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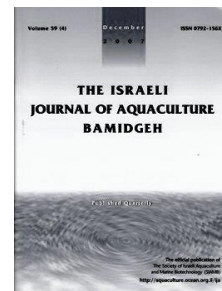
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## The Effects of Dietary Magnesium (Mg) Supplementation on Growth Performance of Adult Japanese Seabass (*Lateolabrax japonicas*)

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### Abstract

Japanese seabass, *Lateolabrax japonicus* (mean initial weight 204.5 g) were fed one of six casein-dextrin-based diets containing graded levels of magnesium (Mg) (184, 278, 487, 929, 1299, and 1737 mg/kg) for 10 weeks with the water-borne Mg concentration of 985 mg/L. Magnesium sulphate was used as the Mg source in the diets. The experiment was carried out in floating sea cages. Growth, survival rate, superoxide dismutase (SOD) activities, glutathione peroxidase (GPx), malondialdehyde (MDA) contents and tissue mineral contents were measured to investigate the effect of dietary magnesium in seabass. After 10 weeks, dietary magnesium supplementation did not improve the growth performance or feed efficiency of adult seabass. On the contrary, negative effects on growth performance, decreased glutathione peroxidase (GPx) and increased malondialdehyde (MDA) contents were observed in seabass fed Mg diets with 929, 1299 mg/kg and 1737 mg/kg, and feed efficiency (FE) exhibited a decreasing tendency with increasing Mg supplemental levels, which indicates the Mg requirement of seabass was met in fish fed the basal diet. Survival, hepatosomatic index, viscerosomatic index, condition factor, and Mg and Ca concentration in whole-body, vertebrae, and scales were not affected by dietary Mg supplementation ( $P < 0.05$ ).

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## Introduction

Magnesium (Mg) is an essential element involved in several biochemical processes. Mg is mainly bound to nucleic acids, negatively charged phospholipids and proteins (Vormann 2003) and an essential cofactor of many enzyme systems such as phosphokinase, thiokinases, phosphases, pyrophosphatase, carboxylase, and oxidase of pyruvic acid, alkaline phosphatase and amino acid acyl synthetase (Shils, 1994). Deficiency symptoms include retarded growth, anorexia, sluggishness, high mortality, reduced ash content, reduced magnesium and calcium concentration of the whole body, and of the vertebrae (Lall, 2002). Calcinosis of the kidney, vertebrae deformity, and degeneration of muscle fibers, epithelial cells of pyloric caeca and gill filaments, were also observed (Lall, 1989).

The dietary requirements of rainbow trout, *Salmo gairdneri* (Cowey et al., 1977; Ogino et al., 1978; Knox et al., 1981), carp, *Cyprinus carpio* L. (Ogino and Chiou., 1976), Channel catfish, *Ictalurus punctatus* (Raf.), fingerlings (Gatlin et al., 1982), eel, *Anguilla japonica* T & S. (Nose and Arai, 1979), guppy, *Poecilia reticulata* (Peters) (Shim and Ng, 1988) and tilapia, *Oreochromis niloticus* (L.) (Dabrowska et al., 1989; Reigh et al., 1991) have been estimated in fresh water, and range from 400 to 800 mg/kg dry diet. For marine species, Mg supplementation in the diets may not be necessary (Lall and Bishop, 1977; Sakamoto and Yone, 1979). When red sea bream (*Chrysophrys major*) were fed diets with and without supplemental Mg (660 and 120 mg/kg of the diet respectively) over a 60-day period, it was found that Mg supplementation in the diet was not essential (Sakamoto and Yone, 1979). A similar study in grouper (*Epinephelus coioides*) showed that a high dietary Mg level of 2.8 g/kg had no negative effects on growth, feed efficiency (FE), and Mg concentration in scales and vertebrae of fish (Ye et al., 2010). Based on weight gain, magnesium requirement of *L. vannamei* cultured in low-salinity (2‰) water was found to be 0.26 to 0.35% of the diet (Cheng et al., 2005). Another study in low-salinity water did not improve growth of *L. vannamei* with supplementation of 150 and 300mg/kg in their feed (Roy et al., 2007).

Japanese seabass *Lateolabrax japonicus* is a carnivorous species and one of the most commercially valuable aquaculture species in Asia. Japanese seabass is recognized as a potential candidate for worldwide aquaculture because of its good taste, rapid growth and adaptability to a wide range of environmental factors such as salinity and temperature. Based on previous Mg studies on other fish, it might be necessary to supplement Mg in the diet of seabass (Zhao et al. in press), however the study on Mg requirement was only conducted on juvenile seabass. To our knowledge, no information is available on dietary magnesium requirements in adult seabass. The present investigation was undertaken to investigate: (i) the effects of dietary magnesium supplementation on the growth performance, morphometry, and tissue Mg, Ca bioaccumulation in adult seabass and (ii) whether there are adverse impacts on adult seabass fed high Mg supplemented diets. Growth, survival rate, feed efficiency, superoxide dismutase (SOD) activities, and malondialdehyde (MDA) content were measured in this study to evaluate the effect of dietary magnesium on adult seabass.

## Materials and Methods

**Experimental diets.** The composition of the basal diet is shown Table 1. Casein (Huaan Biological Products Co., Ltd, Linxia, China), gelatin (Yixin Biological Technology Co., Ltd., Shandong, China) and fish meal were used as the main protein source. Fish oil (Haiyang Industrial Company Ltd, Rongcheng, China) and soybean oil (Luhua Company, Shandong, China) were used as lipid source. Six experimental diets were formulated to contain graded levels of Mg by supplementing the basal diet with 0, 200, 400, 800, 1000 and 1500 mg/kg Mg in the form of Mg sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) (Sinopharm Chemical Reagent Co., Ltd, SCR, Shanghai, China). The corresponding levels of dietary Mg, analyzed by the inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; VISTA-MPX, VARIAN), were 184, 278, 487, 929, 1299 and 1737 mg/kg Mg. Ingredients were ground into fine powder through 200  $\mu\text{m}$  mesh. All ingredients were thoroughly mixed with the oils, and water was added to produce a stiff dough. The dough was then pelleted with an experimental feed mill and dried for 12 h in a ventilated oven

at 45 °C. After drying, the diets were broken up and sieved into 5.0×6.0 mm pellets, and stored at –20 °C until use.

Table 1. Formulation and proximate composition of the experimental diets (% dry matter)

Ingredients	Diet-1	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6
Casein	40	40	40	40	40	40
Gelatin	10	10	10	10	10	10
Fish meal	10	10	10	10	10	10
Fish oil	6	6	6	6	6	6
Soybean oil	2	2	2	2	2	2
Corn starch	20	20	20	20	20	20
Dextrin	5.85	5.85	5.85	5.85	5.85	5.85
Soybean lecithin	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix <sup>1</sup>	1	1	1	1	1	1
Mineral premix, magnesium-free <sup>2</sup>	1	1	1	1	1	1
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
Ascorbic acid	0.5	0.5	0.5	0.5	0.5	0.5
Attractant	0.5	0.5	0.5	0.5	0.5	0.5
Mold inhibitor	0.1	0.1	0.1	0.1	0.1	0.1
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
Monocalcium phosphate	1	1	1	1	1	1
Proximate chemical composition						
Crude protein	45.21	45.44	45.94	45.48	45.74	45.32
Crude lipid	8.67	8.79	8.68	9.01	8.75	8.64
Analysed Mg content (mg/kg)	184	278	486	929	1299	1737

<sup>1</sup> Vitamin premix (mg/kg diet): retinol acetate, 32; cholecalciferol, 5; menadione sodium bisulfite, 5.1;  $\alpha$ -tocopherol, 120; thiamin-HCl, 25; riboflavin, 36.7; pyridoxine-HCl, 20; vitamin B<sub>12</sub>, 0.1; D-pantothenic acid calcium, 60; niacin acid, 200; folic acid, 20; biotin, 1.2; inositol, 792; ascorbic acid, 2000; choline chloride, 4000; cellulose, 6683.

<sup>2</sup> Mineral premix, magnesium-free (mg/ kg diet): FeSO<sub>4</sub>·7H<sub>2</sub>O, 119; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 76; MnSO<sub>4</sub>·7H<sub>2</sub>O, 44; CoCl<sub>2</sub>·6H<sub>2</sub>O, 2; Na<sub>2</sub>SeO<sub>3</sub>, 0.45; Ca (IO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, 2.35; cellulose, 8039.

**Experimental procedures.** Japanese seabass (*Lateolabrax japonicus*) were obtained from a commercial farm in Ningbo, China. Prior to the start of the experiment, the adult seabass were acclimated in floating sea cages (3.0×3.0×3.0 m), and fed the control diet for 2 weeks.

At the initiation of the experiment, the fish were fasted for 24 h and weighed after anesthetization with eugenol (1:10,000) (Shanghai Reagent, China). Fish of similar size (204.5±1.0g) were randomly distributed into 18 sea cages (1.5×1.5×2.0 m). Each cage was stocked with 18 fish and diets were randomly assigned to triplicate cages. Fish were hand-fed to apparent satiation twice daily (05:30 and 17:00). The feeding trial lasted for 10 weeks. During the experimental period, the temperature ranged from 25 to 31 °C, salinity from 28‰ to 33‰ and dissolved oxygen was approximately 7 mg/L. The Mg concentration in seawater was 985 mg/L.

**Sample collection and chemical analysis.** At the termination of the experiment, the fish were fasted for 24 h before harvest. Total number and body weight of fish in each cage were measured. After that, three fish per cage were randomly selected for determination of whole-body composition, Mg, and Ca, concentration. Contents of the moisture, crude protein, crude lipid, and ash, in the whole-body were analyzed by standard procedures (AOAC, 1995). Another 4 fish per cage were randomly selected to collect serum samples for analysis of superoxide dismutase activity (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA). Then the hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor (CF) of the sampled fish was calculated. The scales of the four fish were then removed and pooled. Fish carcasses were cooked in a microwave oven for 6 min, and the surrounding tissues were removed from the vertebrae. Scales and vertebrae were rinsed with deionized water, oven dried, and ground for mineral analysis. Another 3 fish per cage were randomly chosen to analyze the SOD activity, GPx activity, and MDA content in liver. The SOD activity, GPx activity, and MDA content were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Scales and vertebrae were rinsed with deionized water, oven dried and ground

for mineral analysis. Approximately 0.10-0.15 g dried and finely ground samples were digested in 15 mL 65–68% nitric acid and 2 mL 72% perchloric acid using Kjeldahl flasks. After digestion, Mg and Ca concentrations in the whole body, vertebra and scale were determined by the inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; VISTA-MPX, VARIAN).

*Calculations and statistical analysis.* The calculation equations were as follows:

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

Survival (%) =  $100 \times (\text{final number of fish}) / (\text{initial number of fish})$ .

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

Hepatosomatic index (HSI, %) =  $(\text{hepatic weight} / \text{body weight}) \times 100$ .

Viscerosomatic index (VSI, %) =  $(\text{viscera weight} / \text{body weight}) \times 100$ .

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

The results are presented as means  $\pm$  SE of three replicates. Results were analyzed by one-way ANOVA (SPSS 15.0 for Windows; SPSS Inc., Chicago, IL, USA). When statistically significant ( $P < 0.05$ ) differences between treatments were identified, multiple comparisons among means were performed with Duncan's multiple range test ( $P < 0.05$ ). Regression analysis of the measured parameters did not reveal any significant correlation to dietary Mg.

## Results

*Growth and feed utilization.* The growth and feed utilization data are presented in Table 2.

Table 2. Survival, growth and body indices of seabass fed the experimental diets with different levels of magnesium for 10 weeks (means  $\pm$  SE,  $n = 3$ ).

Dietary Mg levels FBW <sup>1</sup> (g) (mg/kg)		WGR <sup>2</sup> (%)	Survival (%)	FE <sup>3</sup> (%)	HSI <sup>4</sup>	VSI <sup>5</sup>	CF <sup>6</sup>
184	383.33 $\pm$ 5.24 <sup>ab</sup>	88.40 $\pm$ 2.57 <sup>ab</sup>	96.30 $\pm$ 1.85	0.88 $\pm$ 0.03 <sup>ab</sup>	1.07 $\pm$ 0.09	9.20 $\pm$ 0.25	1.21 $\pm$ 0.05
278	396.33 $\pm$ 3.67 <sup>a</sup>	94.28 $\pm$ 1.80 <sup>a</sup>	98.15 $\pm$ 1.80	0.97 $\pm$ 0.01 <sup>a</sup>	1.04 $\pm$ 0.05	9.23 $\pm$ 0.32	1.13 $\pm$ 0.05
487	379.67 $\pm$ 4.18 <sup>b</sup>	86.11 $\pm$ 2.05 <sup>b</sup>	88.89 $\pm$ 3.22	0.86 $\pm$ 0.03 <sup>bc</sup>	1.00 $\pm$ 0.06	8.97 $\pm$ 0.03	1.13 $\pm$ 0.06
929	363.67 $\pm$ 4.41 <sup>c</sup>	78.29 $\pm$ 2.16 <sup>c</sup>	96.30 $\pm$ 3.71	0.90 $\pm$ 0.05 <sup>ab</sup>	1.09 $\pm$ 0.02	9.25 $\pm$ 0.17	1.22 $\pm$ 0.02
1299	358.66 $\pm$ 4.74 <sup>c</sup>	75.49 $\pm$ 2.31 <sup>c</sup>	94.44 $\pm$ 5.63	0.88 $\pm$ 0.02 <sup>b</sup>	0.99 $\pm$ 0.02	8.63 $\pm$ 0.69	1.12 $\pm$ 0.05
1737	351.67 $\pm$ 4.67 <sup>c</sup>	72.39 $\pm$ 2.29 <sup>c</sup>	92.59 $\pm$ 4.97	0.77 $\pm$ 0.02 <sup>c</sup>	1.10 $\pm$ 0.06	9.34 $\pm$ 0.53	1.13 $\pm$ 0.05
One-way ANOVA							
F value	14.272	14.272	0.768	5.494	1.001	0.394	1.005
P value	0.000	0.000	0.590	0.007	0.458	0.844	0.456

Values with different superscripts in the same column are significantly different ( $P < 0.05$ ).

<sup>1</sup> FBW: Final body weight.

<sup>2</sup> WGR: Weight gain rate.

<sup>3</sup> FE: Feed efficiency ratio.

<sup>4</sup> HSI: hepatosomatic index.

<sup>5</sup> VSI: viscerosomatic index.

<sup>6</sup> CF: condition factor.

There were significant differences ( $P < 0.05$ ) in weight gain rate (WGR) and final bodyweight (FBW). Fish fed the diet with 278 mg/kg of dietary magnesium showed the significantly highest WGR and FBW, there was no significant difference between 184 mg/kg dietary magnesium and 278 mg/kg of dietary magnesium ( $P > 0.05$ ). WGR and FBW significantly decreased as dietary magnesium increased from 278 to 929 mg/kg ( $P < 0.05$ ), after which there was no further decrease. A significant adverse effect on growth performance was observed in fish fed the diet with 929 mg/kg (Diet 4). Survival was not significantly affected by dietary magnesium levels ( $P > 0.05$ ), they ranged from 88.89% to 98.15%. Feed efficiency (FE) exhibited a decreasing tendency with the increasing Mg supplemental levels.

*Body composition and morphometry index.* Body composition is listed in Table 3. There were no significant differences in contents of crude protein, crude lipid among the all treatments ( $P < 0.05$ ). These contents ranged from 12.73% to 13.56% and 6.82% to 7.48%, respectively. The moisture level from feed containing 487 mg/kg dietary

magnesium was lower than that of 1299 mg/kg and 1737 mg/kg dietary magnesium ( $P<0.05$ ), and not significantly different compared with that of 184, 487 and 929 mg/kg dietary magnesium ( $P>0.05$ ). With feed of 184 mg/kg dietary magnesium, ash level was lower than with 487 mg/kg dietary magnesium ( $P<0.05$ ), and was not significantly different compared with other groups ( $P>0.05$ ).

Table 3. The whole-body compositions of adult seabass fed the experimental diets with different levels of magnesium for 10 weeks (means  $\pm$  SE,  $n = 3$ ).

Dietary Mg levels (mg/kg)	Moisture(%)	Crude protein(%)	Crude lipid(%)	Ash(%)
184 mg/kg	74.88 $\pm$ 0.51 <sup>ab</sup>	13.38 $\pm$ 0.046	7.78 $\pm$ 0.58	3.37 $\pm$ 0.04 <sup>a</sup>
278 mg/kg	74.83 $\pm$ 0.67 <sup>ab</sup>	13.56 $\pm$ 0.037	7.23 $\pm$ 0.86	3.71 $\pm$ 0.08 <sup>b</sup>
487 mg/kg	74.36 $\pm$ 0.56 <sup>b</sup>	13.33 $\pm$ 0.19	7.26 $\pm$ 0.53	3.63 $\pm$ 0.14 <sup>ab</sup>
929 mg/kg	75.04 $\pm$ 0.58 <sup>ab</sup>	13.11 $\pm$ 0.41	6.82 $\pm$ 0.27	3.61 $\pm$ 0.08 <sup>ab</sup>
1299 mg/kg	76.09 $\pm$ 0.45 <sup>a</sup>	12.69 $\pm$ 0.46	7.48 $\pm$ 0.78	3.62 $\pm$ 0.13 <sup>ab</sup>
1737mg/kg	75.62 $\pm$ 0.61 <sup>a</sup>	12.73 $\pm$ 0.18	7.07 $\pm$ 0.49	3.55 $\pm$ 0.06 <sup>ab</sup>
One-way ANOVA				
F value	2.519	1.731	0.289	1.433
P value	0.018	0.202	0.910	0.012

Values with different superscripts in the same column are significantly different ( $P<0.05$ ).

There were no significant differences in the morphometry index among all treatments ( $P>0.05$ ) (Table 2). Hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (CF) ranged from 0.99%-1.10%, 8.63%-9.34%, 1.12-1.22, respectively.

**Magnesium and calcium concentration.** Magnesium and calcium concentration in whole body, scales, and vertebrae, of adult seabass were not significantly affected by dietary Mg supplements (Table 4).

Table 4. Mg and Ca concentration in whole body, scales and vertebrae of adult seabass fed the experimental diets with different levels of magnesium for 10 weeks (means  $\pm$  SE,  $n = 3$ ).

Dietary Mg levels (mg/kg)	Mg (mg/g)			Ca (mg/g)		
	Whole-body	Vertebrae	Scales	Whole-body	Vertebrae	Scales
184mg/kg	1.93 $\pm$ 0.08	2.77 $\pm$ 0.12	1.87 $\pm$ 0.07	56.69 $\pm$ 4.61	126.73 $\pm$ 10.61	123.72 $\pm$ 5.26
278 mg/kg	1.99 $\pm$ 0.13	3.02 $\pm$ 0.06	1.92 $\pm$ 0.04	54.61 $\pm$ 2.81	132.32 $\pm$ 4.59	126.15 $\pm$ 9.03
487 mg/kg	2.30 $\pm$ 0.04	2.84 $\pm$ 0.07	2.02 $\pm$ 0.11	57.18 $\pm$ 1.92	140.17 $\pm$ 8.91	133.82 $\pm$ 4.59
929 mg/kg	2.16 $\pm$ 0.10	2.92 $\pm$ 0.13	1.99 $\pm$ 0.08	57.28 $\pm$ 5.41	136.54 $\pm$ 3.63	130.23 $\pm$ 8.52
1299 mg/kg	2.23 $\pm$ 0.07	3.04 $\pm$ 0.09	2.12 $\pm$ 0.13	58.81 $\pm$ 3.12	133.98 $\pm$ 7.81	129.40 $\pm$ 2.96
1737mg/kg	2.14 $\pm$ 0.12	3.07 $\pm$ 0.11	2.07 $\pm$ 0.06	55.11 $\pm$ 2.37	143.81 $\pm$ 6.28	131.91 $\pm$ 5.62
One-way ANOVA						
F value	0.027	0.439	0.438	0.712	2.184	1.583
P value	0.175	0.681	0.158	0.263	0.327	0.634

Values with different superscripts in the same column are significantly different ( $P<0.05$ ).

**Serum and liver enzymatic activities.** SOD activity in the liver was not significantly affected by dietary magnesium levels ( $P>0.05$ ). The SOD activity in serum increased with increasing Mg supplemental levels. MDA content (Table 5) in liver from Diet 2 (278 mg/kg dietary magnesium) was lower ( $P<0.05$ ) than that with Diet 6 (1737 mg/kg dietary magnesium), was not significantly different compared with other diets ( $P>0.05$ ). Serum MDA content exhibited significant decrease ( $P<0.05$ ) from the dietary magnesium concentration of 184 mg/kg (Diet 1) to 278 mg/kg (Diet 2) and then a significant increase ( $P<0.05$ ) with the increasing dietary magnesium concentration (Diet 2–Diet 6). GPx activities in serum and liver exhibited an increasing tendency from the dietary magnesium concentration of 184 mg/kg (Diet 1) to 278 mg/kg (Diet 2) and then a decrease with the increasing dietary magnesium concentration (Diet 2–Diet 6)(Table 5).



Table 5. The superoxide dismutase (SOD) activities, malondialdehyde (MDA) contents and glutathione peroxidase (GPx) in serum and liver of adult seabass fed the experimental diets with different levels of magnesium for 10 weeks (means  $\pm$  SE, n = 3).

Dietary Mg levels (mg/kg)	SOD activity		MDA content		GPx activity	
	Serum( U/mL)	Liver(U/mg prot)	Serum (nmol/mL)	Liver (nmol/mg prot)	Serum( U/mL)	Liver (U/mg prot)
184mg/kg	44.70 $\pm$ 2.97 <sup>ab</sup>	69.26 $\pm$ 2.58	31.58 $\pm$ 5.37 <sup>c</sup>	0.59 $\pm$ 0.05 <sup>ab</sup>	507.71 $\pm$ 11.30 <sup>ab</sup>	19.13 $\pm$ 3.87 <sup>b</sup>
278 mg/kg	38.55 $\pm$ 5.53 <sup>b</sup>	68.26 $\pm$ 4.10	15.72 $\pm$ 3.54 <sup>d</sup>	0.53 $\pm$ 0.06 <sup>b</sup>	529.82 $\pm$ 4.71 <sup>a</sup>	29.47 $\pm$ 1.72 <sup>a</sup>
487 mg/kg	48.93 $\pm$ 4.15 <sup>ab</sup>	72.71 $\pm$ 3.45	35.85 $\pm$ 4.5 <sup>bc</sup>	0.65 $\pm$ 0.07 <sup>ab</sup>	506.90 $\pm$ 6.72 <sup>ab</sup>	19.72 $\pm$ 3.84 <sup>b</sup>
929 mg/kg	42.90 $\pm$ 5.39 <sup>ab</sup>	67.67 $\pm$ 2.22	42.7 $\pm$ 6.18 <sup>bc</sup>	0.70 $\pm$ 0.11 <sup>ab</sup>	472.81 $\pm$ 15.32 <sup>b</sup>	18.05 $\pm$ 0.75 <sup>b</sup>
1299 mg/kg	44.28 $\pm$ 2.14 <sup>ab</sup>	66.73 $\pm$ 3.77	50.77 $\pm$ 3.18 <sup>b</sup>	0.79 $\pm$ 0.06 <sup>ab</sup>	482.62 $\pm$ 7.90 <sup>b</sup>	20.09 $\pm$ 1.02 <sup>b</sup>
1737mg/kg	53.82 $\pm$ 4.57 <sup>a</sup>	75.21 $\pm$ 4.47	65.8 $\pm$ 4.68 <sup>a</sup>	0.82 $\pm$ 0.07 <sup>a</sup>	479.53 $\pm$ 16.61 <sup>b</sup>	16.34 $\pm$ 1.54 <sup>b</sup>
One-way ANOVA						
F value	1.493	0.872	13.331	1.919	3.728	3.484
P value	0.026	0.028	0.000	0.016	0.029	0.036

Values with different superscripts in the same column are significantly different ( $P < 0.05$ ).

### Discussion

Adult seabass tolerated the semi-purified diet very well and fish growth and body weight increased from 204.5 g to an average weight of 372.2 g during the 10-week experimental period. The basal diet containing 184 mg/kg Mg was not significantly better, than most of the other diets in terms of growth, feed efficiency, survival, whole body, scales, and vertebrae Mg and Ca content. This indicates that Mg content of the basal diet (184 mg/kg) satisfies the Mg requirement of seabass for maintaining growth and the scales and vertebrae Mg concentration. For juvenile seabass, dietary Mg supplement had no significant effect on growth, feed efficiency, and Mg concentration in muscle, serum and vertebrae (Zhao et al. in press). When Mg supplement levels increased there was a significant negative effect on growth performance in fish (Table 2). This suggests that excess Mg may cause a toxic effect on seabass growth. Similar reactions were seen when tilapia were fed diets with excess Mg (Dabrowska et al., 1989).

In this study, Mg concentration of the seawater was 985 mg/L. This undoubtedly led to relatively higher absorption of Mg from the water. The Mg requirement of rainbow trout fed a diet containing 78 mg/kg Mg could be satisfied with 46 mg/L Mg in the water (Shearer and Asgard 1992). For red sea bream, the dietary Mg supplement to the basal diet containing 120 mg/kg Mg had no significant effect on growth, feed efficiency, whole blood and vertebrae Mg concentrations (Sakamoto and Yone, 1979). For grouper (*Epinephelus coioides*) the dietary Mg supplement to the basal diet containing 242 mg/kg Mg had no significant effect on growth, feed efficiency, and Mg concentration in scales and vertebrae of grouper. This indicates that the Mg requirement of grouper was met in fish fed the basal diet. For Atlantic salmon (*Salmo salar* L.) reared in seawater-treated fresh water, which contained 54 mg/L Mg, Mg supplement to the basal diet (196 mg/kg Mg) did not significantly affect the growth and feed efficiency; however, a minimum Mg supplement level of 100 mg/kg dry diet (in total, 326 mg/kg) was needed to maintain Mg concentration in the whole body and serum and for proper bone mineralization (El-Mowafi and Maage, 1998).

As Mg concentration is high in seawater and ingredients commonly used in fish feeds are a rich source of Mg, it may not be necessary to supplement Mg in the diet of seabass. Hepatopancreas magnesium levels decreased in *L.vannamei* in response to the withdrawal of magnesium from a semi-purified diet, however, weight gain and magnesium levels of carapace were unaffected in sea water (Davis et al. 1992). Magnesium requirements of *L. vannamei* cultured in low-salinity (2‰) water were estimated to be 0.26-0.35% of diet based on weight gain (Cheng et al., 2005). Dietary magnesium supplementation did not improve the growth performance, including feed intake, weight gain and feed conversion efficiency of juvenile gibel carp, where the water-borne Mg concentration was from 10.6 to 12.7 mg/L (Han et al. 2012). Dietary Mg content of 500 mg/kg was required for tilapia (*Oreochromis aureus*) reared in freshwater

with the Mg level of 0.1 mg/kg (Reigh et al. 1991). However a waterborne Mg concentration of about 4.8 mg/L was sufficient for tilapia (*Oreochromis mossambicus*) fed a low magnesium diet (Van der Velden et al., 1991).

Our data showed that extra Mg supplementation to the basal diet did not affect Mg and Ca concentrations in whole body, scales, and vertebrae. Similar responses have also been reported for Mg, and Ca concentrations of scales and vertebrae of grouper *Epinephelus coioides* (Ye et al., 2010). Mg concentrations in whole body, vertebrae, scales, muscle, and plasma exhibited similar and consistent responses to dietary levels of Mg i.e. Mg content increased significantly as dietary levels of Mg increased until a plateau was reached for grass carp (Wang et al., 2011).

Dietary Mg content had no significant effect on CF, VSI, HSI in this study. There have been differing reports of the effect of dietary minerals on morphometry. Zinc deficiency in rainbow trout caused short body dwarfism (Satoh et al., 1983). CF, VSI and mesenteric fat index (MFI) were statistically significant between treatments with different dietary Mg levels in grouper (Ye et al. 2010). Dietary Mg supplement to the basal diet containing 120 mg/kg Mg had no significant effect on CF and HSI of red sea bream (Sakamoto and Yone, 1979). Dietary Mg content had no significant effect on CF, VSI, HIS of grass carp (Liang et al., 2011).

To protect against free radicals, organisms have developed a variety of antioxidant defenses against free radicals. These include metal sequestering proteins, utilizing compounds such as vitamin C and E, glutathione, and specialized antioxidant enzymes such as GPx, SOD. MDA is the final product of lipid peroxidation, and demonstrates membrane injury caused by free radicals (Traystman et al., 1991). In this study, fish fed the diet with 278 mg/kg Mg showed higher GPx activity than fish fed the other diets. In fact it decreased as the dietary Mg level increased. MDA level from Diet 2 (278 mg/kg dietary magnesium) was lower than in fish fed the other diets, and it increased as dietary Mg levels increased. The antioxidant capacity of seabass fed a diet supplemented with 278 mg/kg Mg was greater. A possible explanation for the effectiveness of using Mg against peroxidation is that Mg can eliminate free radicals by increasing antioxidant activities such as SOD, GPx (Shivakumar and Kumar, 1997). Hepatopancreas MDA content in gibel carp fed a diet with 220 mg/kg Mg was higher than in fish fed diets with 1600 or 3200 mg Mg/kg (Han et al. 2012). In grass carp the serum MDA content was higher in fish fed diets supplemented with 0 and 150 mg/kg Mg than that in fish fed diets with  $\geq 300$  mg/kg Mg (Wang et al. 2011). In our study SOD activity in the liver was not significantly affected by dietary magnesium levels, this was not consistent with the previous two studies mentioned above. The SOD activity in serum increased with increasing Mg supplemental levels. These results are difficult to explain.

In conclusion, dietary magnesium supplementation did not improve the growth performance, weight gain, and feed efficiency of adult seabass. The survival, hepatosomatic index, viscerosomatic index, condition factor, Mg, and Ca concentration in whole-body, vertebrae, and scales were not affected by dietary Mg supplementation ( $P < 0.05$ ). A dietary Mg level higher than about 930 mg/kg could lead to reduced growth performance in adult seabass.

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