Research Article

Impact of Graded Levels of Dietary Protein on Elemental Concentration of Genetically Improved Farmed Tilapia (GIFT) from Pakistan

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Abstract | The present investigation conducted in 90 days feeding trial evaluated the effect of three dietary protein feeds (15% CP, 20%CP, 25%CP) composed of plant protein ingredients, on elemental concentration in Genetically Improved Farmed Tilapia (GIFT) fingerlings, a developed strain of Nile tilapia (*Oreochromis niloticus*). Feed preparation criteria was based on cost effectiveness and local availability of cheaper plant protein feed ingredients. Ten specimens were randomly selected from each treatment hapa for evaluating the concentrations of selected essential and non-essential elements in GIFT fingerlings fed with dietary protein levels of 15% (T1), 20% (T2) and 25% (T3) crude protein. Flame Atomic Absorption Spectrometer was used for the analysis of whole-body elemental concentrations in relation to fish size under the influence of various dietary protein levels. The concentrations of K, Na, Ca, Mg, Fe, Zn, Pb and Cd were quantified for T1, T2 and T3 fish samples. An increasing trend was observed in the concentrations of studied elements with the increase of dietary crude protein in fish feed. The elemental concentration in the carcasses of studied GIFT yielded similar accumulation pattern for three treatment groups T1, T2 and T3, i.e., K > Na > Ca > Mg > Fe > Zn > Pb > Cd. Linear and multiple regression was applied to check the significance of accumulated elements. The relationship among the element concentrations and weight and length of fishes was also examined. A positive association between the elements and weight and length of the sampled fish was noted except for Fe in T1 with body weight. A common trend of positive and negative allometry was observed for most of the elemental concentrations with wet body weight and total length respectively. The level of non-essential toxic elements, Cd and Pb, did not exceed the permitted levels of international standard.

Received | May 22, 2021; **Accepted** | July 07, 2021; **Published** | August 24, 2021
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Citation | Kousar A., M. Naeem, S. Masud, A. Ishtiaq, Z. Naeem and Rabia Iqbal. 2021. Impact of graded levels of dietary protein on elemental concentration of Genetically Improved Farmed Tilapia (GIFT) from Pakistan. *Sarhad Journal of Agriculture,* 37(4): 1156-1166.
DOI | https://dx.doi.org/10.17582/journal.sja/2021/37.4.1156.1166
Keywords | Dietary protein, Elemental concentration, GIFT fingerlings, Hapas, Regression analysis

Introduction

In flourishing fish industry, one of the major impediment is the cost of fish feed ingredients of the total production budget (Cheikyula *et al*., 2020) because fish meal is the preferred gold standard component of fish feed but highly expensive and limited in supply (Radhakrishnan *et al*., 2016). Although, now it is considered both environmentally and ecologically unsustainable and requires to find alternative proteins due to economic pressure on the aquaculture industry (Turchini *et al*., 2019). For successful aquaculture, there is a need to replace fish meal for the preparation of fish feeds, particularly for omnivorous fish species. Plant based protein sources can cope the need of the time due to increasing fish culture as population is increasing day by day (Daniel, 2018; Zettle *et al*., 2019). Alternative plant protein sources like pulses, grains and oilseeds are widely used for economic fish feed preparations (Abdel-Warith *et al*., 2019).

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Fish is considered a very valuable source of protein and essential nutrients including minerals and vitamins for balanced nutrition and good health of humans (Ahmed *et al*., 2017; Bennett *et al*., 2018). They also possess polyunsaturated fatty acids which provide protection against cardiac issues. Improper handling in fisheries units and level of pollutants in fish demand attention because of adverse effects of pollutants on human health, the consumers of fish (Das *et al*., 2017).

Heavy metals are of particular concern due to their non-biodegradable nature and bioaccumulation ability. Heavy metals pollute the aquatic environment because these are toxic and become harmful for fish due to being accumulated and bio-magnified inhabiting such environments. Heavy metal pollution imposes dreadful consequences leading to disturbance in the equilibrium of environment and fish health (Adamu *et al*., 2016; Alhassan *et al*., 2016). However, the presence of heavy metals in fish, not only impairs the health of the consumers, but also obstruct the positive effect of beneficial elements. If accumulation of these elements is higher than the requirement of organisms, then deposition of such elements occur in their tissues (Aldogachi *et al*., 2016). These elements are metabolized to a limited extent because mostly heavy metals are unable to biodegrade (Rajkowska and Protasowicki, 2013) and thus have long lasting toxic effects (Sthanadar *et al*., 2015). Therefore, contamination due to heavy metals has adverse impact on the aquatic organisms as well as on the environment (Agrahari *et al*., 2017).

It is fact that all heavy metals are not lethal. Sodium is a principal positive ion in cells (Soetan *et al*., 2010). Calcium is a mineral that must be constantly taken up to build bone and maintain the blood level of calcium. Normal clotting of blood needs calcium. It is also required for muscle, neuron and cellular function. Many enzymes as well as carbohydrate and protein metabolism need magnesium (Soetan *et al*., 2010). Few heavy metals, such as iron and zinc act as trace elements and are vital for the maintenance of human metabolism and fish (Aziz *et al*., 2017). Iron is an integral component of haemoglobin, myoglobin, enzymes and cytochromes. Zinc is an essential element of many enzymes and metalloproteins in the metabolism of fish (Olsson *et al*., 1998).

Other metals, such as lead and cadmium do not have known role in the normal fish physiology and are non-essential metals (Hanan, 2007). Presence of cadmium, for a long time, in the bodies of animals and humans, can be carcinogenic (Jaishankar *et al*., 2014). It can cause severe pain in bones and lead to accumulation in kidneys and may be a cause of kidney failure (NIFES, 2016). Lead affects the children's intelligence and neurophysiological development (WHO, 1995).

Bio-accumulation of metals in the cultured fish occurs through the skin and gills by diffusion from the polluted water as well as with ingested food (Das *et al*., 2017). Deposition of metals above their threshold level, may cause stress and severely affect the fish physiology (Roy *et al*., 2011). Aquatic habitats deposit high amounts of metals in sediments and with the passage of time transfer to the fish (Shovon *et al*., 2017). Fish gills, gonads, kidney and liver accumulate more metals due to their active metabolism (Ahmed *et al*., 2017). Feed ingredients also have an imperative role in the aggregation of element components in the fish body (Iqbal *et al*., 2019).

The main objective of the current study was to evaluate the impact of three cost-benefit fish feeds composed of different combinations of plant protein-based ingredients on elemental concentration in GIFT fed with three different dietary proteins.

Materials and Methods

The current experimental work was carried out for 90 days at Tawakkal Fish Hatchery, Muzaffer Garh, South Punjab, Pakistan.

Experimental layout and feed formulation

The mono sex (male) Genetically Improved Farmed Tilapia (GIFT) fingerlings, an improved strain of Nile tilapia (*Oreochromis niloticus*), were selected for the evaluation of elemental concentration to check the influence of graded dietary protein feeds. Three crude protein feeds (15% CP, 20% CP, 25%CP) were fed to GIFT) fingerlings, after acclimatization for two weeks by feeding on fish meal @ 10% fish body weight in cemented tanks. After acclimatization, fish specimens having mean initial weight of 1.61 ± 0.08 g and mean initial length 4.31 ± 0.39 cm were transferred to hapas (8x6x3ft), placed in earthen ponds having water level of 3 to 4 ft, fed with experimental feed @ 5% fish body weight. Feed was provided to GIFT fingerlings twice a day between 8:00-9:00 am and 7:00- 8:00 pm, for 90 days.

Analysis of elemental concentration in GIFT
At the end of feeding trial, GIFT samples were packed in polythene bags filled with water and oxygen and transferred to fishery laboratory situated in Institute of Pure and Applied Biology (IP&AB), Bahauddin Zakariya University, Multan (Pakistan). A total of 30 fish samples, 10 fish from each treatment group were randomly collected. Wet body weight and total length of all the collected fish samples were measured using digital electric balance (SHIMADZU ELB-300 Japan) to the nearest of 0.01g, and length (cm) measured using a scale. Each fish was placed on aluminum foil plate and dried (at 70ºC, whole fish) until a constant weight in drying oven (Memmert, W. Germany). After drying, pestle and mortar was used for grinding the whole fish samples to obtain its powder form.

Dried fish powder was ashed in muffle furnace at 500ºC for 24 hours. One gram ash content of each fish sample was dissolved in 10 ml solution of 70% HNO_3. All samples were diluted using deionized water up to 25 ml and filtered using Whatman 42 filter papers. Prepared samples were kept in the labelled plastic bottles. To analyze the elemental concentration in fish samples, a total of 8 essential and non-essential elements were selected. Atomic absorption spectrometer (Agilent 240 A A) was used to estimate the concentrations of sodium, calcium, magnesium, potassium, iron, zinc, lead and cadmium. Two replicate analyses were carried out for each and every sample. Concentrations of elements were computed using Spectr A A Software.

Statistical analysis of data
Mean values and their standard deviations were calculated for each element. Statistical analyses including regression, computation of correlations and standard error were applied using excel. Predictive models were developed using linear regression to check the significance of accumulated elements. MINITAB software was used for the analysis of multiple regression. Regression analyses were performed between values of fish size and whole-body elemental concentration of the fish. As variations in elemental concentration are related to fish size, relationship between these variables was determined using the multiple regression equation given below:

$$Y = a + b_1W + b_2TL$$

Where;
Y represents elements, wet body weight and total length are represented by W and TL, respectively, b1 and b2 are slopes and a is intercept.

Table 1: *Values of grand means and standard deviations of elemental concentrations in carcasses of GIFT (Oreochromis niloticus, whole fish) (n = 10).*

Elements	Treatments	Dry weight (μg/g) Mean ± S.D.	Wet weight (μg/g) Mean ± S.D.
Na	T1 (15%)	4101.25 ± 857.59	858.02 ± 202.88
	T2 (20%)	4870.88 ± 953.88	966.20 ± 192.76
	T3 (25%)	5528.98±1691.43	1195.32 ± 405.17
K	T1 (15%)	9090.00 ± 1023.45	1900.4 8± 289.54
	T2 (20%)	9840.00 ± 1048.26	2052.86 ± 282.76
	T3 (25%)	10137.50 ± 2245.91	2207.17 ± 639.99
Ca	T1 (15%)	5296.62 ± 730.49	1104.69 ± 172.78
	T2 (20%)	6057.88 ± 526.98	1202.04 ± 130.32
	T3 (25%)	7860.38 ± 1147.83	1687.23 ± 282.15
Mg	T1 (15%)	871.23 ± 183.95	181.61 ± 39.91
	T2 (20%)	989.04 ± 163.53	195.47 ± 27.02
	T3 (25%)	1349.04 ± 216.59	292.37 ± 67.53
Fe	T1 (15%)	607.67 ± 386.56	125.26 ± 80.58
	T2 (20%)	896.93 ± 118.81	178.56 ± 30.77
	T3 (25%)	921.38 ± 2 57.49	198.81 ± 60.97
Zn	T1 (15%)	169.33 ± 47.57	35.26 ± 10.14
	T2 (20%)	182.18 ± 37.05	36.32 ± 8.32
	T3 (25%)	206.83 ± 20.32	44.70 ± 7.64
Cd	T1 (15%)	0.41 ± 0.85	0.10 ± 0.02
	T2 (20%)	0.43 ± 0.20	0.10 ± 0.04
	T3 (25%)	0.46 ± 0.18	0.11 ± 0.08
Pb	T1 (15%)	1.45 ± 0.42	0.31 ± 0.97
	T2 (20%)	1.49 ± 0.27	0.30 ± 0.05
	T3 (25%)	1.65 ± 0.39	0.40 ± 0.10

S.D. = Standard Deviation

Results and Discussion

The mean values and standard deviations of various elemental concentrations observed on the basis of wet and dry weight in the carcasses of farm-raised GIFT are summarized in Table 1. In the present study, on the basis of obtained results, the accumulation pattern of studied elements in the treated GIFT in descending order, on dry and wet weight basis, was as follows: K > Na > Ca > Mg > Fe > Zn > Pb > Cd. These results indicate that concentration of potassium (K) was highest in the studied GIFT (*O. niloticus*) samples than any other element. The maximum concentration value of K in the GIFT belonging to treatment group fed with 25% CP diet (T3) was 10137.50 ± 2245.91μg/g and 2207.17 ± 639.99 μg/g on dry and wet weight basis, respectively. Whereas the minimum recorded

Table 2: *Relationship between log-transformed data of body burden element (μg/g) and wet body weight (g) of GIFT (Oreochromis niloticus) (n = 10).*

Elements	Treatments	Correlation coefficient (r)	Intercept (a)	Slope (b)	Standard Error (b)	t -value when b=1
Na	T1 (15%)	0.930***	0.889	2.795	0.389	7.173
	T2 (20%)	0.778**	2.156	1.768	0.504	3.505
	T3 (25%)	0.866**	1.429	2.383	0.485	4.909
K	T1 (15%)	0.896***	2.238	1.915	0.336	5.705
	T2 (20%)	0.860**	3.137	1.141	0.239	4.770
	T3 (25%)	0.937***	1.356	2.672	0.352	7.594
Ca	T1 (15%)	0.749*	2.560	1.422	0.445	3.199
	T2 (20%)	0.718*	3.123	0.958	0.328	2.919
	T3 (25%)	0.922***	2.207	1.860	0.275	6.754
Mg	T1 (15%)	0.721*	2.121	1.268	1.101	1.152
	T2 (20%)	0.794**	1.777	1.477	0.399	3.698
	T3 (25%)	0.761*	1.681	1.657	0.499	3.319
Fe	T1 (15%)	0.191ns	4.322	-1.039	1.892	-0.549
	T2 (20%)	0.503ns	2.439	0.819	0.497	1.648
	T3 (25%)	0.858**	0.729	2. 317	0.491	4.719
Zn	T1 (15%)	0.832**	-0.709	2.975	0.702	4.241
	T2 (20%)	0.067ns	2.483	0.128	0.667	0.191
	T3 (25%)	0.808**	0.985	1.558	0.402	3.874
Cd	T1 (15%)	0.671*	-1.401	1.364	0.532	2.563
	T2 (20%)	0.190ns	-0.990	0.925	1.685	0.549
	T3 (25%)	0.714*	-3.799	3.363	1.166	2.884
Pb	T1 (15%)	0.177ns	0.022	0.515	1.013	0.508
	T2 (20%)	0.815**	-1.347	1.766	0.444	3.977
	T3 (25%)	0.918***	-1.906	2.266	0.346	6.549

*Non-Significant= ns, * P < 0.05, **P < 0.01, *** P < 0.001.*

concentration of Cd amounted to 0.41 ± 0.85μg/g in sampled GIFT belonging to treatment group fed with 15% CP diet (T1) on dry weight basis and 0.10 ± 0.02μg/g and 0.10±0.04μg/g on wet weight basis in T1 and T2 treatment groups, respectively. An increasing trend in the elemental concentration was noted in the treated fish with the increasing dietary proteins level (25% CP > 20% CP > 15%CP).

Relationship between fish body weight and elemental concentration

In GIFT samples, values of correlation coefficient 'r' showed diverse correlation among various log-transformed values of elements and fish weight in three treatments groups (Table 2). In the present study, sodium, potassium and magnesium were found to show positive allometry with increasing body weight in all the treatment groups as the value of slope b was more than 1. While The studied GIFT fed with 15% and 25% CP diet showed positive allometry in calcium, zinc and cadmium, but negative allometry (b < 1) in 20% fed GIFT. Iron showed isometry in 15% CP fed fish (T1) and positive allometry in 25% CP fed fish (T3), but negative allometry was found in 20% CP fed GIFT (T2). Lead yielded negative allometry in T1, but positive allometry in T2 and T3 GIFT. The regression parameters of the relationships between log-transformed data of elemental concentrations and fish body weight are given in Table 2.

Relationship between total length and elemental concentration

Regression analysis to assess the total length dependence of these elements revealed weak correlation between log transformed data of total length and most of the essential metals in three treated groups (Table 3). The relationship between total length and concentrations of Fe in T1 and Cd was found to be non-significant in T1, T2 and T3. Lead had non-significant relationship in T1, while having significant relationship in T2 and T3 fish.

As variations were observed to be related to the total length of the body, regression analysis revealed that the slope 'b' value of log-log regression of the relationship between total length and total metal body burden was less than 3 (b < 3) exhibiting negative allometry in T1, T2 and T3 fish, except for Na and Pb

Table 3: *Relationship between log-transformed data of body burden element (μg/g) and total length (cm) of GIFT (Oreochromis niloticus) (n = 10).*

Elements	Treatments	Correlation coefficient (r)	Intercept (a)	Slope (b)	Standard error b	t-value when b = 3
Na	T1 (15%)	0.721*	1.242	2.829	0.960	2.947
	T2 (20%)	0.815**	0.273	3.103	0.781	3.974
	T3 (25%)	0.869**	1.345	2.819	0.569	4.959
K	T1 (15%)	0.559ns	2.854	1.563	0.818	1.911
	T2 (20%)	0.795**	2.625	1.783	0.481	3.705
	T3 (25%)	0.872***	1.454	2.935	0.582	5.046
Ca	T1 (15%)	0.666*	2.529	1.652	0.653	2.529
	T2 (20%)	0.709*	2.574	1.620	0.570	2.842
	T3 (25%)	0.931***	2.126	2.217	0.307	7.221
Mg	T1 (15%)	0.377ns	2.121	1.268	1.101	1.152
	T2 (20%)	0.775**	0.959	2.468	0.712	3.467
	T3 (25%)	0.656*	1.904	1.686	0.685	2.461
Fe	T1 (15%)	0.251ns	0.883	0.035	0.048	0.734
	T2 (20%)	0.496ns	1.989	1.366	0.845	1.617
	T3 (25%)	0.863**	0.726	2.664	0.619	4.303
Zn	T1 (15%)	0.725*	0.581	0.155	0.052	2.973
	T2 (20%)	0.805**	2.369	0.258	1.127	0.228
	T3 (25%)	0.761*	1.045	1.732	0.523	3.313
Cd	T1 (15%)	0.505ns	0.966	0.190	0.115	1.654
	T2 (20%)	0.137ns	-1.094	1.125	2.877	0.391
	T3 (25%)	0.541ns	-2.923	3.009	1.653	1.821
Pb	T1 (15%)	0.103ns	0.977	0.027	0.093	0.292
	T2 (20%)	0.837**	-2.438	3.067	0.709	4.323
	T3 (25%)	0.856**	-1.777	2.478	0.542	4.575

*Non-Significant= ns, *P < 0.05, **P < 0.01, ***P < 0.001.*

in T2 and Cd in T3 GIFT fed with 20% and 25% dietary proteins, respectively, showing isometric increase with total length. Regression parameters of all these relationships are summarized in Table 3.

Multiple Regression Analysis (MRA)

When multiple predictive equations were applied to determine elemental concentrations in fish body weight and total length, significant correlations were observed in most of the cases (Table 4).

For the people of impoverished third world countries, fish is considered a cheap source among animal proteins. It is considered as a main source of many minerals that are very important for humans (El-Shehawy *et al*., 2016). The mean concentrations of essential and non-essential elements obtained in GIFT (*O. niloticus*) showed similarities as well as variations in comparison with the values already reported in the literature for the same and other fish species. These inter-specific variations may be related to the nature of their habitat, feeding habits (Ansari *et al*., 2006; Jim *et al*., 2017), season (Obot *et al*., 2016) and the differences in ecological requirements, swimming behaviours and the metabolic activities among different fish species (Kalay *et al*., 1999). On the basis of results of present study, the accumulation order of elements in studied GIFT was: K > Na > Ca > Mg > Fe > Zn> Pb > Cd; this trend is in agreement with the findings of Tsegay *et al*. (2016) in *O. niloticus* although Cd and Pb was not considered in their investigation. Iqbal *et al*. (2019) has reported same accumulation pattern of all these elements (K > Ca > Na > Mg > Fe > Zn> Pb > Cd), in the whole fish body of hybrid (*Catla* x *Labeo*), except for Ca which showed higher levels than Na. Similar pattern of elemental accumulation was also reported by Naeem *et al*. (2010) in *O. mykiss*, except for Ca which was highest in their study. Potassium is found to be the most abundant element in GIFT (*O. niloticus*) and this observation is supported by the results of Adefemi (2011) who had reported potassium as the most abundant metal in all the fish samples of *Tilapia mosambis* and is not known to be toxic to fish. The bio-accumulation of K could be very beneficial to man since they are essential minerals in human nutrition (Adeyeye, 1996). Jim *et al*. (2017) had also reported maximum concentrations of K in *O. niloticus* studied from different ecological regions. Observed

Table 4: *Multiple regression analysis data concerning wet body weight (W, g) and total length (TL, cm) with element concentration (wet body weight, μgg⁻¹) for GIFT (Oreochromis niloticus) (n = 10).*

Relationships	Treatments	r	b	b_1 ± S.E.	b_2 ± S.E.	r^2
K = $a+b_1W+b_2TL$	T1 (15%)	0.797**	949	199.5 ± 62.0	-179 ± 112	0.635
	T2 (20%)	0.421ns	3068	194 ± 169	-365 ± 360	0.177
	T3 (25%)	0.893***	-857	381 ± 159	-256 ± 264	0.797
Na= $a + b_1 W + b_2 TL$	T1 (15%)	0.842**	-518	106.2 ± 38.7	-7.4 ± 70.0	0.709
	T2 (20%)	0.611ns	-1521	-117 ± 178	412 ± 378	0.373
	T3 (25%)	0.755*	-1100	-27 ± 147	253 ± 243	0.570
Ca = $a + b_1W + b_2 TL$	T1(15%)	0.404ns	353	-2.9 ± 56.0	80 ± 101	0.163
	T2(20%)	0.481ns	809	-63 ± 152	121 ± 323	0.256
	T3(25%)	0.791**	32	-8.4 ± 95.4	167 ± 158	0.625
Mg = $a + b_1W + b_2TL$	T1(15%)	0.588ns	152	21.8 ± 11.4	-27.2 ± 20.7	0.346
	T2(20%)	0.481ns	71	7.9 ± 27.7	3.2 ± 58.9	0.231
	T3(25%)	0.604ns	275	55.0 ± 29.7	-76.8 ± 49.3	0.365
Fe = $a + b_1W + b_2TL$	T1(15%)	0.819**	-190	-55.5 ± 16.4	108.5 ± 29.6	0.670
	T2(20%)	0.028ns	184	-4.0 ± 35.7	4.4 ± 75.9	0.0008
	T3(25%)	0.681*	-92	7.1 ± 24.6	17.1 ± 40.9	0.465
Zn = $a + b_1 W + b_2TL$	T1 (15%)	0.685*	-29.9	3.18 ± 2.62	2.19 ± 4.73	0.469
	T2 (20%)	0.414ns	52.6	-3.93 ± 8.83	3.2 ± 18.8	0.171
	T3 (25%)	0.471ns	30.1	3.32 ± 3.72	-3.38±6.18	0.222
Cd = $a+b_1W+a+b_2TL$	T1(15%)	0.767**	0.07	0.003 ± 0.006	-0.0002 ± 0.011	0.589
	T2(20%)	0.336ns	-0.43	0.042 ± 0.045	-0.089 ± 0.095	0.113
	T3(25%)	0.869**	-079	0.087 ± 0.021	-0.121 ± 0.034	0.756
Pb= $a + b_1 W + b_2TL$	T1 (15%)	0.754*	0.538	-0.0093±0.034	-0.0100 ± 0.061	0.569
	T2 (20%)	0.621ns	-0.296	-0.0155 ± 0.049	0.083 ± 0.105	0.386
	T3 (25%)	0.843**	-0.027	0.0615 ± 0.029	-0.0481 ± 0.049	0.712

*Multiple correlation coefficient (r), intercept (a), Regression coefficient (b1, b2), Standard error (S.E.), Non-significant (ns), *P < 0.05. **P < 0.01, ***P < 0.001*

levels of Na in the present study are in line with the reported values of Salam *et al.* (1993) in *Labeo rohita* but higher than Salam *et al.* (1996) and Tsegay *et al.* (2016) in *O. niloticus*. The obtained levels of Ca in studied GIFT are in agreement with Salam *et al.* (1993) in *Labeo rohita* but is lower than the reported value by Salam *et al.* (1996) in *O. niloticus*. Magnesium concentration in studied GIFT (T3) is similar to the findings of Salam *et al.* (1996) in *O. niloticus* and Naeem *et al.* (2010) in *Aristichthys nobilis* but is lower than the value reported by Salam *et al.* (1993) in *L. rohita*. Naeem *et al.* (2012a) had reported much higher level of Mg in *Mystus bleekeri*. Adefemi (2011) had also reported higher concentrations of K, Na, Ca and Mg in *Tilapia mosambis* similar to the findings of the present study. This similarity in body concentrations suggest that the incorporation of essential elements into the body from dietary and waterborne sources is closely regulated (Shearer, 1984).

The higher concentrations of Fe and Zn observed in this study may be because of their essential role in fish metabolism. The international standards for Zn range are approximately 192-480 μg/g (Yamazaki *et al*., 1996). The observed values of Zn concentration (T1, T2, T3) observed in the present study are within the international standard range. The whole-body Fe concentration is higher than Zn (Fe > Zn) in studied GIFT. Merciai *et al.* (2014) observed higher concentrations of Fe than Zn in the muscles of the fish they studied. Similarly, Jakhrani *et al.* (2017) had reported higher levels of Fe than Zn in homogeneous and dried samples of liver, gills and muscles of *L. rohita* and *Cirrhinus mrigala*. Mannan *et al.* (2018) studied the bioaccumulation of heavy metals in fish before and after feeding with synthetic feed for six months and reported higher levels of Cu, Cr, Cd, Pb, Fe and Zn. Metal accumulation in these fish tissues varies according to the rates of uptake, storage and elimination (Langston, 1990). It is also known that the metabolic activity of younger individual is normally higher than that of an older individual which may lead to more metal accumulation (Widianarko *et al*., 2000). Different levels of heavy metals accumulate in different organs which could be attributed to the variations in their physiological roles to maintain homeostasis, feeding habit, regulatory ability and behaviour of each fish (Adaka *et al*., 2017) and may contribute to whole body concentrations of various metals.

Cadmium is not essential for fish and its contamination in fish diet induces hazardous effects leading to retarded growth and abnormal protein pattern, such as in Nile tilapia (El-Serafy *et al*., 2013). The acceptable level of Cd is 0.5 µg/g (FAO, 1983) cited in Adaka *et al*. (2017). The obtained levels of Cd in the present study had low mean values. Ayeloja *et al*. (2014) had also reported lowest concentration of Cd in *O. niloticus* from Eleyele reservoir, Nigeria, as compared to other studied fishes in the same habitat. The range of Pb concentrations in T1, T2 and T3 is below the permitted level of Pb (2.0 µg/g) in fish flesh (FAO, 2010). Iqbal *et al*. (2019) has also reported mean Cd and Pb concentrations with in normal range in hybrid (*Catla catla* x *Labeo rohita*) fed on 15%, 20% and 25% dietary protein levels of plant origin.

Effect of body weight of fish on elemental concentration

Influence of fish body weight on whole body elemental concentration exhibited positive correlation, except for Fe in 15% fed fish (T1) and this observation agrees with some previous reports (Salam *et al*., 2002; Ansari *et al*., 2006; Naeem *et al*., 2010; Fand, 2011). In the present study, Na, K, Mg in T1, T2 and T3 while calcium in T1 and T3, and Pb in T2 andT3 exhibited positive allometry in relation to wet body weight in studied GIFT. These findings are in agreement with Salam *et al*. (1996) in *O. nilotica* for Na, K and Pb showing positive allometry with body weight. The present study also revealed negative allometry in Ca and Zn (T2), Cd (T2) and Pb (T1) and isometric increase in Fe (T1) in relation to body weight and this is in general agreement with Naeem *et al*. (2010) report on *Onchorynchus mykiss*. Negative allometry in whole body Zn with body weight also coincides with results of Naeem *et al*. (2012 a) in *M. bleekeri*. Positive allometric increase in Fe in T3 indicating significant proportional increase is in agreement with Naeem *et al*. (2011) in *A. nobilis*. Report of Ansari *et al*. (2006) indicating positive allometry in Ca, negative allometric growth in Fe and Zn in *Puntius chola* is in line with positive allometry in Ca (T1, T3) and negative allometry in Fe and Zn (T2) in GIFT in relation to body weight. Significant proportional increase in Zn (T1, T3) indicating positive allometry with body weight agrees with result of Naeem *et al*. (2012a) in *M. bleekeri*. These variations may be due to differences in foraging methods, metabolic rates and size of fish (Naeem *et al*., 2011) age, geographical distribution, and species-specific factors (Bawuro *et al*., 2018).

Cadmium showed least significant correlation with body weight and positive allometry in T1 and T3 fish, whereas, insignificant relation with body weight and negative allometry in T2 coincides with observations of Naeem *et al*. (2012 a) in *Mystus bleekeri*. This observation also agrees with reports of negative allometry between whole body Cd and body weight in *O. mykiss* and *A. nobilis* (Naeem *et al*., 2010, 2011). A non-significant relationship between Pb and body weight in T1, significant in T2 and highly significant correlation in T3 fish was observed. Lead exhibited negative allometry in T1 and positive allometry in T2 and T3 fish. Naeem *et al*. (2010) had also reported negative allometric increase in Pb with body weight in *O. mykiss*, although, Naeem *et al*. (2011) had discovered isometric increase of Pb with body weight in *A. nobilis*. These differences may be due to interspecific variations (Naeem *et al*., 2012b).

Effect of Total length on elemental concentration

Regression parameters revealed significant positive correlation for all metals, except for K, Mg and Pb (T1), Fe (T1, T2) and Cd (T1, T2, T3) which showed non-significant positive relationship with total length. Naeem *et al*. (2011) in Na and Fe had reported highly significant correlation between elemental concentration and total length of the studied fish, while Salam *et al*. (1996) in *O. niloticus* documented no influence of total length on concentration of element composition, except for Na, K, Sn and Pb which showed positive allometry with total length. Naeem *et al*. (2010) had also reported highly significant correlation in Na, K, Ca, Zn and Pb but non-significant relationship in Mg, Cd, Cu and Mn with total length in the studied *O. mykiss*. Similarly, Iqbal *et al*. (2019) observed non-significant relationship between all studied elements and total body length in hybrid (*Catla catla* x *Labeo rohita*) fed on 15%, 20% and 25% dietary protein levels. These variations, in the deposition of elements with increase in body length, may be the result of differences in fish species (Obot *et al*., 2016; Bawuro *et al*., 2018), fish age (Bawuro *et al*., 2018) and fish size (Naeem *et al*., 2011).

The present study revealed that all elements exhibited negative allometry, except for Na and Pb (T2) and Cd (T3) which showed isometric increase with total length. These results agree with those of Naeem *et al.* (2010) who had reported negative allometry (b < 3) in Na, K, Ca, Zn and Pb in *O. mykiss.* although Naeem *et al.* (2011) had documented positive allometry in Na. K, Ca, Mg, Fe, Zn, Cd and Pb with total length in *A. nobilis.* Negative allometry in Zn and Mg in relation to total length in the present study is in agreement with results of Naeem *et al.* (2011) in female *O. niloticus*. Ansari *et al.* (2006) had also reported negative allometry in Zn and Fe with total length in *Cirrhinus mrigala* and *Puntius chola,* respectively similar to the findings of present study. Cadmium (T1, T2) and Pb (T1, T3) indicated negative allometry with total length of sampled fish and this observation agrees with the opinion of Naeem *et al.* (2010). Isometric increase in Cd (T3) and Pb (T2) with total length is in line with Naeem *et al.* (2011). It is suggestive of some elements showing constant concentrations, while others exhibiting linear increase or decrease with the increase in the size of fish (Naeem *et al.*, 2010), or being site-specific (Chatta *et al.*, 2017) or species specific (Naeem *et al.*, 2012a; Obot *et al.*, 2016; Bawuro *et al.*, 2018).

Multiple regression analysis

Application of multiple predictive equations for the determination of elemental concentrations from wet body weight and total length revealved that non-significant correlations were observed for most of the elements in the various treatment groups. Naeem *et al.* (2011) had reported non-significant correlation in Ca, Zn, Cd and Pb which agrees with the findings of present study: Ca (T1, T2), Zn (T2, T3), Cd and Pb (T2). Current study findings also showed significant correlations with K, Na, Cd, Fe and Pb (T1, T3), Zn (T1) which is in agreement with the results of Naeem *et al.* (2010).

Conclusions and Recommendations

In the current investigation, graded dietary protein feed levels 15% (T1), 20% (T2) and 25% (T3) showed their influence by increasing the concentrations of studied elements with increasing level of crude protein in the diet. The maximum concentrations of whole-body elements were displayed in fish fed with 25% crude protein diet (T3). Variations in the ranges of elemental concentration may be due to changes in ratios of feed ingredients in three feeds (T1, T2, T3). A positive association between the elements and wet body weight as well as length of the sampled fish was investigated except for Fe in T1 with body weight. Predictive regression equations in the present study indicated that body size of studied GIFT showed its influence on the concentration of studied elements. As common trend of positive and negative allometry was observed for different elemental concentrations with fish body weight and total length. The level of non-essential toxic elements Cd and Pb has not exceeded the permitted levels of international standard. Further studies should be carried out in mono and polyculture in ponds instead of hapas to examine the impact of plant protein diets on elemental concentration of GIFT.

Novelty Statement

This study will be helpful for aquaculturists in the formulation of less expensive plant protein feeds and their no detrimental impact on fish health for the propagation of GIFT culture.

Author's Contribution

Anila Kousar performed experiment, did statistical analysis, and wrote the manuscript. Muhammad Naeem provided guidance for experimental layout, supervised the research work and helped in writing the manuscript. Samrah Masud helped in data analysis and writing the manuscript. Abir Ishtiaq helped in statistical analysis and reviewing of the manuscript. Zara Naeem helped in lab analysis. Rabia Iqbal assisted in lab work and statistical analysis of data.

Conflict of interest

The authors declare that they have no conflict of interest.

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