Methionine Modulated Bioavailability of Inorganic Zinc (ZnSO₄. 7H₂O) 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 (ZnSO₄. 7H₂O) 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 (D₁) and four experimental diets (D₂ to D₅) were formulated. In treatment diets D₂ and D₃ ZnSO₄.7H₂O was added @88.4 and 176.8mg/kg, while in D₄ and D₅ ZnSO₄.7H₂O 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 (D₁ to D₅) 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 D₅. 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 D₁ to D₅. 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 digestibility 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 (Ashmead 1993). During production process of fish meal, calcareous compound present in fish converts into calcium phosphates and their derivatives such as casein, dextrin, gelatin, fish meal, sodium alginate, soybean meal, oil, carboxy methyl cellulose, Zn free mineral, vitamin mix, zinc sulphate (ZnSO4.7H2O), 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:

\[
\text{SGR} = \frac{\ln \text{Final body wt. (g)} - \ln \text{Initial body wt. (g)}}{\text{Number of days}} \times 100
\]

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

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

uptake of Zinc as ZnSO4. 7H2O 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 x30cm) @ 11 fish/ aquarium. Five diets including one control (D1) and four experimental diets (D2 to D5) 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 (ZnSO4.7H2O), 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:
tive effect in terms of behavioral changes.

Fish, being an aquatic vertebrate, is directly influenced by water quality 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 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.

#### 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.

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

| Ingredients                  | Diets                                |
|------------------------------|--------------------------------------|
|                              | D₁ Control (0mg Zn kg⁻¹) D₂ Zn kg⁻¹ (20mg Zn kg⁻¹) D₃ Zn kg⁻¹ (40mg Zn kg⁻¹) D₄ Zn kg⁻¹ (20mg Zn kg⁻¹) D₅ Zn kg⁻¹ (40mg Zn kg⁻¹) |
| Casein                       | D₁ 20 D₂ 20 D₃ 20 D₄ 20 D₅ 20         |
| Dextrin                      | D₁ 30 D₂ 30 D₃ 30 D₄ 30 D₅ 30         |
| Gelatin                      | D₁ 8 D₂ 8 D₃ 8 D₄ 8 D₅ 8             |
| Fish Meal                    | D₁ 10 D₂ 10 D₃ 10 D₄ 10 D₅ 10         |
| Sodium Alginate              | D₁ 12 D₂ 12 D₃ 12 D₄ 12 D₅ 12         |
| Soybean Meal                 | D₁ 8 D₂ 8 D₃ 8 D₄ 8 D₅ 8             |
| Soybean Oil                  | D₁ 2 D₂ 2 D₃ 2 D₄ 2 D₅ 2             |
| Carboxy Methyl Cellulose     | D₁ 5 D₂ 5 D₃ 5 D₄ 5 D₅ 5             |
| Zinc Free Mineral*           | D₁ 3 D₂ 3 D₃ 3 D₄ 3 D₅ 3             |
| Vitamin Mix**                | D₁ 2 D₂ 2 D₃ 2 D₄ 2 D₅ 2             |
| ZnSO₄7H₂O (mg/kg diet)        | D₁ 0 176.8 D₃ 176.8 D₄ 176.8          |
| Tri Calcium Phosphate***     | D₁ 0 D₂ 2 D₃ 2 D₄ 2 D₅ 2             |
| DL Methionine***             | D₁ 0 D₂ 0 D₃ 1 D₄ 1 D₅ 1             |

* FeSO₄. H₂O: 41.16, CuSO₄.0.51, CaCO₃: 20.58, KIO₃: 10.56, MgSO₄: 44.50, MnSO₄.H₂O: 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%

#### 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 (D₁ to D₅) 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 D₅, followed by D₄, 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.
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 |
|---------------------------------|-------|
|                                 | D₁    | D₂    | D₃    | D₄    | D₅    |
| 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 D₂, D₃ and TCP with methionine in D₄ and D₅.

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 D₅ diet, however in D₁ diet fed fish lowest growth rate was recorded, which may be possibly due to negative effect of tricalcium phosphate and no element/carryer was added to cater the negative effect of TCP, however in treatment D₂ and D₃ 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 D₃ 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 | D₁    | D₂    | D₃    | D₄    | D₅    |
|------------|-------|-------|-------|-------|-------|
| 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±0.05 | 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 –
2.73, ash 1.82 – 2.61 and carbohydrate 1.13 – 1.92% among fish fed with experimental diets i.e. D1 to D5. Soluble protein content was recorded significantly higher (P ≤ 0.05) in fish flesh fed with diet D5, 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 | D1 | D2 | D3 | D4 | D5 |
|----------------|---------|----|----|----|----|----|
| Moisture       | 42.10±0.27 | 41.70±0.35 | 41.80±0.49 | 42.00±0.52 | 41.60±0.54 |
| Total proteins | 11.60±0.08 | 11.60±0.08 | 11.60±0.08 | 11.60±0.08 | 11.60±0.08 |
| Total lipids   | 2.60±0.21 | 2.60±0.21 | 2.60±0.21 | 2.60±0.21 | 2.60±0.21 |
| Ash            | 1.60±0.05 | 1.60±0.05 | 1.60±0.05 | 1.60±0.05 | 1.60±0.05 |
| Total carbohydrates | 0.77±0.27 | 0.77±0.27 | 0.77±0.27 | 0.77±0.27 | 0.77±0.27 |

* Values with different alphabetical superscripts differ significantly within row (P ≤ 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 D1 -D5, 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 ≤ 0.05) concentration of zinc in fish flesh fed with D5 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 D4.

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

| Fish organs | Initial | D1 | D2 | D3 | D4 | D5 |
|-------------|---------|----|----|----|----|----|
| Flesh       | 21.20±0.01 | 21.20±0.01 | 21.20±0.01 | 21.20±0.01 | 21.20±0.01 |
| Liver       | 35.60±0.01 | 35.60±0.01 | 35.60±0.01 | 35.60±0.01 | 35.60±0.01 |
| Bone        | 96.42±0.01 | 96.42±0.01 | 96.42±0.01 | 96.42±0.01 | 96.42±0.01 |

* Values with different alphabetical superscripts differ significantly within row (P ≤ 0.05)

IV. CONCLUSION

In the present study, high NWG, SGR, FCR, PER and soluble protein in fish fed with diet D5 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 D5, which gives an indication that methionine, helps to improve zinc absorption and accumulation in body even though from its inorganic source i.e. ZnSO4. 7H2O and mitigates the negative effect of TCP up to certain extent.

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