Bioavailability and tissue distribution of amino acid-chelated trace elements in rainbow trout

*Oncorhynchus mykiss*

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ABSTRACT: Feeding trials were conducted to determine the availability of amino acid-chelated trace elements to rainbow trout. Three practical diets were supplemented with trace element mix either all from sulfates (Tr-Sf), Zn and Mn from sulfates added with Cu from amino acid chelates (Cu-Am) or a mixture of trace elements from amino acid chelates (Tr-Am). Rainbow trout weighing 1.11 g were fed the experimental diets for 15 weeks. Growth, feed gain ratio (FGR), tissue distribution, retention of the elements and plasma alkaline phosphatase (ALP) activity were compared between the treatments. Absorption of the elements was determined using larger fish of approximately 95 g fed the same experimental diets. Growth and FGR were not significantly influenced by the chemical form of the elements. The highest concentration of Cu was measured in the liver, whereas highest concentrations of Zn and Mn were in bone. Plasma ALP activity was significantly higher in the Tr-Am group. The absorption of elements from the Tr-Am diet was higher but not significantly different from the other two diets. These results suggest that trace elements from Tr-Am seem to be more available than from inorganic sources tested.

KEY WORDS: alkaline phosphatase, amino acid chelates, bioavailability, elemental deposition, *Oncorhynchus mykiss*, trace elements.

INTRODUCTION

Some of the trace elements that are considered to be essential include zinc (Zn), manganese (Mn), and copper (Cu).1 Prominent among the factors that affect their absorption, tissue deposition and regulation of the body processes is their chemical form. Chelates, a form of mineral bound to a carbon-based or organic substance, are known to have higher availability than inorganic salts because of their stability and low molecular weight. Binding of the ion to a low molecular weight ligand (LMWL) permits a better understanding of Zn transport.2 The LMWL in the intestinal tract may also facilitate the uptake of Mn.3 The higher bioavailability of Mn from Mn proteinate than from MnO and MnSO4 suggests that chelate is the preferred chemical form for intestinal absorption.4 Considering Cu, amino acid complexes of Cu are widely used in animal nutrition and it has been shown that copper–lysine was as effective as sulfate in chicks, but its availability was only two-thirds of the inorganic salt in lambs.5 As for Zn, its availability from a Zn–amino acid complex for pigs and Zn–methionine for chicks was not significantly different from that of inorganic sources.6,7 However, Wedekind et al. reported that the availability of organic Zn could be greater than Zn sulfate.8 Likewise, studies on catfish exhibited that Zn–methionine was more efficient than Zn–sulfate in preventing deficiency symptoms.9 There are still species-dependent inconsistencies in the availability of trace elements from organic sources. In aquatic organisms, such as fish, studies on the availability of trace elements from various sources are limited.

In the present study, we further elucidate the bioavailability of amino acid-chelated trace elements and their effect on growth and tissue deposition in rainbow trout.

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MATERIALS AND METHODS

Experimental diet, fish and feeding

Three diets were formulated and supplemented with trace elements either in sulfate form (Kokusan Chemical Works, Tokyo, Japan), amino acid-chelated Cu combined with Zn and Mn in sulfate form or a combination of trace elements from an amino acid chelate (Eisai, Tokyo, Japan) designated as Tr-Sf, Cu-Am and Tr-Am, respectively (Tables 1–3). The elements from either of the sources were supplemented at 40 mg Zn, 20 mg Mn and 4 mg Cu/g test diet. Proximate and mineral compositions of the diets are shown in Table 4.

TriPLICATE groups of rainbow trout (30 fish/tank) with an average weight of 1.11 ± 0.17 g were fed the experimental diets three times daily to near satiation for 15 weeks. Fish were reared in 60 L glass rectangular tanks in a partly recirculating system at a flow rate of 500–600 mL/min and provided with sufficient aeration. The feeding trial was performed in a completely randomized design.

Sample collection and chemical analyses

Fish were weighed individually before the start of the experiment and every 3 weeks thereafter. At the end of the trial, 10 fish/tank were selected at random for whole-body and bone analyses. Plasma, tissue, muscle and bone samples for mineral analysis were taken from the same fish. Fish were dissected and tissues were separately excised and washed with 0.85% NaCl and stored at -20°C for the analysis. Muscle samples were taken from the dorsolateral portion of the fish. Another set of 5 fish/tank was sampled randomly for the analysis of plasma alkaline phosphatase (ALP).

All samples were prepared and analyzed as described by Satoh et al.10 and Apines et al.11 Except for phosphorus, mineral concentrations were measured by a Polarized Zeeman Atomic Absorption Spectrophotometer (Hitachi Z-5010; Hitachi, Tokyo, Japan).

Alkaline phosphatase

Alkaline phosphatase (EC 3.1.3.1) activity was determined colorimetrically using p-nitrophenyl phosphate (Sigma, St Louis, MO, USA) as a substrate following the method of Snedeker and Greger12 with some modifications. Briefly, 15 µL diluted sample was added to the substrate and

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Table 1  Composition of the experimental diets (g/kg)

<table>
<thead>
<tr>
<th>Diet code</th>
<th>Tr-Sf</th>
<th>Cu-Am</th>
<th>Tr-Am</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet*</td>
<td>931</td>
<td>931</td>
<td>931</td>
</tr>
<tr>
<td>Complete mineral mixture</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cu-free mineral mix</td>
<td>–</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>Zn-, Mn- and Cu-free mineral mix</td>
<td>–</td>
<td>–</td>
<td>10</td>
</tr>
<tr>
<td>Zn, Mn-sulfate + Cu-AA chelate mix†</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Zn, Mn, Cu-AA chelate mix‡</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>49</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Cr2O3 (50%)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

*See Table 2.
†4 mg Cu/g (0.4 g amino acid (AA)-chelated Cu/9.6 g dextrin).
‡40 mg Zn, 20 mg Mn, 4 mg Cu/g (4 g AA-chelated Zn, 2 g AA-chelated Mn, 0.4 g AA-chelated Cu/3.6 g dextrin).

Tr-Sf, trace element mix all from sulfates; Cu-Am, Zn and Mn from sulfates added with Cu from amino acid chelates; Tr-Am, mixture of trace elements from amino acid chelates.

Table 2  Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jack mackerel meal</td>
<td>270</td>
</tr>
<tr>
<td>Defatted soybean meal</td>
<td>200</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>150</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>60</td>
</tr>
<tr>
<td>Pregelatinized starch</td>
<td>50</td>
</tr>
<tr>
<td>Dextrin</td>
<td>50</td>
</tr>
<tr>
<td>Pollock liver oil</td>
<td>50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>60</td>
</tr>
<tr>
<td>Vitamin mixture*</td>
<td>15</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin E (50%)</td>
<td>1</td>
</tr>
<tr>
<td>NaH2PO4.2H2O</td>
<td>20</td>
</tr>
</tbody>
</table>

*Vitamin mix (mg/100 g diet): vitamin B1 6.0; vitamin B2 10.0; vitamin B6 4.0; vitamin B12 0.01; vitamin C 500; vitamin K3 5.0; niacin 40.0; Ca-pantothenate 10.0; inositol 200; biotin 0.6; folic acid 1.5; p-aminobenzoic acid 5.0; vitamin A 4000 IU; vitamin D3 4000 IU.
incubated for 30 min. Then, 2 mL of 3 mol/L NaOH was added to stop the reaction. The change in absorbance at 405 nm due to the formation of p-nitrophenol is directly proportional to ALP activity.

Absorption study

Rainbow trout of approximately 100 ± 18 g were stocked in 60 L glass rectangular tanks with 20 fish in each tank. Feeding of the experimental diets was conducted in duplicate. Rearing conditions were the same as mentioned above. Methods for feces collection were the same as described by Apines et al.11 Chromic oxide was digested following the method of Furukawa and Tsukahara13 and the concentration was computed from the absorbance as determined by visible light spectrophotometry (UV 265 FW; Shimadzu, Kyoto, Japan).

Kinetics of plasma trace elements

Uptake of trace elements was determined using fish weighing approximately 100 g. Fish were acclimatized to the experimental diets for 1 week. Plasma samples were taken from five fish after 3 days of starvation to serve as a control. Fish were again fed the diets and plasma was collected from three fish for each treatment at 0.5, 6, 12, 24, 36, 48, 60 and 72 h after feeding.

Statistical analysis

Means were compared by one-way ANOVA using SYSTAT 8.0 software program (SPSS Inc., Chicago, IL, USA). Differences between means were determined by Tukey’s test, except for ALP activity, which was analyzed by Fisher’s Least Significant Difference test. All tests used a significance level of P = 0.05.

RESULTS

Growth and feed utilization

Growth of fish did not differ significantly among the groups throughout the culture period (Table 5).
Likewise, feed consumption and feed gain ratio were not affected by the treatments.

Plasma mineral contents

Plasma trace element uptake was found to vary with time (Fig. 1). The concentration of Cu peaked between 6 and 12 h after feeding, whereas the highest Zn concentration was attained between 36 and 60 h post-prandial. The concentration of Mn peaked at approximately 24 h, beyond which time dropped gradually. Plasma trace element concentrations were not significantly influenced by the dietary treatments over the 15 week feeding trial (Table 5).

Whole-body and tissue distribution of the trace elements

Whole-body Cu and Mn levels were similar in all treatments, but the Zn content was significantly higher in the Cu-Am group (Table 5). Among the tissues examined, Cu was significantly higher in the liver; however, there were no significant differences between sources (Fig. 2). The Cu content in bone was significantly higher for the Cu-Am group compared with the other groups.

The Mn level was significantly higher in bone compared with other tissues, with the Cu-Am diet again showing a greater accumulation (Fig. 3). Heart and liver Mn content was significantly higher in the Tr-Am and Cu-Am groups, respectively.

Fish bones contained significantly high amounts of Zn, but the levels were not affected by the treatments (Fig. 4). Mineral sources did not significantly effect the concentration of Zn in the tissues.

Alkaline phosphatase activity

Fish in the Tr-Am group exhibited a significantly higher ALP activity than did fish in the other two groups (Fig. 5).

Absorption and retention

Absorption of Cu and Mn was slightly higher in the Tr-Am group, but the differences between the groups were not significant (Table 5). In contrast, the absorption of Zn in the Cu-Am group was sig-
Retention of Cu was not affected by the treatments. However, Mn and Zn retention in the Tr-Sf and Cu-Am groups, respectively, was significantly high (Table 5).

**DISCUSSION**

Growth was not significantly affected by the chemical form of the elements, confirming our previous result that different sources of Zn had no effect on the weight gain of fish.\(^{11}\) In addition, in channel catfish,\(^{14}\) as well as in heifers,\(^{15}\) the chemical form of Zn and Cu, respectively, did not affect weight gain. In contrast, Mn sources significantly affected the growth of rainbow trout in our previous study, with amino acid chelates showing the best growth.\(^{16}\) The effect of mineral sources on growth in the present study may not be apparent, but may produce subtle changes in the metabolism and body regulatory mechanisms of the fish.

The present study also examined the plasma mineral content, a commonly used indicator of mineral status. In humans, Cu deficiency always results in lower levels of Cu in the plasma.\(^{17}\) In the present study, kinetics of trace element uptake in the plasma varied for each metal. The faster absorption of Cu compared with Mn and Zn may be due to the lesser binding capacity of Cu with mineral inhibitors in the small intestine when compared with Zn and Mn.\(^{18}\) In the case of Zn, its slow passage from the intestinal lumen into the
circulation may be due to its binding to intracellular ligands.\textsuperscript{18} However, after 15 weeks of feeding, we found no significant differences in plasma mineral concentrations among the sources. Gomes and Kaushik\textsuperscript{14} also found that Zn sources did not affect the plasma Zn concentration of the fish. Likewise, in heifers, different sources of Cu did not affect the plasma Cu concentration of the animal.\textsuperscript{15} In most species, plasma/serum and whole-blood Cu concentrations are similar, whereas in birds, fish and marsupials, the concentrations are approximately half those of other species.\textsuperscript{19}

Whole-body Cu and Mn contents of the fish did not vary significantly among the treatments, suggesting that the source from which they were ingested had no influence on whole-body deposition. However, Zn had a significantly higher level in the Cu-Am group, showing that its chemical form played a role in its availability.

The highest concentration of Cu is normally found in the liver, brain, heart and hair.\textsuperscript{1} A significantly higher Cu content of the liver was observed in the present study, as has been found in humans,\textsuperscript{20} confirming that it is the main storage and regulatory organ of this element.\textsuperscript{21} After its hepatic uptake, Cu may be stored within hepatocytes, secreted into plasma or excreted in the bile, which represents the major excretory route for this

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**Fig. 3** Tissue Mn distribution in rainbow trout fed trace elements from different sources (n = pooled samples of five fish). Means with different letters are significantly different (P < 0.05).

**Fig. 4** Tissue Zn distribution in rainbow trout fed trace elements from different sources (n = pooled samples of five fish). Means are not significantly different (P > 0.05).
element. The higher liver concentration of Cu in ruminants compared with non-ruminants is thought to reflect a higher retention of absorbed Cu rather than a difference in dietary uptake of Cu or its absorption. Thus, the high levels of Cu we found in rainbow trout may also reflect its high retention in the liver tissue; however, no significant differences were observed among the sources. Second to liver, bone also contained a considerable amount of Cu, with the Cu-Am group showing a significantly higher deposition than the rest, probably indicating a better availability of the amino acid chelate from this group.

The accumulation of Mn in the present study was found to be highest in bone, with the Cu-Am group exhibiting a significantly higher level among the three groups. Similarly in chicks, liver and bone Mn accumulation appeared to be an excellent indicator of Mn availability. In rats, it has been reported that Mn inhibits loss of bone mass. In sheep, however, liver was the most responsive to dietary Mn, followed by kidney and bone. In the present study too, bone, kidney and liver had a considerably higher Mn level compared with other organs. Similarly, heart and liver Mn contents were also affected by the treatments, indicating that the different forms of Mn affected its tissue distribution.

A significantly higher bone Zn content in the present study was consistent with the findings in higher vertebrates and fish that bone is the reservoir for Zn. In catfish, dietary Zn affected bone but not liver Zn content, indicating the preferential storage of the element in this tissue. There is a growing evidence that Zn is important in the regulation of bone metabolism by stimulating bone formation and inhibiting bone resorption. In the present study, Zn deposition in bones from an amino acid chelate was slightly higher than from inorganic sources, but this difference was not statistically significant. The same pattern of was observed as in the previous study. The results also indicated that tissues vary in their capacity to absorb Zn and may not always be affected by its chemical form.

Being cofactors of many enzymes, a deficiency of minerals can cause degradation of enzymes involved in the regulation of body processes. Zinc has been shown to have a role in enzymes such as ALP, a Zn-dependent enzyme found on the surface of osteoblasts and in the circulation. In the present study, the Zn amino acid chelate-supplemented group showed a significantly higher ALP activity than the other groups, suggesting a better utilization of the elements in this group. This finding supports our previous results, where fish supplemented with a Zn amino acid chelate exhibited significantly higher ALP activity than fish supplemented with other Zn compounds. In rats, the addition of Zn also resulted in a significant increase in ALP activity in an in vitro system. Similarly, Zn has also been found to increase the half-life of skeletal ALP activity. Zinc deficiency may induce structural changes in the enzyme that favor degradation, resulting in increased turnover rates and lower activity in the tissues.

Absorption of Zn was significantly higher again in the Cu-Am group, which is in contrast with our previous result. Although not significantly different, absorption of Cu and Mn in the present study tended to be higher in the Tr-Am group, indicating that chelates of amino acids for these elements are better utilized by fish compared with the metal salts and the results agree with those of our previous study. In rats, Mn absorption was also enhanced by the presence of LMWL. In the present study, absorption of Cu, Mn and Zn ranged from 80 to 82%, from 73 to 78% and from 37 to 44%, respectively. However, in humans, absorption of Cu was only 30–40% due to the lower digestibility of Cu from vegetables, which is their main source.

The retention of Cu and Mn was not affected by the treatments in the present study. However, Zn from the Cu-Am group showed a significantly higher retention, indicating that the chemical form of Zn plays a role in its availability and retention. Interestingly, Zn in this group is in the form of sulfate (inorganic source). This indicates that amino acid-chelated trace elements, when combined, may not always give a better performance, which still needs to be clarified. Among the elements, Mn exhibited a significantly lower retention, which conforms with reports in higher animals that Mn has the fastest turnover rate with the least amount being retained in the tissues.
Based on the results, trace elements from amino acid chelates seem to be more available than those from inorganic sources tested. Furthermore, among the three elements, Zn seems to be more available in the Cu-Am group, as indicated by its absorption and retention, as well as whole-body Zn concentration. Studies are needed to further elucidate the effect of combined amino acid-chelated trace elements in fish diet.

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