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Dietary manganese requirement for juvenile cobia, *Rachycentron canadum* L.

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Abstract

A 10-week feeding trial was conducted to estimate the optimum dietary manganese requirement for juvenile cobia, *Rachycentron canadum* L. The basal diet was formulated to contain 501 g kg^{-1} crude protein from vitamin-free casein, gelatin and fish protein concentrate. Manganese sulphate was added to the basal diet at 0 (control group), 6, 12, 18, 24 and 36 mg Mn kg^{-1} diet providing 5.98, 7.23, 16.05, 23.87, 28.87 and 41.29 mg Mn kg^{-1} diet, respectively. Each diet was randomly fed to three replicate groups of cobia for 10 weeks, and each tank was stocked with 30 fish (initial weight, 6.27 ± 0.03 g). The manganese concentration in rearing water was monitored during the feeding period and was < 0.01 mg L^{-1}. Dietary manganese level significantly influenced survival ratio (SR), specific growth ratio (SGR), feed efficiency ratio (FER) and the manganese concentrations in the whole body, vertebra and liver of cobia. When the dietary manganese level rose from 5.98 mg kg^{-1} to 23.87 mg kg^{-1}, the superoxide dismutase (SOD; EC 1.15.1.1) activities in liver also increased ($P < 0.05$). But there was no significant change in SOD activities for the groups fed with diets containing manganese level higher than 23.87 mg kg^{-1}. On the basis of broken-line regression of SGR, manganese concentration in whole body and vertebra the manganese requirements of juvenile cobia were 21.72 mg kg^{-1}, 22.38 mg kg^{-1} and 24.93 mg kg^{-1} diet in the form of manganese sulphate, respectively.

KEY WORDS: fish nutrition, growth, manganese, *Rachycentron canadum* L., requirement

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Introduction

Cobia is a carnivorous pelagic fish that can grow from fingerling to 4–6 kg in 1 year in offshore net cage systems (Liao *et al.* 2004). The white flesh of this fish, which is suitable for sashimi consumption, is highly prized (Chou *et al.* 2004). Excellent flesh quality, rapid growth and adaptability to culture conditions confer highly desirable characteristics for global commercial aquaculture on cobia (Holt *et al.* 2007). Following successful aquaculture development in Taiwan of China (Liao *et al.* 2004), cobia is extensively farmed in cages in China, Vietnam and Philippines. Recently, its production has been initiated in EU, Brazil, etc. Among the technical limitations in global cobia farming, development of sustainable, high-quality feeds for cobia is one of the major objectives identified by International Initiative of Sustainable and Biosecure Aquafarming, established in 2005 to accelerate commercial viability of cobia culture through international collaborations (Holt *et al.* 2007). The nutritional value of several plant protein sources has been evaluated for potential use in cobia formulated feeds (Chou *et al.* 2004; Lunger *et al.* 2006). Dietary requirements for some nutriments including crude protein (Chou *et al.* 2001; Craig *et al.* 2006), lipid (Wang *et al.* 2005), methionine (Zhou *et al.* 2006), lysine (Zhou *et al.* 2007) and choline (Mai *et al.* 2009) have been reported. However, the dietary requirements for minerals have only been reported for a few elements, such as zinc, iron, copper (Qiao 2007) and selenium (Liu *et al.* 2010).

Manganese is known to be an essential trace element for growth, reproduction and prevention of skeletal abnormalities in terrestrial animals and fish. It serves as a cofactor for many enzymes (e.g. glycosyl transferases) and as an integral constituent of certain metalloenzymes such as arginase, pyruvate carboxylase and Mn superoxide dismutase. Mn deficiency has been shown to produce impaired growth, skeletal abnormalities and reduced reproduction together with

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defects in lipid and carbohydrate metabolism in mammals and chicken (Lall 2002; Leach 1976).

The requirement of Mn has been quantified in some fish species, such as common carp, *Cyprinus carpio* L. and rainbow trout, *Salmo gairdneri* (Ogino & Yang 1980), channel catfish, *Ictalurus punctatus* fingerling (Gatlin & Wilson 1984), Atlantic salmon, *Salmo salar* (Maage *et al*. 2000), juvenile gible carp, *Carassius auratus gibelio* (Pan *et al*. 2008), juvenile tilapia, *Oreochromis niloticus* × *O. aureus* (Lin *et al*. 2008), grass carp, *Ctenopharyngodon idella* fingerling (Wang & Zhao 1994) and grouper, *Epinephelus malabaricus* (Ye *et al*. 2008) at levels of 12–13, 12–13, 2.4, 7.5, 13.77, 7, 15 and 19 mg kg^{-1}diet, respectively. The optimum dietary Mn requirement for juvenile cobia, however, has not been reported. Therefore, this study was designed to determine the requirement of dietary Mn in the form of manganese sulphate for juvenile cobia, to determine the optimal Mn levels in commercial diets.

Materials and methods

Experimental diets

The basal diet, using casein, gelatin and fish protein concentrate as protein sources, fish oil and lecithin as lipid sources, was formulated to contain 501 g kg^{-1} crude protein and 112 g kg^{-1} crude lipid (Table 1), which satisfied the protein and lipid requirements of this fish (Chou *et al*. 2001; Craig *et al*. 2006). The experimental diets were formulated with a manganese-free mineral premix. Graded levels (0.0, 6.0, 12.0, 18.0, 24.0 and 36.0 mg manganese (Mn) kg^{-1} diet) of manganese sulphate (purity 990 g kg^{-1}; Shanghai Reagent Corp., Shanghai, China) were supplemented to the basal diet. The actual levels of dietary manganese in experimental diet, analysed by ICP atomic emission spectrophotometry (Vista-mpx, Varian, USA), were 5.98, 7.23, 16.05, 23.87, 28.87 and 41.29 mg Mn kg^{-1}, respectively (Table 1). Ingredients were ground to a fine powder and sieved through a 246-μm screen. All ingredients were thoroughly mixed with menhaden fish oil, and deionized water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill and dried for 24 h in a ventilated oven at 38 °C. The diets were then broken up and sieved into proper pellet size (2.5 × 5.0 mm) and stored at −20 °C until used.

Experimental procedure

The experimental fish were obtained from a commercial farm in Sanya, Hainan, China. The cobia was reared in flow-through plastic tanks. Initially, all fish were daily fed the basal diet to apparent satiation twice (08:00 and 17:00 h). After 21 days, all fish readily accepted the starter diets, and then were converted to the experimental diets. Water flow rate was maintained at approximately 2 L min^{-1} to maintain optimal water quality throughout the study. Before commencing the feeding trial, fish were fasted for 24 h, and then weighed after being anesthetized with eugenol (1 : 10 000) (Shanghai Reagent Corp., Shanghai, China). Fish of similar size (6.27 ± 0.03 g) were distributed into 18 tanks at a density of 30 fish per tank. Each experimental diet was randomly assigned to three tanks. Fish were hand-fed to apparent satiation twice (08:00 and 17:00 h) daily for 10 weeks. The remaining feed and faeces were removed by siphoning immediately after feeding. During the trial period, water temperature ranged from 27.5 to 31 °C and the salinity of seawater ranged from 24 to 26 g L^{-1}. Air stone in each tank maintained dissolved oxygen concentration was 7 mg L^{-1} or more. Fish were reared under natural light. The manganese concentration in

Table 1 Formulation and proximate composition of the basal diet [1] (g kg^{-1} dry matter)

Ingredients	g kg^{-1}
Casein	420
Gelatin	50
Fish oil	115
Fish protein concentrate	40
Dextrin	250
Lecithin	25
Vitamin mix[2]	15
Mn-free mineral mix[3]	40
Choline chloride	2
Ascorbic acid	3
Ethoxyquin	0.5
Attractant[4]	20
Microcrystalline cellulose (MCC)	19.5
Proximate analysis (g kg^{-1}, on a dry weight basis)	
Crude protein	501
Crude lipid	132

[1] $MnSO_4.H_2O$ was added to the basal diet at 0, 6, 12, 18, 24 and 36 mg Mn kg^{-1} diet.
[2] Vitamin premix, 15 [(mg kg^{-1} diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine–HCl, 20 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; folic acid, 20 mg; niacin acid, 200 mg; biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol, 120 mg; ethoxyquin, 150 mg; microcrystalline cellulose, 135 117 mg].
[3] Mineral premix, 40 [(mg kg^{-1} diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2{\cdot}6H_2O$ (1%), 50 mg; $CuSO_4{\cdot}5H_2O$, 10 mg; $FeSO_4{\cdot}H_2O$, 80 mg; $ZnSO_4{\cdot}H_2O$, 50 mg; Na_2SeO_3(1%),100 mg; $MgSO_4{\cdot}7H_2O$, 1,200 mg; $Ca(H_2PO_4)_2{\cdot}H_2O$, 8000 mg; NaCl, 100 mg; microcrystalline cellulose, 30.4 g].
[4] Attractant, taurine, 2 g;betain–HCl, 9 g; glycine, 9 g.

rearing water was monitored during the feeding period and was < 0.01 mg L^{-1}. Cobia finished their ration within 1–2 min after feeding, thus manganese sulphate leached off into water was very low and negligible.

Analysis and measurements

At the termination of the feeding trial, five fish randomly selected from the sampled fish of each tank were used to remove liver and vertebra. Livers were obtained by surgically. After heating the fish in a microwave oven for 50 s, vertebrae were easily removed from fish, then lightly scrubbed, and finally washed with distilled water to remove flesh. The vertebrae were dried for 2 h at 100 °C, ether extracted in a Soxhlet apparatus for 12 h (AOAC 1995) to remove lipid, and dried again (Mai *et al*. 2006). The vertebra and a part of liver samples were used for manganese analysis by ICP atomic emission spectrometer (Vista-mpx, Varian, USA), and the rest part of liver samples was used for the superoxide dismutase (SOD) activities analysis by the method of Knox *et al*. (1981). The rest of the trial samples were pooled for individual proximate composition analysis. Fish body and diet composition were performed by standard methods (AOAC 1995). Dry matter was determined by drying at 105 °C for 24 h, crude protein by the Kjeldahl method, crude fat after extraction with ether by the Soxhlet method and ash by combustion at 550 °C.

Calculations and statistical analysis

The following variables were calculated:

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

Feed efficiency ratio (FER) = wet weight gain g/dry feed fed g (Hardy & Barrows 2002).

$$\text{Specific growth ratio (SGR, \%)} = [(\text{Ln final weight - Ln initial weight}) / \text{Rearing period (days)}] \times 100$$

All data from the feeding trial were subjected to Levene's test of equality of error variances and one-way ANOVA followed by Tukey's test using SPSS® (SPSS, Inc, Chicago, IL, USA). All treatment effects were considered significant at a P value of 0.05 or less. Response indices that were significantly influenced by dietary manganese level also were subjected to linear regression analysis against dietary manganese. Broken-line regression analysis was performed on SGR, manganese concentrations in whole body and vertebra to establish the dietary requirement for manganese (Robbins *et al*. 1979). The equation used in the model is

$$Y = L + U(R - XLR)$$

where Y is the parameter (SGR, manganese concentration in liver or vertebra) chosen to estimate the requirement, L is the ordinate and R is the abscissa of the breakpoint. R is taken as the estimated requirement. X_{LR} means $X < R$, and U is the slope of the line for X_{LR}. By definition $R - X_{LR} = 0$ when $X > R$.

Results

Growth performance

In this study, both SGR and FER showed a significantly positive correlation with dietary manganese levels below 23.87 mg kg^{-1} and reached a plateau when dietary manganese levels were higher than 23.87 mg kg^{-1} (Table 2). The minimum dietary requirement for manganese is estimated to be 21.72 mg kg^{-1} by broken-line regression analysis on the basis of SGR for juvenile cobia under the experimental conditions (Fig. 1). Fish fed the basal diet had significantly lower SGR compared to those fed the other experimental diets ($P < 0.05$). After the 10-week feeding trial, survival ratio of cobia fed the basal diet averaged 79.97%, which was lower than those fed the manganese-supplemented diets ($P < 0.05$). Similarly, FER of cobia fed the basal diet were significantly lower than those fed diets containing added manganese ($P < 0.05$).

Whole-body composition

The carcass crude protein content of cobia increased (from 160.7 g kg^{-1} to 171.2 g kg^{-1}) with an increase in dietary manganese level from 5.98 to 28.87 mg kg^{-1}($P < 0.05$) and then slightly decreased for the groups fed diets containing higher levels of manganese. The crude lipid content of cobia also increased (from 46.9 g kg^{-1} to 60.9 g kg^{-1}) with an increase in dietary manganese level from 5.98 to 23.87 mg kg^{-1}($P < 0.05$). The carcass ash content of cobia fed basal diets was higher than other groups (Table 3).

Manganese concentration in liver, vertebra and whole body

The manganese concentration in cobia fresh liver was progressively increased with increasing concentration of dietary manganese within the range of 0.76–3.92 mg kg^{-1}

Table 2 Effects of different dietary manganese level on specific growth ratio (SGR), feed efficiency ratio (FER) and survival ratio (SR) of juvenile cobia[1]

Level Mn in diet (mg kg^{-1})	Initial body weight (g)	Final body weight (g)	SGR (%. d^{-1})	FER	SR (%)
0.0 (5.98)	6.27 ± 0.011	24.63 ± 1.098	1.97 ± 0.061^{a}	0.43 ± 0.009^{a}	79.97 ± 1.934^{a}
6.0 (7.23)	6.24 ± 0.012	30.56 ± 1.212	2.26 ± 0.052^{b}	0.51 ± 0.018^{b}	85.50 ± 1.100^{b}
12.0 (16.05)	6.27 ± 0.032	37.76 ± 1.882	2.57 ± 0.068^{c}	0.57 ± 0.012^{bc}	87.87 ± 1.073^{bc}
18.0 (23.87)	6.29 ± 0.023	47.67 ± 1.499	2.89 ± 0.052^{d}	0.63 ± 0.009^{d}	91.10 ± 1.100^{c}
24.0 (28.87)	6.23 ± 0.009	46.11 ± 1.808	2.86 ± 0.056^{d}	0.64 ± 0.015^{d}	90.33 ± 0.333^{bc}
36.0 (41.29)	6.28 ± 0.020	45.99 ± 1.625	2.85 ± 0.055^{d}	0.62 ± 0.012^{cd}	89.67 ± 0.333^{bc}
ANOVA					
F value			43.866	40.784	13.779
P value			<0.001	<0.001	<0.001

ANOVA, analysis of variance.
Values in a column that do not have the same superscript are significantly different at $P \leq 0.05$, based on Tukey's test.
[1] Values are means ± SEM of three replicate aquaria.

(Table 4). The manganese concentration of vertebrae and whole body also significantly increased with increasing dietary manganese ($P < 0.05$). On the basis of linear regression of whole-body manganese concentration ($P < 0.05$, $R^2 = 0.896$), a minimum dietary requirement for manganese in the form of manganese sulphate was estimated to be 22.38 mg kg^{-1} diet (Fig. 2). Similarly, manganese concentration in cobia vertebra reached a plateau when dietary manganese was higher than 24.93 mg kg^{-1} diet (Fig. 3).

Enzyme activities in liver

A significant increase in total SOD activity has been observed in the liver of cobia fed diets containing with 5.98, 7.23 and 16.05 mg Mn kg^{-1}, and the total SOD activity reached a plateau in fish fed diets with 23.87, 28.87 and 41.29 mg Mn kg^{-1}. Cu–Zn SOD and Mn SOD activities in cobia liver showed the same trend and has the lowest enzyme activity in fish fed diet with 5.98 mg Mn kg^{-1} (Table 5).

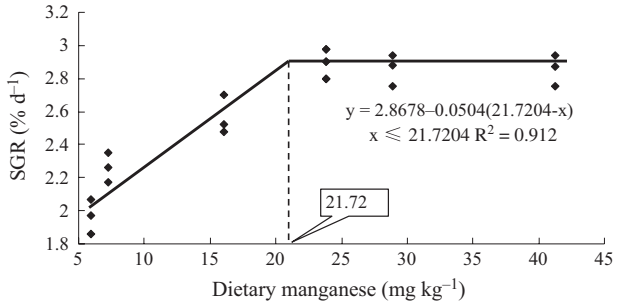

Figure 1 Relationship between dietary manganese level and SGR of cobia fed the six diets for10 weeks. Each point represents the mean of three groups of fish within a treatment with 6 fish per group. Requirements derived with the broken-line method for specific growth rate is 21.72 mg kg^{-1}diet.

Discussion

Fish readily accepted the experimental diet from the beginning of the experiment and maintained normal behaviour throughout the experimental period. In this study, the growth response of juvenile cobia was significantly affected by the supplementation of dietary manganese, and a positive relationship was found between the growth and the dietary manganese levels. Weight gain was lower in fish fed the basal diet owing to insufficient manganese. This result is similar to the reports on rainbow trout (Ogino & Yang 1980), grass carp fingerling (Wang & Zhao 1994) and juvenile gible carp (Pan *et al.* 2008). Fish fed insufficient manganese diet had typical manganese-deficient symptoms such as cataracts and skeletal abnormalities (dwarfism) in some studies (Lall 2002). In this study, cobia did not have these typical manganese-deficient symptoms. The manganese-deficient symptom has also not been observed in study of Atlantic salmon (Lorentzen *et al.* 1996), grouper (Ye *et al.* 2008) and tilapia (Lin *et al.* 2008).

In the present study, broken-line analysis was employed to establish the relationship between SGR and dietary manganese. Based on the SGR, the minimum requirement of dietary manganese for the optimal growth of juvenile cobia was 21.72 mg Mn kg^{-1}(Fig. 1). This result is much higher than that reports for channel catfish fingerling (2.4 mg Mn kg^{-1}, Gatlin & Wilson 1984) Atlantic salmon (7.5 mg Mn kg^{-1}, Maage *et al.* 2000), juvenile gible carp (13.77 mg Mn kg^{-1}, Pan *et al.* 2008), juvenile tilapia (7 mg Mn kg^{-1},Lin *et al.* 2008), grass carp fingerling (15 mg Mn kg^{-1}, Wang & Zhao 1994) common carp and

Table 3 Effect of different dietary manganese level on whole-body composition of cobia[1]

Dietary manganese (mg.kg-1)	Whole-body composition (g kg^{-1} fresh weight)			
	Moisture	Crude protein	Crude lipid	Ash
0.0 (5.98)	751.6 ± 3.1^{b}	160.7 ± 0.7^{a}	46.9 ± 1.6^{a}	40.8 ± 1.0^{c}
6.0 (7.23)	746.4 ± 1.6^{ab}	163.6 ± 0.7^{ab}	53.6 ± 0.6^{b}	36.4 ± 0.4^{abc}
12.0 (16.05)	739.1 ± 1.9^{ab}	165.2 ± 1.6^{abc}	56.8 ± 0.4^{bc}	38.9 ± 1.5^{bc}
18.0 (23.87)	734.4 ± 2.5^{a}	170.3 ± 1.7^{bc}	60.9 ± 0. 6^{c}	34.4 ± 0. 2^{a}
24.0 (28.87)	736.3 ± 0. 6^{a}	171.2 ± 1.3^{c}	59.2 ± 1.1^{c}	33.3 ± 0. 4^{a}
36.0 (41.29)	734.3 ± 5.4^{a}	170.7 ± 2.5^{bc}	59.8 ± 1.8^{c}	35.2 ± 1.2^{ab}
ANOVA				
F-value	5.99	8.136	20.54	9.56
P-value	<0.001	<0.001	<0.001	<0.001

ANOVA, analysis of variances.
Values in a column that do not have the same superscript are significantly different at $P \leq 0.05$, based on Tukey's test. ANOVA, analysis of variances.
[1] Values are means ± SEM of three replicate.

Table 4 Manganese concentrations in liver, vertebra and whole body[1] (dry matter basis)

Level Mn in diet (mg kg^{-1})	Liver(fresh) (mg kg^{-1})	Vertebra (mg kg^{-1})	Whole body mg kg^{-1})
0.0 (5.98)	0.76 ± 0.06^{a}	15.62 ± 0.51^{a}	9.81 ± 0.31^{a}
6.0 (7.23)	0.92 ± 0.02^{ab}	20.42 ± 1.59^{b}	11.71 ± 0.34^{b}
12.0(16.05)	1.08 ± 0.02^{b}	26.11 ± 1.04^{c}	13.15 ± 0.28^{b}
18.0 (23.87)	1.31 ± 0.01^{c}	32.00 ± 1.02^{d}	14.82 ± 0.40^{c}
24.0 (28.87)	2.37 ± 0.04^{d}	31.78 ± 0.88^{d}	14.90 ± 0.28^{c}
36.0 (41.29)	3.92 ± 0.06^{e}	34.56 ± 0.58^{d}	15.15 ± 0.31^{c}
ANOVA			
F-value	913.121	54.4898	44.812
P-value	<0.001	<0.001	<0.001

ANOVA, analysis of variances.
Values in a column that do not have the same superscript are significantly different at $P \leq 0.05$, based on Tukey's test. ANOVA, analysis of variances.
[1] Values are means ± SEM of three replicate.

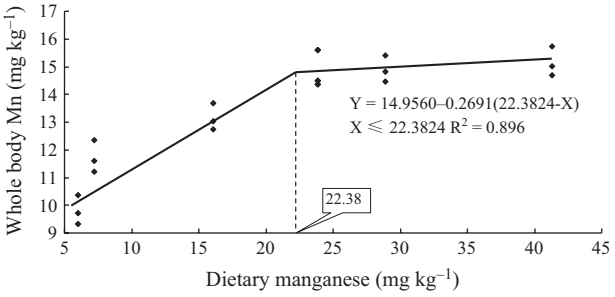

Figure 2 Relationship between dietary manganese level and whole body manganese of cobia fed the six diets for 10 weeks. Each point represents the mean of three groups of fish within a treatment with 6 fish per group. Requirements derived with the broken-line method for specific body manganese concentration is 22.38 mg kg^{-1}diet.

rainbow trout (12–13 mg kg^{-1}, Ogino & Yang 1980), and somewhat higher than the report of grouper (19 mg Mn kg^{-1}, Ye *et al.* 2008). These differences in the estimated manganese requirements of different species are probably real species specific, variations in intestinal manganese absorption rate and feed efficiency (Shearer 1995). Moreover, the disparity probably comes from the methods of data analysis, manganese forms and their availability.

There are also some special reasons for the different results. In the present study, the water temperature varied from 27.5 to 31 °C and in the range of 25 to 32 °C, which is optimal water temperature for cobia (Guo *et al.* 2007). However, this temperature is higher than optimal water temperature of other fish (except tilapia and grouper's), such as rainbow trout, carp, Atlantic salmon and juvenile gible carp. Such high temperature increased the oxidation pressure to cobia, and produced some antioxidant response (Parihar *et al.* 1997). This is probably one possibility why manganese requirement of cobia and grouper is higher than rainbow trout, carp, Atlantic salmon and juvenile gible carp. After 10-week experiment, the final body weight of fish fed the diet with 23.87 mg Mn kg^{-1} was 7.6 times of its initial body weight. In the present study, the SGR of juvenile cobia is higher than that in other fish, such as grouper (Ye *et al.* 2008) and juvenile tilapia (Lin *et al.* 2008). The juvenile cobia grows so fast that it needs more mineral nutrients (Qiao 2007; Liu *et al.* 2010) including manganese.

The requirements of dietary manganese based on the whole-body manganese and vertebrae manganese were 22.38 (Fig. 2) and 24.93 mg Mn kg^{-1}(Fig. 3), respectively.

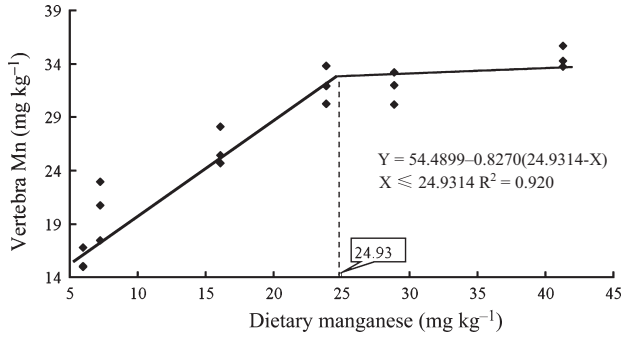

Figure 3 Relationship between dietary manganese level and vertebra manganese of cobia fed the six diets for 10 weeks. Each point represents the mean of three groups of fish within a treatment with 6 fish per group. Requirements derived with the broken-line method for specific vertebra manganese concentration is 24.93 mg kg^{-1}diet.

Table 5 Total superoxide dismutase (T-SOD), Cu–Zn superoxide dismutase (Cu–Zn SOD) and Mn superoxide dismutase (Mn SOD) activities in liver of cobia fed different diets for 10 weeks[1]

Level Mn in diet(mg kg^{-1})	Hepatic total SOD activity (U mg^{-1} protein)	Hepatic Cu–Zn SOD activity (U mg^{-1} protein)	Hepatic Mn SOD activity (U mg^{-1} protein)
0.0 (5.98)	97.83 ± 2.15^{a}	65.58 ± 0.69^{a}	32.25 ± 2.84^{a}
6.0 (7.23)	103.02 ± 2.95^{ab}	65.82 ± 1.23^{a}	37.20 ± 1.72^{ab}
12.0 (16.05)	111.93 ± 2.38^{b}	67.22 ± 1.06^{ab}	44.71 ± 1.93^{bc}
18.0 (23.87)	130.00 ± 1.50^{c}	71.85 ± 0.62^{c}	58.15 ± 1.20^{d}
24.0 (28.87)	128.17 ± 3.01^{c}	70.57 ± 0.45^{bc}	57.60 ± 2.77^{d}
36.0 (41.29)	125.03 ± 2.55^{c}	71.80 ± 0.91^{c}	53.23 ± 3.46^{cd}
ANOVA			
F value	30.70	11.49	19.93
P value	<0.001	<0.001	<0.001

ANOVA, analysis of variances.
Values in a column that do not have the same superscript are significantly different at $P \leq 0.05$, based on Tukey's test. ANOVA, analysis of variances.
[1] Values are means ± SEM of three replicate.

These results were higher than the requirement value (21.72 mg Mn kg^{-1}) based on SGR. This finding suggested that vertebrae, liver and other body tissues have a capacity to buffer changes in manganese supply, and manganese deposition need not be at its maximum for the highest weight gain, as what was found in phosphorus requirement of juvenile large yellow croaker, *Pseudosciaena crocea* R. (Mai *et al.* 2006) and juvenile Japanese seabass, *Lateolabrax japonicus* (Zhang *et al.* 2006).

Hepatic total SOD activity has been found to decrease when dietary manganese was deficient (NRC 1993). Similar results have also been reported in rainbow trout (Knox *et al.* 1981), Atlantic salmon (Maage *et al.* 2000) and juvenile gible carp (Pan *et al.* 2008). And the present study also supports this point. The activities of Cu–Zn SOD and Mn SOD were all suppressed at low dietary manganese level, and this had been confirmed by the findings of Knox *et al.* (1981) who reported that hepatic Cu–Zn SOD activity was suppressed in the Mn-deficient trout because the Cu and Zn concentration in Mn-deficient trout liver was lower. Results of the present study showed that manganese deficiency suppressed cobia growth and reduced SOD activities. This clearly indicated that cobia have a requirement for Mn that cannot be met by Mn in the unsupplemented diet, thus dietary supplementation is necessary. On the basis of broken-line regression of SGR, manganese concentration in whole body and vertebra and the manganese requirements of juvenile cobia were 21.72 mg kg^{-1}, 22.38 mg kg^{-1} and 24.93 mg kg^{-1} diet in the form of manganese sulphate, respectively.

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