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Comparative Study of Proximate Composition, Minerals, Vitamins and Fatty Acid Contents in *Labeo rohita* (Hamilton, 1822) Reared In Freshwater and Treated Wastewater

S. Chanda[1], B.N. Paul[1*], P. Singh[1,] K. Ghosh[2] and S.S. Giri[3]

[1]Regional Research Station, Central Institute of Freshwater Aquaculture, Rahara, Kolkata- 700 118, West Bengal
[2]Aquaculture Laboratory, Department of Zoology, The University of Burdwan, Golapbagh, Burdwan- 713 104
[3]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751002, Odisha
*Correspondence: bnpaulcifa@gmail.com

ABSTRACT

The study was conducted to investigate the variation in proximate composition, mineral contents, vitamin contents and fatty acid profile of *Labeo rohita* reared in Freshwater (FW) and wastewater (WW) of different weight ranges. The samples were collected from different location of West Bengal and were categorized based on body weight as 1-500(g) as BW1 and 501-2000 (g) as BW2 for both (FW and WW) reared system and they were grouped into 4 treatments viz., TR1(BW1FW), TR2(BW1WW), TR3(BW2FW) and TR4(BW2WW). The protein content was significantly higher (P<0.05) in TR4(BW2WW) reared in WW. Sodium content was significantly higher (P<0.05) in TR1(BW1FW), reared in Freshwater whereas Potassium content significantly higher (P<0.05) in TR4(BW2WW). Iron and Zinc content in rohu was significantly higher (P<0.05) in TR2 and TR3. All the fat-soluble vitamins content (A,D,E &K) were significantly higher (P<0.05) in TR1(BW1FW) reared in Freshwater. The SFA content in rohu sample was significantly (P<0.05) higher in TR2 whereas palmitic acid was significantly higher (P<0.05) in TR1 and TR2.MUFA and oleic acid content in rohu is significantly higher (P<0.05) higher in TR4. PUFA, α linoleic acid, EPA and DHA are significantly higher (P<0.05) TR4 having weight range (501-2000g) reared in WW. The protein, PUFA, α linoleic acid, EPA, DHA, MUFA, Oleic acid and potassium is higher in rohu of weight range (501- 2000g) reared in WW (TR4(BW2WW). Sodium and Vitamin A,D ,E and K is higher in rohu of weight range (1-500g) reared in FW (TR1(BW1FW).

KEYWORDS: Fatty acids, Mineral, Proximate composition, Rohu, Vitamin

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INTRODUCTION

The fisheries and aquaculture sectors are vital in ensuring global food and nutritional security. As per FAO (2022), the global production of aquatic animals was a staggering 178 million tonnes in 2020, with aquaculture contributing significant 87.5 million tones. The present practice in India is bispecies aquaculture, in which 80% to 90% of the population is shared by rohu (Giri, 2024). Globally, more than 1 billion people rely on fish for consumption and livelihood (FAO, 2016). Fish are excellent sources of essential nutrients like protein, amino acids and fatty acids (Zula and Desta, 2021). It plays a major role in the creation of growth hormones of the body (Holecek, 2020). The protein, fat and moisture content range between (15-30), (0-25) and (50-80) % respectively (Chakma et al., 2020). Apart from that minerals play a pivotal role in maintaining acid-base and water balance, formation of teeth structure and bones and accelerating metabolic reactions in the human body (Zhang et al., 2020). In catla the protein, zinc and selenium were in WW reared catla compared to FW as reported earlier (Paul et al., 2018). Fish consumption plays an important role in preventing coronary artery disease in humans, lowering the risk of breast cancer, asthma, inflammatory bowel disease, rheumatoid arthritis etc (Ullah et al., 2022).

Fish is a source of macro minerals (Ca, Mg, Na, K, P, Cl, S) and micro minerals (Fe, Zn, Mn, Cu,I, Co, Ni, F, V, Cr and etc.) minerals. The analysis of chemical composition of fish gives assessment of food composition of fish, its physiological condition and can serve as a guide for any future feed for fish in captivity (Dempson et al., 2004). Hence, this study was undertaken to find out the differences in chemical composition of fish reared in fresh and wastewater conditions.

MATERIALS AND METHODS

The samples were collected from different places of West Bengal viz., Rahara fish farm of ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga (Odisha), Malancha, Berhampur, Barasat, Mudiyali Fishery co-operative, Pandua, Bongaon, Nazat; from each location 20 nos. of rohu samples were collected from each Freshwater (FW) and Wastewater (WW) rearing systems. Weight of the fish samples were noted down at the time of collection which was divided into BW1 (1-500g) and BW2 (501-2000g). As per the weight ranges BW1 (1-500g) and BW 2 (501-200g) and water sources Freshwater (FW) and Wastewater (WW); grouped to four treatments viz., TR1(BW1FW), TR2(BW1WW), TR3 (BW2 FW) TR4 (BW2WW). The collected fish samples were immediately stored in an ice container and after brought into the laboratory prepared for evisceration and the head was removed. The representative portion of the edible part was taken and homogenized in a mixer for further analysis. The sampling procedure and sample preparation for analysis were followed as per the methodology of the Outreach Project on Nutrient Profiling of ICAR (Sankar et al., 2010). The sample preparation for vitamin analysis was done as per (Sankar et al., 2010). 10 g of Fish tissue was grinded with anhydrous sodium sulphate and extracted oil using 2:1 chloroform: methanol after adding BHA. Finally, the non-saponifiable matter was filtered through 0.45µ syringe filter and stored under refrigerator. Vitamins were quantified by injecting 5µl of prepared sample in High pressure liquid chromatography (HPLC). The HPLC consisting of a quaternary gradient pump, programmable variable wavelength, UV detector was used for the analysis. The wavelength used for eluting different vitamins is as follows. 265nm for vitamin D, 325nm for vitamin A, 291nm for vitamin E and 250nm for vitamin K. The vitamin content in the unknown sample was determined from the linear graph drawn for the standard.

Proximate composition of fish tissue was analyzed as per (AOAC, 2005). The mineral (Na, K, Fe, Mn, Zn and Se) assay was done as per (AOAC, 2005; Paul et al., 2014). Fatty acid profile was analyzed as per standard protocol of Gas Chromatography. Pooled samples were extracted as per (Folch et al., 1957) using chloroform: methanol in 2: 1 ratio (v/v) as solvent system that contained 0.01% butylated hydroxyl anisole as an antioxidant. Fatty Acid Methyl Esters were prepared by the transmethylation with boron trifluoride in methanol from lipids fraction according to (Metcalfe et al., 1966). The FAMEs were quantified by injecting 1 µL (50:1 split ratio) into a Gas Chromatograph/GC (Perkin Elmer, CLARUS 480). The oven temperature was programmed from an initial temperature at 30^0C rising to 140^0C (hold time 4 minute) and up to 200^0C. Nitrogen gas was used as a carrier gas. The injection port and the flame ionization detector were maintained at 260^0C and 300^0C. GC operating software "Total Chrom" was followed. Identification of individual fatty acids was done by comparison of retention time to those of standards (SUPELCO, Cat No. 47885-U) and quantification by comparing with respective areas as per the method of Paul et al., (2015b). Data were subjected to statistical analysis (Snedecor and Cochran, 1994) by one way ANOVA, and the least significant difference was used for comparison of the mean values.

RESULTS AND DISCUSSION

The proximate composition, mineral concentration and vitamin content of rohu of different weight ranges of WW and FW are presented in Table 1. The moisture and protein content ranged from 76.96-79.09 and 13.59-16.52 respectively. The crude protein content of rohu was significantly higher ($P<0.05$) higher in TR1 and TR2. Moisture and lipid content of rohu did not differ significantly among the treatments.

The moisture content of IMC ranged from 76 to 77(%) as reported by (Ullah et al., 2022) was in agreement with our result. The moisture content of the rohu in the present study was also within the ranged reported by (Paul et al., 2016; Shakir et al., 2013). The moisture content of the fish in the present study was higher than earlier reports in carps (Jabeen and Chaudhary, 2011) However, Sankar and Ramachandran, 2001 reported the 77-81% moisture content in IMC, which was higher than our findings.

In the present study protein content in the rohu muscles ranged from 13.59 to 16.52, was in the range of protein levels for carp (FAO, 2008). The protein content of rohu as reported in our study was in agreement with earlier reports (Paul et al., 2016; Shekhar et al., 2004). (Hossain et al., 2015) reported protein content in *Puntius gonionotus* is 16.7% which is in agreement with our results

Table 1. Nutrient composition, mineral concentration and vitamin content of rohu of different weight ranges collected from freshwater (FW) and wastewater (WW).

Particulars	TR1 (BW1FW)	TR2 (BW1WW)	TR3(BW2FW)	TR4 (BW2WW)
Nutrient	Composition	(% WW basis)		
Moisture ^{NS}	76.96 ±1.11	79.09±0.33	76.54±0.56	76.69±0.57
Crude Protein	13.59^{a} ±0.15	14.76^{b}±0.21	15.79^{bc}±0.66	16.52^{c}±0.62
Crude lipid	1.80 ±0.26	1.93±0.30	2.04±0.2	2.16±0.23
Ash	3.35^{b} ±0.34	3.38^{b}±0.07	2.33^{a}±0.23	2.57^{a}±0.14
Mineral	content (ppm)			
Sodium (Na)	189.54^{b}±7.94	214.94^{c}±2.94	164.73^{a}±5.78	189.44^{b}±4.77
Potassium (K)	262.73^{b} ±2.60	268.61^{bc} ±2.53	241.77^{a}±5.92	278.93^{c} ±3.96
Iron (Fe)	2.59^{c} ±0.55	1.34^{b}±0.22	1.73^{b}±0.22	1.2^{a}±0.07
Manganese (Mn)	0.43±0.23	0.55±0.02	0.45±0.07	0.53±0.03
Zinc (Zn)	3.66^{c} ±0.34	1.9^{b} ±0.2	1.53^{b} ±0.07	1.30^{a}±0.04
Selenium (Se)	0.80^{c}±0.18	0.54^{ab}±0.06	0.76^{bc}±0.15	0.44^{a}±0.09
Fat soluble Vitamin	Content			
Vitamin A (I.U/100g)	19.63^{c} ±1.18	11.73^{b}±1.74	4.20^{a}±0.59	7.67^{a}±1.62
Vitamin D(I.U/100g)	208.40^{c}±4.17	30.80^{a}±1.68	31.60^{a}±1.02	62.40^{b}±1.24
Vitamin E(I.U/100g)	0.88^{b}±0.21	0.16^{a}±0.18	0.54^{bc}±0.16	0.7^{c}±0.18
Vitamin K (µg/100g)	1.45^{c}±0.48	0.16^{a}±0.08	0.41^{bc}±0.03	0.99^{c}±0.17

Data are expressed as Mean ±SE. Values bearing different superscripts in a row differ significantly (P<0.05)

Felts et al (1996) stated that 16.60-19.59% of protein content was found in IMC. The data stated were higher than our findings. In the present study the fat content of FW and WW reared rohu for both the weight ranges varied from 1.80-2.16%. Paul *et al*. reported lipid content in rohuas 1.30-2.94% and Shakir et al.(2013) reported 1.0- 2.71 % lipid in IMC which is in agreement with our present study. The crude lipid content of the present study was below the range as reported (Shekhar et al., 2004) fat content of rohu was 1.2-1.5%. However, Hossain et al. (1999) have shown higher crude lipid content than our findings.

Fish muscles and bones behave as an excellent source of dispensable minerals and about 65% of minerals are stocked in the skeleton, particularly vertebra (Njinkoue et al., 2016). Generally, the ash content of the fish samples indicates its potential as a source of minerals such as zinc, magnesium, iron, potassium and sodium (Bolawa et al., 2011). The ash content in the present study was ranged from 2.33-3.38 which is in agreement with the findings of (Paul et al., 2016 and Paul et al., 2015a). Jabeen and Chaudhary (2011) found a higher content of ash in *L. rohita* than the values obtained in our study. Whereas Shakir et al. (2013) reported a lower ash content in IMC than our findings. Sodium and potassium content of rohu was significantly higher (P<0.05) in TR1 and TR4 as given in Table 1. Iron and zinc content of rohu was significantly higher (P<0.05) higher in TR2 and TR3. Selenium content was significantly higher (P<0.05) in Tr1.

The higher concentration of potassium and lower concentration of sodium found in the present study making it an excellent source of these minerals to utilize for the improvement of public health, particularly in the prevention of the cardiovascular disease (Perez et al., 2014). The current findings was also in corroboration with Paul et al. (2016) who recorded higher concentration of potassium and lower sodium level in rohu.

Iron plays an important role in oxidation-reduction reaction and electron transport associated with cellular respiration (Paul and Mukhopadhyay, 2001). Iron is important for the formation of haemoglobin, necessary to form red blood cells (Oksuz et al., 2011). The level of Fe in rohu in the

present study was comparable to magur and singhi (Paul et al., 2015a) and Indian Major Carp (Paul et al., 2016) but higher than freshwater small indigenous fishes (Jabeen et al*.,* 2015 and Hossain et al., 2015).

Manganese is mainly used as co-factor for the enzymes kinase, peptidase, arginase, succinic decarboxylase. It has also been implicated in oxidative phosphorylation. It is responsible for normal functioning of brain and proper metabolism of lipid and carbohydrate (Chanda et al., 2015). The Mn content in the studied fish was similar to the values reported by (Paul et al., 2015a).

Zinc has a structural role in nucleoproteins and involved in prostaglandin metabolism (Lall, 2002). The Zn contents in the current study were under the recommended maximum limits of 50mg/kg in fish (WHO, 1985). The concentration of zinc in rohu was in comparison with our earlier findings (Paul et al., 2016). The concentration of Zn in the studied fish was lower than the values as reported earlier (Jabeen et al., 2015).

Selenium has shown to decrease the toxicity of methyl mercury and cadmium (Watanabe et al., 1997). It is implicated in the metabolism of tocopherol compounds (Chanda et al.,2015). Lower levels of selenium have been correlated with increased risk of cancer and renal disease (Chanda et al., 2015). Minerals are essential building blocks for many enzymes and metabolic processes as well as contributing to fish growth and a deficiency of these critical nutrients causes lower productivity and disease (Marichamy et al., 2012).

Vitamin A ranged from 4.20-19.63 (I.U/100g) in FW reared rohu and ranged varied from 7.67-11.73 in WW reared rohu for both the weight ranges. Vitamin A, D, E and K was significantly higher in TR1 having weight range (1-500g) reared in Freshwater. Fish acts as a good source of fat-soluble vitamins viz., A, D, E and K. Vitamin A also play a major role in immunity, growth, oxidation resistance, glucose and lipid metabolism, erythropoiesis and in the regulation of iron metabolism (NRC, 2011). Most of the vitamin A in fish is concentrated in the eyes and viscera. This distribution makes the cleaning practice extremely important for the retention of vitamin A. Cleaning practice depends on the fish species, size of the fish and the person cleaning the fish. This distribution makes the cleaning practice extremely important for the retention of vitamin A. Cleaning practices depend on the fish species, size of fish and the person cleaning the fish (Roos et al., 2002). Fat soluble vitamin content in fish flesh is affected by the level of fat (Ozyurt et al., 2009). Vitamin A content of rohu in the present study was found to be higher than salmon, mackerel and dogfish (Dias et al., 2003), rainbow trout 74.33 IU/100g (Stancheva et al., 2010); common carp 75.06 IU/100g and European catfish 21.0 IU/100g (Ozyurt et al., 2009).

Vitamin D content varies between 0.5 and 30 mg/100 g fish muscle in various species (Mattila et al., 1995). Deficiency of vitamin D leads to rickets, osteomalacia, low bone mineral density and thereby osteoporosis. The form of vitamin D found in fish is vitamin D_3 (Cholecalciferol). Vitamin D content in the present study was lower than earlier reports (Hansen et al., 1998).

In the present study vitamin E content ranged from 0.16-0.88 IU/100g, was comparable with common carp (0.46 mg/100g) and European catfish (0.80mg/100g) but lower than pike perch (0.94 mg/100g) (Ozyurt et al., 2009). Earlier studies have shown that vitamin E protects highly unsaturated fatty acids such as DHA and EPA from attack and oxidation by free radicals, reduce lipid peroxidation and help accumulation of polyunsaturated fatty acids (Lebold et al., 2011).

Vitamin K belongs to the lipid soluble vitamins which naturally occur as phylloquinone (vitamin K_1) and menaquinone (vitamin K_2). Our body needs vitamin K for modification in post translational stages of certain proteins required for blood coagulation and in metabolic pathways in bone and other tissues (Halver, 2002).

Fatty acid profile of different weight ranges of rohu reared in FW and WW is presented in Table 2. Perusal of Table 2 reveals that saturated fatty acid (SFA) in rohu is significantly ($P<0.05$) higher in TR 2 followed by TR 1. The predominant fatty acid among SFA; palmitic acid significantly higher ($P<0.05$) higher in TR1 and TT2 in rohu sample. The other fatty acid (stearic acid) is significantly higher in TR 4.SFA is higher in rohu reared in wastewater of weight range (1-500 g).

Table 2. Fatty acid profile (% of total fatty acid) of rohu different weight ranges collected from freshwater (FW) and wastewater (WW)

Particulars	TR1 (BW1FW)	TR2 (BW1WW)	TR3(BW2FW)	TR4 (BW2WW)
Lauric acid	0.03^{a}±0.005	0.13^{b}±0.05	0.16^{b} ±0.0.02	1.24^{c}±0.12
Tridecanoic acid	0.11^{c}±0.01	20.06^{b}±0.05	0.26^{d}±0.045	0.02^{a}±0.01
Myristic acid	1.82^{a}±0.19	2.53^{b}±0.49	1.99^{a} ±0.035	ND
Pentadecanoic acid	1.23^{b}±0.18	50.70^{a}±0.055	ND	ND
Palmitic acid	68.03^{c}±1.79	70.42^{c} ±1.48	57.44^{b}±2.26	34.27^{a}±1.91
Heptadecanoic acid	0.71^{a}±0.09	0.92^{ab} ±0.10	1.97^{bc} ±0.07	2.04^{c} ±0.93
Stearic acid	1.22^{a}±1.06	4.01^{b}±0.03	4.81^{b}±0.50	9.68^{c}±0.44
Arachidic acid	1.70^{b}±1.44	0.16^{a}±0.005	0.27^{a}±0.01	0.38^{a}±0.02
Heneicosanoic acid	1.72^{b} ±0.44	1.10^{a}±0.10	3.36^{c} ±0.04	3.14^{c} ±0.18
Σ SFA	76.49^{c}±1.82	81.85^{d}±0.63	70.69^{b}±2.63	51.14^{a}±0.07
Myristoleic acid	0.44^{b}±0.38	0.06^{a}±0.005	0.05^{a}±0.01	0.02^{a}±0.01
Pentadecanoic acid	1.41^{b}±0.07	ND	0.02^{a}±0.01	0.04^{a}±0.005
Palmitoleic acid	1.62^{b}±0.08	2.82^{c}±0.32	ND	0.77^{a} ±0.09
Heptadecanoic acid	0.22^{a}±0.19	0.26^{a}±0.18	0.52^{b} ±0.04	0.98^{c} ±0.04
Oleic acid	5.97^{b}±0.12	0.05^{a}±0.01	10.28^{b}±0.74	27.82^{c}±3.31
Eicosanoic acid	ND	0.88^{b}±0.06	0.31^{a}±0.03	0.91^{b}±0.08
Erucic acid	1.57^{a}±0.07	2.97^{b}±0.37	ND	1.25^{a}±0.09
ΣMUFA	11.22^{b}±0.47	7.06^{a}±0.54	11.32^{b}±0.90	29.35^{c}±3.16
Linoleic acid	4.04^{b}±0.20	6.64^{c}±0.12	8.07^{d}±0.61	0.12^{a}±0.005
α-Linolenic acid	3.30^{a}±0.18^{b}	2.73^{a}±0.13	7.16^{b}±0.84	10.32^{c}±0.70
ϒ-Linolenic acid	0.69 ±0.40	0.89 ±0.53	0.37 ±0.03	0.45 ±0.07
EPA	1.29^{b}±0.14	0.14^{a}±0.005	0.99^{a}±0.03	3.58^{c}±1.57
DHA	0.81^{b}±0.26	0.84^{b}±0.08	0.47^{a}±0.07	4.42^{c}±0.17
ΣPUFA	13.01^{a}±2.33	12.24^{a}±0.46	18.72^{b}±1.73	20.78^{b}±1.05
EPA+DHA	2.1^{a}±0.93	0.99^{a}±0.09	1.46^{a}±0.1	8.0^{b}±1.70

Data are expressed as Mean ±SE. Values bearing different superscripts in a row differ significantly (P<0.05). EPA: Eicosapentanoic acid, DHA: Docosahexanoic acid, SFA: Saturated Fatty acid, MUFA: Monounsaturated fatty acid and PUFA: Polyunsaturated fatty acid

Fatty acid composition of aquatic animals is influenced by intrinsic variables like species, sex, age and size and extrinsic factors such as diet, salinity, temperature, geographical regions and the general rearing conditions (Rahman et al., 1995). Fatty acids in fishes are derived from two main sources, viz., biosynthesis and diet (Kamler et al., 2001). The chain length varies from C14-C24 of varying degrees of unsaturation, from saturated to polyunsaturated (Swapna et al., 2010). The fish lipid is a rich source of polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which is beneficial for human improvement in several types of disease (Panchal and Brown, 2021). In this study the PUFA content was lesser than the SFA and MUFA. Other researchers have also shown the freshwater fish have lower PUFAs (Paul et al., 2015b) because freshwater fishes feed largely on vegetation and plant materials (Vlieg and Body, 1988). Palmitic acid content among the SFA was maximum in the studied fish which is in agreement with earlier reports (Paul et al., 2015b). The monounsaturated fatty acid(MUFA) and oleic acid is significantly (P<0.05) higher in TR4 having weight range (5001-2000g) reared in wastewater. These data confirmed earlier observations of (Gutierrezand de Silva, 1993). The MUFA contains 31-39% of the total fatty acids in the major carps reported by (Sankar and Ramachandran, 2001) which was higher than our findings. Perusal of Table 2 reveals that the Polyunsaturated fatty acid (PUFA), α linolenic acid, EPA and DHA is significantly (P<0.05) higher in TR 4 having weight range (5001-2000g) reared in wastewater. (Ward and Singh, 2005) reported that α-Linolenic acid is the most abundant fatty acids among PUFA which was in comparable with our findings. In the present findings rohu contained

good amount of long chain PUFA which was in agreement with (Memon et al., 2011). In present study, linoleic acid range was 0.12-8.07, similar to result obtained by (Swapna et al., 2010). The maximum amount of PUFA was estimated to be 18.72-20.78 in WW reared rohu which was similar to result reported by (Jakhar et al., 2012; Swapna et al.,2010). Fish muscles contain higher levels of ω-3 PUFAs which are known to be anti-atherosclerotic, anti-thrombotic and anti-arrhythmic (Givens et al., 2006). DHA and EPA had been reported to have preventive effects on human coronary artery disease.

CONCLUSION

The aim of this paper was to investigate the proximate composition, mineral concentration, vitamin and fatty acids composition of rohu reared in freshwater and wastewater. The variation in nutrient composition was observed in fish reared in freshwater and wastewater environment. Natural and artificial diet, aquatic environment, habitat, physiology and morphology of fish that influence the nutrient composition of fish. Results of the presently reported study indicated that the protein, PUFA, α linoleic acid, EPA, DHA, MUFA, Oleic acid and potassium is higher in rohu of weight range (501- 2000g) reared in wastewater. Sodium and Vitamin A,D,E and K is higher in rohu of weight range (1-500g) reared in freshwater.

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