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EFFECT OF GRADED LEVELS OF DIETERY PLANT PROTEIN ON ELEMENTAL CONCENTRATION OF HYBRID (*Catla catla*♂ x *Labeo rohita*♀) FROM PAKISTAN

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ABSTRACT

The written report under discussion was conducted in 90 days in order to analyse the impact of three dietary protein feeds (15% CP, 20% CP, and 25% CP) on elemental concentration (Fe, K, Na, Zn, Ca, Mg, Pb, Cd) in hybrid fry (*Catla catla*♂ x *Labeo rohita*♀) by using Flame Atomic Absorption Spectrometry. Criteria for preparation of feed was based on cost effectiveness and local availability of ingredients. An increasing trend of essential elements was noted with increasing protein level. Accumulation trend in all treatment groups in decreasing order is "K"> "Ca"> "Na"> "Mg"> "Fe"> "Zn"> "Pb"> "Cd". Regression analysis between different elements and body weight exhibited significant correlation in group fed on 15% protein (T1) and 25% protein (T3) except for Pb (Lead) and Cd (Cadmium) respectively, while the subjects fed on diet containing 20% protein (T2) showed no correlations in different metals except in Ca (Calcium). The study revealed that metals in different groups exhibited diverse allometric relation with increasing body weight. Analysis of hybrid body length with total metal concentration revealed that all metals evaluated in the study showed allometrically negative and non significant correlation in all the groups fed on different diets. The multiple regression analysis showed no relation among hybrid body weight and length against elemental concentration in all three feeding groups except in some elements (Na, Pb, Cd). The results indicate, hybrid fed with plant protein feeds showed the levels of elements (essential and non essential) within the permitted levels of international standards.

Keywords: Crude protein (CP), hybrid, elemental concentration, Allometry, Multiple regression

INTRODUCTION

It is to be noted that Aquaculture required replacement of fish meal in the preparation of fish diets, especially for non-carnivorous species because of increasing cost day by day. Therefore, in lodge to prepare economic diets, alternative plant protein sources such as grains, pulses and oilseeds are widely used [1]. Positive impacts of good compounds in fish are badly impressed by the presence of toxic compounds in water body and fish body and also have an adverse consequence on human health when humans consume such fishes according to [2].

Heavy metals having non biodegradable nature and bioaccumulation ability in various tissues are badly disturbing fish health and surrounding ecosystem [3]. Metal accumulation in fish body is more than in water and sediment reported by [4].

It is a universal fact that all elements are not lethal as iron is required for hemoglobin and myoglobin and are therefore also found in two important proteins (ferritin and hemosiderin) in fish liver, only long term exposure of iron can be lethal [5, 6]. It is thus understood that low concentration of heavy metals can be dangerous if consumed for a long period. Similarly, if essential elements are practiced up in excessive quantity, they can work out to be extremely dangerous. Among important metals, lead is considered a toxic heart, delivering the lowest rate of evacuation and its poisoning causes anemia, colic, encephalopathy, abdominal pain and also acts as immunosuppressive in human [7]. A probable carcinogenic agent in human is cadmium, which also causes renal failure, kidney and liver diseases, anemia and skeletal weakening [8].

Referable to the rapid increase in industrialization, Pakistan is adversely facing a serious problem of aquatic pollution of heavy metals due to industrial effluents and domestic sewage in freshwater bodies especially in the state of Punjab. Moreover, heavy metals slow down the development rate of fish, increase mortality and also affect reproduction and physiological functions [9]. It is a fact that there exist three common passages or ways of heavy metals in the fish's body, namely gills, body surface and digestive tract [10]. Then, feed ingredients also have an imperative role in the aggregation of components in fish body.

Many surveys have been planned to develop a balanced diet containing different combinations of plant protein sources. The primary purpose of the present work was to develop three different cost benefit protein diets to aquaculture by using different combinations of constituents and to examine the potential shock of these diets on the elemental concentration of hybrid (*Catla* x *Labeo*) in hatchery environment.

MATERIALS AND METHODS

The present experimental study was conducted for three months (90 days) at Tawakkal Fish Hatchery, near District Muzaffargarh in Punjab (Pakistan).

Experimental Design

The hybrid fry (*Catla catla*♂ x *Labeo rohita*♀) were chosen for elemental composition by providing three different protein feeds consisting of 15%, 20% and 25% crude protein levels, because deep body of *Catla catla* and narrow head of *Labeo rohita* are ideal morphometric characters which can be gained in same fish by hybridization. Furthermore, the fry of hybrid were shifted in three hapas after acclimatization. The size of each hapa was 8X6X3 feet and placed in earthen ponds. Acclimatization period was 15 days in cement tanks and fish meal was fed to fry during this period. Each treatment feed was given in duplicates. Holding in view the rate of 5% with regard to body weight, the feed was provided to fish just one time during a daytime.

Methodology of feed preparation

Three diets containing protein levels as 15 % is called T1, 20% is called T2 and 25% is called T3 were prepared from different ingredients, most important plant protein sources are rice polish, wheat brawn, sunflower meal etc. The following steps are followed in feed preparation

1- Drying of all ingredients in oven at 600ºC.
2- Mixing of all ingredients in grinder for 40 minutes. **3-** Stirring of the powdered form feeds contents in feed mixing machine for 30 minutes. **4-** Storage of feed in polyethylene bags

Analysis of elemental composition in hybrid (*Catla catla*♂ x *Labeo rohita*♀)

After completing feeding trial of 90 days (3 months), fishes were shifted to ***Institute of Pure and Applied Biology (IP and AB), Bahauddin Zikriya University (BZU) Multan.*** Information on hybrid body weight in "gm" and total length in "cm" was noted after washing the fish's body. Steps for preparation of the sample for atomic absorption analysis are presented under,

1- Until gained constant weight, drying of each fish at 50-60°C. **2-** Digestion of each fish at 500°C for 6 hours. **3-** Dissolution of each sample in solution of 10 ml containing 70% HNO_3 in conical flask. **4-** Dryness of each sample at 82-100°C on hot plate. **5-** Dilution of each sample using deionized water. **6-** Determination of different elemental concentration using Atomic Absorption Spectrometer (Agilent Technologies 240 AA Series with spectra software) using the absorbance method of each element in flame atomization mode. Body weight and total length both influenced variations in elemental concentration so the interrelationships are examined by applying regression plus multiple regression equations. Multiple regression analysis was done by applying the following equation

Y = a+b1 (W) + b2 (X)

Where intercept is "**a"**, the regression coefficients are "**b1"** and "**b2"**, body weight is "**W",** total length is "**X"** and different body elemental contents are "**Y"**. Correlation coefficients r was also defined for these variables. Multiple regression (analysis) was performed by "MINITAB" statistical software.

RESULTS

All information regarding average values in all three feeding groups in hybrid (*Catla catla*♂ x *Labeo rohita*♀) is given in Table 1**.** Potassium (K) showed highest and increasing trend in average values of all treatment groups on dry weight basis, while Cadmium (Cd) observed minimum and decreasing trend in all feeds. Values of Iron (Fe), Sodium (Na) , Zinc (Zn), Calcium (Ca) and Magnesium (Mg) in all feeds in dry weight basis follow an increasing trend because 25% protein feed showed highest mean values in above mentioned elements as compared to 20% and 15% protein feed.

Hybrid Body weight relation with metal concentration

The value of "r" (correlation coefficient) among different elements and fish weight showed diverse correlations in different treatment groups in Table 2. When analysis of log total metal quantity was performed with log fish weight, it was observed that different elements in different feeding groups revealed increasing trend means "b">1.0, while others showed negative allometric correlation means "b"<1.0 showed in Table 2.

Hybrid Body length relation with metal concentration

Analysis of log length with log value of metal concentration results showed that all metals (Fe, K, Na, Zn, Ca, Mg, Pb, Cd) showed negative allometry "b"<"3.0" and no correlation showed in Table 3. Multiple regression analysis in Table 4 is between hybrid weight, length and different elemental variables showed non significant correlation in all parameters except in case of Fe, Na and Cd in first group (15% protein) and in case of Pb in third group (25% protein).

Table 1: Mean values of elemental concentration with standard error in whole body of hybrid (*Catla x Labeo*) (whole fish) (n = 10)

Elements	Treatment Groups	Mean ± S.E In dry weight (μgg^{-1})	Mean ± S.E In wet weight (μgg^{-1})
Fe	T1 (15%)	436.12±19.03	84.89±6.43
	T2 (20%)	548.14±78.54	104.79±13.11
	T3 (25%)	638.25±26.99	132.62±5.91
K	T1 (15%)	8277.5±347.70	1610.79±114.04
	T2 (20%)	9267.5 ±466.82	1777.29±110.17
	T3 (25%)	9857.5±251.67	2049.44±93.43
Na	T1 (15%)	4332.5±384.78	842.12±78.61
	T2 (20%)	4772.5±325.20	915.22 ±70.22
	T3 (25%)	5100±248.32	1060.52±68.42
Zn	T1 (15%)	184.37±5.50	35.88±2.36
	T2 (20%)	205.01±15.83	39.24±2.14
	T3 (25%)	222.12±12.53	46.78±2.69
Ca	T1 (15%)	5100±48.59	992.05±48.94
	T2 (20%)	5299.1±114.10	1015.89±31.43
	T3 (25%)	5511.46±125.31	1145.53±40.53
Mg	T1 (15%)	1911.07±131.52	372.15±36.26
	T2 (20%)	2104.2±168.71	403.19±30.71
	T3 (25%)	2329.61±119.31	484.18±27.99
Pb	T1 (15%)	1.92±0.23	0.37±0.04
	T2 (20%)	1.88±0.24	0.36±0.04
	T3 (25%)	1.85±0.15	0.38±0.03
Cd	T1 (15%)	0.54±0.194	0.105±0.039
	T2 (20%)	0.53±0.22	0.102±0.044
	T3 (25%)	0.50±0.15	0.105±0.03

S.D (Standard Deviation)

Table 2: Regression results of wet body weight in gm (log) with body elemental concentration in µg (log) of hybrid (Catla x Labeo) (n = 10)

Elements	Treatment Groups	Coefficient correlation (r)	Intercept (a)	Slope (b)	Standard error (b)	Value of t (when b=1)
Fe	T1 (15%)	0.847**	0.909	2.032	0.450	4.514
	T2 (20%)	0.069^{ns}	3.355	-0.242	1.236	-0.196
	T3 (25%)	0.779**	2.366	0.786	0.223	3.520
K	T1 (15%)	0.838**	2.338	1.880	0.451	4.171
	T2 (20%)	0.400^{ns}	3.518	0.750	0.607	1.235
	T3 (25%)	0.850**	3.213	1.086	0.238	4.559
Na	T1 (15%)	0.716*	2.087	1.848	0.636	2.903
	T2 (20%)	0.407^{ns}	3.004	0.960	0.762	1.259
	T3 (25%)	0.861**	2.508	1.454	0.304	4.781
Zn	T1 (15%)	0.682*	1.212	1.347	0.510	2.639
	T2 (20%)	0.303^{ns}	2.170	0.464	0.515	0.900
	T3 (25%)	0.880***	1.208	1.405	0.268	5.243
Ca	T1 (15%)	0.811**	2.599	1.403	0.358	3.915
	T2 (20%)	0.858**	2.650	1.331	0.282	4.718
	T3 (25%)	0.932***	2.781	1.244	0.171	7.280
Mg	T1 (15%)	0.788**	1.365	2.220	0.613	3.623
	T2 (20%)	0.554^{ns}	2.119	1.450	0.771	1.881
	T3 (25%)	0.742*	2.732	0.958	0.306	3.133
Pb	T1 (15%)	0.030^{ns}	0.491	0.066	0.782	0.085
	T2 (20%)	0.193^{ns}	-0.163	0.737	1.324	0.557
	T3 (25%)	0.877***	-1.358	1.827	0.355	5.152
Cd	T1 (15%)	0.296	-2.742	2.756	3.147	0.876
	T2 (20%)	0.118^{ns}	1.619	-1.459	4.328	-0.337
	T3 (25%)	0.046^{ns}	-0.122	0.230	1.779	0.129

(Coefficient of correlation (r), Intercept (a), Slope (b), Standard error (SE), Highly significant correlation=***P<0.001, Significant correlation=**P<0.01, Non significant correlation=>0.05)

Table 3: Regression results of total length in cm (log) with body elemental concentration in µg (log) of hybrid (Catla x Labeo) (n=10)

Elements	Treatment Groups	Coefficient correlation (r)	Intercept (a)	Slope (b)	Standard error (b)	Value of t (when b=3)
Fe	T1 (15%)	0.461^{ns}	2.536	0.435	0.296	1.468
	T2 (20%)	0.080^{ns}	3.358	-0.281	1.234	-0.228
	T3 (25%)	0.427^{ns}	2.905	0.355	0.266	1.334
K	T1 (15%)	0.445^{ns}	3.847	0.398	0.283	1.404
	T2 (20%)	0.126^{ns}	4.547	-0.235	0.657	-0.358
	T3 (25%)	0.443	3.992	0.458	0.336	1.360
Na	T1 (15%)	0.630^{ns}	3.214	0.800	0.221	3.617
	T2 (20%)	0.040^{ns}	3.949	0.094	0.833	1.113
	T3 (25%)	0.386^{ns}	3.625	0.538	0.455	1.182
Zn	T1 (15%)	0.350^{ns}	2.304	0.272	0.257	1.055
	T2 (20%)	0.009^{ns}	2.683	-0.014	0.541	-0.026
	T3 (25%)	0.507^{ns}	2.140	0.668	0.402	1.662
Ca	T1 (15%)	0.379^{ns}	3.758	0.258	0.223	1.157
	T2 (20%)	0.335^{ns}	3.594	0.520	0.517	1.006
	T3 (25%)	0.521^{ns}	3.623	0.574	0.333	1.725
Mg	T1 (15%)	0.414^{ns}	3.156	0.459	0.357	1.286
	T2 (20%)	0.153^{ns}	3.303	0.402	0.915	0.439
	T3 (25%)	0.375^{ns}	3.423	0.400	0.349	1.144
Pb	T1 (15%)	0.384^{ns}	0.266	0.334	0.284	1.175
	T2 (20%)	0.027^{ns}	0.532	0.104	1.348	0.077
	T3 (25%)	0.413^{ns}	0.011	0.711	0.554	1.283
Cd	T1 (15%)	0.137^{ns}	-0.458	0.501	1.285	0.390
	T2 (20%)	0.226^{ns}	2.665	-2.779	4.244	-0.655
	T3 (25%)	0.096^{ns}	-0.258	0.398	1.464	0.272

(Coefficient of correlation (r), Intercept (a), Slope (b), Standard error (SE), Highly significant correlation=*P<0.001, Significant correlation=**P<0.01, Non significant correlation=>0.05)**

Table 4: Multiple regression results of wet body weight in gm and total length in cm with elements burdrn in µgg^{-1} in wet body weight of hybrid (*Catla x Labeo*) (n=10)

Relationships	Treatment Groups	Coefficient correlation (r)	Intercept (a)	Value of b1+Standard error	Value of b2+Standard error	(r^2)
Fe = a + b_1 W + b_2 TL	T1 (15%)	0.684*	-6.4	12.43±5.35	-260±2.87	0.468
	T2 (20%)	0.351^{ns}	238	-9.8±13.0	-1.8±18.5	0.123
	T3 (25%)	0.321^{ns}	161.3	-194±2.76	-0.18±3.20	0.103
K = a + b_1 W + b_2 TL	T1 (15%)	0.618^{ns}	-11	202±102	-46.0±54.8	0.383
	T2 (20%)	0.482^{ns}	3033	42±102	-201±145	0.233
	T3 (25%)	0.152^{ns}	1.962	18.1±45.5	-16.2±52.7	0.023
Na= a + b_1 W + b_2 TL	T1 (15%)	0.670*	450	-11.2±66.6	67.2±35.7	0.449
	T2 (20%)	0.238^{ns}	1214	23.9±72.4	-67±103	0.057
	T3 (25%)	0.503^{ns}	671	44.4±29.1	-22.1±33.7	0.254
Zn = a + b_1 W + b_2 TL	T1 (15%)	0.344^{ns}	18.3	2.46±2.53	-0.85±1.36	0.119
	T2 (20%)	0.404^{ns}	66.6	-0.88±2.09	-1.93±2.96	0.164
	T3 (25%)	0.449^{ns}	28.6	1.24±1.19	0.11±1.37	0.202
Ca = a + b_1 W + b_2 TL	T1 (15%)	0.547^{ns}	433	81.0±46.7	-30.5±25.0	0.300
	T2 (20%)	0.505^{ns}	855	44.4±28.8	-42.3±40.9	0.256
	T3 (25%)	0.426^{ns}	894	19.2±18.1	-1.3±20.9	0.182
Mg =a + b_1 W + b_2 TL	T1 (15%)	0.607^{ns}	-136	61.5±32.9	-12.0±17.6	0.369
	T2 (20%)	0.309^{ns}	331	26.3±31.0	-27.6±44.0	0.096
	T3 (25%)	0.083^{ns}	517	-0.2±13.7	-2.9±15.9	0.007
Pb= a + b_1 W + b_2 TL	T1 (15%)	0.618^{ns}	0.877	-0.0758±0.0368	0.0312±0.0197	0.382
	T2 (20%)	0.618^{ns}	0.532	-0.0040±0.0492	-0.0141±0.0699	0.013
	T3 (25%)	0.663*	0.099	0.0266±0.0121	-0.0081±0.0140	0.44
Cd = a + b_1 W + b_2 TL	T1 (15%)	0.741*	-0.073	0.0277±0.0440	-0.0121±0.0236	0.055
	T2 (20%)	0.624^{ns}	0.384	-0.0096±00466	-0.0191±0.0662	0.039
	T3 (25%)	0.608^{ns}	0.169	-0.0079±0.0152	0.0045±0.0176	0.037

(Coefficient of correlation (r), Intercept (a), Regresssion coefficients (b1, b2), Standard error (SE), Highly significant correlation=*P<0.001, Significant correlation=**P<0.01, Non significant correlation=>0.05)**

DISSCUSION

Fishes are an important source of metals for humans and like other organisms, humans cannot destroy these metals instead accumulate within the body soft and hard tissues as the liver, muscles, and bone, which can be dangerous for human health, so it is very necessary to utilize pollutant free fish but unfortunately, no uniform or single source of standards are available for most metals in marine and freshwater fishes [11]. The present study also provided a look of elemental concentration in hybrid (*Catla x Labeo)* by providing three different protein diets.

Values of Na and K in hybrid of the present study are in the range values of the researcher [12] but the values of Mg, Fe and Zn of the present study are higher than [12], while values of Ca, Pb and Cd of the present study are lower than [12]. Present study values of Na, Mg, Pb and Cd on dry weight basis for whole fish are similar to the investigator [13] in *Labeo rohita,* while values of K, Mg and Fe are similar to the range values of [14] in *Catla catla.*

Values of Fe and Zn on dry weight basis of whole fish in current case, having similarity to the range values of [15] in *Puntius chola*. Present study values of Pb and Cd in all (T1, T2, T3) feeds are smaller than values gained by [16] in muscle tissues (Pb=11.63$\pm$3.07, Cd=0.79$\pm$0.48) of *Catla catla.* Present study values of Fe, Zn and Cd are higher and value of Pb is lower when compared with values of [17] in *Wallagu atto*.

The difference in results of the present study and previous reports can be explained on the basis of many factors influenced on accumulation of heavy metals in fish tissues as environmental conditions (temperature, salinity and pH), biological factors (sex, age, size and species), seasonal changes and handling of fish during transportation. Physical and chemical properties of elements are also important for bioaccumulation [18, 19, 20]. With respect to the present study, variations in concentration of different elements may be due to difference in the ratios of ingredients in three dietary protein feeds.

According to studies of [21], ranges of zinc, lead and cadmium in fish are 192-480, 2.4-48 µg/g and 0.1-2.8 µg/g and present study values are under these normal ranges for zinc (184-222 µg/g) and cadmium (0.50-0.54) on dry weight basis of whole fish and less than above mentioned values for lead (1.85-1.94 µg/g) in all (T1, T2, T3) feeds. Mean lead concentration of present study for all (T1, T2, T3) feeds are less than obtained in *Catla catla* (12.29 µg/g) and *Labeo rohita* (10.72

μg/g) [22]. Present study reported normal levels of Cd and Pb in all feeds which are permitted by [23, 24] in fish flesh may be due to pollution free environment of hatchery.

Regression was usually done to judge relation of these elements with fish weight and length to predict "isometric" and "allometric" increase of elements with respect to weight and length [25, 26].

Fe, Zn, Ca in 1st group (15%), Ca in 2nd group (20%) and Zn, Ca in 3rd group (25%) showed allometrically positive relation "b">"1.0" while Pb in 1st group (15%), Fe, K, Zn, Na in 2nd group (20%) and Fe, Na, Mg in 3rd group (25%) observed negative allometric relation "b"<"1.0" similar to the results of many researchers [12, 17, 15, 27, 28, 29, 30]. Present study hybrid length showed allometrically negative relation "b"<"3.0" in all metals similar to the results of [15] and [28].

When analysis of body weight with different elements was performed, it is suggested that Fe, K, Na, Zn, Ca and Mg showed significant correlations in the first group (15% protein) and a third group (25% protein) having similarity to the observations of [12, 26, 28]. Significant correlation of body weight with Mg and Zn in the first group (15% protein) and a third group (25% protein) feed and non significant relation to body weight with Cd in all feeds in hybrid case is found similar to the observation of [30] in *Mystus bleekri.*

The present study relationship of different metals with fish length suggested no correlation in the case of all metals contrary to the results of [12, 26, 28] who showed a significantly higher correlation of metal concentration with fish length. It has seen that concentration of different elements increased or decreased while others remained constant with increase in size, the possible reason is that some elements accumulation rate is higher than others to increase in fish size and may also be influenced by metabolic rate of fish, its foraging behavior, habitat of fish, temperature, seasonal changes and physical and chemical changes of water [12, 31].

It can be further noted that analysis of multiple regression between body weight, length and different metal showed an insignificant relation in the second group (20% protein) and a third group (25% protein) in case of Na, Cd and Zn have similarity to the results of [12].

CONCLUSION

It is concluded that the decreasing trend of elements in all three groups is as "K"> "Ca"> "Na"> "Mg"> "Fe"> "Zn"> "Pb"> "Cd". Dietary protein feeds increase essential elementals concentration with increasing protein level as 25% feed observed maximum gain. Non essential (Pb, Cd) elemental concentration values of three feeds (T1, T2, T3) are within normal ranges proved by many studies and international standards given by many researchers. The existing study also concluded that different metals increased or decreased with body weight, but total length have no influence on the elemental concentration of hybrid. Variations in elemental concentration ranges may be due to changes in ratios of feed ingredients in three feeds (T1, T2, T3). Such an analysis demand further investigations in terms of decreased or increased crude protein levels like 10% or 30% etc. to observe variations in elemental concentrations.

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