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Absorption, body distribution, and excretion of dietary zinc by rainbow trout (*Salmo gairdneri*)

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Abstract

Rainbow trout (*Salmo gairdneri*) were held in metabolizable energy chambers at Standard Environmental Temperature (15°C) for 72h following a single feeding of a semi-purified test diet containing tracer quantities of a radioisotope of zinc (^{65}Zn) and different combinations of dietary calcium level and zinc source. Gill wastes, urine, and feces were separately collected. After 72h, the fish were killed, and samples of the following tissues removed: eyes, skin, muscle, blood, bone, liver, bile, kidney, gill, spleen, stomach, pyloric caeca, intestine, gonad, and remaining carcass. Radioactivity in the tissues and wastes was determined and the body distribution of the ingested zinc was quantified. Approximately 58% of the administered dose of ^{65}Zn was recovered. Of the recovered dose, 43.2% was present in the gastro-intestinal tract, 27% in the feces, 14% in the gill water, 16% in the body of the fish, and less than 1% in the urine. Of individual tissues, the gill, liver, kidney, and spleen had concentrations of ^{65}Zn higher than blood, while the remaining tissues had lower concentrations. Body and tissue levels were increased but not significantly by feeding ^{65}Zn as an amino acid chelate, compared to feeding as inorganic ^{65}Zn, while dietary calcium level had no effect. The results of this study indicate that the gills play a major role in excretion of dietary zinc, while the urine plays a minor role.

Introduction

Mineral studies in fish are difficult to conduct because of the complications which arise from fishes' aquatic existence. Fish can obtain some minerals by swallowing water along with food and by direct absorption by the gills (NRC 1981). For example, it is known that calcium is taken from water since fish must be raised in low-calcium water to produce deficiency signs. However, many essential minerals, such as zinc, are not taken from water in sufficient amounts to meet the needs of growing fish and must therefore be supplied by the diet to prevent deficiency signs (Ogino 1978; Ketola 1979; Hardy and Shearer 1985).

Most zinc feeding studies involve some assessment of the zinc status of the fish, such as evaluating whole body levels or zinc concentrations of specific tissues (Spinelli *et al.* 1982; Wekell *et al.* 1983; Jeng and Sun 1981; Hardy and Shearer 1985). While the measurements of zinc status used in these studies reflect retention in fish, they do not differentiate between absorption and excretion. In order to measure zinc absorption in fish, the zinc concentration of the food and feces must be known. To measure excretion, zinc levels in urine

and/or gill wastes must be determined.

Seperate collection of feces, urine, and gill wastes in fish can be made using a metabolizable energy chamber (Smith 1971). These chambers are primarily used to measure the metabolizable energy content of feed ingredients. However, they are ideally suited for use in studies on the absorption, retention, and excretion of a pulsed dietary component. In such studies, one must be able to identify the amount of the component found in the feces, urine, gill wastes, and body originating from a single feeding. The use of radionuclides permits the differentiation between existing levels in tissues of the dietary component of interest before the experiment and the amount retained or lost by the fish after a single feeding.

Rainbow trout can tolerate dietary zinc levels of 1700 μg/g without apparent toxicity and without accumulating zinc in the tissues other than liver, blood, and gills (Wekell *et al.* 1983). The high levels of zinc in blood and gills suggested that regulation of tissue zinc concentration in rainbow trout might be due in part to excretion of zinc by the gills, as suggested by Nakatani (1966). The first part of this study was conducted to measure absorption, retention, and excretion via the urine and gills of ^{65}Zn by rainbow trout after a single feeding.

The dietary zinc requirement of rainbow trout is reported to be 15–30 ppm in semi-purified diets (Ogino and Yang 1978). However, the dietary level required to prevent deficiency signs is much higher in practical diets containing compounds that reduce the availability of dietary zinc. Recent reports have shown that dietary calcium and phosphorus levels alone (Hardy and Shearer 1985) or in combination with phytic acid (Richardson *et al.* 1985) reduce the availability of dietary zinc in rainbow trout diets. The proposed mechanism for this reduction is that calcium and phosphorus form insoluble precipitates during passage from the acid stomach to the alkaline intestine which complex with zinc, preventing intestinal absorption. Dietary phytic acid prevents intestinal absorption even further. Chelated forms of zinc do not form such complexes and thus are available to the fish. The second part of this study was conducted to determine if dietary calcium/phosphorus level and zinc source ($ZnSO_4$ vs Zn-amino acid chelate) affected absorption, retention, or excretion of ^{65}Zn from a single feeding. A number of individual tissues were analyzed for ^{65}Zn content to determine if difference due to diet could be detected.

Materials and methods

In order to separately collect feces, urine, and gill excretions from rainbow trout, six plexiglass chambers were constructed using the specifications of Smith (1971). These chambers were first used to conduct a material balance study to determine the extent to which dietary ^{65}Zn was absorbed, retained in the body, and excreted in the urine or by the gills. For subsequent studies to determine the effects of the dietary treatments, the static head reservoirs of the chambers, required to collect gill waste, were changed to flow-through reservoirs. Diets containing two levels of calcium/phosphorus (1 and 4% calcium with 1.9 and 2.6% phosphorus, respectively) in combination with two dietary zinc sources, $ZnSO_4$ and Zn-amino acid chelate, to make four dietary treatments were tested. Eight to twelve fish per dietary treatment successfully completed the 72h collection period after feeding, although several were later omitted from the analysis, as described later. After 72h, each fish was sacrificed and tissue samples were removed for analysis of ^{65}Zn content.

Experimental animals

Test subjects, obtained from the University of Washington experimental fish hatchery, were male and female 2-year-old rainbow trout (*Salmo gairdneri*) with similar mean weight (382 ± 11 g). Prior to the experiment, all fish were held in dechlorinated, aerated, municipal water (15° ± 1°C). The fish were fed once daily to satiation with a commercial trout feed containing 130 μg zinc/kg diet.

Fish placement in chambers

The chambers were supplied with a mixture of chilled, dechlorinated, municipal water and ambient-temperature Lake Washington water (3 l/min). The two water sources were oxygenated and mixed to maintain a temperature of 15 ± 1°C in the chambers. Six fish were placed in the six chambers each week and held without feeding. After 2 days, the fish were anesthetized in a solution of ethyl m-aminobenzoate methanesulfonate (MS-222, 100 mg/l) buffered to pH 7.4 with $CaCO_3$, and their urinary bladders were catheterized with 13 mm O.D. poly-ethylene tubing (PE50, Clay Adams, Inc.). One end of the tubing was tapered, flamed, lubricated with petroleum jelly, and inserted through the urogenital papilla into the urinary bladder. The catheter was held in place by two sutures through the base of the anal fin. A rubber diaphragm, fashioned from the cuff end of a size 6 surgical glove (Surgicose) isolated the water in the head reservoir of the chamber from the water in the tail reservoir which contained fecal waste. The diaphragm was sutured to the dorsal fin and was covered by a second diaphragm which was not attached to the fish. This arrangement provided for a water-tight seal between the head and tail reservoirs of the metabolizable energy chamber. Water flowed through the head reservoir, while the tail reservoir was static. The tail reservoir was surrounded by a coil of tubing containing 15° ± 1°C water to maintain the water temperature inside. The fish were held in the chambers, unfed, for an additional 24h prior to administration of the experimental diets. Any fish that did not adjust to the chambers, that struggled and shed its catheter or diaphragm, or that did not produce urine was removed at this point and omitted from the study.

Diet preparation

Four experimental diets were prepared differing only in the level of $Ca_3(PO_4)_2$ and zinc source (Table 1). ^{65}Zn Cl (specific activity of 4.79 mCi/mg) was obtained from New England Nuclear and was added to the diets at a level of 8 μ Ci/g feed. To produce the ^{65}Zn-amino acid chelate, 0.5 ml of dilute $^{65}ZnCl$ in 0.5 N HCL (2.0 mCi/ml) was combined with 0.5 ml of $ZnSO_4$ solution (2.5 mg Zn/ml) and 49 ml of amino acid chelate solution. The mixture was allowed to stand for 2h and was then heated to boiling and mixed with the other dietary ingredients. The diet mixture was extruded through a large syringe to form pellets, and stored frozen at −80°C until used. The basal diet contained 35 ppm Zn and the Zn supplements added an additional 25 ppm to make a total of 60 ppm Zn in each diet.

Table 1. Composition of experimental diets

Ingredient	g/kg diet
Vitamin-free casein	260.0
Gelatin	52.5
Pre-gelatinized wheat starch	29.5
Carboxymethylcellulose	10.0
Dextrin[1]	73.5, 37.5
Vitamin premix[2]	7.5
Choline choride (70%)	2.5
Cod liver oil	52.5
$Ca_3(PO_4)_2$[3]	12.0, 48.0
Mineral solution[4]	490
Zinc supplement[5]	10

[1] Dextrin replaced as required by $Ca_3(PO_4)_2$.

[2] Vitamin premix supplied the following per kg diet: 141 mg D-calcium pantothenate; 41 mg pyridoxine-HCl; 111 mg riboflavin; 293 mg nicaninamide; 17 mg folic acid; 57 mg thiamin mononitrate; 0.79 mg biotin; 0.08 mg vitamin B^{12}; 15 mg menadione sodium bisulfite; 668 mg alpha tocopheryl acetate; 8,800 I.U. vitamin A palmatate or acetate; 352 mg myoinositol; 1,188 mg ascorbic acid; and 660 I.U. vitamin D_3.

[3] $Ca_3(PO_4)_2$ added to provide 1% calcium in diets 1 and 2, and 4% to diets 3 and 4.

[4] Mineral solution supplied the following kg per diet: 573 mg Mn; 333 mg Fe; 125 mg Cu; 22 mg Co; 6,430 mg Mg.

[5] $ZnSO_4$ solution added 25 ppm Zn to diets 1 and 3. Zn-amino acid chelate solution added 25 ppm Zn to diets 2 and 4.

Feeding and collection procedures

After three days in the chambers, fish that had adjusted to the experimental conditions were anesthetized and force-fed 0.25% of their body weight with one of the four test diets. They ingested 20 nCi

^{65}Zn/g body weight, and were held in the chambers for 72h after being fed. The fish were watched carefully for any sign of regurgitation, and those that regurgitated the pellets were discarded.

A 72h collection period was chosen based on a preliminary study of rate of passage of diet 1 through rainbow trout held in the chambers. To measure rate of passage, 1% chromic oxide was added to the diet and the fish were force-fed a measured amount of diet. Tail reservoir water containing feces was collected after 48h and 72h and the chromic oxide concentration was determined (Furakawa and Tsukahara 1966). The results showed that the chromic oxide was completely recovered, with approximately one-half of the chromic oxide appearing in the tail water after 48h and the remainder at 72h.

The relative importance of the various excretory pathways for Zn was evaluated in a material balance study by determining the distribution of ^{65}Zn among head reservoir water (gill excretions), tail reservoir water (fecal waste), and urine. Six fish were held for 72h in static water in chambers maintained at 15° ± 1°C by passing chilled water through coils of plastic tubing placed in the head reservoir and around the tail reservoir. The oxygen content of the head reservoir water was maintained near saturation by bubbling O_2. Three water samples (5.0 ml each) were taken from the head reservoir at 12h intervals, at which time the water in the reservoir was changed. All other fish were maintained for 72h in chambers with head reservoirs supplied with flowing water (3 l/min, 11.0 ppm O_2) at a temperature of 15° ± 1°C. For all fish, tail reservoir water (feces) and urine were collected after 72h, the volume of water or urine was recorded, and the samples were frozen at −80°C until processed for determination of specific activity.

Seventy-two hours after feeding, the fish were killed with concentrated anesthetic and dissected to obtain tissue samples for determination of specific activity. Prior to dissection, a blood sample was obtained from the caudal vessels with a syringe containing 0.2 ml of 1.19 mg/ml ammonium heparin as an anticoagulant (blood volumes were corrected for dilution). In the following order, samples of skin, muscle, eye, gill, spleen, liver, bile, esophagus and stomach, pyloric caeca, intestine, gonad, kidney, bone, and remaining carcass were obtained. Skin, muscle, eye, gill, and bone were subsampled as follows: a 4 cm^2 area of skin between the dorsal fin and lateral line on the right side of the body was excised; the mass of skeletal muscle defined dorsoventrally by the dorsal fin and the lateral line, and laterally by the body surface and axial skeleton was excised; the right eye was excised; the vertebral column beneath the dorsal fin was excised. All other tissues were sampled in their entirety. Gastrointestinal (GI) tissues were clamped with hemostatic forceps to prevent contamination of the carcass with gut contents. Fish with undigested food present in the GI tract were discarded. All tissue samples were frozen at −80°C until processed for liquid scintillation spectrometric analysis.

Analysis of tissues and excretory products

All blood, tissue, urine, and water samples were prepared for analysis in glass liquid scintillation counting (LSC) vials with polyethylene-lined caps, using Insta-Gel scintillation cocktail (Packard Instruments). A 200- μl aliquot of blood sample was dispensed into a 20 ml LSC vial, to which was added 0.75 ml of a mixture (1:2, v/v) of a tissue solubilizer (Soluene 350, Packard Instruments) and isopropanol. The resulting mixture was incubated in a water bath with occasional swirling for 1h at 40°C, then removed from the bath and allowed to cool to room temperature. When cool, 0.5 ml of 30% H_2O_2 was added dropwise to the vial with swirling, and the vial was capped loosely and incubated for 15 minutes at ambient temperature, followed by a 30 minute incubation at 40°C. After cooling to room temperature, 15 ml of acidified Insta-Gel scintillation cocktail (0.5 N HCL/Insta-Gel; 1:9 v/v) was added.

Thawed tissue samples were weighed to the nearest 0.1 g and homogenized in five volumes of 0.15 M NaCl with a Brinkman Polytron tissue homogenizer (Brinkman Instruments). Three samples of homogenate for each tissue type were dispensed into LSC vials and were processed for

LSC as described above for blood samples, with the exception that 1.0 ml of Soluene 350 was used without isopropanol, and 0.2 ml of 30% H_2O_2 was used. For the various tissue types, the volumes of 10% tissue homogenate were as follows: muscle and remaining carcass, 1000 μl; bone and skin, 750 μl; eye, gonad, kidney, liver, gill, and spleen, 500 μl; stomach, pyloric caeca, and intestine, 250 μl. Samples of bile (50 μl) were dispensed without dilution or homogenization. Thawed samples of water, containing gill or fecal wastes or urine, were sub-sampled for LSC. A 10ml aliquot of water or urine was added to an LSC vial containing 10ml of Insta-Gel scintillation cocktail. Water samples containing fecal wastes were homogenized prior to being sampled for LSC. The volume of tissue homogenate, water, or urine processed for LSC was determined empirically to yield maximum gross counts per minute (CPM) with an acceptable degree of quench (less than 50%).

Liquid scintillation counting was used to measure radioactivity in the samples because a previous study indicated that aqueous suspensions of ^{65}Zn in biological material could be counted with reasonable efficiency using this method (Handler and Romberger 1973). Radioactivity of samples was measured at 60% efficiency with a Packard liquid scintillation counter, Model A300C, with automatic correction for sample luminescence. All samples were counted for 10 minutes two times. Sample counts were adjusted for quenching of radioactivity using individual quench curves. These curves were prepared for each sample type with a known specific activity of radionuclide and increasing volumes of tissue homogenate, urine, or water. Samples were processed for counting as described above. Background radioactivity was measured and quench curves were prepared using samples of tissue, water, or urine, taken from control fish which were fed non-radioactive food (diet 1), but which were otherwise treated as experimental fish. For all samples, background counts were subtracted and the average net CPM of three replicate samples per samples type was used as an index of the specific activity of the tissue, urine, or water.

Data analysis

The products of the specific activity of the tissue, urine, or water and the sample weights or volumes were used as an index of total radioactivity. These data were normalized to 100% recovery of the dose administered to an 'ideal' fish of 380g body weight. Minitab® (Ryan *et al.* 1982) was used to conduct one-way analysis of variance (ANOVA1) to test for significant differences in the percentage distribution and specific activity of ^{65}Zn in tissue, urine, or gill water samples for the four fish in the material balance study. ANOVA1 was always followed by a Student-Newman-Keuls (SNK) multiple range test (Zar 1974) to evaluate significant differences between means. ANOVA1 and SNK testing was done to evaluate significant differences in the percentage distribution and specific activity of ^{65}Zn in tissues, urine, and fecal waste of fish the experimental diets. The effect of dietary treatment on the body burden (less gastrointestinal tract) of ^{65}Zn remaining 72h after the fish were fed was evaluated by two-way analysis of variance (ANOVA2) with correction for unequal samples sizes using the multiple regression analysis of Kleinbaum and Kupper (1978). The data for the fish in the material balance study (which were fed diet 1) and the fish fed diet 1 in the dietary treatment study were pooled for the ANOVA2 since ANOVA1 did not indicate any significant difference between these two groups of fish in their body burden or percentage distribution of ^{65}Zn. The level of statistical significance used for all analyses was $P \leq 0.05$.

Results

Material balance study

Six rainbow trout were used in the study, but two were discarded for reasons given earlier. In the four remaining trout, 57.9 ± 5.4% (mean ± SE) of the administered dose of ^{65}Zn was recovered. The individual data for percentage distribution and specific activity of ^{65}Zn in the various tissue, urine, or water samples were normalized to 100% recovery of the administered dose of ^{65}Zn for the 'ideal' fish

of 380g and the adjusted data were used to calculate the percentage of ^{65}Zn unabsorbed in the gastrointestinal (GI) tract (feces), absorbed in the GI tract, absorbed and retained in the body (less GI tract), and absorbed and excreted (in gill waste and urine) (Table 2). The largest proportion of the administered dose of ^{65}Zn was recovered in the GI tract, followed by the feces, body (less GI tract), and gill water in order of decreasing percentage distribution. The percentage of ^{65}Zn recovered in the urine was negligible. The highest recovery of ^{65}Zn in the gill water occurred 48h after the fish were fed (Figure 1). There were no significant differences in the percentage of ^{65}Zn recovered between the GI tract, fecal waste, body, or gill water, presumably due to the small sample size (N = 4) and the large amount of variation between individual fish. The percentage of ^{65}Zn recovered in urine was significantly less than that measured in the other sample types, and it was not significantly different from zero.

The percentage distribution and specific activity of ^{65}Zn recovered in the sampled tissue (less GI tract) indicated that liver contained the highest average concentration of ^{65}Zn (Table 3). While the body tissue remaining after bleeding and dissection contained over 55% of the ^{65}Zn found in the fish tissues, this category accounted for over 87% of the weight of the samples taken from the fish and consisted primarily of muscle, bone, and skin on a weight basis. Five sampled tissues, liver, gill, kidney, spleen, and skin, had average concentrations of ^{65}Zn higher than that of blood (Table 3). Six sampled tissue, gonad, eye, body remainder, bone, muscle, and bile, had average concentrations of ^{65}Zn lower than that of blood. These differences were not statistically significant, probably for reasons mentioned above, but they were verified by later analyses (see below).

Table 2. Percentage distribution of ^{65}Zn after 72h in rainbow trout used in the material balance study[1]

	Percentage ± SE
Total gastrointestinal (GI) tract	43.2 ± 16.2
– Stomach	(4.1 ± 1.4)
– Pyloric caeca	(14.1 ± 2.3)
– Intestine	(25.0 ± 12.5)
Fecal waste	27.2 ± 9.7
Urine	0.2 ± 0.1
Gill water	13.7 ± 11.3
Body burden (less GI)	15.6 ± 5.7
Total absorbed	72.8
Total absorbed and excreted	13.9
Total retained	58.8

[1] Normalized to 100% recovery and mean fish weight of 380g.

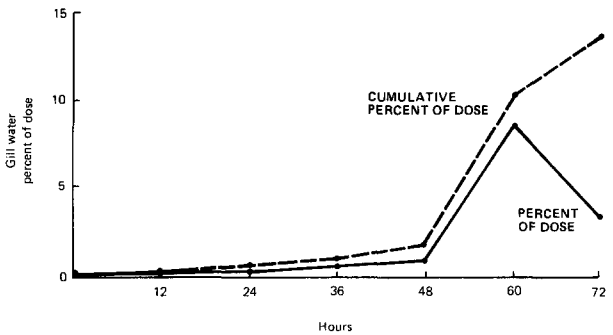


Fig. 1. Percent dose and cumulative percent of dose of ^{65}Zn recovered in gill water of rainbow trout over 72h following a single feeding of diet containing ^{65}Zn.

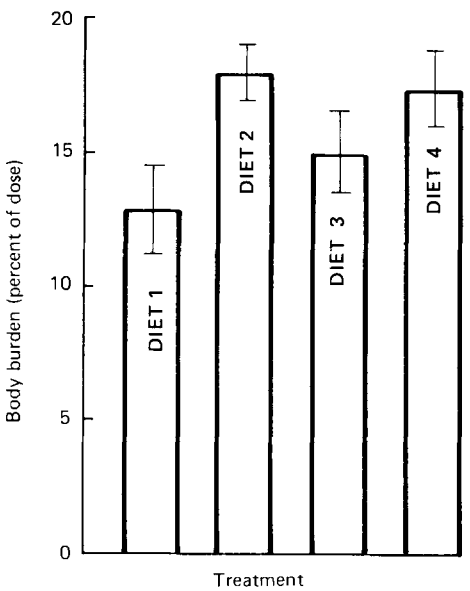
Dietary treatment study

The percentage distribution and specific activity of ^{65}Zn in the tissues of rainbow trout fed diet 1 did not significantly differ from those of the four fish used in the material balance study. Since the material balance fish were also fed diet 1, the data from the two groups were combined for analysis of dietary treatment effects. The percentage of the recovered dose of ^{65}Zn measured in the bodies of the fish minus the GI tract was not significantly affected by dietary Zn source ($0.1 < P < 0.05$), although there was a consistently higher recovery of ^{65}Zn as an amino acid chelate than as $ZnSO_4$ (Figure 2). Increasing the level of calcium/phosphorus in the diet did not change the percentage of ^{65}Zn recovered as an amino acid chelate or as $ZnSO_4$ ($P > 0.75$). No significant interaction between dietary factors (calcium level and Zn source) was detected ($P > 0.75$). The percentage of ^{65}Zn recovered in the feces of fish fed diets containing 4% calcium and those fed the diets containing

Table 3. The percentage distribution and concentration of ^{65}Zn in tissue samples of rainbow trout used in the material balance study[1]

Sample	Sample weight (g ± SE)	Body burden (% ± SE)	Tissue concentration (nCi/g ± SE)
Blood	1.9 ± 0.1	1.7 ± 0.1	5.1 ± 1.5
Gill	2.3 ± 0.3	5.7 ± 0.8	19.9 ± 8.3
Whole liver	4.5 ± 0.5	15.1 ± 2.4	26.3 ± 11.7
Whole kidney	3.4 ± 0.4	8.0 ± 0.6	17.4 ± 6.1
Whole spleen	0.5 ± 0.1	1.0 ± 0.2	14.8 ± 5.8
Bile	0.8 ± 0.2	0.2 ± 0.1	1.7 ± 0.7
Skin	4.2 ± 0.3	7.9 ± 4.0	14.3 ± 8.3
Muscle	17.3 ± 4.7	0.7 ± 0.1	0.3 ± 0.1
Whole gonad	4.2 ± 3.4	1.7 ± 1.1	5.6 ± 2.1
Whole eye (1)	3.9 ± 0.3	1.2 ± 0.3	2.4 ± 1.0
Bone	2.7 ± 0.4	0.5 ± 0.1	1.2 ± 0.4
Body remainder[2]	306.0 ± 17.9	55.1 ± 4.4	1.3 ± 0.5

[1] Normalized to 100% recovery and mean fish weight of 380g.
[2] Indicates carcass remaining after bleeding and dissection.



DIET	BODY BURDEN (PERCENT DOSE)	N
1	12.8 ± 2.7	11
2	18.0 ± 1.8	8
3	14.3 ± 2.0	5
4	17.3 ± 2.6	7

ANOVA results

Zinc $F_{(1,28)} = 2.89$ $P \approx 0.10$

Calcium $F_{(1,28)} = 0.02$ $P > 0.75$

Interaction $F_{(1,27)} = 0.18$ $P > 0.75$

Fig. 2. ^{65}Zn body burden in rainbow trout calculated as a percentage of dose (± SE). Body burden was determined 72h after a single feeding of the experimental diets.

1% calcium did not differ significantly ($P > 0.1$). No significant differences ($P > 0.05$) due to dietary treatment in concentration of ^{65}Zn were found in any of the individual tissue or urine samples (Table 4). Body remainder, which made up 85% of the weight of the fish after removal of the GI tract, had higher average concentrations of ^{65}Zn in fish fed the diets containing the amino acid chelate. This resulted in the apparent differences in recovery of ^{65}Zn in the body. The primary components of body remainder (muscle, bone, and skin) showed similar trends in ^{65}Zn concentration. The average

Table 4. Specific activity of tissue samples of rainbow trout 72h after a single feeding of the experimental diets[1].

Sample	Specific activity (nCi/g ± SE)			
	Diet 1	Diet 2	Diet 3	Diet 4
Blood	8.3 ± 2.2	12.5 ± 2.9	6.6 ± 3.3	6.2 ± 1.5
Gill	32.5 ± 9.3	25.9 ± 4.6	15.1 ± 4.4	25.1 ± 3.1
Liver	28.0 ± 4.8	33.1 ± 5.5	19.4 ± 5.6	25.7 ± 4.0
Kidney	18.6 ± 2.9	25.3 ± 3.8	17.5 ± 4.4	28.6 ± 5.2
Spleen	15.4 ± 2.6	15.4 ± 3.1	11.6 ± 3.1	11.0 ± 1.6
Bile	9.2 ± 3.8	3.0 ± 0.9	2.2 ± 0.9	1.7 ± 0.5
Skin	14.3 ± 3.8	14.6 ± 2.8	6.2 ± 2.3	13.2 ± 4.5
Muscle	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
Gonad	8.0 ± 1.8	10.7 ± 1.7	5.8 ± 2.0	10.9 ± 2.4
Eye	2.9 ± 0.5	4.7 ± 1.7	2.7 ± 1.5	3.3 ± 0.7
Bone	2.0 ± 0.3	2.6 ± 0.7	1.5 ± 0.7	2.3 ± 0.4
Body remainder[2]	2.2 ± 0.6	2.9 ± 0.5	1.8 ± 0.7	2.8 ± 0.5
Stomach	98.6 ± 39.0	63.2 ± 31.0	12.6 ± 4.1	35.6 ± 16.4
Pyloric caeca	159.8 ± 26.5	109.7 ± 19.1	45.2 ± 5.5	57.4 ± 10.7
Intestine	243.1 ± 40.2	193.7 ± 72.8	255.9 ± 75.8	127.0 ± 33.1

[1] Normalized to 100% recovery and a mean fish weight of 380g.
[2] Indicates carcass remaining after bleeding and dissection.

level of ^{65}Zn recovered in the gonads was generally higher in males than in females (data not shown), but no significant differences ($P > 0.05$) between the sexes or between dietary treatments were found. Although high levels of radioactivity were recovered in the stomach, pyloric caeca, and the intestines, no differences ($P > 0.05$) in activity due to dietary treatment were found.

Because tissue concentrations of ^{65}Zn did not differ among the dietary treatments, the data for all fish were pooled to provide a more precise evaluation of the distribution of ^{65}Zn among tissues (Fig. 3). The results verified those obtained with the fish used in the material balance study that the specific activity of gill, liver, kidney, spleen, and skin was significantly greater than that of blood, and body remainder was significantly less than that of blood. The remaining tissues did not differ significantly from blood in specific activity.

Discussion

The percentage recovery of the administered dose of ^{65}Zn in the material balance study is comparable

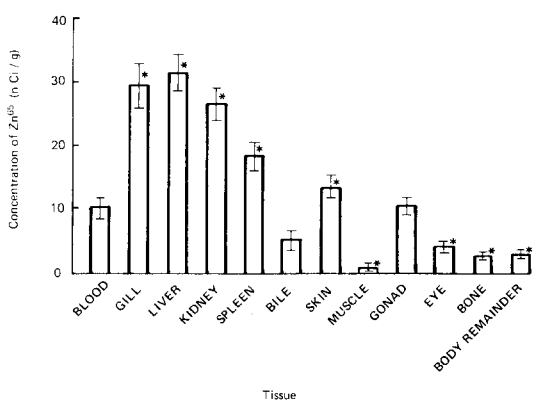


Fig. 3. Concentration (mean ± SE) of ^{65}Zn in tissues of all of the rainbow trout used in the study. Asterisks indicate values significantly different ($P < 0.05$) from that of blood.

to the percent recovery of labelled compounds obtained in a similar study with fish of 21.5–53.0% (Thomas and Rice 1981). In our study, a portion of the administered dose could have been lost through ^{65}Zn remaining on the surface of the metabolizable energy chamber, through losses of body fluids during dissection of the fish, or through potential errors associated with counting small subsamples of large volumes of gill water and large amounts of ground body tissue remaining after dissection.

The percentage distribution of the recovered dose indicated that 27% was present in the feces after 72h. In a preliminary study of the diet passage rate through the trout in the metabolizable energy chamber using chromic oxide as an indicator, nearly 100% of the chromic oxide was found in the fecal water after 72h. The absence of undigested food in the GI tract of the fish fed the labeled diet suggested that complete passage of food had occurred after 72h. In a previous study, apparent Zn retention values of 35–47% were calculated for growing rainbow trout fed a similar diet (Hardy and Shearer 1985). In the present study, a value of 58.8% ^{65}Zn retention was obtained. Zn turnover, which has been estimated to be about 1% per day in rainbow trout (Shearer and Hardy, unpublished data), would not significantly influence these values. The discrepancy between these values is not great considering the short-term nature of the present study and the differences in experimental conditions, i.e., daily feeding in low-density rearing tanks *vs* a single feeding in a metabolizable energy chamber. Had the sampling of the rainbow trout been extended, the Zn retention values might have been closer to those previously observed.

Nakatani (1966) and Byran (1971) suggested that excretion of ingested Zn occurs through the urine, GI tract, and gills. The present study appears to be the first to show that rainbow trout excrete Zn of dietary origin through the gills. Nearly 14% of the recovered dose of ^{65}Zn was found in the gill water after 72h. The actual level may have been higher, since no attempt was made to measure ^{65}Zn levels remaining in the head chamber after gill water was drained. Nakatani (1966) attempted to determine material balance in rainbow trout held in a metabolism chamber for 4 and 7 days. He did not collect gill water and he sutured the anus to prevent defecation. Between 52% and 65% of the administered dose of ^{65}Zn was unaccounted for, and Nakatani (1966) speculated that excretion by the gill tissue was responsible. Recovery of ^{65}Zn in the urine of the rainbow trout in the present study amounted to less than 1% of the recovered dose. Nakatani (1966) found that only 0.12% of the administered dose of ^{65}Zn was found in the urine after 96h; thus, our results corroborate those of Nakatani (1966). In rainbow trout in fresh water, the urine is a minor route of excretion and thus unimportant in the regulation of body Zn levels.

The percentage of the administered dose of ^{65}Zn measured in the body plus GI tract of the fish in the material balance study was 34.1%, with 25.0% found in the GI tract and 9.1% found in the body less GI tract. These values are lower than those reported by Nakatani (1966), who found 47.9% of administered dose in the body plus GI tract after 96h. The GI tract contained 23.5% and the body (less GI tract) contained 24.3%. Nakatani, however, prevented fecal excretion in his fish. Adjusting Nakatani's data for the fecal losses we observed in our study reduces the percentage recovery in the body plus GI tract to 40.3%, which is close to the value we found. The presence of a large recovered dose in the GI tract may indicate that short-term storage of Zn as metallothionein is induced prior to transport through the blood to the liver and other tissues (Richards and Cousins 1977). In addition, it suggests that the intestinal epithelial cell may play a major role in regulation of plasma Zn levels in rainbow trout, as is the case in mammals (Cousins 1979).

The substantial differences in ^{65}Zn concentration between tissues of rainbow trout examined in the material balance study were verified in our later analyses (Fig. 3). Similar differences were found in trout (Nakatani 1966), in carp (Saiki and Mori 1955) and in rats (Heth *et al.* 1965). These differences may reflect the degree of vascularization and rates of zinc turnover in these tissues. The concentrations of ^{65}Zn in liver, gill, kidney, and spleen were higher than that of blood, suggesting active transport of ^{65}Zn from the blood to these tissues. Active transport of ^{65}Zn into gill tissue supports

the Zn excretory function of gills indicated by the results of our material balance study. The relatively high concentration of ^{65}Zn in the kidney was also found in plaice by Pentreath (1973), who suggested that urine was a major route of Zn excretion. As mentioned above, the concentration of ^{65}Zn in the kidney may not reflect a zinc-excretory function of this tissue. The concentration of ^{65}Zn in the skin was higher than that of other cartilagenous tissues, but the skin may have been contaminated by exposure to ^{65}Zn in the fecal water. This contamination may have slightly increased the concentration of ^{65}Zn in the body remainder, as skin was included in this category and made up about 5% of its total weight. The relatively low level of ^{65}Zn found in the bile suggests that biliary excretion and possibly enterohepatic recycling of Zn did not occur to a significant extent during the 72h test period, as suggested by Lovegrove and Eddy (1982).

Increased dietary calcium/phosphorus levels are known to reduce Zn bioavailability in salmonids, as measured indirectly by reduced whole body levels of Zn in rainbow trout (Hardy and Shearer 1985) and by reduced serum Zn levels in juvenile chinook salmon (Richardson *et al.* 1985). In the present study, dietary calcium/phosphorus level did not change the percentage of the recovered ^{65}Zn dose found in the tissues of the fish or in the feces. Dietary Zn source, which influenced whole body zinc levels in trout fed diets containing elevated levels of calcium/phosphorus in an earlier report (Hardy and Shearer 1985), resulted in an increase in the percentage of the recovered dose found in the body of the fish, but the increase was not significant at the 5% level. The limitation of approaching the problem of how dietary calcium/phosphorus levels and dietary Zn source influence whole body zinc levels in fish using confinement chambers and sampling at a particular time after dosing the fish is that a high degree of variability exists between fish in the rate at which absorption and excretion of dietary components occur. This difference increases the variation among fish within treatments, making detection of significant differences due to diet more difficult. Although we discarded any fish which, upon dissection, was found to have undigested food in the GI tract, variation in specific activity of tissues among fish within dietary treatments remained high. Nevertheless, the use of metabolizable energy chambers for studying the rate of labelled dietary nutrients has merit, especially where routes of excretion, absorption efficiency, and tissue distribution are unknown.

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Note

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References cited

Byran, G.W. 1971. The effects of heavy metals (other than mercury) on marine and estuarine organisms. Proc. Roy. Soc. Lond. B. 177: 389–410.

Cousins, R.J. 1979. Regulatory aspects of zinc metabolism in liver and intestine. Nutr. Rev. 37: 97–103.

Furakawa, A. and Tsukahara, H. 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull. Jpn. Soc. Sci. Fish. 32: 502–506.

Handler, L.H. and Romberger, J.A. 1973. The use of a solubilizer-scintillator mixture for counting aqueous systems containing high- and low-energy radionuclides. Int. J. of Appl. Rad. Isotopes 24: 129–134.

Hardy, R.W. and Shearer, K.D. 1985. Effect of dietary calcium phosphate and zinc supplementation on whole body zinc concentration of rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 42: 181–184.

Heth, D.A. Becker, W.M. and Hoekstra, W.G. 1966. Effect of calcium, phosphorus, and zinc or $zinc^{65}$ absorption and turnover in rats fed semi-purified diets. J. Nutr. 88: 331–337.

Jeng, S.S. and Sun, L.T. 1981. Effects of dietary levels on zinc concentrations in tissues of common carp. J. Nutr. 111: 134–140.

Ketola, H.G. 1979. Influence of dietary zinc on cataracts in rainbow trout (*Salmo gairdneri*). J. Nutr. 109: 965–969.

Kleinbaum, D.G. and Kupper, L.L. 1978. Applied Regression Analysis and other Multivariable Methods. Duxbury Press, North Scituate, Mass.

Lovegrove, S.M. and Eddy, B. 1982. Uptake and accumulation of zinc in juvenile rainbow trout, *Salmo gairdneri*. Environ. Biol. Fish. 7: 285–289.

Nakatani, R.E. 1966. Biological response of rainbow trout (*Salmo gairdneri*) ingesting Zn^{65}. *In* Proceeding on the Disposal of Radioactive Wastes in Seas, Oceans, and Surface Waters. pp. 809–823. Edited by A. Guillon. International Atomic Energy Agency, Vienna.

National Research Council. 1981. Nutrient Requirements of Coldwater Fishes. National Academy of Science, Washington, DC.

Ogino, C. and Yang, G.Y. 1978. Requirement of rainbow trout for dietary zinc. Bull. Jap. Soc. Sci. Fish. 44: 1015–1018.

Pentreath, R.J. 1973. The accumulation and retention of ^{65}Zn and ^{54}Mn by the plaice, *Pleuronectes platessa* L. J. Exp. Mar. Biol. Ecol. 12: 1–18.

Richard, M.P. and Cousins, R.J. 1977. Isolation of an intestinal metallothionein induced by parenteral zinc. Biochem. Biophys. Res. Commun. 75: 286–294.

Richardson, N.L., Higgs, D.A., Beames, R.M. and McBride, J.R. 1985. Influence of dietary calcium, phosphorus, zinc, and sodium phytate level on cataract incidence, growth, and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). J. Nutr. 115: 553–567.

Ryan, T.A., Joiner, B.L. and Ryan, B.F. 1982. Minitab Reference Manual. Minitab Inc., University Park, PA.

Saiki, M. and Mori, T. 1955. Studies on the distribution of administered radioactive zinc in the tissues of fishes. Bull. Jap. Soc. Sci. Fish. 21: 945–949.

Smith, R.R. 1971. A method of measuring digestibility and metabolizable energy of feeds. Prog. Fish-Cult. 33: 132–134.

Spinelli, J., Houle, C.R. and Wekell, J.C. 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture 30: 71–83.

Thomas, R.E. and Rice, S.D. 1981. Excretion of aromatic hydrocarbons and their metabolites by freshwater and seawater Dolly Varden char. *In* Biological Monitoring of Marine Pollutants. pp. 425–449. Edited by F.J. Vernberg, A. Calabresa, F.P. Thurberg and W.B. Bernberg. Academic Press, New York.

Wekell, J.C., Shearer, K.D and Houle, C.R. 1983. High zinc supplementation of rainbow trout diets. Prog. Fish-Cult. 45: 144–147.

Zar, J.H. 1974. Biostatistical Analysis. Prentice-Hall Inc., Englewood Cliffs, NJ.