Aquaculture 454 (2016) 118–124

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Dietary copper requirement of juvenile Russian sturgeon *Acipenser gueldenstaedtii*

Hewei Wang [a], Erchao Li [a], Haoyong Zhu [a], Zhenyu Du [a], Jianguang Qin [b], Liqiao Chen [a,*]

[a] Laboratory of Aquaculture Nutrition and Environmental Health, School of Life Sciences, Shanghai 200241, PR China
[b] School of Biological Sciences, Flinders University, Adelaide, SA 5001, Australia

ARTICLE INFO

Article history:
Received 11 September 2015
Received in revised form 16 December 2015
Accepted 17 December 2015
Available online 19 December 2015

Keywords:
Russian sturgeon
Copper
Requirement
Growth
Antioxidant ability

ABSTRACT

To quantify the dietary copper (Cu) requirement of Russian sturgeon *Acipenser gueldenstaedtii*, copper sulfate ($CuSO_4 \cdot 5H_2O$) was added into the basal semi-purified diet at seven levels 0, 1, 2, 4, 6, 8 and 16 mg Cu kg^{-1} diet yielding the actual dietary values of 0.3, 1.5, 2.4, 4.4, 6.2, 8.3 and 16.1 mg Cu kg^{-1} diet, respectively. Each diet was fed to sturgeon (11.84 ± 0.07 g) in triplicate in a flow-through system for 8 weeks. The Cu concentration in the rearing water was 0.8–1.2 μg L^{-1} during the trial period. The weight gain rate (WGR) and feed efficiency were significantly higher in fish fed 2.4 to 8.3 mg Cu kg^{-1} than those fed 0.3, 1.5 or 16.1 mg Cu kg^{-1}. The highest WGR was found in fish fed 6.2 mg Cu kg^{-1}. The whole-body Cu concentrations were highest in fish fed 6.2 to 16.1 mg Cu kg^{-1}. The liver Cu concentration was highest in fish fed 16.1 mg Cu kg^{-1} diet and lowest in fish fed diets Cu levels range from 0.3 to 4.4 mg Cu kg^{-1}. Hepatic copper–zinc superoxide dismutase (Cu–Zn SOD) activities, total antioxidant capacity and serum ceruloplasmin activity were significantly higher in fish fed 6.2 and 8.3 mg Cu kg^{-1} diet than those in other treatments. The lowest hepatic malondialdehyde values were observed in fish fed 6.2 and 8.3 mg Cu kg^{-1}. No significant differences were in survival, hepatosomatic index, viscerosomatic index and condition factor among all the treatments. There were also no significant differences in whole body moisture, crude protein, crude lipid and ash between all the treatments. Analysis of broken-line regression based on the WGR, whole-body Cu concentration, hepatic Cu–Zn SOD and serum ceruloplasmin activity of the fish fed different diets indicates that the minimum Cu concentration in the semi-purified diet for juvenile Russian sturgeon (12–70 g) is at 7–8 mg kg^{-1}.

Statement of relevance

The dietary copper (Cu) requirement of juvenile Russian sturgeon was investigated. Dietary Cu deficiency and over-loading significantly reduced growth, feed utilization and antioxidant capacity. The results suggest that the appropriate dietary Cu content is considerable in Russian sturgeon farming. Minimum dietary Cu level for this species is recommend as 7–8 mg Cu kg^{-1} in this study. These information will be useful in developing a practical diet for Russian sturgeon culture.

1. Introduction

Copper (Cu) is an essential trace element and a redox-active transition metal related to oxidative damage in fish (Gropper et al., 2005; Lall, 2002). Cu is a cofactor required for structural and catalytic properties of superoxide dismutase, ceruloplasmin, cytochrome oxidase, lysyl oxidase, dopamine hydroxylase and tyrosinase (Watanabe et al., 1997). These enzymes are involved in a series of biological processes for oxidation–reduction reaction, iron utilization, cellular energy production, collagen synthesis and brain neurotransmitters (Lall, 2002). On the other hand, a high Cu concentration may cause oxidative damage to lipids, proteins, and DNA and lead to neurodegenerative disorders (Gaetke and Chow, 2003).

Fish can obtain Cu from both diet and water, but the former provides most Cu consumed by fish (Kamunde et al., 2002). Until now, minimum dietary Cu requirement has been determined in rainbow trout *Oncorhynchus mykiss*, common carp *Cyprinus carpio* (Ogino and Yang, 1980), channel catfish *Ictalurus punctatus* (Gatlin and Wilson, 1986), Atlantic salmon *Salmo salar* (Berntssen et al., 1999), hybrid tilapia (Shiau and Ning, 2003), grouper *Epinephelus malabaricus* (Lin et al., 2008), yellow catfish *Pelteobagrus fulvidraco* (Tan et al., 2011), and large yellow croaker *Larimichthys croceus* (Cao et al., 2014). It has been shown that Cu deficiency decreases the activity of ceruloplasmin and copper–zinc superoxide dismutase (Cu–Zn SOD) in grouper (Lin et al., 2008). In contrast, excessive Cu in diet can be toxic and overloaded Cu

* Corresponding author.
E-mail address: lqchen@bio.ecnu.edu.cn (L. Chen).

http://dx.doi.org/10.1016/j.aquaculture.2015.12.018
0044-8486/

can also cause intestinal apoptosis and slow growth in Atlantic salmon (Lundebye et al., 1999). Therefore, identification of minimum dietary Cu requirement is important for aquacultured species during grow-out.

There are 27 extant species of sturgeons and paddlefishes (other Acipenseriformes) on the earth, all of them face extinction. 15 of the 27 species are artificial cultured to their valuable meat and caviar (Memis et al., 2006; Pikitch et al., 2005). Eleven species and hybrids are being cultured since 1998 in China. Since 2000, China has been the world's largest producer of sturgeon (Wei et al., 2011). The rapid increase of sturgeon production in aquaculture occurred at the end of the last century, which coincides with the massive decline of wild caught stocks. The modern aquaculture production started only during and after the early nineteen-eighties. (Bronzi et al., 2011). The production of the cultured sturgeon in 2013 has reached 75,000 t in the world (FAO, Food and Agriculture Organization of the United Nations, 2015) and was more than 64,000 t in China (China Fishery Statistical Yearbook, 2014). Russian sturgeon accounted for 10% of the annual sturgeon production in China from 2010 to 2012 (Shen et al., 2014). Under culture conditions, the nutritional requirements on protein (Stuart and Hung, 1989), lipid (Şener et al., 2005), carbohydrate (Hung et al., 1989), and trace elements (Wen et al., 2008; Xu et al., 2011) have been studied in various sturgeon species. However, our knowledge on dietary Cu requirement in sturgeon is very limited. Until now, only the dietary Cu requirement for juvenile beluga *Huso huso* has been determined (Mohseni et al., 2014). The objective of this study was to determine the dietary Cu requirement of juvenile Russian sturgeon *Acipenser gueldenstaedtii* (Bronzi et al., 2011).

2. Materials and methods

2.1. Experimental diets

Basal diet formulation and composition analysis (AOAC, 1995) are shown in Table 1. Casein and gelatin were used as the dietary protein sources and fish oil and soybean oil were used as the main dietary lipid source. Corn starch was used as the carbohydrate source. The basal diet was supplemented with $CuSO_4 \cdot 5H_2O$ (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) at 0, 1, 2, 4, 6, 8 and 16 mg kg^{-1} diet, respectively, resulting in final dietary Cu concentrations of 0.3, 1.5, 2.4, 4.4, 6.2, 8.3 and 16.1 mg kg^{-1}, respectively. Dietary Cu concentration was analyzed using an inductively coupled plasma-atomic emission spectrophotometer (ICP-OES Optima 5300DV; PerkinElmer Corporation) at the Shanghai Academy of Public Measurement, China. Diets were processed into 2.5 mm diameter strips, air dried, ground and sieved to appropriate size and stored at −20 °C before feeding.

Table 1
Formulation and proximate composition of the basal diet (% dry matter).

Ingredient	%
Casein, vitamin-free[a]	40
Gelatin[b]	10
Corn starch[c]	30
Fish oil[d]	5
Soybean oil[e]	5
Choline chloride	0.5
Taurine	0.5
Monocalcium phosphate	3
Vitamin premix[f]	1
Mineral premix, copper-free[g]	1
Cellulose	4
Proximate composition	
Crude protein	43.1
Crude lipid	9.8
Moisture	10.2
Ash	3.9

[a] Casein, vitamin-free: crude protein 92% (Sigma-Aldrich Trading Co., Ltd., Shanghai, China).
[b] Gelatin: Sangon Biotech, Shanghai, China.
[c] Corn starch: Sinopharm Chemical Reagent Co., Ltd., Shanghai, China.
[d] Fish oil: Xiamen Xinsha Pharmaceutical Co., Ltd., Xiamen, China.
[e] Soybean oil: Kerry Oils & Grains Industrial Co., Ltd., Shanghai, China.
[f] Vitamin premix (mg kg^{-1} diet): thiamin hydrochloride, 50; riboflavin, 200; pyridoxine hydrochloride, 50; vitamin B_{12}, 0.1; nicotinic acid, 200; calcium pantothenate, 100; folic acid, 20; biotin, 5; inositol, 800; ascorbic acid, 1000; menadione sodium bisulfite, 10; retinol acetate, 15; cholecalciferol, 10; alpha-tocopherol, 200; cellulose, 7340.
[g] Mineral premix, copper-free (mg kg^{-1} diet): $ZnSO_4 \cdot 7H_2O$, 100; $FeSO_4 \cdot 7H_2O$, 700; $MnSO_4 \cdot H_2O$, 80; $CoCl_2 \cdot 6H_2O$, 20; KI, 8; Na_2SeO_3, 3; $MgSO_4 \cdot 7H_2O$, 4000; NaCl, 1000; KCl, 1000; $AlCl_3 \cdot 6H_2O$, 15; cellulose, 3074.

2.2. Experimental procedure

Russian sturgeon juveniles were obtained from a farm in Quzhou, China. Before starting the experiment, fish were acclimated to the laboratory condition for two weeks in circular fiberglass tanks (diameter: 2 m, height: 0.51 m) in a flow-through system with continuous aeration, and fed with the basal diet during acclimation.

Prior to the feeding trial, fish were fasted for 24 h. Juvenile sturgeon (11.84 ± 0.07 g) were randomly assigned to 21 aquariums (40 × 45 × 100 cm) in a flow-through system at a density of 20 fish per aquarium. Each diet was fed to fish in three randomly chosen aquaria. The diet was divided into two equal meals fed at 08:30 and 16:30 h by hand for 8 weeks. Fish were daily fed 3% of their body weight. Fish were weighed once every 2 weeks and the daily ration was adjusted according to weight gain. Feed intake was completed within 2–3 min after delivery, thus leaching of Cu into water was negligible. Dissolved Cu in water was collected 10 min before and after feeding and was 0.8–1.2 μg L^{-1} throughout the experiment. Excess feed and feces were siphoned daily. During the experimental period, the water temperature was 19.0–21.4 °C, dissolved O_2 7 mg L^{-1}, pH 7.2 and ammonia 0.1 mg L^{-1}. A photoperiod of 12 h light (08:00 to 20:00 h) and 12 h dark was used during the study.

2.3. Sample collection and analysis

At the termination of the experiment, fish were fasted for 24 h and anesthetized with MS222 at 150 mg L^{-1} before handling. Then total number and weight of fish in each aquarium were counted and weighted to calculate the body weight gain rate and survival rate. Subsequently, three fish per aquarium were randomly selected to collect blood sample from the caudal vessels. Serum was separated by centrifugation of blood at 1500 ×*g* for 10 min at 4 °C using a centrifuge (5804R, Eppendorf, Hamburg, Germany). And then, the serum samples were frozen in liquid nitrogen and then stored at −80 °C for ceruloplasmin activity determination. After blood collecting, the liver was rapidly removed from the same fish and frozen in liquid nitrogen and then stored at −80 °C for determining liver Cu concentration, Cu–Zn SOD activity, total antioxidant capacity (T-AOC) and malondialdehyde (MDA). Viscerosomatic index and hepatosomatic index were calculated after blood and liver were collected. Another six fish per aquarium were randomly selected, pooled and then stored in −20 °C for determining the whole-body composition and Cu concentration.

Growth performance of the juvenile Russian sturgeon was evaluated using weight gain rate (WGR), feed efficiency (FE), survival rate (SR), condition factor (CF), hepatosomatic index (HSI) and viscerosomatic index (VSI) and calculated as in the following:

$$\text{Weight gain rate (WGR, \%)} = 100 \times [(\text{final body weight} - \text{initial body weight})/\text{initial body weight}].$$

$$\text{Feed efficiency (FE)} = (\text{final body weight} - \text{initial body weight})/\text{feed intake}.$$

$$\text{Survival rate (SR, \%)} = 100 \times (\text{final amount of fish})/(\text{initial amount of fish}).$$