonly used commercial fish feeds.

2) In this study, concentrations of Cu, Cd, Pb, Cr and Ni were determined in collected fish feeds, fingerling samples and cultured fish but another heavy metal could also accumulate at ahigher amount than the studied metals.

3) The current study is totally laboratory based, so field studies are needed toclarify the actual accumulation of heavy metals in cultured fish.

4) The culture of Thai koi in aquarium is not very conductive. In that case, further experiment could be conducted with farmers like monosex tilapia; thaipangus etc. and feed prepared from quality ingredients like improved blood meal could provide more production and economic return.

5) Proper management and initiative measures should be taken immediately for reducing heavy metal pollution and enhancement of our aquatic resources.

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Appendices

Appendix I

Table 1: Condition factor (K) during rearing period (Mean ± SEM).

	Condition factor		
	30th day	60th day	90th day
Mega feed	2.1527±0.00866	2.3932±.26777	2.5237±.04513
Madinafeed	1.8186±0.0065	2.4553±.17986	2.3392±.04446
Quality feed	2.0565±.0667	1.5876±.09613	1.9359±.06482
Saudi Bangla	2.3746±.32305	2.6085±.19401	3.0459±.36667
Handmade feed (B3)	3.1336±1984	2.1881±.25374	2.2154±.27829

Tukey HSD (Condition Factor 30th day)

Treatment	N	Subset for alpha = 0.05	
		1	2
Madina feed	2	1.8186	
Quality feed	2	2.0565	
Mega feed	2	2.1527	
Saudi Bangla feed	2	2.3746	2.3746
Handmade feed(B3)	2		3.1336
Sig.		.285	.120

ANOVA

Condition Factor 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.026	4	.506	8.540	.019
Within Groups	.297	5	.059		
Total	2.323	9			

Tukey HSD (Condition Factor 60th day)

Treatment	N	Subset for alpha = 0.05
		1
Quality feed	2	1.5876
Handmade feed(B3)	2	2.1881
Mega feed	2	2.3932
Madina feed	2	2.4553
Saudi Bangla feed	2	2.6085
Sig.		.083

ANOVA

Condition Factor 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.268	4	.317	3.680	.093
Within Groups	.431	5	.086		
Total	1.698	9			

Tukey HSD (Condition Factor 90th day)

Treatment	N	Subset for alpha = 0.05

		1
Quality feed	2	1.9359
Handmade feed(B3)	2	2.2154
Madina feed	2	2.3392
Mega feed	2	2.5237
Saudi Bangla feed	2	3.0259
Sig.		.069

ANOVA

Condition Factor 90th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.320	4	.330	3.748	.090
Within Groups	.440	5	.088		
Total	1.760	9			

Appendix II

Table-2: Average Daily Gain (ADG) during rearing period (Mean ± SEM).

	Average daily gain		
	30th day	60th day	90th day
Mega feed	0.0187±.0016	.0410±.00090	.0366±.0041
Madinafeed	0.0311±.02823	.0803±.01487	.0564±.006
Quality feed	0.0385±.01210	.1569±.04257	.2821±.0458
Saudi Bangla	0.0692±.02090	.0404±.00453	.0874±.0344
Handmade feed (B3)	.1122±.00068	.0408±.00077	.1044±.0074

Tukey HSD (Average daily gain 30th day)

Treatment	N	Subset for alpha = 0.05
		1
Mega feed	2	.0187
Madina feed	2	.0311
Quality feed	2	.0385
Saudi Bangla feed	2	.0692
Handmade feed(B3)	2	.1122
Sig.		.052

ANOVA

Average daily gain 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.011	4	.003	5.082	.052
Within Groups	.003	5	.001		
Total	.014	9			

Tukey HSD (Average daily gain 60th day)

Treatment	N	Subset for alpha = 0.05	
		1	2
Saudi Bangla feed	2	.0404	
Handmade feed(B3)	2	.0408	
Mega feed	2	.0410	
Madina feed	2	.0803	.0803
Quality feed	2		.1569
Sig.		.658	.190

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.020	4	.005	6.213	.035
Within Groups	.004	5	.001		
Total	.025	9			

Tukey HSD Average daily gain 90th day

Treatment	N	Subset for alpha = 0.05	
		1	2
Mega feed	2	.0366	
Madina feed	2	.0564	
Saudi Bangla feed	2	.0874	
Handmade feed(B3)	2	.1044	
Quality feed	2		.2821
Sig.		.443	1.000

ANOVA

Average daily gain 90th days

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.077	4	.019	14.138	.006
Within Groups	.007	5	.001		
Total	.084	9			

Appendix III

Table 3: Food Conversion Ratio during rearing period (Mean ± SEM)

Treatment	Food conversion ratio		
	30th day	60th day	90th day
Mega feed	3.74±0.375	1.53±.09	2.06±0.24
Madinafeed	3.16±.275	1.35±.235	1.77±0.17
Quality feed	1.85±.61	1.39±0.145	1.29±0.13
Saudi Bangla	1.64±.18	1.93±0.65	2.18±0.34
Handmade feed (B3)	1.45±.14	3.3±0.28	1.65±0.11

Food conversion ratio 30th day

Tukey HSD

Food conversion ratio

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
Handmade feed (B3)	2	1.4550	
Saudi Bangla feed	2	1.6400	
Quality feed	2	1.8500	1.8500
Madina feed	2	3.1850	3.1850
Mega feed	2		3.7350
Sig.		.088	.065

ANOVA

Food conversion ratio

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.336	4	2.084	8.154	.020
Within Groups	1.278	5	.256		
Total	9.614	9			

Food conversion ratio 60th day

Food conversion ratio

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
Madina feed	2	1.3450	
Quality feed	2	1.3850	1.3850
Mega feed	2	1.5250	1.5250
Saudi Bangla feed	2	1.9300	1.9300
Handmade feed (B3)	2		3.3000
Sig.		.748	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

ANOVA

Food conversion ratio

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.349	4	1.337	5.721	.042
Within Groups	1.169	5	.234		

Total	6.518	9			

Food conversion ratio 90th day

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
Quality feed	2	1.2900
Handmade feed (B3)	2	1.6450
Madina feed	2	1.7750
Mega feed	2	2.0600
Saudi Bangla feed	2	2.1800
Sig.		.143

ANOVA

Food conversion ratio

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.993	4	.248	2.716	.151
Within Groups	.457	5	.091		
Total	1.449	9			

Appendix IV

Table 4: Specific growth rate (SGR) during rearing period (Mean ± SEM).

	Specific growth rate		
	30th day	60th day	90thday
Mega feed	1.9775±.15168	2.2825±.08518	1.2192±.011878
Madinafeed	.6435±.26239	4.6836±.39264	1.4290±.04891
Quality feed	3.3761±.72001	4.3230±1.14015	2.773±.03871
Saudi Bangla	4.3171±1.06954	1.2235±.30030	1.6595±.64189
Handmade feed (B3)	4.5921±.10413	.8013±.01023	1.4539 ±.07510

ANOVA

Specific growth rate 30th day (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22.015	4	5.504	7.796	.022
Within Groups	3.530	5	.706		
Total	25.546	9			

Specific growth rate 30th day (%)

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2

Madina feed	2	.6435	
Mega feed	2	1.9775	1.9775
Quality feed	2	3.3761	3.3761
Saudi Bangla feed	2		4.3171
Handmade feed(B3)	2		4.5921
Sig.		.105	.121

ANOVA

Specific growth rate (%) 90th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.036	4	.759	4.355	.069
Within Groups	.871	5	.174		
Total	3.907	9			

Specific growth rate 90th day (%)

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
Mega feed	2	1.2192
Madina feed	2	1.4290
Handmade feed(B3)	2	1.4539
Saudi Bangla feed	2	1.6595
Quality feed	2	2.7730
Sig.		.066

Specific growth rate 60th day (%)

Tukey HSD

Treatment	N	Subset for alpha = 0.05		
		1	2	3

Handmade feed(B3)	2	.8013		
Saudi Bangla feed	2	1.2235	1.2235	
Mega feed	2	2.2825	2.2825	2.2825
Quality feed	2		4.3230	4.3230
Madina feed	2			4.6836
Sig.		.427	.054	.129

ANOVA

Specific growth rate 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.043	4	6.261	10.087	.013
Within Groups	3.103	5	.621		
Total	28.146	9			

Appendix V

ANOVA

Temperature

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.034	4	.759	13.791	.007
Within Groups	.275	5	.055		
Total	3.309	9			

Temparature

Tukey HSD

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Madina feed	2	26.2500		
Mega feed	2	26.7500	26.7500	
Quality feed	2	27.1000	27.1000	27.1000
Saudi Bangla feed	2		27.6000	27.6000
Handmade feed (B3)	2			27.7500
Sig.		.072	.072	.172

ANOVA

PH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.031	4	.008	1.485	.333
Within Groups	.026	5	.005		

Total	.056	9			

PH

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
Mega feed	2	8.0100
Madina feed	2	8.0750
Saudi Bangla feed	2	8.1000
Quality feed	2	8.1150
Handmade feed (B3)	2	8.1800
Sig.		.260

ANOVA

DO

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.066	4	.516	.462	.763
Within Groups	5.593	5	1.119		
Total	7.659	9			

DO

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
Mega feed	2	5.1650
Handmade feed (B3)	2	5.8000
Madina feed	2	6.0550
Saudi Bangla feed	2	6.3700
Quality feed	2	6.4050
Sig.		.767