

Effects of dietary magnesium supplementation on the growth performance, body composition, and immune indices of juvenile Persian sturgeon, *Acipenser persicus*

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ABSTRACT

The present experiment was carried out to investigate the effects of different levels of dietary magnesium on growth performance, proximate composition, and immune response of juvenile Persian sturgeon, *Acipenser persicus*. Fish were fed with six different rations including T1: 32, T2: 185, T3: 380, T4: 592, T5: 790, and T6: 935 mg kg⁻¹ Mg in diet for 8 weeks. At the end of the experiment, samples were collected for estimating the proximate composition and immunological parameters. Results of the present study showed that dietary Mg level significantly influences on growth performance and proximate body composition. Body weight, BWI, and DG were increased by up to 592 mg kg⁻¹ dietary Mg, while decreased by its higher levels. The significant increase in lysozyme activity (by 790 mg kg⁻¹ of dietary Mg) and immunoglobulin (by 935 mg kg⁻¹) were observed in fish fed with different levels of magnesium supplementation compared to control group, which may be due to immune enhancement. The dietary Mg level of 592 mg kg⁻¹ was suitable for Persian sturgeon based on the growth performance.

Keywords: Magnesium requirement, Diet, Persian sturgeon, *Acipenser persicus*.

Article type: Research Article.

INTRODUCTION

Magnesium (Mg), as an essential mineral, is the third most abundant ion in ocean water with the concentration of more than 50 mmol L⁻¹ (Rankin & Davenport 1981). It plays an important role in activation of enzymes (at least 300 enzymatic reactions) involved in the normal metabolism of lipids, proteins, and carbohydrates (Davis & Gatlin, 1996; Davis & Lawrence 1997; Lall 2002; Vormann 2003). It is essential for the maintenance of intra- and extracellular homeostasis (Houston 1985), transferring phosphate groups, and controlling ATP-dependent ion pumps (Bijvelds *et al.* 1998). Mg also plays a key role in immune mechanism (Lall 2002; Tam *et al.* 2003), and is a main component of bone in fish (Houston 1985). The deficiency signs of Mg in fish include anorexia, sluggishness, reduced growth, muscle flaccidity, vertebral deformity, high mortality which have been reported for several species (Lall 2002). Since the level of Mg in freshwater is apparently insufficient to meet the metabolic requirements of fish, therefore adding the element in the diet of fish reared in fresh water is so unavoidable (Steffens 1989). Fish can uptake magnesium both from water and food (Bijvelds *et al.* 1997; Lall 2002). The necessity of Mg has been recognized for several species of fresh water fish such as common carp, *Cyprinus carpio* (Ogino & Chiou 1976); rainbow trout, *Oncorhynchus mykiss* (Ogino *et al.* 1978; Knox *et al.* 1981; Shearer 1989); channel catfish, *Ictalurus punctatus* (Gatlin *et al.* 1982; Lim and Klesius 2003); guppy, *Poecilia reticulata* (Shim

& Ng 1988); tilapia, *Oreochromis niloticus* (Dabrowska *et al.* 1989); far eastern catfish, *Silurus asotus* (Yoon *et al.* 2014); Japanese seabass, *Lateolabrax japonicus* (Huang *et al.* 2016); white shrimp, *Litopenaeus vannamei* (Roy *et al.* 2009); blue tilapia, *Oreochromis aureus* (Reigh *et al.* 1991); common carp, *Cyprinus carpio* (Kandeep 2013) and Pacific white shrimp, *Litopenaeus vannamei* (Jahan *et al.* 2018). Dietary Mg requirements vary for different species or even for the same species at different environment (Han *et al.* 2011). The extra Mg from the diet could be either reabsorb or secret as a function of nutritional status (Liang *et al.* 2011). A reduction in growth was observed when high levels of Mg were fed in a purified diet (Shearer 1989). Therefore, estimation of dietary Mg requirements in cultured aquatic animals is a priority. In order to assess the dietary mineral supplementation for aquatic animals, growth performance, and whole body composition are the main parameters to be measured. The effect of the element on immune function should also be taken into consideration, but this has been seldom addressed. Obviously, changes in immune parameters should be considered as important criteria to assess the nutritive value of Mg for fish meal. Persian sturgeon, *Acipenser persicus* is one of the most important sturgeon species in the south coast of the Caspian Sea, caught for both meat and caviar production. It is considered as an endangered species by the International Union for Conservation of Nature and Natural resources (Sturgeon Specialist Group 1996) due to overfishing and destruction of habitat, which is the subject of restoration and restocking schemes (Mohseni *et al.* 2008; Williot *et al.* 2001). There are many studies about sturgeons around the world (Madadi & Khara, 2016; Badan-Ara Marzdashti *et al.* 2018; Shirangi *et al.* 2019; Fathollahi *et al.* 2020; Barimani *et al.* 2021). However, no study was found about Mg requirements in these fish species. Given the previous Mg studies for other freshwater fish, it is necessary to supplement Mg in the diet of this commercially-important fish species. To date, no study has been conducted about the effects of minerals on sturgeons. This study was performed to investigate the effects of Mg levels on growth performance, survival, body composition, and immune parameters of Persian sturgeon.

MATERIALS AND METHODS

Diet preparation

Composition and the chemical proximate of the basal diet is given in Table 1. Mg sulphate ($\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$) was supplemented to the basal diet at the levels of 0 (T_0), 150 (T_{150}), 350 (T_{350}), 550 (T_{550}), 750 (T_{750}), and 900 (T_{900}) (Karl *et al.* 1992; Allen *et al.* 2005; Roy *et al.* 2009; Liang *et al.* 2012; Han *et al.* 2012). Fish meal and soybean meal were used as the protein sources, fish oil as the lipid source, wheat flour and corn gluten as carbohydrate sources of the basal diet. The analysed Mg contents in the six diets were: 32 (0), 185 (150), 380 (350), 592 (550), 790 (750), 935 (900). All dry ingredients were thoroughly mixed for 20 min, then fish oil was added and mixed for 15 min. Double deionized water was added to make a homogenous ingredients. The pellets (2 mm in diameter) were made using an experimental pellet-making machine and air-dried at 30 °C for 24 hours, then stored at 4 °C until used. Control diet was prepared in the same way without adding magnesium.

Fish husbandry and feeding trial

Juvenile Persian sturgeon were obtained from Dr. Dadman International Sturgeon Research Institute, Rasht, Guilan Province, Iran, then were randomly distributed into 18 circular fiberglass tanks at a density of 20 fish per tank (50 L), where three tanks were assigned to each diet. Fish were allowed to become acclimatized to the experimental condition for 2 weeks prior to beginning the experiments in circular fiberglass tanks. During the acclimation, fish were fed twice daily with the basal diet. Fish were kept under natural light/dark cycle at 12:12 hours. The tanks were supplied with constant flow freshwater of $0.4 \pm 0.2 \text{ L sec}^{-1}$ and supplemental aeration was provided to maintain dissolved oxygen levels near saturation. Water temperature was maintained at 15 °C throughout the trial. Mean dissolved oxygen, and pH were $7.1 \pm 0.2 \text{ mg L}^{-1}$ and 7.7 ± 0.1 , respectively. Magnesium concentrations of waterborne were 2.5 to 3.7 mg L^{-1} during the trial period.

Before initiation of the experiment, fish were deprived of feed for one day. Fifteen fish per tank randomly sampled for analysis. The initial body weight and length were $4.8 \pm 0.6 \text{ g}$ (mean \pm SE), and $9.93 \pm 0.3 \text{ cm}$, respectively. The experimental diets were carefully hand-fed to triplicate tanks four times per day (08.00, 12.00, 16.00, 20.00 hours) till apparent satiation, and feeding trial lasted for 8 weeks. One hour after feeding, the faeces were removed by siphoning and feed intake of each tank was measured (Abdel-Tawwab *et al.* 2006). During the experimental period, fifteen fish per each replicate was randomly weighed every 14 days.

Table 1. Ingredient and chemical composition of the basal diet.

Ingredients	Amount (g kg ⁻¹ dry weight)
Fish meal	540
Wheat meal	180
Corn meal	50
Milk powder	50
Fish oil	40
Soybean meal	80
Yeast	30
Mineral premix ¹	13
Vitamin premix ²	17
Ascorbic phosphate ester	1
Proximate chemical composition (%)	
Crude protein	40
Crude fat	19
Ash	9

¹Mineral mix (g kg⁻¹ diet): Iron, 5 mg; Zinc, 15mg; Copper, 3 mg; Calcium lactate, 327 mg; NaCl, 43.5 mg; Potassium Iodate, 0.3 mg.

²Vitamin mix (mg kg⁻¹ of diet): Vitamin A , 5000 IU ; Vitamin D3 , 500 IU; Vitamin E , 3 mg; Vitamin K₃ , 1.5 mg ; Vitamin B₂ , 1 mg; Calcium pantothenate, 4 mg; Vitamin B₃, 15 mg, Vitamin B₆, 0.3 mg.

Sampling and analytical methods

At the end of the trial, fish in each tank were anesthetized with 0.5 g L⁻¹ clove powder (Yarmohammadi *et al.* 2012), then body weight (to the nearest 0.01 g) and total length (to the nearest 1 mm) of all fish were individually measured 24 hours after the last feeding to evacuate their gut before measuring. After weighing and measuring length, fish were returned to their tank for recovery. Body weight increase (BWI), condition factor (CF), specific growth rate (SGR), daily growth rate (DG), feed conversion ratio (FCR), protein efficiency ratio (PER) were calculated as follows:

$$\text{BWI (\%)} = 100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$$

$$\text{CF} = 100 \times (\text{body weight}) / \text{total length}^3$$

$$\text{SGR (\% day}^{-1}\text{)} = 100 \times (\text{Ln final body weight} - \text{Ln initial body weight}) / \text{days of the trial}$$

$$\text{DG} = 100 \times (\text{final body weight} - \text{initial body weight} / \text{initial body weight} - \text{days of the trial})$$

$$\text{FCR} = \text{feed intake} / \text{weight gain}$$

$$\text{PER} = \text{Wet weight gain (g)} / \text{Protein intake (g)}$$

Fifteen fish per group (five fish in each replicate) were sacrificed with an overdose of 1000 mg L⁻¹ clove solution. Thereafter, samples immediately frozen at -20 °C. Fish composition were analysed according to AOAC (1995) standard methods. To find the moisture content, samples were placed in oven drying at 105 °C to reach constant weight. Crude fat was measured by Soxhlet method, based on extraction of the lipids. Crude protein content was assayed by Kjeldahl method by measuring the total nitrogen (N × 6.25). In order to measure ash content, samples were burned for 6 h in a Gallenkamp furnace at 550 °C. To assess the immune parameters, five fish were randomly collected from each replicate. The blood was withdrawn from the caudal vessel using a 2 mL syringe. The sampling lasted less than 1 min for each fish after anaesthetization. Blood were transferred to test tubes, centrifuged at 1600 g for 10 min, then plasma separated and stored in Eppendorf tubes at -80 °C for subsequent assays of immune parameters including immunoglobulin (IgM), and lysozyme concentrations. IgM was determined by immune turbidimetric method, measured by spectrophotometer at 360 nm absorbance. Lysozyme was measured by turbidimetric assay as described by Ellis (1990). 1.75 mL⁻¹ of *Micrococcus lysodeikticus* suspension (Sigma; containing 0.375 mg mL⁻¹, 0.05 M PBS, pH 6.2) were mixed with 250 mL of each sample and the optical density was measured after 15 and 180 seconds by spectrophotometer at 670 nm. PBS was used as a blank and results were expressed according to amounts of lysozyme (mg) per one mg of sample calibrated by standard curve determined with hens' egg white lysozyme (Sigma) in sterile sodium phosphate buffer.

Statistical analyses

Data are presented as means ± standard error (SE). To evaluate normality of data and homogeneity of variances, one Sample Shapiro-Wilk and Levene's test were used, respectively. All data subjected to one-way analysis of

variance to determine whether significant differences occurred among treatments. When significant differences were observed, Duncan's multiple range tests by $p < 0.05$ were performed (Zar 2010).

RESULTS

Growth performance

All measured growth parameters are reported in Table 2. The body weight was significantly different ($p < 0.05$) among the treatments. T₅₅₀ exhibited the most body weight compared to all other treatments. No significant difference was observed in body length among treatments ($p > 0.05$). CF in fish fed the basal diet was significantly higher than in the other feeding regime ($p < 0.05$), whereas there was no significant difference in CF among other treatments. T₅₅₀ exhibited the most BWI, SGR, and DG compared to all other treatments. FCR was significantly improved in T₅₅₀ with the level of 559 mg kg⁻¹ magnesium compared to other treatments. PER in T₉₀₀ was significantly higher than in the other treatments.

Body composition

The proximate composition of carcass at the end of the experiment is shown in Table 3. Significant differences ($p < 0.05$) were observed among the groups for all body composition including moisture, ash, crude fat, and crude protein at the end of the trial.

Immune parameters

Lysozyme level was significantly changed among treatments ($p < 0.05$; Fig. 1). The highest level and lowest level of lysozyme were found in T₇₅₀ and T₀, respectively. Significant differences in IgM concentrations were recorded among the treatments after 56 days ($p < 0.05$; Fig. 2). The highest and lowest level of IgM were found in T₉₀₀ and T₇₅₀, respectively.

Table 2. Changes in final weight, final length, body weight increase (BWI), condition factor (CF), specific growth rate (SGR), daily growth rate (DG), feed conversion ratio (FCR), and protein efficiency ratio (PER), in Persian sturgeon *Acipenser persicus* after 8 weeks of applying different experimental diets.

Growth performance	Mg supplementation levels (mg Kg ⁻¹)					
	0 (32)	185 (150)	380 (350)	592 (550)	790 (750)	935 (900)
Initial weight (g)	4.7 ± 0.35	4.8 ± 1.9	4.5 ± 3.6	4.2 ± 0.06	4.1 ± 0.5	4.2 ± 4.9
Final weight (g)	10.9 ± 1.4 ^{ab}	11.2 ± 1.2 ^a	11.6 ± 1.5 ^a	12.3 ± 1.1 ^a	10.6 ± 1.0 ^{ab}	9.7 ± 0.8 ^b
Initial length (cm)	9.6 ± 0.5	10.2 ± 0.3	10.7 ± 0.3	9.8 ± 0.9	10.0 ± 0.7	9.3 ± 0.2
Final length (cm)	13.3 ± 1.8	14.1 ± 1.6	14.3 ± 2.2	14.5 ± 2.0	13.6 ± 1.0	13.8 ± 2.0
BWI ¹ (%)	122.4 ± 1.7 ^{bc}	128.6 ± 1.8 ^{bc}	136.1 ± 1.9 ^b	150.3 ± 2.1 ^a	117.0 ± 1.6 ^c	98.0 ± 1.4 ^d
CF ²	0.5 ± 0.1 ^a	0.4 ± 0.0 ^{ab}	0.4 ± 0.0 ^{ab}	0.4 ± 0.0 ^{ab}	0.4 ± 0.0 ^{ab}	0.4 ± 0.0 ^{ab}
SGR ³ (% day ⁻¹)	1.5 ± 0.01 ^{ab}	1.5 ± 0.02 ^{ab}	1.6 ± 0.02 ^a	1.7 ± 0.01 ^a	1.4 ± 0.01 ^{ab}	1.2 ± 0.02 ^b
DG ⁴ (%)	1.7 ± 0.02 ^{ab}	1.8 ± 0.03 ^{ab}	1.9 ± 0.03 ^a	2.1 ± 0.02 ^a	1.6 ± 0.02 ^{ab}	1.4 ± 0.03 ^b
FCR ⁵	1.5 ± 0.01 ^{ab}	1.6 ± 0.02 ^{ab}	1.6 ± 0.02 ^{ab}	1.7 ± 0.01 ^a	1.5 ± 0.01 ^{ab}	1.3 ± 0.02 ^b
PER ⁶	2.1 ± 0.01 ^{ab}	2.1 ± 0.01 ^{ab}	2.1 ± 0.01 ^{ab}	2.0 ± 0.01 ^b	2.2 ± 0.01 ^{ab}	2.4 ± 0.02 ^a

Note: Values are mean ± SE from all fish of each treatment (n = 60). Different superscripts in the same row indicate significant differences ($p < 0.05$) between feeding strategies by One-Way ANOVA and Duncan's tests.

Table 3. Changes in proximate composition (as % of wet weight) in Persian sturgeon, *Acipenser persicus* after 8 weeks of applying different experimental diets.

Proximate composition	Mg supplementation levels (mg Kg ⁻¹)					
	0 (32)	150 (185)	350 (380)	592 (550)	790 (750)	935 (900)
Moisture (%)	78.7 ± 0.07 ^{ab}	82.6 ± 0.1 ^a	82.3 ± 0.7 ^a	77.2 ± 0.2 ^b	81.7 ± 0.09 ^a	82.2 ± 0.6 ^a
Crude fat (%)	10.0 ± 0.0 ^{cd}	21.6 ± 1.2 ^a	16.8 ± 0.6 ^b	8.9 ± 0.5 ^{cd}	7.5 ± 0.1 ^d	10.5 ± 0.5 ^c
Crude protein (%)	57.5 ± 0.3 ^c	63.1 ± 0.1 ^b	64.2 ± 0.6 ^a	67.02 ± 0.2 ^a	68.07 ± 0.6 ^a	58.6 ± 0.1 ^c
Ash (%)	14.0 ± 1.0 ^{bc}	14.1 ± 0.7 ^{bc}	16.1 ± 1.0 ^b	13.6 ± 0.5 ^{bc}	15.0 ± 1.0 ^b	20.4 ± 0.5 ^a

Note: Values are mean ± SE from all fish of each treatment (n = 15). Different superscripts in the same row indicate significant differences ($p < 0.05$) between feeding strategies by One-Way ANOVA and Duncan's tests.

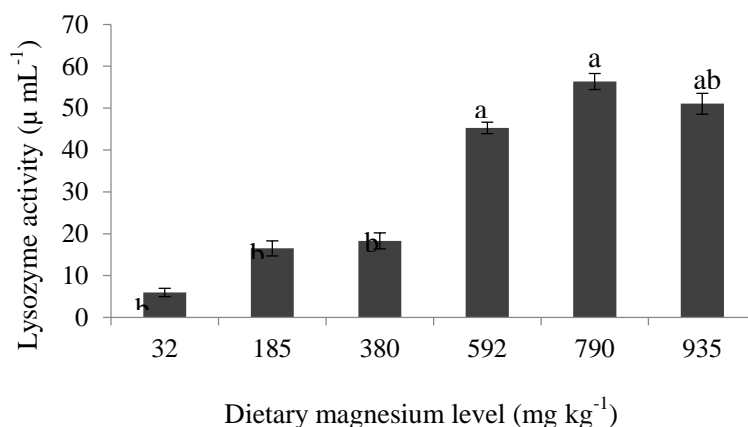


Fig. 1. Serum lysozyme activity of Persian sturgeon, *Acipenser persicus* fed different Magnesium supplemented diets for 8 weeks. Data are presented as mean \pm SE (n = 15). Bars with different superscript (s) are significantly different ($p < 0.05$).

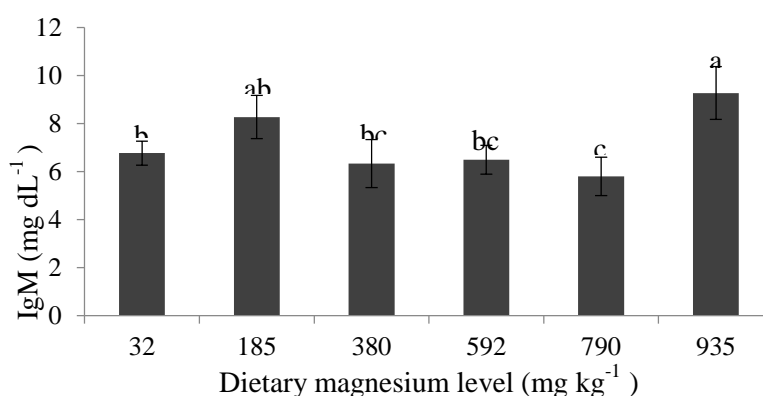


Fig. 2. Serum IgM of Persian sturgeon, *Acipenser persicus* fed different Magnesium supplemented diets for 8 weeks. Data are presented as mean \pm SE (n = 15). Bars with different superscript (s) are significantly different ($p < 0.05$).

DISCUSSION

The present study was carried out to determine which dietary level of Mg is optimum for growth, body composition and immune parameters in Persian sturgeon. Unfortunately, poor and inconsistent knowledge is available on the growth and immune response of sturgeon fed with dietary Mg or mineral supplementation. Indeed, the effects of dietary magnesium on growth patterns, proximate composition and immune parameters of this species was assessed for the first time in this study. Results of the present study showed that dietary Mg level significantly influence on growth performance and feed utilization. Mg concentration of waterborne was 2.5 to 3.7 mg L⁻¹ during the experiment, which was insufficient to meet the metabolic requirement of Persian sturgeon. In the present study, body weight, BWI, and DG were increased T₅₅₀, then decreased in the higher levels of dietary Mg. Although no significant difference in body length was revealed after 8 weeks, T₅₅₀ exhibited the highest body length. CF in T₀ was significantly the highest. FCR and PER enhanced in T₉₀₀, indicating a higher utilization of the dietary protein for growth. In channel catfish, *Ictalurus punctatus*, a Mg dietary level of 400 mg kg⁻¹ was required for optimum growth and survival (Lim & Klesius 2003). In addition, Japanese seabass, *Lateolabrax japonicus*, (Huang et al. 2016); common carp, *Cyprinus carpio* (Kandeep 2013) and Pacific white shrimp, *Litopenaeus vannamei* (Jahan et al. 2018) required 520 mg kg⁻¹, 0.4 g 100 g⁻¹ and 150 mg kg⁻¹ respectively. Dietary Mg significantly influenced on the growth performance in grass carp, *Ctenopharyngodon idella*, and a dietary Mg of 637 mg kg⁻¹ level was sufficient for its optimum growth (Liang et al. 2011). On the other hand, in juvenile Gibel carp, *Carassius auratus*, dietary magnesium did not enhance the growth performance, so that, Mg requirement of fish could be met by Mg uptake from the rearing water (Han et al. 2011). These contradicting results suggest that Mg requirement changes depending on species, and Mg concentration of rearing water could significantly affect the magnesium requirement of fish (Shearer and Asgard 1992). It has been found that tilapia, *Oreochromis aureus* reared in freshwater with the Mg level of 0.1 mg L⁻¹ required a dietary Mg of 500 mg kg⁻¹ (Reigh et al. 1991), whereas waterborne Mg concentration of 4.8 mg L⁻¹ was sufficient for tilapia fed low Mg diet (Van der Velden et al. 1991a). It seems that when tilapia fed low Mg diets, it can absorb Mg from water (Van der

Velden *et al.* 1991a). In the present study, Persian sturgeon reared in freshwater, whereas the main habitat of this species is brackish water (Caspian, Azov, Black and Baltic Seas; Billard & Lecointre 2001). Therefore, the low waterborne Mg concentration in the present study is not sufficient for the optimum growth. From the point view of body composition, T₅₅₀ and T₇₅₀ exhibited the lowest and highest rate (%) of body lipid and protein, respectively revealing that the main source of energy was lipid in these treatments. Nutritional modification has recently been used to reduce the severity of infection (Chandra, 1996; Scrimshaw & San Giovanni 1997). Lysozyme occurs predominantly in fish mucus and serum (Ellis 1999). An increase in the lysozyme concentration in fish bloodstream could be the result of infections or invasion by foreign material (Siwicki & Studnicka 1987; Moyner *et al.* 1993). In the present study, the significant increase in lysozyme activity of fish fed with different magnesium supplementation was observed compared to control group, suggesting an immune enhancement. Dietary magnesium has been shown to be important in disease resistance and immune response because of its necessity in the control of both physiological functions and structural components of the body, including antibody protein synthesis (Karppanen 1993). Several factors such as nutritional status, seasonal variation, sexual maturation, salinity, pH, water temperature, stresses and infections are involved in variation of lysozyme activity (Saurabh & Sahoo, 2008). Serum immunoglobulins provide disease protection in animals and human beings which are major components of the humoral immune system (Watts *et al.* 2001). The IgM results showed significantly higher values in fish fed with the different diets of Mg, compared to the control group exhibiting that the Mg supplementation can stimulate the antibody production in fish (Panigrahi *et al.* 2004). The increased lysozyme and IgM contents measured in blood serum of fish fed with the different diet of magnesium supplementation, are signs of an inflammatory response which has previously been classified as a subacute enteritis (Baeverfjord and Krogdahl, 1996), or maybe as a result of a hypersensitivity reaction (Pedersen, 1989; Rumsey *et al.* 1994; Baeverfjord and Krogdahl 1996).

CONCLUSION

In conclusion, dietary magnesium supplementation enhances the growth performance of juvenile Persian sturgeon. Based on the growth performance, the dietary suitable Mg level for Persian sturgeon was 592 mg kg⁻¹. The lysozyme activity and IgM were increased in blood serum of fish fed with the different dietary magnesium supplementation.

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