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Dietary magnesium requirement on dietary minerals and physiological function of juvenile hybrid sturgeon (*Acipenser schrenckii*♀ × *Acipenser baerii*♂)

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Abstract
Sturgeons are an economically important freshwater aquacultural fish in China and elsewhere. Research was conducted to study the magnesium requirement of juvenile hybrid sturgeon (*Acipenser schrenckii*♀ × *Acipenser baerii*♂) based on mineral composition, proximate chemical analysis, antioxidant enzyme levels, and growth metrics. Different levels of magnesium supplements (43.2, 157.3, 326.5, 549.6, 743.9, 938.4, and 1118.2 mg kg^{-1}) were fed to juvenile sturgeon for 8 weeks. Five hundred twenty-five juvenile hybrid sturgeons (an average initial body weight of 7.65 g) were randomly divided into 7 groups with 3 replicates each (25 fish per replicate, tanks of 100×50×50 cm, dissolved oxygen ≥ 5.0 mg L^{-1}, 12 light:12 dark) and fed 4 times per day with the experimental diets containing 40.78% crude protein and 10.03% crude fat. The body tissues and blood of fish were then sampled and analyzed. Growth performance was not significantly different between treatments ($P>0.05$). The optimal dietary magnesium requirement for hybrid sturgeon was estimated to be 355.16, 573.6, or 584.6 mg kg^{-1} dietary magnesium based on whole-body Mg retention, the whole-body or vertebrae magnesium content versus dietary magnesium levels. The whole-body calcium to phosphorus ratio of the 43.2 and 326.5 mg kg^{-1} groups was significantly higher than that of the 938.4 mg kg^{-1} group ($P< 0.05$). A dietary magnesium concentration of 350–700 mg kg^{-1} improved the antioxidant capacity by decreasing the serum malondialdehyde and enhancing serum superoxide dismutase, glutathione peroxidase, and catalase activities.

Keywords Hybrid sturgeon · Magnesium · Mineral composition · Proximate chemical analysis · Antioxidant enzymes

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Introduction

As an essential mineral, magnesium (Mg) has a crucial role in physiology in fish, such as the maintenance of intra- and extracellular homeostasis; numerous enzymatic processes (at least 300 enzymatic steps); nucleic acid, carbohydrate, lipid, and protein metabolism; skeletal tissue metabolism; osmoregulation; neuromuscular transmission; ion balance and exchange (Davis and Gatlin 1996; Lall 2002; Vormann 2003); tissue mineral metabolism (Zhang et al. 2016); and immune system regulation (Tam et al. 2003). Anorexia, sluggishness, reduced growth, reduced tissue Mg content, and vertebral deformity were the signs of Mg deficiency in fish (Lall 2002).

The Mg requirements of fish can be met by its uptake from water, the diet, or both (Bijvelds et al. 1997). For marine species, dietary Mg supplementation may not be necessary because Mg requirements can be met by uptake from seawater, where Mg concentrations are high (Sakamoto and Yone 1979; Ye et al. 2010; Liang et al. 2015). In contrast, if the concentration of Mg in freshwater is low (1–4 mg kg^{-1}), its uptake from the environment is considered insufficient to satisfy metabolic requirements (Lall 2002), and dietary requirements of Mg have been reported for several species of freshwater fish (Ogino and Chiou 1976; Gatlin et al. 1982; Shim and Ng 1988; Reigh et al. 1991; Han et al. 2012; Liang et al. 2011; Mohammad and Khan 2018).

Sturgeons are important aquatic products, not only for preserving genetic resources and extending biological diversity, but also for breeding and consumption. They are particularly important as freshwater aquaculture species with high nutritional value (CFSY, 2018). Balancing the intake of trace elements is important to ensure the health and production of sturgeon in aquaculture systems and needs further study. Thus, the aim of the current study was to estimate the dietary Mg requirement of juvenile hybrid sturgeon (*Acipenser schrenckii*♀ × *Acipenser baerii*♂) using growth indices supported by growth performance, body composition, mineral concentration, Mg balance, and plasma antioxidant function analyses.

Materials and methods

Ethics statement

All procedures involving animals were conducted according to the Guidelines for the Care and Use of Laboratory Animals of Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences (CAFS). The procedures were reviewed and approved by the Committee for the Welfare and Ethics of Laboratory Animals of Heilongjiang River Fisheries Research Institute, CAFS.

Diet preparation

The ingredients and composition of the control diet used in the study are shown in Table 1. The experimental diets were supplemented with 0 (control diet), 100, 300, 500, 700, 900, and 1100 mg kg^{-1} Mg (as Mg sulfate; Sigma-Aldrich, St. Louis, MO, USA) with corresponding amounts of cellulose removed. Diets comprising dry ingredients were mixed thoroughly with distilled water and the pellets were then crushed into desirable particle sizes. The feeds were then dried and stored at −20 °C until use. The element analysis showed final Mg

Table 1 Ingredients and proximate composition of the control diet

Ingredients	% dry diet
Casein	40.20
Gelatin	8.00
Corn starch	35.68
Fish oil	5.00
Soybean oil	5.00
Cellulose	3.30
Monocalcium phosphate	2.00
Choline chloride	0.20
Betaine	0.10
Butylated hydroxytoluene	0.02
Mineral premix[a]	0.20
Vitamin premix[b]	0.30
Proximate composition (%)	
Crude protein	40.78
Crude fat	10.03
Crude ash	3.74

[a] The mineral premix provided the following per kg of diet: zinc, 60 mg; copper, 3 mg; iron, 25 mg; manganese, 15 mg; iodine, 0.6 mg; and selenium, 0.30 mg

[b] The vitamin premix provided the following per kg of diet: vitamin A, 8000 IU; vitamin C, 500 mg; vitamin D, 33,000 IU; vitamin E, 60 mg; vitamin K, 35 mg; vitamin B1, 15 mg; vitamin B2, 30 mg; vitamin B6, 15 mg; vitamin B12, 0.5 mg; choline chloride, 5000 mg; nicotinic acid, 175 mg; D-biotin, 2.5 mg; inositol, 1000 mg; folic acid, 5 mg; and pantothenic acid, 50 mg

concentrations of 43.2, 157.3, 326.5, 549.6, 743.9, 938.4, and 1118.2 mg kg^{-1} dry diet, and the distilled water was found to contain 2.8 mg L^{-1} Mg. The concentrations of other essential elements in the diet, calcium (Ca) and phosphorus (P), are shown in Table 2. Element analysis of the experimental diets following the method of Ye et al. (2010) was performed to determine the final Mg concentrations. Feed samples of approximately 0.10–0.15 g were digested with 15 mL 65–68% nitric acid and 2 mL 72% perchloric acid using Kjeldahl flasks. After digestion, samples were diluted to 50 mL and their Ca, P, and Mg contents analyzed by using an inductively coupled plasma atomic emission spectrophotometer. The standard solution was provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The analysis of Mg in water was performed using an atomic absorption spectrometer (Nthunya et al., 2018). The analysis was performed under an air pressure of 28 psi, a fuel (acetylene) pressure of 8 psi, and a sample uptake of 4 mL. The samples were filtered using 0.45-μm filters and 0.4 mL of NaCl-

Table 2 Mg supplement and mineral content analysis of experimental diets

Mg (mg kg^{-1})	Analyzed content		
	Mg (mg kg^{-1})	Ca (g kg^{-1})	P (g kg^{-1})
0	43.2±2.1	7.71±0.24	6.85±0.14
100	157.3±1.8	8.28±0.25	6.77±0.16
300	326.5±11.8	8.06±0.23	7.03±0.20
500	549.6±16.9	7.53±0.17	6.98±0.15
700	743.9±22.0	8.02±0.20	6.54±0.09
900	938.4±12.9	8.47±0.24	6.76±0.09
1100	1118.2±32.6	8.25±0.18	6.79±0.13

Values represent means ± SD, $n = 3$

HCl was added to each sample before analysis. The percentage absorption was converted to absorbance, which was subsequently used to calculate the concentration of each element. Proximate analyses (crude protein, crude fat, and crude ash) of diets were performed according to methods of the Association of Official Analytical Chemists (AOAC, 1995). In brief, the dry matter content was dried to constant weight at 105 °C and the crude protein content was determined by the Kjeldahl method after acid digestion. The crude fat content was determined through the ether extraction method using a Soxhlet device, and the samples were placed into a muffle furnace at 550 °C for 8 h for ash content analysis.

Fish and experimental conditions

Juvenile hybrid sturgeons were obtained from the Fangshan Breeding Center (Beijing, China). The fish were transferred to an indoor aquarium and acclimated for 2 weeks, during which they were fed a control diet. The temperature ranged from 21 to 23 °C, the dissolved oxygen was not less than 5.0 mg L^{-1}, the photoperiod was 12 light:12 dark, and the water was changed every 3 days. The tanks were filled with a continuously aerated mixture of tap water. After 2 weeks, 28 juvenile hybrid sturgeons (an average weight of 7.65 g) were then randomly assigned into one of 21 tanks of 100×50×50 cm. Three fish per tank were used to analyze the initial whole-body Mg at the beginning of the trial. The remaining 25 fish were weighed in-bulk (initial body weight (IBW)) and formed the experimental group in each tank. Seven diets were randomly assigned to three replicate tanks. The diets were fed by hand to apparent satiation at 07:00 h, 11:00 h, 16:00 h, and 19:00 h based on visual observations of the fish feeding behavior. Water quality was regularly monitored weekly and no differences were recorded among tanks throughout the experimental period.

During the 8-week experimental period, the feed conversion ratio (FCR) was determined by feed intake (g)/body weight gain. Uneaten feed particles were dried and weighed and used for correction of the feed consumption rate.

At the end of the trial, all of the fish were deprived of feed for 24 h and then the fish from each replicate were weighed (final body weight (FBW)). Weight gain rate (WG, %) was calculated as 100× [final weight (g) − initial weight (g)]/initial weight (g).

Sample collection and analyses

At the end of the 8-week feeding trial, six fish per tank were anesthetized with MS-222 (250 mg/L) and three of these were then used to analyze the whole-body composition; the other three were used to analyze the whole-body Mg, Ca, and P concentrations. All six fish were stored at −80 °C before use. Another three fish per tank were anesthetized and dissected on an ice plate, and their vertebrae were removed from the surrounding tissues and rinsed with distilled water, dried, ground, and stored at −40 °C for Mg, Ca, and P analyses. Blood of another six fish per tank was taken immediately following euthanasia and collected by 1-mL disposable heparinized syringes through the caudal vasculature of each fish, transferred into a disposable Eppendorf tube, centrifuged at 4000×*g* (4 °C) for 15 min to obtain the serum samples (two mixed into one), and then immediately stored at –20 °C until analysis.

The whole-body crude protein, crude fat, dry matter, and ash contents were determined following the procedures of AOAC (1995) as the same descriptions as proximate analyses of diets. The Mg, Ca, and P concentrations in whole body and vertebrae were measured using the

method of Ye et al. (2010). The indexes correlated with whole-body Mg retention (Lin et al., 2013) were calculated as follows:

$$\text{Initial whole-body Mg content (mg)} = \text{initial whole-body Mg concentration (mg/g)} \times \text{initial body weight (g)}$$

$$\text{Total Mg fed (mg)} = \text{feed Mg concentration (mg/g)} \times \text{total feed intake (g)}$$

$$\text{Final whole-body content (mg)} = \text{final whole-body Mg concentration (mg/g)} \times \text{final body weight (g)}$$

$$\text{Whole-body Mg retention (\%)} = [\text{final whole-body Mg content (mg)} - \text{initial whole-body Mg content (mg)}] \times 100/\text{total Mg fed (mg)}$$

The serum was used to determine the superoxide dismutase (SOD) (A001-3-2, hydroxylamine method), glutathione peroxidase (GPx) (A005-1, colorimetric method), and catalase (CAT) (A007-1-1, ultraviolet) activities and malondialdehyde (MDA) (A003-1, thiobarbituric acid method) content. All of these indices were determined using commercial kits supplied by Nanjing Jiancheng Bioengineering Institute. The activities and content were measured according to the kit protocols provided in the manufacturer's instructions.

Statistical analyses

The data were subjected to one-way analysis of variance (ANOVA) using SPSS 22.0 (SPSS, Chicago, IL, USA) and are presented as the means ± SD. Differences in the means between dietary treatments were evaluated using Duncan's multiple range tests. The optimum dietary Mg requirements, including relationship between the whole-body Mg concentration and dietary Mg as well as relationship between the vertebrae Mg concentration and dietary Mg, were estimated using the broken line model (Robbins et al. 1979). The relationships between whole-body Mg retention and dietary Mg levels were estimated by power function analysis.

Results

Growth performance

The growth performance of juvenile hybrid sturgeon fed the experimental diets for 8 weeks is shown in Table 3. The effects of the dietary Mg levels on IBW, FBW, WG, and FCR were not significantly different between treatments ($P > 0.05$).

Table 3 Growth performances of juvenile hybrid sturgeon fed experimental diets containing different Mg concentrations for 8 weeks

Mg (mg kg^{-1})	IBW (g)	FBW (g)	WG (%)	FCR
0 (43.2)	7.68±0.16	17.06±1.64	121.95±20.08	1.34±0.05
100 (157.3)	7.67±0.08	17.90±1.77	133.32±15.37	1.37±0.03
300 (326.5)	7.62±0.10	19.83±1.65	160.12±21.16	1.34±0.85
500 (549.6)	7.63±0.05	20.29±1.88	166.01±16.38	1.32±0.12
700 (743.9)	7.65±0.24	20.29±1.44	165.47±21.60	1.30±0.08
900 (938.4)	7.63±0.16	19.99±1.94	162.16±18.65	1.32±0.11
1100 (1118.2)	7.63±0.23	20.00±1.90	162.58±12.23	1.37±0.11

Values represent means ± SD, $n = 3$

IBW initial body weight, *FBW* final body weight, *WG* weight gain rate, *FCR* feed conversion ratio

Whole-body composition

The whole-body compositions of juvenile hybrid sturgeon fed the experimental diets containing different Mg levels for 8 weeks are shown in Table 4. Compared with 43.2 mg kg^{-1} (control), 743.9 mg kg^{-1} Mg significantly decreased the body moisture of hybrid sturgeon and significantly increased the crude protein content ($P< 0.05$). The fish fed 43.2 mg kg^{-1} exhibited lower crude fat levels than the other groups ($P< 0.05$). Fish provided with diets containing 549.6–1118.2 mg kg^{-1} had higher ash concentrations than the group given the control feed (43.2 mg kg^{-1}).

Whole-body and vertebrae mineral concentration

The whole-body and vertebrae Ca and P concentrations of juvenile hybrid sturgeon fed the experimental diets containing different Mg levels for 8 weeks are shown in Table 5. The whole-body Ca and P concentrations exhibited no significant differences among all the groups ($P>0.05$). The Ca:P ratios in the 43.2 and 326.5 mg kg^{-1} groups were significantly higher than those in the 938.4 mg kg^{-1} group ($P< 0.05$). The Ca concentrations of the vertebrae in the 157.3–743.9 mg kg^{-1} groups were significantly higher than those in the 1118.2 mg kg^{-1} group ($P< 0.05$). The Ca:P ratio in the 43.2 and 157.3 mg kg^{-1} groups was significantly higher than that in the 938.4 and 1118.2 mg kg^{-1} groups ($P< 0.05$) and significantly higher in the 326.5 mg kg^{-1} group than in the 1118.2 mg kg^{-1} group ($P< 0.05$).

Table 4 Whole-body composition (%) of juvenile hybrid sturgeon fed experimental diets containing different Mg concentrations for 8 weeks

Mg (mg kg^{-1})	Moisture	Crude protein	Crude fat	Ash
0 (43.2)	80.07±0.44^{a}	12.99±0.42^{b}	3.75±0.19^{b}	2.86±0.05^{b}
100 (157.3)	79.25±0.47^{ab}	13.42±0.33^{ab}	4.36±0.08^{a}	3.17±0.07^{ab}
300 (326.5)	78.37±0.64^{ab}	14.11±0.62^{ab}	4.48±0.10^{a}	3.19±0.10^{ab}
500 (549.6)	77.90±0.57^{ab}	14.44±0.19^{ab}	4.64±0.17^{a}	3.46±0.09^{a}
700 (743.9)	77.13±0.37^{b}	14.77±0.06^{a}	4.86±0.12^{a}	3.49±0.03^{a}
900 (938.4)	78.79±1.58^{ab}	13.90±0.94^{ab}	4.54±0.29^{a}	3.38±0.24^{a}
1100 (1118.2)	78.04±0.64^{ab}	14.46±0.42^{ab}	4.61±0.02^{a}	3.37±0.16^{a}

Values represent means ± SD, $n = 3$. Means in the same column with different superscript letters are significantly different ($P< 0.05$)

Table 5 Whole-body and vertebrae Ca and P concentrations of juvenile hybrid sturgeon fed experimental diets containing different Mg concentrations for 8 weeks

Mg (mg kg^{-1})	Whole-body			Vertebrae		
	Ca (g kg^{-1})	P (g kg^{-1})	Ca:P ratio	Ca (g kg^{-1})	P (g kg^{-1})	Ca:P ratio
0 (43.2)	5.88±0.18	5.81±0.49	21.14±0.69^{a}	161.40±8.92^{ab}	141.85±9.16	81.41±8.59^{a}
100 (157.3)	5.94±0.54	5.46±0.46	18.54±1.53^{ab}	172.09±14.25^{a}	136.55±7.27	80.04±2.93^{a}
300 (326.5)	6.68±0.20	5.75±0.42	20.97±1.71^{a}	171.81±7.50^{a}	137.22±11.01	76.73±7.36^{ab}
500 (549.6)	6.54±0.26	6.46±0.29	17.20±1.80^{ab}	172.33±11.24^{a}	164.10±10.60	64.30±7.26^{abc}
700 (743.9)	6.79±0.09	6.41±0.29	17.78±1.27^{ab}	170.32±18.70^{a}	164.12±11.05	66.95±4.89^{abc}
900 (938.4)	6.09±0.10	6.47±0.40	16.05±1.14^{b}	158.60±13.08^{ab}	162.93±9.63	60.32±0.21^{bc}
1100 (1118.2)	6.60±0.36	6.37±0.44	17.19±1.44^{ab}	128.35±11.18^{b}	164.83±9.92	50.33±5.21^{c}

Values represent means ± SD, n = 3. Means in the same column with different superscript letters are significantly different (P< 0.05)

Whole-body and vertebrae Mg concentrations

Whole-body and vertebrae Mg concentrations are shown in Figs. 1 and 2. The Mg concentration in the 43.2 mg kg^{-1} group was significantly lower than that in the 549.6–1118.2 mg kg^{-1} groups (P< 0.05). The whole-body concentrations of Mg increased with increasing Mg concentrations and then stabilized after a dietary Mg concentration of 549.6 mg kg^{-1} (Fig. 1). The Mg concentrations of the vertebrae in the 938.4 and 1118.2 mg kg^{-1} groups were significantly higher than those in the 43.2 mg kg^{-1} group (P< 0.05) (Fig. 2). The optimal dietary Mg requirement for juvenile hybrid sturgeon was estimated to be 573.6 (Fig. 1) and 584.6 mg kg^{-1} (Fig. 2) dietary Mg.

Whole-body Mg balance

The whole-body Mg balances of juvenile hybrid sturgeon fed the experimental diets containing different Mg levels for 8 weeks are shown in Supplementary material Table S1. The average initial whole-body Mg content of hybrid sturgeon was 6.95–7.65 mg. The final whole-

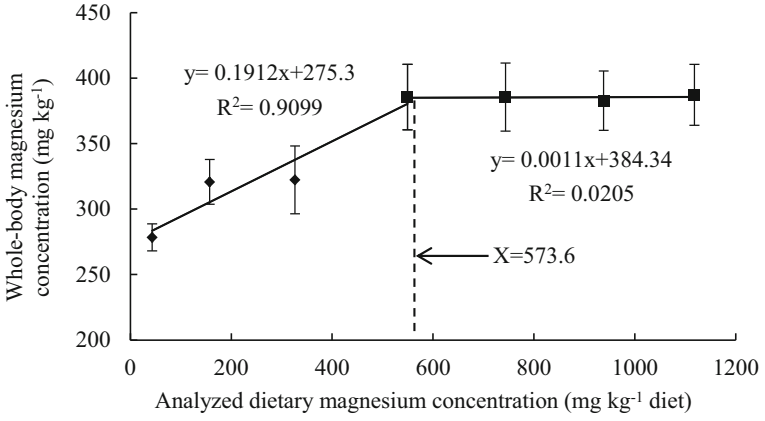

Fig. 1 Relationship between whole-body Mg concentration and dietary Mg levels of juvenile hybrid sturgeon fed experimental diets for 8 weeks. The breakpoint of the broken line is 573.6 mg kg^{-1} dietary Mg

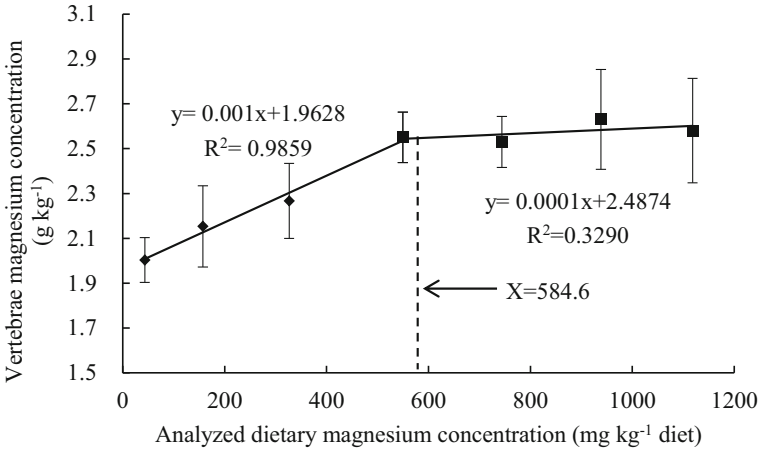

Fig. 2 Relationship between the vertebrae Mg concentration and dietary Mg levels of juvenile hybrid sturgeon fed experimental diets for 8 weeks. The breakpoint of the broken line is 584.6 mg kg^{-1} dietary Mg

body Mg contents increased as the dietary Mg levels increased. Whole-body Mg retention decreased as the dietary Mg levels increased.

The dietary Mg requirement for juvenile hybrid sturgeon was estimated and is shown in Fig. 3. Dietary Mg of 355.16 mg kg^{-1} was a concentration between the whole-body Mg retention and the dietary Mg levels, when the whole-body Mg retention was 100%.

Antioxidant capacity

The serum antioxidant levels of hybrid sturgeon fed the experimental diets containing different Mg levels for 8 weeks are shown in Table 6. Serum SOD in the 549.6 to 1118.2 mg kg^{-1} groups was significantly higher than that in the 43.2 mg kg^{-1} group ($P< 0.05$) and significantly higher in the 549.6 to 938.4 mg kg^{-1} groups than in the 157.3 mg kg^{-1} group ($P< 0.05$). Serum GPx in the 743.9 and 938.4 mg kg^{-1} groups was significantly higher than that in the other

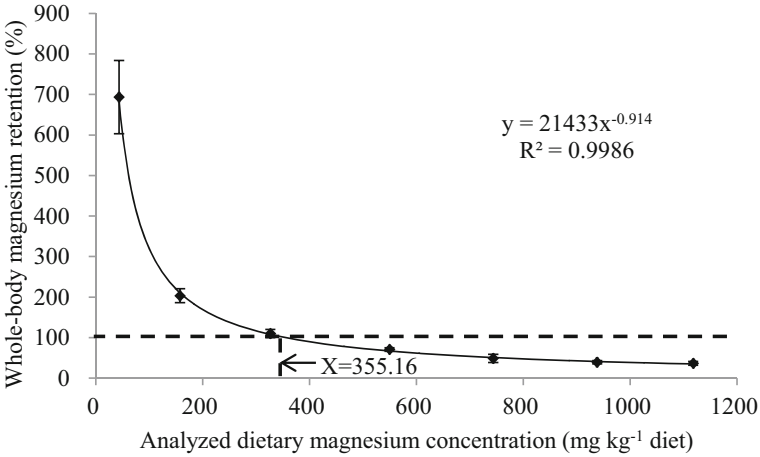

Fig. 3 Relationship between the whole-body Mg retention and dietary Mg levels of juvenile hybrid sturgeon fed experimental diets for 8 weeks

Table 6 Serum antioxidants of hybrid sturgeon fed experimental diets containing different Mg concentrations for 8 weeks

Mg (mg kg^{-1})	SOD (U mL^{-1})	GPx (U mL^{-1})	CAT (U mL^{-1})	MDA (nmol mL^{-1})
0 (43.2)	86.37±9.72^{d}	690.76±16.21^{d}	1.90±0.14^{c}	133.26±12.45^{a}
100 (157.3)	111.93±13.02^{cd}	796.64±45.47^{d}	1.91±0.08^{c}	117.80±10.50^{a}
300 (326.5)	125.84±12.63^{bcd}	931.09±23.71^{c}	2.21±0.09^{c}	73.57±5.77^{b}
500 (549.6)	187.63±6.96^{a}	1168.07±59.47^{b}	6.08±0.67^{a}	36.46±3.22^{c}
700 (743.9)	184.07±16.26^{a}	1712.61±30.85^{a}	6.05±0.26^{a}	50.98±7.62^{bc}
900 (938.4)	161.10±17.97^{ab}	1650.42±54.64^{a}	3.85±0.25^{b}	69.28±8.45^{b}
1100 (1118.2)	150.42±14.10^{abc}	1050.42±49.60^{bc}	4.41±0.27^{b}	72.48±6.78^{b}

Values represent means ± SD, $n = 3$. Means in the same column with different superscript letters are significantly different ($P< 0.05$)

‡ *U* activity unit of enzyme

groups ($P< 0.05$) and was significantly higher in the 326.5, 549.6, and 1118.2 mg kg^{-1} groups than in the 43.2 and 157.3 mg kg^{-1} groups ($P< 0.05$). Serum CAT was significantly higher in the 549.6 and 743.9 mg kg^{-1} groups than in the other groups ($P< 0.05$) and significantly higher in the 938.4 and 1118.2 mg kg^{-1} groups than in the 43.2, 157.3, and 326.5 mg kg^{-1} groups ($P< 0.05$). Serum MDA in the 43.2 and 157.3 mg kg^{-1} groups was significantly higher than that in the other groups ($P< 0.05$) and significantly higher in the 326.5, 938.4, and 1118.2 mg kg^{-1} groups than in the 549.6 mg kg^{-1} groups ($P< 0.05$).

Discussion

The requirements of Mg previously reported for freshwater fish were 326–1400 mg kg^{-1} diet (Ogino et al. 1978; Knox et al. 1981; Gatlin et al. 1982; Shim and Ng 1988; Shearer 1989; Reigh et al. 1991; El-Mowafi and Maage 1998), which was the basis for setting the doses of Mg in this study. An isotopic study found that when Mg in food was insufficient, fish were able to absorb some Mg from water; when the Mg concentration in food is too high, fish will excrete redundant Mg via the skin (Bijvelds et al. 1996; Wang et al., 2011). These processes could regulate the Mg balance. In the current study, the whole-body Mg concentration (573.6 mg kg^{-1} diet of Mg), the vertebrae Mg concentration (584.6 mg kg^{-1} diet of Mg), and the whole-body Mg retention (355.16 mg kg^{-1} diet Mg) were used to estimate the dietary Mg requirement of grass carp. This value is similar to that reported for other fish from 326 to 745 mg kg^{-1} diet (Ogino and Chiou 1976; Ogino et al. 1978; Knox et al. 1981; Shim and Ng 1988; Reigh et al. 1991; El-Mowafi and Maage 1998; Duan et al., 2012).

There are differences in reports on the effect of Mg requirement on growth performance. Reigh et al. (1991) reported a positive response to 30–650 mg kg^{-1} diet Mg supplementation of blue tilapia (*Oreochromis aureus*) fed casein-based diets in terms of their growth, feed, and protein efficiency. A lack of Mg in the feed can dull appetite, slow growth, increase mortality, and significantly decrease the production performance in aquatic animals (Ogino and Chiou 1976; Cowey 1976; Cowey et al. 1976; Cowey et al. 1977; Knox et al. 1981; Gatlin et al. 1982; Shim and Ng 1988; Shearer 1989; Reigh et al. 1991; Wei et al. 2017; Mohammad and Khan 2018). Not all fish respond well in terms of growth to dietary Mg, such as Atlantic salmon (*Salmo salar L.*) (El-Mowafi and Maage 1998), tilapia (*Oreochromis mossambicus* (Peters)) (Velden et al. 1991), grass carp (*Ctenopharyngodon*

idella) (Wang et al., 2011), and gibel carp (Han et al. 2012). In the current study, dietary Mg (from 43.2 to 1118.2 mg kg^{-1}) did not significantly impact the growth performance of juvenile hybrid sturgeon.

Wang et al. (2011) found that adding an appropriate amount of Mg sulfate to the diet significantly increased the whole-body crude protein in fish. Compared with the 43.2 mg kg^{-1} diet Mg group, treatment groups, such as 743.9 mg kg^{-1} diet Mg in whole-body crude protein contents, 157.3–1118.2 mg kg^{-1} diet Mg in whole-body crude fat contents, and 549.6–1118.2 mg kg^{-1} diet Mg in whole-body ash contents, were significantly increased in the current study.

The whole-body Ca and P concentrations in rainbow trout (*Oncorhynchus mykiss*) fed low Mg diets were considerably higher than those fed normal diets (Shearer 1984), suggesting that Ca and P levels associated with Mg status. The Ca content in the bones or whole body of fish fed with low-Mg diets was significantly higher than that in guppy (*Poecilia reticulata* (Peters)), rainbow trout, and blue tilapia fed with normal diets (Shim and Ng 1988; Shearer 1989; Reigh et al. 1991). In our results, whole-body Ca and P concentrations were not influenced by Mg concentrations, and 938.4 mg kg^{-1} diet Mg in Ca:P ratio of whole-body mineral concentrations was significantly lower than 938.4 mg kg^{-1} diet Mg, which may be due to the difference in species. The vertebrae Ca content was affected by the dietary Mg levels (Ogino and Chiou, 1976). Our results showed that moderate Mg concentrations (157.3–743.9 mg kg^{-1}) increased vertebrae Ca concentrations compared with 1118.2 mg kg^{-1} diet Mg, and the Ca:P ratios in the lower Mg concentration groups of vertebrae (dietary 43.2 and 157.3 mg kg^{-1}) were higher than those in 938.4 and 1118.2 mg kg^{-1} diet Mg, which indicated that low Mg concentrations were beneficial to the deposition of Ca and P.

A variety of antioxidant defense systems have evolved in organisms. Antioxidant systems include enzymes, such as SOD, CAT, and GPx, to constantly suppress and remove the production of oxidative stress. In the current study, serum SOD and CAT activities were significantly higher in the 549.6 and 743.9 mg kg^{-1} groups than in the other groups and the serum GPx activities were significantly higher in the 743.9 and 938.4 mg kg^{-1} groups than in the other groups. These results indicated that appropriate Mg levels enhanced the antioxidant capacity; this observation is supported by Garcia et al. (1998), who found that Mg can reduce the production of free radicals. Mg can eradicate free radicals by enhancing antioxidant enzyme activities, such as those of SOD, CAT, and GPx, and this phenomenon might explain the effectiveness of Mg against peroxidation (Mohammad and Khan 2018). Wang et al. (2011) documented that grass carp fed imbalanced Mg levels were susceptible to lipid peroxidation as a result of reduced serum SOD and GPx activities, suggesting reduced antioxidant capacity in response to dietary Mg deficiency (Mohammad and Khan 2018). As an essential cofactor in glutathione biosynthesis, Mg can inhibit lipid peroxidation by preventing exhaustion of glutathione, which showed similar results as the serum GPx activity in response to low-Mg diets (Zhang et al. 2016). In the current study, the activities of SOD, CAT, and GPx were positively correlated with the concentration of Mg. MDA is the final product of lipid peroxidation and an important indicator of membrane injury caused by free radicals (Longbaf Dezfouli et al. 2019). Mohammad and Khan (2018) reported that the antioxidant status of Rohu improved with the inclusion of dietary Mg levels up to 460 mg kg^{-1} diet and then stabilized in groups fed higher levels of Mg, whereas the reverse pattern was observed for the MDA content. In the current study, the MDA contents in the 549.6 mg kg^{-1} groups were significantly lower than those in the 43.2, 157.3, 326.5, 938.4, and 1118.2 mg kg^{-1} groups, suggesting that the antioxidative effect of Mg results from its ability to increase the activity of radical-scavenging enzymes and decrease the peroxidation of lipids.

Conclusion

In summary, although dietary Mg concentrations had no effect on growth performance, the optimal amount of Mg supplementation improved dietary crude protein content, crude fat content, and whole-body Mg concentration; enhanced serum SOD, GPx, and CAT activities; and decreased serum MDA contents in juvenile hybrid sturgeon. Thus, a dietary Mg concentration of 350–700 mg kg^{-1} is suggested as a recommended dosage for the aquaculture of juvenile hybrid sturgeon.

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Author contribution Zhang and Wang conceived and designed the experiments; Zhang, Fan, and Wu performed the experiments; Zhang and Li analyzed the data; and Zhang, Xu, and Liu wrote and modified the paper.

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Data availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Conflict of interest The authors declare no competing interests.

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