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Comparative study of proximate composition and mineral contents in *Cyprinus carpio* and *Hypothalmichthys molitrix* collected from River Sutluj

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Abstract

The current study was carried out to estimate the chemical composition and mineral contents in *Cyprinus carpio* (common carp) and *Hypophathalmic molitrix* (silver carp). The main objectives were to find the effect of feeding habitat on the fish growth, to determine the nutrients and minerals composition in the fish body. The samples of fish carcass were analyzed by standard methods (AOAC, 1995). The maximum percentage of protein, fat, moisture and ash in *C. carpio* were 67.19±0.56, 19.96±0.44, 0.29±0.05 and 8.97±0.57 respectively while in *H. molitrix were* 67.58±2.17, 19.63±1.67, 0.64±0.08 and 12.33±0 respectively. The maximum percentage of Na, K, Ca, Cr, Zn, Mn in *C. carpio* 2.121±0.837, 10.362±4.227, 0.514±0.231, 0.072±0.017, 0.036±0.014 and 6.163±2.494 while in *H. molitrix were* 0.847±0.306, 14.052±5.769, 0.441±0.344, 0.023±0.009, Cr was 0.036±0.014 and 4.231±1.703 respectively. The Co-Stat computer software (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis. The present study showed important information and significant difference ($P<0.05$) between the studied fish species regarding proximate composition and mineral contents.

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Introduction

Aquaculture has been known as the most famous for fast manufacturing food industry in the whole world. The increasing demand, inadequate and unbalanced supply, high rate of fish meal with the development of aquaculture made it essential to find out substitute protein sources (Hussain *et al.,* 2014). Fish is easily digestible because of short muscles and deficiency of scleroprotein and elastin (Cirkovic *et al.,* 2012). Protein of fish is more beneficial for human health and it has free amino acids (Buchtova *et al.,* 2010). The nutrient quality is dependent on their kind of food and feeding behaviors. Watermann (2000) conducted a research on the proximate composition of fishes and explored that the measurement of proximate contents like protein, carbohydrates, lipids, moisture and ash contents is often necessary to ensure that they meet the requirements of food regulations and commercial specifications. Some workers (Achionye-Nze and Omoridion, 2002; Abdullahi and Balogun, 2006) have earlier, investigated the proximate and mineral contents of some fishes in rivers and reservoir and reported that freshwater fishes are good sources of minerals and protein.

Body composition of any edible animal, including fish is a key indicator of its biological and functional condition, but it is time consuming process (Ali *et al.*, 2005). Every component of the body works as good indicator for specific contents (Chatta *et al.,* 1993) moisture content and water indicates its energy contents relatively such as lipids and protein, in other words water is inverse proportion to these two such as lower percentage of water mean a greater amount of lipids as well as protein and vice versa (Anthony *et al.*, 2000) however these are not fixed values as these differ considerably inter specifically and intraspecific ally. It also varies on the basis of age, size, physical activity, feeding season, reproductive status, fishing season and sexual condition (Deegan, 1986).

River Sutluj is the longest river and flows through the historic crossroad region of Punjab in northern India and Pakistan. Sulemanki Headworks is on the River Sutluj in the Punjab province of Pakistan and is used for irrigation and flood control. It receives the Beas River and forms 65 miles (105 km) of the India-Pakistan border before entering Pakistan and flowing another 220 miles (350 km) to join the Chenab River west of Bahawalpur (Tabinda *et al.*, 2013).

Hypophthalmichthys molitrix is a warm water fish (Frimodt, 1995). Points out that this species is famous for being consumed. This fish can resist against disease, stress and it develops more rapidly. This fish is highly produced because of its ability to survive under rough environment. This fish belongs to cyprinid and lives in fresh water of China and Siberia. The range of production of silver carp is more than that of grass carp through the whole world. Silver carp is commonly produced with some other carps like Asian carp and catla in polyculture (Kolar *et al.,* 2005). The average length of silver carp is 60-100 cm (24-39 in) while its maximum length is 140 cm (55 in). The weight of this carp is 50 kg (110 lb) (Maccracken, 2016). This carp belongs to filter feeder and possess a particular device for feeding and filters very tiny particles of 4 µm. Zooplankton and detritus are also the food of silver carp (Willink, 2009).

Cyprinus carpio is one of the most widely developed in freshwater throughout the world. The range of production of this carp in freshwater is 3.6 million tonnes. Tokur *et al.* (2006) gave the reason behind this production is rapid growth, easy farming. The value and efficiency of food are also the reasons of this fast production. *C. carpio* is one of the most economically important fish species in aquaculture and stock enhancement (Kim *et al.,* 2001). The *C. carpio* is cyprinid fish and live in medium or lower pressure of water in rivers and their habitations are mostly grassy zones with a dark bottom. Carp fry consume zooplankton and when they grow up then they behave as benthetic feeders and feed on other animals and plants. Tycon, (1991) points out that 3% of the cyprinids are produce in intensive systems.

The nutritive needs of *C. carpio* for amino acids, lipids, protein, minerals, fatty acids, vitamins, carbohydrates and their energy ratios have been explored by many scholars and reviewed (Satoh, 1991). This research was needed to study the body composition and mineral contents of common carp and silver carp due to their economical and nutritive worth, which will help to choose best fish in future as source of important nutrients and minerals. The main objectives of this research work was to find out the nutrients, minerals composition of fish and comparison of the components in the fish samples collected from different water sources.

Materials and methods

Procedure of sampling

A fish net was used to capture common carp and silver carp from Sulemanki head works of River Sutluj, Okara, Pakistan and local Fish Seed Hatchery, Okara, Pakistan. These fishes were stored in thin plastic bags containing dry ice and was labeled. These labeled bags were transported to research laboratory, Department of Zoology, GC University, Faislabad for further analysis.

Chemical analysis of fish carcass

Body composition and mineral contents from carcass of these fish species was analyzed by standard methods (AOAC, 1995). Moisture was determined by oven-drying at 105°C for 12 h; crude protein (N × 6.25) by micro kjeldahl apparatus; crude fat by petroleum ether extraction method through Soxtec HT2 1045 system; crude fiber as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH; Ash, by ignition at 650°C for 12 h in electric furnace (Eyela-TMF 3100) to constant weight. Gross energy was determined with the help of oxygen bomb calorimeter.

Mineral estimation

The fish carcass samples of fish were digested in boiling nitric acid and perchloric acid mixture (2:1) according to AOAC, 1995. After appropriate dilution, mineral contents were estimated by using Atomic Absorption Spectrophotometer. The phosphorus was analyzed calorimetrically (UV/VIS spectrophotometer) at 350nm.

Proximate analyses

The analysis included in this group, also known as Weende proximate analysis, are applied firstly to materials to be used in formulating a diet as a protein or energy source and to finished feedstuffs, as a control to check that they meet the specifications or requirements established during formulation. These analyses were show the moisture, crude protein (total nitrogen), crude lipids, ash and nitrogen-free extract content of the sample. A fuller description of these analysis can be found in Osborne and Voogt (1978), MAFF (1982) and AOAC (1984).

Moisture

In balancing the ration, it is essential to know the water content of each component; also, moisture in prepared feed must be monitored because levels over 8% favour the presence of insects, and over 14% there is the risk of contamination by fungi and bacteria (Cockerell *et al.,* 1971). The method is based on drying a sample in an oven and determining moisture content by the weight difference between dry and wet material. Weigh out approx. 5-10 g of previously ground sample. Placed sample in drying oven at 105°C for at least 12 h. Let sample cool in dryer. Weight again, taking care not to expose the sample to the atmosphere.

Crude protein

Because of its cost this is the most important dietary nutrient in a commercial operation; proper evaluation of it means that the quality of protein intake or of the feed being provided can be controlled. Analysis is by Kjeldahl's method, which evaluates the total nitrogen content of the sample after it has been digested in sulphuric acid with a mercury or selenium catalyst. Weight out 1 g of sample and placed in the Kjeldahl flask; added 10g potassium sulphate, 0.7 g mercuric oxide and 20 ml concentrated sulphuric acid. Placed the flask tilted at an angle in the digester, bring to boiling point and retained until the solution is cleared; continue to heat 30 minutes more. If foam is too abundant, added a little paraffin wax. Left to cool, gradually adding approximately 90 ml distilled, de-ionized water. When cold added 25 ml sodium sulphate solution and stir. Added one glass bead and 80 ml of 40% sodium hydroxide solution, keeping the flask tilted. Two layers will form. Quickly connected the flask to the distillation unit, heat and collect 50 ml of distillate containing ammonia in 50 ml of indicator solution. At the end of distillation, removed the receptor flask, rinse the end of the condenser and titrate the solution with the standard perchlorhydric acid solution.

Crude lipids

In this method, the fats are extracted from the sample with petroleum ether and evaluated as a percentage of the weight before the solvent is evaporated. Removed extraction flasks from the kiln without touching them with the fingers, cooled in a dryer and weigh to within milligrams. Weigh 3 to 5 g of dry sample to within milligrams in an extraction thimble, handling it with tongs and placed in the extraction unit. Connected the flask containing petroleum ether at 2/3 of total volume to the extractor. Bring to boil and adjust heat to obtain about 10 refluxes per hour. The length of the extraction will depend on the quantity of lipids in the sample. Very fatty materials will take 6 hours. When finished, evaporate the ether by distillation or in a roto evaporator. Cooled the flask in a dryer and weigh them to within milligrams. The defatted sample can be used in determining crude fibre.

Ash

This method is used to determine ash content in feedstuffs by calcination. Ash is considered as the total mineral or inorganic content of the sample. Placed 2.5 to 5 g of dry sample in a crucible previously calcined and brought to constant weight. Placed the crucible in a furnace and heat at 550°C for 12 hours; left to cool and transfer to a dryer. Carefully weigh the crucible again with the ash.

Statistical analysis

Data of nutrient (crude protein, crude fat and apparent gross energy) and mineral contents were subjected to one-way analysis of variance (Steel *et al.,* 1996). The differences among means were compared by Tukey's Honesty Significant Difference Test and were considered significant at $p<0.05$ (Snedecor and Cochran, 1991). The Co-Stat computer software (Version 6.303, PMB 320, Moneterey, CA, 93940 USA) was used for statistical analysis.

Results and discussions

Analysis of carcass composition in C. carpio and H. molitrix

Apparent Protein % in C. carpio

The current study reveals that the protein (dry) values were observed 66.54±0.39, 67.53±0.36 and 67.51±.33 in hatchery, River I and River II respectively. The results indicated that these values were highly significant ($P<0.05$) from each other.

Apparent fat % in C. carpio

The values of crude fat observed were 20.46±0.37 and 19.69±0.16 and 19.73±0.14 in hatchery, River I and River II respectively. All these values were significantly different ($P<0.05$) from each other. These variations could be due to their different habitats, size and sex of the fish, physiology of the water in which these fish live.

Apparent moisture (%) in C. carpio

Values of moisture observed in *C. carpio* were 0.27±0.02 and 0.27±0.02 and 0.35±0.04 in hatchery, River I and River II respectively. Results showed minimum values of moisture were in hatchery and River I as compared than the maximum value of moisture was in River II. Comparison of means indicated that moisture value in River II was maximum as compared than River I and hatchery. These results revealed that the values of moisture were significantly different ($P<0.05$) from each other. These variations in the mean values of moisture could be due to certain factors such as their different habitats.

Table 1. Carcass composition (%) and mineral analysis in *C. carpio* and *H. molitrix*.

Parameters	*C. carpio*			*H. molitrix*		
	River I	River II	Hatchery	River I	River II	Hatchery
Crude protein	67.51±0.33[a]	67.53±0.36[a]	66.54±0.39[b]	69.76±0.17[a]	68.42±0.12[b]	67.57±0.22[c]
Crude fat	19.69±0.16[b]	19.73±0.14[b]	20.46±0.37[a]	20.57±0.22[a]	17.71±0.33[b]	20.62±0.18[a]
Crude moisture	0.27±0.02[b]	0.35±0.04[a]	0.27±0.02[b]	0.65±0.03[b]	0.55±0.02[c]	0.72±0.03[a]
Ash	8.62±0.24[b]	8.67±0.18[b]	9.63±0.16[a]	11.75±0.19[b]	12.64±0.21[a]	12.59±0.18[a]
% of Na	1.72-2.23[b]	1.36-1.08[a]	3.28-0.14[c]	0.717±0.781[b]	0.555±0.338[a]	1.27±0.165[c]
% of K	8.23-11.33[b]	6.59-5.77[a]	16.27-0.18[c]	11.114±15.555[b]	8.928±7.948[a]	22.113±0.115[c]
% of Ca	0.841±0.241[b]	0.361±0.432[a]	0.34±0.001[c]	0.908±0.062[b]	0.324±0.507[a]	0.089±0.001[c]
% of Mn	0.089±0.053[b]	6.047±0.044[a]	0.77±0.001[c]	0.018±0.025[b]	0.015±0.013[a]	0.036±0.001[c]
% of Cr	0.029±0.036[b]	0.024±0.017[a]	0.055±0.004[c]	0.029±0.037[ab]	0.023±0.018[a]	0.056±0.003[b]
% of Zn	4.914±6.687[a]	3.929±3.361[a]	9.643±0.18[b]	6.606±0.162[b]	2.311±3.721[a]	6.606±0.162[c]

Apparent ash (%) in C. carpio

The values of ash 9.63±0.16 and 8.62±0.24 and 8.67±0.18 were in hatchery, River I and River II respectively. Minimum ash values were in River I and River II as compared than the value 8.67±0.18 of ash in hatchery. Comparison of means indicated that ash value in hatchery was significantly different ($P<0.05$) from the value of ash in River I and River II.

Apparent Protein % in H. molitrix

The values of protein (dry) in *H. molitrix* were observed 67.57±0.22 and 69.76±0.17 and 68.42±0.12 in hatchery, River I and River II. The minimum value of protein 67.57±0.22 was observed in hatchery as compared than the values of protein were in River I and River II. It was clear from the results that these values of protein were significantly different ($P<0.05$) from each other.

Apparent fat % in H. molitrix

The values of fat observed were 20.46±0.37 and 20.57±0.22 in hatchery and River I respectively as compared than the mean values of fat 17.71±0.33 in River II. These concentrations of means were significantly different ($P<0.05$) from value of River II.

Apparent moisture (%) in H. molitrix

The values of moisture were 0.72±0.03, 0.65±0.03 and 0.55±0.02 were in hatchery, River I and River II respectively. The results showed that the maximum 0.72±0.03 value of moisture was in hatchery. Comparison of means indicated that moisture values in River I and River II and in hatchery were significantly different ($P<0.05$) from each other could be due to their different habitats.

Apparent ash % in H. molitrix

The mean concentration values of ash were 12.59±0.18, 11.75±0.1 and 12.64±0.21 were in hatchery, River I and River II respectively. Comparison of means indicated that ash values in hatchery, River I and River II were significantly different ($P<0.05$) from each other.

Analysis of mineral contents in C. carpio

Apparent Na and K (%)

Na values in *C. carpio* were observed 3.28±0.14, 1.72±2.23 and 1.36±1.08 in hatchery, River I and River II respectively. Minimum Na value 3.28±0.14 was in hatchery as compared than the mean values of Na in River I and River II. Comparison of means indicated that Na values in hatchery, River I and River II were significantly different ($P<0.05$) from each other. Variations in mean values of Na could be due to the availability of Na in their different habitats. The values of K observed were 16.27±0.18, 8.23±11.37 and 6.59±5.77 in hatchery, River I and River II respectively. Comparison of means indicated that K values in hatchery, River I and River II were significantly different (P<0.05) from each other due to certain factors such as the availability of K in their different habitats, their different capacity to absorbed K etc.

Apparent Ca and Mn (%)

The values of Ca observed were 0.34±0.001, 0.841±0.241 and 0.361±0.432 were in hatchery, River I and River II respectively. Comparison of means indicated that Ca values in hatchery, River I and River II were significantly different (P<0.05) from each other. It was reveal that values of Mn were 0.077±0.001, 0.089±0.05374 and 0.047±0.044 in hatchery, River I and River II respectively. Comparison of means indicated that Mn values in hatchery, River I and River II were significantly different (P<0.05) from each other due to certain factors such as the availability of Mn in their different habitats, their different capacity to absorbed Mn etc.

Apparent Cr and Zn (%)

In the current study the values of Cr observed were 0.055±0.004, 0.029±0.036 and 0.024±0.017 in hatchery, River I and River II respectively. Comparison of means indicated that Cr values in hatchery, River I and River II were significantly different (P<0.05) from each other. The Zn values observed were 9.643±0.185, 4.914±6.687 and 3.929±3.36 in hatchery, River I and River II respectively. Comparison of means indicated that Zn values in River I and River II and in hatchery were significantly different (P<0.05) from each other.

Apparent Na and K % in the carcass of H. molitrix

Na values were 1.27±0.165, 0.717±0.781 and 0.555±0.338 in hatchery, River I and River II respectively. Comparison of means indicated that Na values in hatchery, River I and River II were significantly different (P<0.05) from each other. It was reveal that K values were 22.113±0.115, 11.114±15.555 and 8.928±7.94 in hatchery, River I and River II respectively. Comparison of means indicated that K values in hatchery, River I and River II were significantly different (P<0.05) from each other due to certain factors such as the availability of K in their different habitats, their different capacity to absorbed K etc.

Apparent Ca and Mn %

From the results mean concentrations of Ca values were 0.089±0.001, 0.908±0.062 and 0.324±0.507 were in hatchery, River I and River II respectively. Comparison of means indicated that Ca values in hatchery, River I and River II were significantly different (P<0.05) from each other. From the results that Mn values were observed 0.036±0.001, 0.908±0.062 and 0.324±0.507 in hatchery, River I and River II respectively. Comparison of means indicated that Mn values in hatchery, River I and River II were significantly different (P<0.05) from each other due to certain factors such as the availability of Mn in their different habitats, their different capacity to absorbed Mn etc.

Apparent Cr and Zn %

From the results mean values of Cr observed were 0.056±0.003, 0.029±0.037 and 0.023±0.018 in hatchery, River I and River II respectively. Comparison of means indicated that Cr values in hatchery, River I and River II were significantly different (P<0.05) from each other. Zn values observed were 6.606±0.162, 6.606±0.162 and 2.311±3.721 were observed in hatchery, River I and River II respectively. Comparison of means indicated that Zn values in hatchery, River I and River II were significantly different (P<0.05) from each other due to certain factors such as the availability of Zn in their different habitats, their different capacity to absorbed Zn etc.

Discussion

Proximate composition (Protein, fat, moisture, ash) and mineral contents showed significant variations among the species and in different specimens of the same species. Various studies have been carried out on the proximate compositions of fish species. Watermann (2000) worked on the proximate composition of fish species and concluded that the measurement of proximate profiles such as protein, lipids, moisture, and ash contents is often necessary to ensure that they meet the requirements of food regulations and commercial specifications. Some workers (Achionye-Nze and Omoridion, 2002; Abdullahi and Balogun, 2006) investigated the proximate/mineral content of some fishes in rivers and reservoir in Nigeria and reported that freshwater fishes are good sources of minerals and protein.

According to Sinclair and Duncan (1972) lipids is the most essential reserve of food. This has led to employ fat indices to measure the association between percent fat and water. According to (Barros *et al.*, 2000 and Yildirim *et al.,* 2003) fat contents are associated closely to gained weight and inversely linked with moisture content of the body. In this study both fish species were having variation between fat contents, which may be attributed to catchment and collection from different geographic locations. (Olagunju *et al.,* 2012) showed the same trend of crude protein for Tilapia (18.8%). Proteins are the second most important fish constituent (FAO, 2005). Fish protein is an excellent source of lysine, methionine and cysteine and can significantly raise the value of cereal based diets, which are poor in these essential amino acids. The amount of protein in fish muscle is usually between 15 and 20%, even though in rare cases it has been found to be as low as 13.5% or as high as 28% (Murray and Burt, 1969).

Different researchers have also reported that moisture contents can vary with sex of the fish (Amer *et al.,* 1991; Islam and Joadder, 2005; Alemu *et al.,* 2013). The slightly lower moisture content of larger fish in the present study can be attributed to muscles containing more organic materials and less water than the young fish. The functions of inorganic elements include the formation of skeleton structure, electron transfer, regulation of acid base equilibrium and osmoregulation. Minerals also are important components of hormones, enzymes and vitamins. They activate complex biochemical mechanisms, control, and regulate the uptake, storage and excretion of various inorganic elements allowing fish to live in a dynamic equilibrium with their aquatic medium (Sinha *et al.,* 1990).

Conclusion

The current research work proved that there is a significant difference ($P<0.05$) between the two studied fish species *Cyprinus carpio* and *Hypophthalmichthys molitrix*. The results provided up to date and important information about differences between the studied fish species regarding proximate composition and mineral contents. From the results it proved that fresh water fishes are good sources of minerals and are protein rich than hatchery samples. The variations could be due to certain factors such as their different habitats, availability of food, water quality etc.

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