Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 4 Issue 11 November 2020

Research Article

Assessment of Essential Metals in Selected Fish Feeds and Cultivated Fish Species in Bangladesh and their Impacts on Human Body

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Received: September 23, 2020

Published: October 21, 2020

Abstract

Cultivated fishes are major protein and microelement sources for all sorts of people in Bangladesh due to its availability and affordability. Essential metals are persistent in the environment and are subject to bioaccumulation in the food chain. Fish is at the top of the aquatic food chain and normal metabolism of fish may accumulate large amounts of different essential metals from water, food or sediment. This study was carried out to determine essential metal concentrations in the flesh of some cultivated fish species and commercial fish feeds available in Bangladesh. The essential metal concentration in fish and consumed fish feed were assessed by Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES) with significant variation ($P < 0.05$). The concentration (mg/kg - dry weight) of Fe, Cu, Zn, Na, K, and Ca were observed 23.33 - 63.3, 12.80 - 20.62, 10.00 - 26.25, 214.2 - 367.68, 285.71 - 485.71, 256.89 - 432.65 in selected commercial fish feeds whereas those were found 33.33 - 80.83, 14.00 - 31.80, 20.60 - 49.25, 250.98 - 500.78, 400.76 - 587.32, 408.16 - 857.14 in cultivated fish species. All analyzed metal concentrations were within the acceptable of World Health Organization (WHO, 2004), Food and Agricultural organization (FAO, 2004), European Union (EU, 2010) and US Environmental Protection Agency (USEPA, 2011) and Total Hazardous Quotient (THQs) value of all the selected metals were lower than 1, which suggesting no remarkable non - carcinogenic health hazards for adult population.

Keywords: Essential Metal; Nutrient; Cultivated Fish; Carcinogenic; Bioaccumulation

Introduction

Fish farming is one of the most important aspects of agriculture with commercial fish contributing tremendously in meeting the upward protein demand of the increasing population through fish. Fish is a very rich and convenient source of nutrient, including microelements. The chemical composition of fish depends on both the kind and degree of the feeding animal [1]. The primary conditions for intensification of any culture depend on the natural and artificial feed. With the progress of technology and increasing demand different types of improved culture technique are now being practiced in Bangladesh. As a result, huge quantity of supplementary feed has to provide to the cultured species. The demand has increased 1.5 times greater than the previous five years [2]. So, the prospect of aqua feed industry is very bright in Bangladesh. It is evident that for the proper nourishment of fish a balanced diet containing energy sources - with all essential fatty acids, protein - with all essential amino acids, vitamins and minerals are very vital [3].

Fishes are one of the most important organisms in the aquatic food chain, which are very sensitive to metals contamination. Various metals are accumulated in fish body in different amounts. These differences result from different affinity of metals to fish tissues, different uptake and deposition and excretion rates. Various species of fish from the same water body may accumulate different amounts of metals [4,5]. Bangladesh is well known for cultivation of fish to fulfill protein demand of the country and in 2014, the country was ranked 6th in global farmed fish production (FAO, 2016). The common sources of fish feed ingredients are maize, rice, rice polish, wheat, soybean grits, mustard oil cake, coconut oil cake, lentil bran, molasses etc. [6]. The accumulation of metals or deficiency of these elements may stimulate an alternate pathway which might produce diseases [7]. Elements such as iron, zinc are essential components of enzymes where they attract or subtract molecules and facilitate their conversion to specific end products [8,9]. Essential metals are required in very trace quantities for the proper functioning of enzyme systems, hemoglobin formation and vitamin synthesis in human. The body uses sodium to maintain fluid levels and it is necessary for the health of the heart, liver, and kidneys. It regulates blood fluids and prevents low blood pressure [10,11]. Calcium is a key nutrient in the human body. More than 99% (1.2 - 1.4 kg) calcium is stored in the bones and teeth [12,13]. Although Copper is an important constituent in a number of different enzymes, high intakes can cause health problems such as liver and kidney damage [14]. Zinc is considered as an "essential trace element" because very small amounts of zinc are necessary for human health. It is used for boosting the immune system [15,16]. Although essential metal play important physiological and biochemical roles in the body as they may be part of biomolecules such as enzymes, which catalyze biochemical reactions in the body and either their deficiency or excess can lead to disturbance of metabolism and therefore causes to create various diseases [17,18]. As part of our ongoing research on accumulation of heavy and essential metals into food chain, we investigated the concentration of essential metals such as Na, K, Ca, Fe, Cu and Zn in selected renowned fish feeds and cultivated fish tissues collected from Khulna District, the southern part of Bangladesh.

Aim of the Study

This study is also aimed at evaluating the risk of metals in fishes. The obtained results have been reported in this article.

Materials and Methods

Sample collection

Seven types of Fish and their respective commercial feeds were collected from the fish cultivating firm in the southern part of Bangladesh. The analyzed fishes were cultivated for 8 to 10 months in individual cultivating firm. Fish samples were collected by considering the selected fish feed, age, length, weight of the fishes.

Digestion and analysis

All collected fish samples were cut into small species and dried them in an oven at 105°C until a constant weight was achieved and finally powdered. Feed samples also were dried in similar way. 1g of the homogenate of each sample (dry weight) was taken into a quick fit round bottom flask and 15 mL mixture of concentrated HNO_3, H_2SO_4 and $HClO_4$ in (4:1:1; v/v) was added into the flask. A condenser was set up with the flask and the mixture was stirred at 85°C for 3 hours until the proper digestion was completed and the solution became clear [19]. Filtration of all digested samples was done with Whitman no. 42 filter paper and the filtrated was transferred into the volumetric flask and finally diluted to 50 mL with deionize water. Samples were stored at ambient temperature until metal analysis. All chemicals used were Merck, Germany analytical grade, including standard stock solutions of known concentrations of different metals. The entire samples were analyzed by Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) with detection limit (mg/kg) for Na (0.001), K (0.010), Ca (0.001), Fe (0.010), Cu (0.001), Zn (0.001). Blank samples were analyzed after seven samples. All analyses were replicated three times. The precision and analytical accuracy of the analyses were checked by the analysis of standard reference material C.P.A Chem., Bulgaria.

Health risk estimation

The target hazard quotient (THQ) is the estimate of the risk level (non-carcinogenic) due to pollutant exposure. To estimate the human health risk from consuming metal - contaminated fish, the target hazard quotient (THQ) was calculated as per USEPA using following formula [20]:

$$THQ = \frac{ED \times EF \times FIR \times CF \times CM}{WAB \times ATn \times RfD} \times 10^{-3}$$

Hazard Index (HI) = $THQ_{Fe} + THQ_{Cu} + THQ_{Zn}$

Where THQ is the target hazard quotient, EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years for Bangladeshi population [21]), FIR is the fish ingestion rate (49.5 g/person/day), CF is the conversion factor (0.208) to convert fresh weight (Fw) to dry weight (Dw) considering 79% of moisture content in fish, CM is the heavy metal concentration in fish (mg/kg, dry weight), WAB is the average body weight (bw) (70 Kg) [21], ATn is the average exposure time for non-carcinogens (EF×ED) (365 days/year for 70 years (i.e. ATn = 25,550 days) as used in characterizing non-cancer risk and RfD is the reference dose of the metal an estimate of the daily exposure to which the human population may be continuously exposed over a lifetime without an appreciable risk of deleterious effects [22].

Statistical analysis

All statistical analyses were performed using the Microsoft Excel (version 2016). Analysis of variance (Two-way ANOVA) and correlation matrix were employed to examine statistical significance of differences in the mean concentration of metals between (or among) group of families of fish feed and fish. A probability level of $P < 0.05$ was considered statistically significant.

Results and Discussion

Table 1 represents seven brands of commercial fish feed those were provided to 7 types of cultivated fish for a certain period in individual cultivating firm including age, length and weight of fishes.

The summary of the concentrations of essential metals such as Fe, Cu, Zn, Na, K and Ca found in different commercial fish feed is presented in table 2. The highest concentration of Fe (63.33 mg/kg) was found in ACI fish feed and the lowest concentration was 23.33 mg/kg in Teer fish feed. The average concentration of Fe in analyzed feed samples found 41.84 mg/kg which was within the acceptable limit according to WHO and USEPA [20,23].

No.	Feed Sample	Names of cultivated fish (Scientific Name)	Cultivated fish (Local Name)	Age (Month)	Length (cm)	Weight (gm)
01	FF1	*Labeo rohita*	Rui Fish	8	58.42	2300
02	FF2	*Hypophthalmichthys molitrix*	Silver Carp	8	55.88	2000
03	FF3	*Puntius sarana*	Sarpunti	5	26.67	350
04	FF4	*Labeo calbasu*	Kaalibaus	7	43.18	1500
05	FF5	*Anabas testudineus*	Koi	4	19.05	200
06	FF6	*Ctenopharyngodon idella*	Grass Carp	8	60.96	2500
07	FF7	*Oreochromis niloticus*	Tilapia	8	33.02	1100

Table 1: Different types of cultivated fish and their commercial fish feeds.

No.	Sample	Brand of fish feed	Fe	Cu	Zn	Na	K	Ca
01	FF1	Fresh Fish Feed	49.56	14.74	12.13	367.68	285.71	273.56
02	FF2	Teer Fish Feed	23.33	11.23	10.00	367.57	412.86	323.78
03	FF3	ACI Fish Feed	63.33	20.62	25.00	308.38	285.71	389.56
04	FF4	Quality Fish Feed	51.33	20.21	25.00	335.14	428.57	256.89
05	FF5	Saudi Bangla Feed	36.67	12.80	26.25	254.54	485.71	375.54
06	FF6	Mega Fish Feed	28.00	18.68	15.63	304.32	328.57	573.49
07	FF7	Mesh Fish Feed	40.67	17.29	25.63	214.32	357.14	432.65
Mean			41.84	16.51	19.95	307.41	369.18	375.07
Standard Limit (mg/kg)		FAO/WHO [23]	100	30	40	700	900	900
		EU [24]	....	30	40	700	500 - 1000	- - -
		USEPA [20]	90 - 100	....	....	...	...	1000

Table 2: Concentration of essential metals (mg/kg, dry weight) in the brands of commercial fish feed.

From the result of elemental concentration, Cu ranged from 11.23 to 20.62 mg/kg. The maximum concentration of Cu (20.62 mg/kg) was found in ACI fish feed and the lowest content (11.23 mg/kg) was in Teer fish feed. The mean concentration of Cu (16.51 mg/kg) which was lower than the maximum permissible limits 30 mg/kg according to EU [24] and also lower than 22.618 to 38.480 mg/kg which was found in previous study [1]. The Zn content in these feeds was ranged from 10.00 - 26.25 mg/kg which is acceptable by FAO and USEPA [23,24]. In current study, the Na content ranged from 214.32 - 367.68 mg/kg which was lower than the results 891.04 - 1079.13 mg/kg found in previous study [1]. The average concentration of Na (307.41 mg/kg) found in the current study was within the acceptable limit by WHO and EU [23,24]. The highest concentration of K (485.71 mg/kg) was found in Saudi Bangla feed whereas lowest (285.71 mg/kg) in Fresh and ACI fish feed. The average concentration of K (369.18 mg/kg) was below the permissible limit (900 mg/kg) by WHO [23]. Calcium content was recorded in this study from 256.89 mg/kg to 573.49 mg/kg. The highest concentration of Ca found in Mega Fish Feed while the lowest content was recorded in Quality Fish Feed. The mean value of Ca (375.07 mg/kg) was within acceptable limit by EU [24].

Table 3 summarizes the concentration of elements determined for fish samples. The seven fish samples were analyzed. The values of elemental concentration for Fe ranged from 33.33 to 80.83 mg/kg, Cu ranged from 14.00 to 31.80 mg/kg, Zn ranged from 20.60 to 49.25 mg/kg, Na ranged from 250.98 to 500.78 mg/kg, K ranged from 400.76 to 587.32 mg/kg and Ca ranged from 408.16 to 857.14 mg/kg. The highest Fe content was found in *Puntius sarana* while lowest was found in *Hypophthalmichthys molitrix* fish. The mean value for Fe was 52.86 mg/kg, which was lower than 185 mg/kg recorded in a previous study [26]. The obtained range of Fe in this study was within the acceptable limits according to WHO and EU [24,25]. The mean value of Cu and Zn was found 23.32 mg/kg and 32.11 m/kg, which were also lower than 31.15 mg/kg and 52.87 mg/kg respectably recorded in a previous study [26]. The highest concentration of Cu was found in *Puntius sarana* and that was lowest in *Anabas testudineus.* The experimented result for Cu and Zn was not exceeded the permissible limit of WHO and EU [24,25]. The recorded mean value of Na and K was 377.09 mg/kg and 523.16 mg/kg which also acceptable by WHO and EU [23,25]. A previous study found mean value of Na and K was 900 mg/kg and 700 mg/kg respectably, which were higher than the current study [27]. The highest concentration of Ca (857.14 mg/kg) found in *Hypophthalmichthys molitrix* fish and the lowest concentration was 408.16 mg/kg in *Labeo calbasu* fish. The average value of Ca was 596.92 mg/kg which is also within the permissible limit set by FAO and EU [24,25].

No.	Sample	Scientific name of fish	Fe	Cu	Zn	Na	K	Ca
01	F1	*Labeo rohita*	62.50	20.00	23.88	400.78	567.43	518.37
02	F2	*Hypophthalmichthys molitrix*	30.00	24.67	25.82	500.78	587.32	857.14
03	F3	*Puntius sarana*	80.83	31.80	28.36	378.76	400.76	634.69
04	F4	*Labeo calbasu*	55.00	22.50	49.25	456.98	576.58	408.16
05	F5	*Anabas testudineus*	41.67	14.00	44.78	300.65	543.78	497.96
06	F6	*Ctenopharyngodon idella*	33.33	21.70	20.60	350.76	498.67	676.41
07	F7	*Oreochromis niloticus*	66.67	28.60	32.09	250.98	487.56	585.71
Mean			52.86	23.32	32.11	377.09	523.16	596.92
Standard Deviation			17.32	5.38	10.06	79.89	61.18	134.79
Standard Limit (mg/kg)		FAO/WHO [23,25]	100	30	40	700	900	900
		EU [24]	100	30	40	700	1000	1000

Table 3: Concentration of essential metals (mg/kg, dry weight) in cultivated fish samples.

Translocation of selected metals from fish feeds into cultivated fishes in current study found that the concentration of essential metals in fish samples were more than their respective commercial fish feed (Figure 1). The mean concentration of Fe in fish samples was 25% more than their consumed fish feeds. In case of Cu and Zn, the increasing rate from feed to fish was 41% and 60% respectively whereas other essential metals Na, K and Ca was also increased in fish species by 27%, 36% and 57% respectably (Figure 1). However, though the concentration rate of selected metals was noticeably high from feed to fish but their final content in selected fishes was within permissible limit.

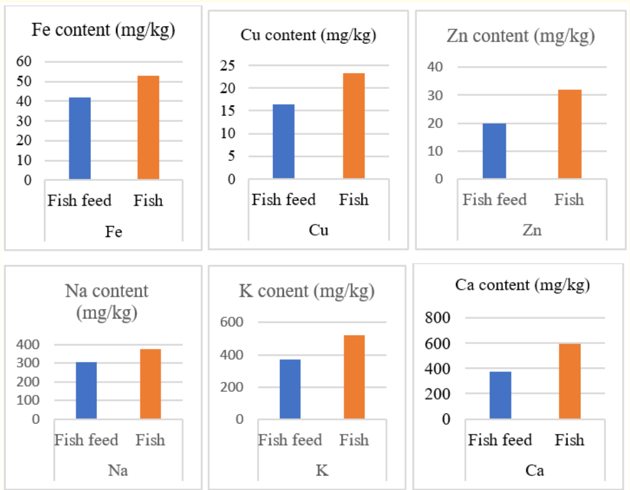

Figure 1: Metal content in fish feed and in cultivated fishes.

Target Hazard Quotient (THQ) of three essential metals such as Fe, Cu and Zn were calculated according to the reported equation [20] and their values are represented in table 4.

		Target Hazard Quotient (THQ)						
Metal	**RfD (mg/kg)**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
Fe	0.7	0.02	0.01	0.03	0.02	0.01	0.01	0.02
Cu	0.04	0.07	0.09	0.10	0.08	0.05	0.07	0.10
Zn	0.3	0.01	0.01	0.02	0.05	0.04	0.01	0.03
Total THQ or HI		0.10	0.11	0.15	0.15	0.10	0.09	0.15

Table 4: THQ value of Fe, Cu and Zn in fish samples.

Table 4 shows total THQ or HI value for individual metal was lower than 1.0, USEPA standard [20] in individual fish species for all selected metals which expressed that no non-carcinogenic risks found for human health due to the consumption of those metals.

The Perason Correlation Coefficient in the present study is used to describe the inter relationships between the elements analyzed at a level of > 0.5 or < - 0.5 which are significantly correlated [28]. Table 5 shows a high possitive correlation between Fe-Cu (0.60) and Na - K (0.70) which indicates the simillar source of these metals in fish samples. By two way factor Anova analysis (MS office 2016), the present study found that F-critical values were higher than F values on contray, P values were greater than α value (0.05) in each individual fish species. Thus, there was a significant (P > 0.05) difference for all metal concentration in each individual fish species. In contrast, F value was higher than F-critical value as a result P value is less than α value (0.05). Thus, There was no significant differences at P < 0.05 for individual metal in all the selected fish species [28].

	Fe	Cu	Zn	Na	K	Ca
Fe	1					
Cu	0.6*	1				
Zn	0.07	-0.63	1			
Na	-0.28	0.04	-0.05	1		
K	-0.62	-0.64	0.3	0.7*	1	
Ca	-0.41	0.4	-0.7	0.3	-0.09	1

Table 5: Correlation matrix of selected metals in cultivated fishes.

Conclusion

Essential metals are very important for body growth that adult can get through daily fish consumption worldwide. This study revealed that the concentration of selected essential metals in commercial fish feeds and cultivated fish samples were within the acceptable limits compared to standard value of WHO/FAO, EU, USEPA. Metal concentration in fish samples were higher than their respective commercial fish feed samples which can be happened due to metabolism rate in fishes. This study found that there are no significant differences (P < 0.05) for individual metal content in all fish samples. The THQ values of analyzed essential metals was less than 1 which indicated that these fish samples had no non carcinogenic impact on human body. So, people can meet the essential metal demand by consuming these fishes for a long time.

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