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Investigation of the effects of dietary protein source on copper and zinc bioavailability in fishmeal and plant-based diets for rainbow trout

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ABSTRACT

Limited research has examined the effects that dietary protein sources have on copper (Cu) and zinc (Zn) absorption, interactions and utilization in rainbow trout. Therefore, the objective of the first trial was to determine what effect Cu source (complex vs. inorganic) and Cu concentration (0, 5, 10, 15, 20 ppm) had on rate and efficiency of gain and Cu tissue concentration in rainbow trout fed both plant-protein and animal-protein based diets. The second trial then examined if interactions occur due to increasing dietary content of Zn (0, 30, 300, 1500 ppm) in rainbow trout fed a plant-based diet. Results from the first trial demonstrated differences in growth and tissue mineral concentrations due to Cu concentration but not source. Trout fed plant-based diets had higher weight gain, improved feed conversion ratio and higher hepatic Cu concentration when compared to fishmeal-fed trout. Results from the second trial show that increasing dietary Zn supplementation increased whole body Zn and Cu at 12 weeks. No significant antagonism between Cu and Zn was observed in rainbow trout fed plant-based diets. Zn deficiency signs were observed at 12 weeks in trout fed a plant-based diet without Cu and Zn supplementation which included mortality, cataracts and caudal fin erosion. Results of these studies indicate that rainbow trout fed plant-based diets require both Cu and Zn supplementation at concentrations higher than those previously reported to maximize growth.

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1. Introduction

Chemical or physical properties of dietary protein sources may interact with other dietary nutrients to change uptake and utilization of those nutrients, specifically, micronutrients. Barrows et al. (2008) found that different vitamin premixes were necessary to optimize growth and feed efficiency for hatchery reared trout when fed plant-based diets compared to those conventionally utilized in traditional fishmeal-based diets. Specifically, higher vitamin concentrations were necessary to maximize growth potential and reduced survival and nutrient retention were found in fish fed plant-based diets supplemented with vitamins to meet required concentrations (NRC, 1993, 2011), indicating that such concentrations were inadequate (Barrows et al., 2008). Similarly, Barrows et al. (2009) observed improved weight gain in trout fed a plant-based diet supplemented with macro-minerals and inositol at concentrations greater than those NRC recommended (Barrows et al., 2009). However, to date, limited research has examined the interaction of plant-based diets on copper (Cu) and zinc (Zn) absorption and utilization in rainbow trout.

The dietary requirement of Cu for rainbow trout has been reported to be 3 μg Cu/g diet (Ogino and Yang, 1980). A common measurement of Cu status in rainbow trout includes whole-body Cu concentration (Clearwater et al., 2002). Fish growth is also used as an indicator since both Cu deficiency and toxicity result in retarded growth (Kamunde et al., 2002). The form of Cu in the diet has also been reported to alter how Cu is absorbed due to bonding with other dietary moieties. Available research reports have not clearly defined as to whether organic or inorganic forms of Cu have greater bioavailability due to reduced random covalent bonding. Studies in rats (Guo et al., 2001) and heifers (Rabiansky et al., 1999) found increased tissue Cu accumulation in animals fed Cu-lysine as compared to those fed $CuSO_4$. In contrast, studies in steers (Ward et al., 1993) and in rainbow trout fed semi-purified diets (Kjoss et al., 2006) found that $CuSO_4$ and Cu-lysine were equivalent in Cu bioavailability.

The dietary Zn requirement for rainbow trout is suggested to be between 15 and 30 mg/kg (Ogino and Yang, 1978). Zinc is

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found in bone and body tissues, of rainbow trout with the highest concentration being in the eyes. Signs of Zn deficiency in rainbow trout include cataracts, erosion of fins and scales, mortality and reduced growth (NRC, 2011). In contrast to Cu, trout fed high concentrations of dietary Zn exhibited no impairment in growth or health (Clearwater et al., 2002; approximately 1000 ppm and Wekell et al., 1983; 1700 ppm).

An antagonistic relationship between Cu and Zn has been reported in terrestrial animals (Ammerman et al., 1995; Skoryna and Waldron-Edwards, 1971). Typical toxicity signs due to excess dietary Zn were anemia, reduced growth, and lower concentrations of Cu and Fe in the tissues. Although less studied, interactions between Cu and Zn in fish have also been previously investigated. Knox et al. (1984) examined the effects of different concentrations of dietary Zn on Cu metabolism in rainbow trout and observed similar growth and feed efficiency across all treatments; however, liver Cu concentration was reduced at the higher concentrations of dietary Zn. Gatlin et al. (1989) followed up the Knox et al. (1984) studying optimal concentrations of Zn in practical diets for fingerling channel catfish. Fish were fed either 100 or 200 ppm Zn over a 12-week period and no negative effect on growth performance or feed efficiency was observed at the higher concentrations. It was suggested that Zn could be supplemented in a practical diet at higher concentrations without any impact on Cu bioavailability.

Dietary mineral requirements for rainbow trout were established nearly 30 years ago (Barrows et al., 2008). Within the last ten years alone, changes in diet processing and formulations have dramatically altered the ingredients being fed to trout thus potentially necessitating re-evaluation of trace mineral needs. Therefore, the objectives of this research were: 1) to determine what effect Cu source (complex vs. inorganic) and concentrations of Cu (0, 5, 10, 15, 20 ppm) in both plant and animal protein-based diets had on rate and efficiency of gain and Cu tissue concentration in rainbow trout and 2) to determine if interactions occur due to increasing diet content of Zn (0, 30, 300, 1500 ppm) on tissue concentration of Cu in rainbow trout fed a plant-based diet.

2. Material and methods

2.1. Fish

A commercially available strain (Trout Lodge, Seattle WA, USA) of juvenile rainbow trout (*Oncorhynchus mykiss*) was used for two experiments. Procedures for the care and handling of the rainbow trout that were used for experiment one were conducted in accordance with the Bozeman Fish Technology Center, Institutional Animal Care and Use Committee.

2.2. Study one

2.2.1. Experimental design

An incomplete factorial treatment arrangement using two Cu sources (CuS or CuLys) and four dietary concentrations of Cu in the fishmeal-based diet (0, 5, 10, or 20) or five dietary concentrations of Cu in the plant-based diet (0, 5, 10, 15 or 20) in both a plant and fishmeal-protein based diet was conducted over a 14-week period at the USFWS, Fish Technology Center in Bozeman, Montana. Thirty-two 168 L tanks were stocked with 18 fish (average initial weight 19.5 g, +/− 1.2 g). Tank was considered the experimental unit for all response variables. Two replicate tanks were randomly assigned to each dietary treatment. All tanks received 9.5 L of water/min via a re-circulating pump system with particulate and biological filtration. Water temperature was held constant at 15 °C and photoperiod was maintained at a 13:11 diurnal cycle.

Table 1
Ingredient composition of plant and fishmeal-based diets (g/100 g).

Ingredients (%DM)	Plant-based	Fishmeal-based
Soy protein concentration[a]	24.64	–
Fish oil, menhaden[b]	15.59	16.75
Corn protein concentrate[c]	17.54	5.50
Wheat flour[d]	16.27	22.72
Soybean meal[e]	13.30	12.00
Menhaden meal[f]	–	33.70
Blood meal[g]	–	7.43
Mono-dical phosphate	2.65	–
L-Lysine	1.99	–
Vitamin premix ARS 702[h]	1.00	1.00
Choline-CL	0.60	0.60
Potassium chloride	0.56	–
Taurine	0.50	–
DL-Methionine	0.50	–
Sodium chloride	0.28	–
Threonine	0.23	–
Stay-C[i]	0.20	0.20
Trace mineral premix[j]	0.10	0.10

[a] Solae, Pro-Fine VF, 693 g/kg crude protein.
[b] Omega Proteins Inc., Virginia Prime menhaden oil.
[c] Cargill, Empyreal 75, 756 g/kg crude protein.
[d] Manildra Milling, 120 g/kg protein.
[e] Archer Daniels Midland Company, 468 g/kg crude protein.
[f] Omega Proteins Inc., Menhaden Special Select, 618 g/kg crude protein.
[g] IDF Inc., 832 g/kg protein.
[h] ARS 702; contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 g; thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5 mg; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5 mg; inositol 600 mg.
[i] DSM Nutritional Products.
[j] Contributed in mg/kg of diet; manganese 13; iodine 5; copper and zinc varied by diet.

2.2.2. Diets

Supplemental Cu was added to the two protein base formulas to create sixteen experimental diets (Table 1). All diets were formulated to contain 40% digestible protein and 20% crude lipid (Table 1). The plant-based diets were supplemented with CuS ($CuSO_4$) or CuLys (CuPLEX, 10% Cu; Zinpro Corporation, Eden Prairie, MN) at 0, 5, 10, 15 and 20 ppm. The fishmeal-based diet series was supplemented with CuS or CuLys at concentrations of 0, 5, 10, and 20 ppm.

Each diet was manufactured as a 3 mm pellet. Diets were processed using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18 s exposure to 127 °C in the extruder barrel. The die plate was water cooled to an average temperature of 60 °C. Pressure at the die head varied from 15 to 30 bar. Pellets were dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland) for 25 min at 102 °C with a 10 min cooling period to a final moisture concentration of less than 10%. A topcoat of fish oil was added to the cooled feed using a vacuum-assisted top-coater (A.J. Mixing, Ontario, Canada). Diets were stored in plastic lined paper bags at room temperature until fed.

2.2.3. Feeding and weighing

Before initiation of experimental diet feeding, fish in each tank were randomly fed one of the two basal diets without Cu supplementation to reduce body stores for two weeks. After this two week adjustment period, fish within each basal diet group were randomly allocated to their respective diet for an additional 12 weeks. Trout were fed to visual satiation twice daily, six days a week. Feed bins were weighed weekly in order to determine average feed intakes. Weight gain was determined by bulk weighing all fish within a tank every three weeks. The following formulae were used:

Weight gain (WG) = Final weight (g) − Initial weight (g) / Initial weight (g) ∗ 100

FCR = Wet diet fed (g) / Wet weight gained (g)

Average daily feed intake = Wet feed fed (g) ∗ 100 / ((Initial tank weight (g) − Final tank weight (g) / 2)) / # of days on feed.