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Methionine Modulated Bioavailability of Inorganic Zinc ($ZnSO_4$. $7H_2O$) in Common Carp (*Cyprinus carpio* L.) Through Diets Containing Tricalcium Phosphate

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Abstract– *The present work has been conducted to study the efficacy of methionine for the intake of dietary inorganic Zinc ($ZnSO_4$. $7H_2O$) in the fingerlings of common carp (Cyprinus carpio L.). The experiment was performed in triplicate for which young ones of common carp of average 3.39±0.68g weight and 6.02±0.25 cm length were stocked in the indoor glass aquaria (60× 30 ×30cm) @11 fish/ aquarium. Five diets including control (D_1) and four experimental diets (D_2 to D_5) were formulated. In treatment diets D_2 and D_3 $ZnSO_4.7H_2O$ was added @88.4 and 176.8mg/kg, while in D_4 and D_5 $ZnSO_4.7H_2O$ was added @88.4 and 176.8mg/kg along with 1% DL Methionine to observe its role in uptake of zinc. Crude protein content in the experimental diets ranged between 36.68 - 39.14%, while zinc concentrations in diets (D_1 to D_5) were recorded 31.80, 57.40, 61.60, 56.60 and 62.80 mg kg^{-1}, respectively. Highest growth w.r.t. net weight gain was recorded (4.01g) and SGR (0.92%), feed conversion ratio (2.42) and protein efficiency ratio (1.08) was also recorded highest in fish fed with diet D_5. Moisture content (%) in fish flesh ranged between 77.20 – 78.90, protein 14.80 – 16.70, lipid 1.75 – 2.73, ash 1.82 – 2.61 and carbohydrate 1.13 – 1.92%, among the fish fed with diet D_1 to D_5. Zinc concentration was recorded significantly high in muscle (36.90 mg kg^{-1}), liver (60.40 mg kg^{-1}) and bone (109.56 mg kg^{-1}). The present study indicates that Zn uptake in different tissues has been significantly improved due to addition of methionine in the formulated diets for young ones of common carp.*

Keywords— Common carp, Cyprinus carpio, Methionine, Tricalcium phosphate, Zinc uptake.

I. INTRODUCTION

Minerals serve as an essential requirement for the variety of functions, as both intra and extra cellular components. Zinc is an essential micro mineral which is directly or indirectly involved in a wide variety of physiological processes including growth, development, reproduction and immune function (Watanabe *et al*., 1997). In fish, its deficiency leads to poor growth, high mortality, erosion of fins and skin, low content in bone (Takeuchi *et al*., 2002). Common carp (*Cyprinus carpio L.*) is an important fresh water carp, cultured across the world as food fish. Success of fish culture depends on rearing of quality seed, fed with nutritionally balanced diet and good management practices. For quality seed production with high survival rate, young ones must be fed with supplementary diets containing all essential nutrients in balanced quantity. Fish meal is always considered as one of the best protein sources containing all essential amino acids, hence are widely used in fish feed. Common carp requires high levels of dietary protein during early stage of life cycle, for this addition of supplementary protein of animal origin in the formulated diets is a common practice. Rendered (recycled) by-products from animal waste serve as cheap source of quality protein (El Seyed 1998), but their application is limited due to low digestibly and restricted bioavailability of nutrients as it tends to bind with organic compounds and forms insoluble complexes, due to presence of certain limiting factors (Cho *et al*., 1982; Gill, 2000). Diets containing animal protein especially fish meal as protein source contains Tricalcium phosphate (TCP), which acts as inhibitory factor for Zn uptake in fish (Davis *et al*., 1993).

Organic compounds or chelated forms are important source of trace minerals, because they protect trace elements from forming insoluble complexes (such as phytate and TCP) in the digestive tract and facilitate transport across the intestinal mucosa (Ashmead1993). During production process of fish meal, calcareous compound present in fish converts into calcium phosphates and their derivatives (Butler and Gross, 2017), even though fish scales also form a variety of calcium phosphate salts (Belouafa *et al*., 2017). Methionine is such sulfur containing amino acids essentially required in fish diets, especially those containing high levels of plant protein source (Mai *et al*., 2006). Being a wide cultivable fish species in India as well as in the world, common carp is still not well studied fish species with respect to response against the inhibitory effect of TCP, more over to find out the possibilities to mitigate the negative effect of TCP through amino acid modulation.

Keeping in view the above facts, the present work had been conducted to study the efficacy of methionine to enhance uptake of Zinc as $ZnSO_4$. $7H_2O$ in the fingerlings of common carp (*Cyprinus carpio L.*).

II. MATERIALS AND METHODS

The experiment was conducted at the Department of Aquaculture, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The experiment was conducted in triplicate through rearing the youngones of common carp of average 3.39±0.68g weight and 6.02±0.25 cm length in the indoor glass aquaria (60× 30 ×30cm) @11 fish/ aquarium. Five diets including one control (D_1) and four experimental diets (D_2 to D_5) were formulated and prepared by mixing different ingredients such as casein, dextrin, gelatin, fish meal, sodium alginate, soybean meal, oil, carboxy methyl cellulose, Zn free mineral, vitamin mix, zinc sulphate ($ZnSO_4.7H_2O$), TCP and DL-methionine as per ratio given in table 1. Fishes were fed with prepared experimental diets in crushed crumble form in two split doses @ 2% (each time) of fish body weight (BW) for 90 days. Physico-chemical parameter of water was observed for temperature, pH, dissolved oxygen, total alkalinity and total hardness as per standard methods given in APHA (2005).

Proximate composition of feed was carried out as per AOAC, 2000, while proximate composition of fish flesh was estimated for soluble protein Lowry *et al*. (1951), lipid Folch *et al*. (1957), moisture and ash AOAC, (2000) methods. Zinc concentration in flesh, liver and bone was analysed through atomic absorption spectrophotometer (Elico) as per standard method as described by Jorhem and Engman, (2000).

Growth, in terms of gain in net weight and length was calculated on the basis of difference in values at the start and termination of experiment. Specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) was calculated as per formula given below:

$$\text{SGR (\% increase in weight /day)} = \frac{\ln \text{Final body wt. (g)} - \ln \text{Initial body wt. (g)}}{\text{Number of days}} \times 100$$

$$\text{FCR} = \frac{\text{Feed given (g)}}{\text{Weight gain (g)}}$$

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

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Table. 1: Composition of feed ingredients in experimental diets (%)

Ingredients	Diets				
	D_1 Control (0mg Zn kg^{-1})	D_2 (20mg Zn kg^{-1})	D_3 (40mgZn kg^{-1})	D_4 (20mg Zn kg^{-1})	D_5 (40mg Zn kg^{-1})
	D_1	D_2	D_3	D_4	D_5
Casein	20	20	20	20	20
Dextrin	30	30	30	30	30
Gelatin	8	8	8	8	8
Fish Meal	10	10	10	10	10
Sodium Alginate	12	12	12	12	12
Soybean Meal	8	8	8	8	8
Soybean Oil	2	2	2	2	2
Carboxy Methyl Cellulose	5	5	5	5	5
Zinc Free Mineral*	3	3	3	3	3
Vitamin Mix**	2	2	2	2	2
$ZnSO_4.7H_2O$ (mg/kg diet)	0	88.4	176.8	88.4	176.8
Tri Calcium Phosphate***	0	2	2	2	2
DL Methionine***	0	0	0	1	1

*$FeSO_4.H_2O$: 41.16, $CuSO_4$:0.51, $CaCO_3$: 20.58, KIO_3: 10.56, $MgSO_4$: 44.50, $MnSO_4.H_2O$: 2.29, NaCl: 17.15(g kg^{-1} diet).

**Vitamin B2: 1.25g, Vitamin B6: 0.5g, Vitamin B12: 6.25 mg, Biotin: 12.5mg, Cal. Pantothenate: 1.25g, Niacinamide: 37.5g, Base: q.s. (g $100g^{-1}$)

***Added as over and above 100%

STATISTICAL ANALYSIS

Data recorded for Physico-chemical parameters of water, Body weight and growth parameters of fish, biochemical composition of flesh and change in zinc concentration (mg kg^{-1}) in different tissues were analysed by two-tailed bivariate Pearson's correlation coefficient for average values, standard deviation and correlation coefficient using one way ANOVA by SPSS 16.00 software.

III. RESULTS AND DISCUSSION

Fish, being an aquatic vertebrate is directly influenced by water quality parameters for their survival and growth. During the experimental period, physico-chemical parameters of water like temperature, pH and dissolved oxygen (DO) varied within the desirable range for fish culture as suggested by Boyd (1990) and Bhatnagar and Devi (2013) for warm water fish species. However, total alkalinity and hardness were slightly higher than optimum range but no negative effect in terms of behavioral changes recorded in fish. Details of the water quality recorded during the study period are given in table 2.

Table. 2: Physico-chemical parameters of water

Parameters	Range
Temperature (°C)	29.1 – 29.6
pH	8.41 – 8.48
Dissolved oxygen (mg l^{-1})	7.96 – 8.64
Total alkalinity (mg l^{-1})	260.00 – 271.00
Total Hardness (mg l^{-1})	275.00 – 287.00

Proximate composition estimation of feed helps to assess nutritional value of feed. Crude protein content in the fish fed with different experimental diets ranged between 36.68-39.14%, while zinc concentration in diets (D_1 to D_5) ranged 31.80, 57.40, 61.60, 56.60 and 62.80 mg kg^{-1}, respectively (Table 3). Among experimental diets, highest crude protein content recorded in D_5, followed by D_4, it may be possibly due to addition of methionine, which is itself a nitrogen containing biomolecule i.e. an essential amino acid. In prepared diets, higher concentration of zinc recorded as compared to rate of addition, which may be due to Zn content already available in different feed ingredients in different ratio, naturally. Garling and Wilson (1976) suggested that 25 – 36% crude protein as optimum level in diets for the warm water fishes, while Jader and Al-Sulevany (2012) reported the highest growth in common carp juveniles, when fed with 30% crude protein. Paul and

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Giri (2015) advocated the protein requirement in-between 25-35% for the optimum growth of fish. Singh *et al*. (2018) reported the highest growth in young ones of common carp @ 35% Crude protein level. In present study, protein content in experimental diets was within the range of protein requirement of common carp suggested by different workers.

Table. 3: Proximate composition (%) of formulated diets (on DM basis)

Proximate composition parameters	**Diets**				
	D_1	D_2	D_3	D_4	D_5
Moisture	8.88	8.98	9.12	8.66	8.54
Crude protein	36.68	37.12	36.28	38.66	39.14
Crude fat	2.12	1.98	2.22	2.44	2.56
Ash	9.14	9.28	11.00	10.62	11.24
Crude fiber	3.41	3.43	3.42	3.52	3.50
NFE	39.76	39.20	37.95	36.10	35.01
Zn (mg kg^{-1})	31.80	57.40	61.60	56.60	62.80

*Variation in proximate composition may be due to addition of TCP in D_2, D_3 and TCP with methionine in D_4 and D_5.

The growth in fish stocked during experimental period was assessed as increment in terms of net weight gain (NWG), specific growth rate (SGR) and efficiency of feed was assessed as feed conversion ratio (FCR) and protein efficiency ratio (PER) for each treatment (Table 4).

In fishes fed with different experimental diets, highest weight gain recorded in fish fed with D_5 diet, however in D_3 diet fed fish lowest growth rate was recorded, which may be possibly due to negative effect of tricalcium phosphate and no element/ carrier was added to cater the negative effect of TCP, however in treatment D_2 and D_4 in between highest and lowest growth rate recorded, which may be either due to less concentration of TCP or negative effect was mitigated due to addition of methionine. Similarly SGR, FCR and PER values also improved in fish fed with D_5 diet, it may be due to addition of methionine. The values recorded in present study were well close to the observations recorded by different workers. Sultana *et al*.(2001) reported the SGR in between 2.53 – 3.24, FCR 1.22 – 1.78 and PER 1.68 – 2.48 in common carp fry fed with 33.34% crude protein @ 5% body weight (BW) for 45 days. Kiaalvandi *et al*.(2011) reported the FCR in between 4.76 – 6.25 and PER 0.38 – 0.47 in common carp juveniles (8.6 g) fed with 26 – 28% crude protein @ 5% BW daily while Jader and Sulevany (2012) recorded the SGR 0.71 – 0.87, FCR 2.27 – 3.01 and PER 0.79 – 1.05 in juveniles of common carp when fed with 25-35% crude protein.

Table.4: Body weight (BW) and growth parameters of fish in different treatments during the experimental period (Mean ±SE)

Parameters	**Diets**				
	D_1	D_2	D_3	D_4	D_5
NWG	2.85±0.69	2.96±0.50	2.19±0.24	3.35±0.89	4.01±0.42
SGR	0.56±0.12	0.72±0.21	0.48±0.65	0.69±0.20	0.92±0.13
FCR	4.54±0.90	5.68±2.86	5.68±1.78	3.71±1.05	2.42±0.30
PER	0.64±0.13	0.71±0.25	0.56±0.13	0.81±0.23	1.08±0.12

The caracas composition of fish flesh analysed to evaluate changes in moisture, soluble protein, lipid, ash and nitrogen free extract (NFE) content w.r.t. growth and different experimental diets given during experimental period. The flesh samples were taken at the time of stocking and termination of experiment. With the progress of experiment, significant improvement in soluble protein, lipid, ash and NFE content recorded (Table 5). After termination of experiment, in fish flesh moisture content (%) ranged between 77.20 – 78.90, protein 14.80 – 16.70, lipid 1.75 –

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2.73, ash 1.82 – 2.61 and carbohydrate 1.13 – 1.92% among fish fed with experimental diets i.e. D_1 to D_5. Soluble protein content was recorded significantly higher ($P \leq 0.05$) in fish flesh fed with diet D_5, which indicates protein is not only digested but also absorbed and assimilated well in the fish flesh.

Table.5: Changes in biochemical composition (%) of common carp flesh (Mean± SE) fed with different diets

Parameters (%)	Initial	Diets				
		D_1	D_2	D_3	D_4	D_5
Moisture	$82.10^{a}\pm0.27$	$78.90^{b}\pm0.24$	$78.10^{cd}\pm0.35$	$78.00^{cd}\pm0.49$	$77.60^{de}\pm0.14$	$77.20^{e}\pm0.02$
Total proteins	$13.60^{d}\pm0.08$	$14.80^{c}\pm0.06$	$15.60^{b}\pm0.00$	$15.60^{b}\pm0.03$	$16.60^{a}\pm0.06$	$16.70^{a}\pm0.15$
Total lipids	$1.97^{cd}\pm0.21$	$2.73^{a}\pm0.06$	$2.27^{bc}\pm0.08$	$2.00^{cd}\pm0.00$	$2.08^{c}\pm0.03$	$1.75^{d}\pm0.05$
Ash	$1.40^{e}\pm0.05$	$1.82^{d}\pm0.07$	$2.52^{ab}\pm0.01$	$2.32^{c}\pm0.01$	$2.48^{b}\pm0.00$	$2.61^{a}\pm0.01$
Total carbohydrates	$0.77^{de}\pm0.27$	$1.67^{abc}\pm0.24$	$1.41^{abc}\pm0.17$	$1.92^{a}\pm0.07$	$1.13^{cd}\pm0.21$	$1.70^{ab}\pm0.31$

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

Zinc concentration in flesh, liver and bone analyzed at the start of experiment and after termination of experiment to access its uptake in different tissues at different concentration levels. Zinc concentration in fish flesh ranged in between 20.10 – 36.90, in liver 20.10 – 62.20 and in bone 89.70 – 109.56 mg kg^{-1} at the time of termination in diets D_1 -D_5, however at the time of stocking it was 18.60, 17.40 and 84.70 mg kg^{-1} in flesh, liver and bone, respectively (Table 6). Significantly high ($P \leq 0.05$) concentration of zinc in fish flesh fed with D_6 diet recorded, it may due to added Zn supplement at higher levels. In addition to this, it may also possible that absorption of zinc might be absorbed through gut in the presence of methionine. In liver, highest levels of Zn concentration recorded in fish fed with diet D_4.

Table. 6: Changes in zinc concentration (mg kg^{-1}) in different tissues of common carp (Mean± SE)

Fish organs	Initial	Diets				
		D_1	D_2	D_3	D_4	D_5
Flesh	$18.60^{f}\pm0.14$	$21.20^{d}\pm0.01$	$20.10^{e}\pm0.02$	$25.40^{c}\pm0.02$	$35.70^{b}\pm0.02$	$36.90^{a}\pm0.03$
Liver	$17.40^{f}\pm0.01$	$20.10^{e}\pm0.01$	$35.60^{d}\pm0.01$	$36.40^{c}\pm0.01$	$62.20^{a}\pm0.00$	$60.40^{b}\pm0.01$
Bone	$84.70^{f}\pm0.005$	$89.70^{e}\pm0.05$	$96.42^{c}\pm0.01$	$94.92^{d}\pm0.02$	$106.70^{b}\pm0.01$	$109.56^{a}\pm0.01$

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

IV. CONCLUSION

In the present study, high NWG, SGR, FCR, PER and soluble protein in fish fed with diet D_5 indicates feed containing even though 1% methionine helps in improving growth parameters, better feed utilization, absorption and assimilation of nutrients particularly protein, in the body of fish. Similarly, Zinc uptake was also improved in fish fed with diet D_5, which gives an indication that methionine, helps to improve zinc absorption and accumulation in body even though from its inorganic source i.e. $ZnSO_4 \cdot 7H_2O$ and mitigates the negative effect of TCP up to certain extent.

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