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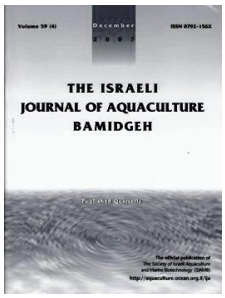
ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

The Israeli Journal of Aquaculture - Bamidgeh, IJA_67.2015.1174, 12 pages



Effects of Supplemented Dietary Curcumin on Growth and Non-Specific Immune Responses in Juvenile Wuchang Bream (*Megalobrama amblycephala*)

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(Received 4.12.14, Accepted 23.1.15)

Key words: *Megalobrama amblycephala*; curcumin; growth; non-specific immune responses

Abstract

The present study was aimed at evaluating the effects of the feed additive curcumin on the growth and non-specific immune responses in juvenile Wuchang bream (*Megalobrama amblycephala*). Six experimental diets were formulated to contain graded curcumin levels (0, 15, 30, 60, 120 and 240 mg/kg of diet, respectively). After 60 days feeding trial, fish fed the 60 mg/kg curcumin diet had significantly higher weight gain rate (WGR) and specific growth rate (SGR), and significantly lower feed conversion ratio (FCR) than the control group. The number of leucocytes (WBC), interleukin-1 beta (IL-1β), tumor necrosis factor-α (TNF-α), alternative pathway of complement (ACH50), and respiratory burst activity increased with increasing dietary curcumin levels up to 60 mg/kg, and thereafter declined. Significantly lower alanine transaminase (ALT) and aspartate transaminase (AST) activities were observed in fish fed the 60 mg/kg curcumin diet compared to those in the control group. Fish fed with the diets containing 60 and 120 mg/kg curcumin had significantly higher TNF-α and ACH50 activities than those in the control, and the fish fed diets supplemented with 60 and 240 mg/kg curcumin had significantly higher IL-1β activities than those in the control. The results suggest that appropriate dietary curcumin supplementation (60 mg/kg curcumin of diet) significantly improved growth and non-specific immune responses in juvenile *M. amblycephala*.

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Introduction

High-density and monoculture production practices have been widely used in aquaculture, leading to rapid development of the aquaculture industry. However, these highly efficient techniques are also responsible for a number of problems such as antibiotic abuse, enhanced feeding, nutritional imbalance, bacterial and viral infections, and accumulation of toxic and hazardous substances (Barton, 2002, Ming et al., 2012).

In aquaculture, antibiotics are commonly used to treat infectious diseases however, inappropriate use of antibiotics may cause antibiotic resistance that can spread in the aquaculture environment and have adverse effects on ecology and public health (Cabello, 2006). The use of antibiotics has become increasingly controlled in the aquaculture industry due to growing concern regarding their effect on the products and on the environment. Alternative environmentally friendly approaches for the prevention and treatment of infectious fish diseases are apparent in Chinese traditional herbal medicine as alternatives to antibiotic feed additives.

Curcumin (*Curcumin*) is a natural yellow acidic phenol extracted from the curcuma plant, genus (*Curcumalonga*), for example turmeric (*Curcuma longa*), curcuma (*Curcuma L*), and acruginous turmeric rhizome, *Rhizoma curcumae Aeruginosae*, (Aggarwal, et al., 2007). Studies have shown that dietary curcumin produces a wide range of pharmacological effects as an antioxidant eliminating free radicals (Toda et al., 1985; Ruby et al., 1995), reduction of inflammation (Gupta S.C. et al., 2011), antibacterial effects, and immunomodulation (Bhuvaneswari & Balasundaram, 2006; Bai et al., 2009; Ganguly et al., 2010). In addition, curcumin improved growth in grass carp (Hu et al., 2003), yellow croaker (Wang & Wu, 2007) and tilapias (Cui et al., 2013), improved activity of digestive enzymes (Hu et al., 2003), and the immune responses of fish (Cui et al., 2013), suggesting that curcumin is a promising alternative to antibiotic feed additives.

Wuchang bream (*Megalobrama amblycephala*) is a major species in Chinese freshwater polyculture systems and annual production of Wuchang bream in China is high (FMBA, 2008; Shen et al., 2010). However, increasing outbreaks of infectious diseases in cultured *M. amblycephalain* have been reported, predominantly in summer, associated with high temperatures (He et al., 2006). The present study aimed to evaluate the effects of dietary curcumin on non-specific immune responses and disease resistance of juvenile *M. amblycephalain*, and to determine the optimal amount of curcumin in feeds.

Materials and Methods

Experimental diets. The formulation and proximate composition of the basal diet are shown in Table 1. The basal diet was supplemented with 0 (the control), 15, 30, 60, 120 and 240 mg/kg curcumin (Henan Zhongda Biological Engineering Co., Henan, China), respectively. To prepare the experimental diets, the ingredients were ground into fine powder through a 60-mesh sieve. Oil and water were added (40%, v/w) into mixed dry ingredients and pelleted into granular feed using a 2.00 mm diameter die in a small feed pellet machine (SLP-45, Fishery Machinery and Instrument Research Institute). The pellets were dried below 25°C for 24 h to reduce moisture to 10%, and then stored at -20°C until use.

Ingredients	*Percentage dry weight*
Fish meal	80.0
Soybean meal	180.0
Rapeseed meal	170.0
Cotton meal	165.0
Rice bran	80.0
Wheat middling	220.0
Soybean oil	40.0
Lecithin	10.0
Choline chloride	5.0
Vitamin premixa[a]	10.0
Mineral premixb[b]	10.0
Powdered zeolite	10.0
Calcium dihydrogen phosphate	20.0
Proximate composition (g/kg)	
Crude protein	312.7
Crude lipid	81.5
Crude ash	110.2
Gross energy (kJ/g)	16.32

a. Vitamin premix (IU or mg/kg premix): Vitamin A, 900000 IU; Vitamin D, 250000 IU; Vitamin C, 5000 mg; Vitamin K3, 220 mg; Vitamin B1, 320 mg; Vitamin B2, 1090 mg; Vitamin B6, 5000 mg; Vitamin B12, 116mg; biotin, 50 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60,000 mg; Inositol, 15000 mg; Niacin acid, 2500 mg.
b. Mineral premix (per kg premix): blue copperas, 2.5 g; green vitriol, 28 g; zinc sulfate heptahydrate, 22 g; Manganese sulfate tetrahydrate, 9 g; sodium selenate, 0.045 g; potassium iodide, 0.026 g; cobalt chloride hexahydrate, 0.1 g.
c Energy, calculated using standard physiological fuel values of 16.7, 16.7 and 37.7 Kj/g for carbohydrate, protein and lipid, respectively.

Fish and animal husbandry. *M. amblycephalain* (initial weight: 3.15 ± 0.022 g) were obtained from the Nanquan fish farm of Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences. Prior to the experiments, fish were fed a commercial diet during the acclimation period. After the acclimation, 540 fish were randomly divided into six groups and distributed into 18 circular tanks (300 L water/tank, 3 tanks/group). Water flow rate in each tank was maintained at approximately 2 L/min. For 60 days fish were hand-fed with the prepared diets three times per day (8:20-9:00, 11:30-12:10, and 16:50-17:30) at a rate of 40-60 g/kg of the fish weight, until apparent satiation. Throughout the experiment, water temperature was controlled at 26 ± 1 °C and the fish were retained under natural photoperiod. pH of water was kept between 7.2-7.8 and ammonia nitrogen concentration was lower than 0.05 mg/L. Dissolved oxygen concentration was higher than 6 mg/L.

Sample collection. At the end of the feeding trial, fish were starved for 24 h to evacuate contents of the alimentary tract. They were then anesthetized with diluted benzocaine (200 mg/L). The total number and weight of fish in each tank was determined. Blood from three fish randomly selected from each tank was collected through caudal venipuncture using 2.5 ml heparinized syringes and was treated in anticoagulation tubes to obtain plasma. 50 μl and 20 μl of blood were used to determine the respiratory burst activity and hemocyte analysis, respectively. The remaining blood was centrifuged at 3000 ×g at 4° C for 10 min to collect plasma that was then stored at -80 °C and used for immunological assays.

Quantification of white blood cell (WBC), red blood cell (RBC), and hemoglobin (HGB). The WBC, RBC, and HGB were measured using an Auto Hematology Analyzer (BC-5300Vet, Mindray, P.R. China) with test kits from Shenzhen Mindray Medical International Co. Ltd. (P.R. China).

Serum biochemical analysis. Serum alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) activities were measured by the IFCC method. The total protein (TP) content was determined by direct assay and the albumin (ALB) level was measured using the Bromcresol green method. All these kits were purchased from Shenzhen Mindray Bio-Medical

Electronics Co., Ltd. Measurements were conducted in a Mindray Auto Bio-chemical Analyzer (BS-400, Mindray, P.R. China).

Respiratory burst activity. The respiratory burst activity of phagocytes was evaluated using nitroblue tetrazolium (NBT) (Shanghai Reagent Corp., China) according to the protocol described by Song et al. (1994) and Ai et al. (2007). The absorbance at 630 nm was measured in a Model Multiskan spectrum (Thermo, USA) using KOH/DMSO as the blank control. Respiratory burst was expressed as NBT reduction in 100 ml of cell suspension.

Enzyme-linked immunosorbent assay (ELISA) for the evaluation of plasma tumor necrosis factor-α (TNF-α) and interleukin-1 beta (IL-1β). The activities of plasma tumor necrosis factor-α (TNF-α) and interleukin-1 beta (IL-1β) were measured by the double antibody sandwich method using the TNF-α and IL-1β ELISA detection kit (IBL, Germany). Optical density for both was measured at 450 nm.

Alternative pathway of complement hemolysis (ACH50) activity. Evaluation of the ACH50 activity was conducted in accordance with the protocol described by Yano et al. (1988) and Matsuyama et al. (1988) with minor modifications. An equal amount of A's solution and ovine blood were mixed and stored at 4℃. The cells were then centrifuged at 400 ×g for 10 minutes, and washed twice with the EGTA-Mg-gelatin veronol buffer. The cells were resuspended in EGTA-Mg-gelatin veronol buffer and adjusted to 2.3 × 109 CFU/ml. The serum (800 µl) was serially diluted with EGTA-Mg-GVB, and ovine red blood cells suspension (400 µl) was added into an EP tube and incubated for 60 min. The control was the complete hemolysis of ovine red blood cells (400 µl ovine red blood cells and 800 µl distilled water), and the blank was a mixture of 800 µl EGTA-Mg-GVB and 400 µl ovine red blood cells. This was described by logarithmic figure (y/(1-y) and x (serum volume). ACH50 activity (U/ml) was determined by the reciprocal of the serum at the 50% hemolysis in the figure. The volume of serum producing 50% hemolysis (ACH50) was determined and the number of ACH50 units/ml was obtained for each group.

Statistical analysis. All data are presented as means ± S.E. (standard error of the mean). Data were transformed logarithmically before being subjected to one-way analysis of variance (ANOVA) using SPSS 13.0. When the overall treatment effects were significantly different, the Duncan multiple range test was conducted to compare the means between different feeding patterns of curcumin treatment. The level of significant difference was set at $P < 0.05$.

Results

Influence of dietary supplement curcumin on the growth performance of M. amblycephala. After 60 days of the feeding trial, fish fed diets supplemented with curcumin exhibited increased weight gain. Fish fed diets supplemented with 60 mg curcumin/kg showed significantly higher (P <0.05) weight gain rate (WGR) and specific growth rate (SGR) than the control (Table 2). During the feeding trial, the FCR of fish fed diets supplemented with curcumin initially decreased and then increased. At the end of the feeding trial, fish fed diets supplemented with 60 mg curcumin/kg diet had significantly lower FCR (P < 0.05) than the control (Table 2). No significant differences (P > 0.05) in VSI, HIS and CF were detected among all groups (Table 2).

Table 2 Growth performance of Wuchang bream (*M. amblycephala*) fed with diets containing different amount of curcumin[1]

Dietary curcumin (mg/kg)	*0*	*15*	*30*	*60*	*120*	*240*
Initial body weight (g)	3.15± 0.01	3.17±0.02	3.14 ± 0.00	3.15 ± 0.01	3.15 ± 0.01	3.14 ± 0.01
Final Body weight (g)	15.79±0.19^{bc}	14.98 ± 0.61^{c}	16.03 ± 0.17^{b}	17.20 ± 0.03^{a}	16.32± 0.09^{b}	14.99 ± 0.13^{c}
WGR[2] (%)	401.17±5.91^{bc}	375.66±19.3	408.97 ± 5.43^{b}	446.00± 1.13^{a}	418.21±2.65^{b}	375.97±4.30^{c}
SGR[3](%/d)	2.70±0.02^{bc}	2.60 ± 0.07^{c}	2.71 ± 0.02^{b}	2.83 ± 0.01^{a}	2.74 ± 0.01^{b}	2.60 ± 0.02^{c}
FCR[4]	2.06±0.04^{a}	1.86 ± 0.06^{ab}	1.86 ± 0.13^{ab}	1.60 ± 0.12^{b}	1.99 ± 0.08^{a}	2.00 ± 0.10^{a}
VSI [5](%)	12.71±0.56	13.47 ± 0.52	12.01 ± 0.51	12.25 ± 0.25	12.08 ± 0.43	13.56 ± 0.56
HST [6](%)	0.97±0.08	1.13 ± 0.07	1.09 ± 0.09	0.93 ± 0.07	1.03 ± 0.09	1.09 ± 0.12
CF [7](%)	2.10 ± 0.05	2.06 ± 0.03	2.10 ± 0.03	2.14 ± 0.08	2.17 ± 0.03	2.11 ± 0.04

[1] Values were presented as means ± S.E. of three replications, values with different superscript letters in the same column were significantly different based on Tukey's test ($P < 0.05$).
[2] Weight gain rate (WGR) = (Final body weight - initial body weight) × 100/initial body weight.
[3] Specific growth rate (SGR) = $(\ln W_t - \ln W_0) \times 100/T$, where W_0 and W_t are the initial and final body weights, and T is the culture period in days.
[4] Feed conversion ratio (FCR) = total diet fed (g)/total wet weight gain (g).
[5] Viscera index (VSI) = (viscera weight (g) × 100)/wet body weight (g).
[6] Hepatosomatic index (HSI) = (liver weight (g) × 100)/wet body weight (g).
[7]Condition factor, (CF) = (wet body weight (g) × 100)/(total body length (cm))^3.

Hematological analyses. No significant differences of RBC and HGB were detected between all groups (Table 3). Fish fed diets supplemented with 60 mg curcumin/kg diet had significantly higher ($P < 0.05$) leucocyte counts (WBC) than the control group and the 240 mg/kg curcumin group (Table.3).

Table 3 Hematological analyses of Wuchang bream (*M. amblycephala*) fed with diets containing different amount of curcumin[1]

Dietary curcumin (mg/kg)	WBC[2] (10^9/L)	RBC[3] (10^{12}/L)	HGB[4] (g/L)
0	181.25 ± 3.35^{bc}	2.05 ± 0.14	84.83 ± 3.92
15	185.69 ± 1.85^{ab}	1.78 ± 0.28	86.17 ± 8.85
30	186.83 ± 2.41^{ab}	2.31 ± 0.29	86.33 ± 4.98
60	190.42 ± 0.47^{a}	1.78 ± 0.12	86.67 ± 5.04
120	184.80 ± 2.04^{ab}	2.01 ± 0.11	85.33 ± 4.93
240	176.11 ± 2.19^{c}	1.94 ± 0.24	85.00 ± 8.33

[1] Values were presented as means ± S.E. of three replications, values with different superscript letters in the same column were significantly different based on Tukey's test ($P < 0.05$).
[2]WBC, white blood cell.
[3]RBC, red blood cell.
[4]RBC, red blood cell

Biochemical indices of blood. Fish fed diets supplemented with 60 mg curcumin/kg diet had the lowest alanine transaminase (ALT) compared to all the other groups (Table.4). It was significantly lower ($P < 0.05$) than the control group and the 15 mg curcumin/kg diet group (Table 4). In addition, curcumin in diets reduced the aspartate transaminase (AST) level, which was relatively lower in all curcumin groups compared with the control. Fish fed diets supplemented with 60 mg curcumin/kg diet had significantly lower AST levels than the control group (Table 4). The lactate dehydrogenase (LDH) level first decreased and then increased with curcumin, but no significant differences in LDH levels were detected among all groups (Table 4). No significant differences ($P > 0.05$) in total protein (TP) and globulin (GLB) content in serum were observed between the treatment groups and the control group, however the albumin (ALB) content in the serum of fish fed diets supplemented with 30 and 60 mg curcumin/kg diet was significantly higher than the control group (Table 4).

Table 4 Biochemical indices of Wuchang bream (*M. amblycephala*) fed with diets containing different amount of curcumin[1]

Dietary curcumin (mg/kg)	0	15	30	60	120	240
ALT2 (IU/ L)	34.31 ± 4.50ab	34.78 ± 3.36a	26.03 ± 1.32abc	21.54 ± 1.43c	25.50 ± 3.49bc	28.60 ± 2.51abc
AST3 (IU L-1)	38.94 ± 2.90ab	35.02 ± 2.03abc	31.43 ± 2.24bc	28.24 ± 3.39c	40.74 ± 3.08a	38.08 ± 2.62ab
LDH4 (IU L-1)	277.65 ± 27.70	230.94 ± 36.67	219.00 ± 23.90	249.22 ± 28.17	318.23 ± 27.88	342.79 ± 26.37
TP5 (g/ L−)	13.63 ± 0.36	15.80 ± 1.07	15.13 ± 0.81	16.19 ± 0.88	15.17 ± 0.49	15.08 ± 0.49
ALB6 (g L−1)	2.75 ± 0.63b	4.43 ± 1.19ab	6.00 ± 0.65a	5.86 ± 0.83a	4.50 ± 0.57ab	4.67 ± 0.75ab
GLB7 (g L-1)	9.60 ± 1.01	10.20 ± 0.77	9.34 ± 0.93	9.24 ± 0.61	10.43 ± 0.64	10.41 ± 0.76

[1]Values were presented as means ± S.E. of three replications, values with different superscript letters in the same column were significantly different based on Tukey's test ($P < 0.05$).
[2]ALT, alanine transaminase.
[3]AST, aspartate transaminase.
[4]LDH, lactate dehydrogenase.
[5]TP, total protein.
[6]ALB, albumin.
[7]GLB, globulin.

Immunological indices. Respiratory burst activity of fish fed diets supplemented with curcumin first increased and then decreased (Fig. 1).

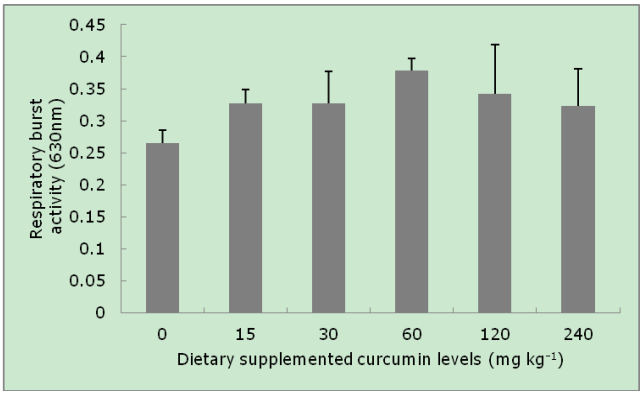

Fig.1 Respiratory burst activity of Wuchang bream (*M. amblycephala*) fed with diets containing different amount of curcumin. Values are means ± S.E. (n = 9).

The Interleukin-1 beta (IL-1β) activity was significantly affected by curcumin supplementation. Fish fed the diet supplemented with 60 mg curcumin/kg had significantly higher IL-1β avidity than the control group (Fig. 2). The tumor necrosis factor-alpha (TNF-α) first increased and then decreased with curcumin supplementation. At the end of feeding trial, all the groups fed curcumin had significantly lower ($P < 0.05$) levels of TNF-α than the control group (Fig. 3). In addition, ACH50 activity also increased at first and then decreased with curcumin feeding. At the end of feeding trial, only the fish fed diets supplemented with 60 and 120 mg curcumin/kg had significantly lower ($P < 0.05$) ACH50 activity than the control group and the other groups (Fig. 4).

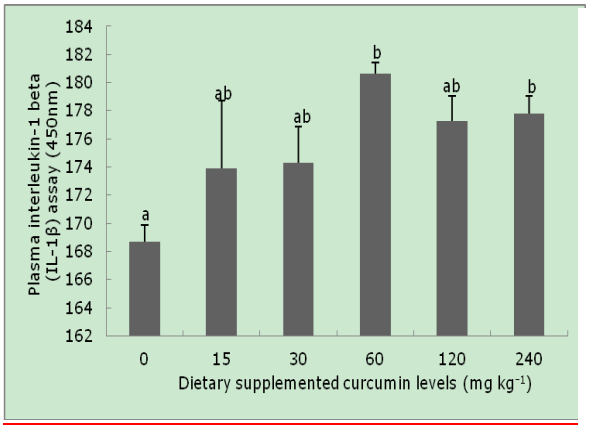

Fig.2 Plasma interleukin-1 beta (IL-1β) assay of Wuchang bream (*M. amblycephala*) fed with diets containing different amount of curcumin. Values are means ± S.E. (n = 9). Bars sharing different letters for each diet are significant different determined by Tukey's test ($P < 0.05$).

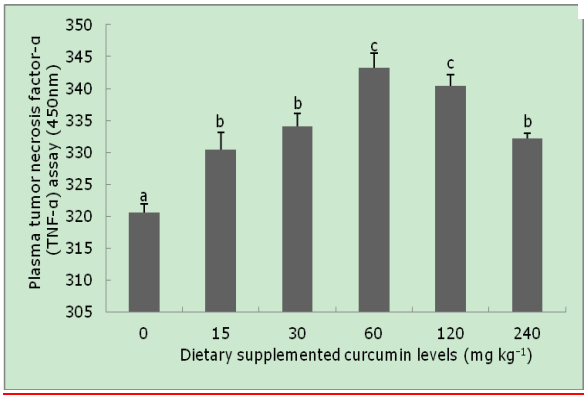

Fig amount of curcumin. Values are means ± S.E. (n = 9). Bars sharing different letters for each diet are significantly different determined by.3 Tumor necrosis factor-α (TNF-α) activity of Wuchang bream (*M. amblycephala*) fed with diets containing different Tukey's test ($P < 0.05$).

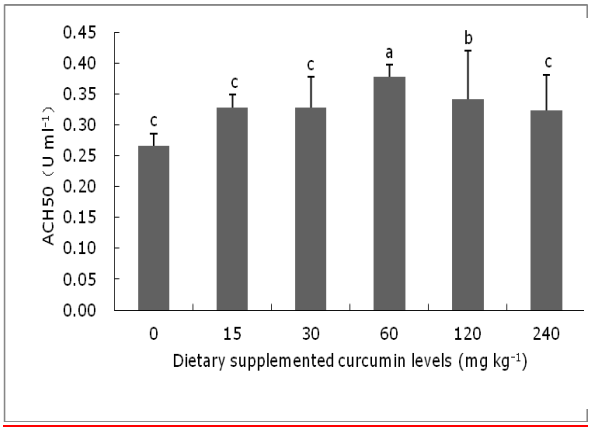

Fig.4 Alternative pathway of complement hemolysis (ACH50) activity of Wuchang bream (*M. amblycephala*) fed with diets containing different amount of curcumin. Values are means ± S.E. (n = 9). Bars sharing different letters for each diet are significantly different determined by Tukey's test ($P < 0.05$).

Discussion

In the present study, we evaluated the effects of curcumin, which is considered to be an immunostimulant on growth performance and immune responses of *M. amblycephalain.* We did this by measuring the weight of fish and the expression of a number of cytokines that enables cell to cell communication in immune responses, and stimulate the movement of cells towards sites of inflammation, infection and trauma. We also evaluated the activities of several enzymes. Our results suggest that curcumin, when used as a dietary supplement for feeding *M. amblycephalain,* can improve both growth performance and immune responses of *M. amblycephalain*

Previous studies have shown that curcumin promoted gastrointestinal motility (Luo, 2012) and enhanced the activity of digestive enzymes in the gut and

pancreas of rats (*Rattus norvegicus*) (Platel & Srinivansan, 1996). It has also been reported that curcumin increased the activity of protease and diastase in the intestinal tract, enhanced protein absorbability, and improved growth of fish (Hu et al., 2003; Wang & Wu, 2007; Cui et al., 2013). The results of our study were consistent with these previous reports. We found that the basal diet supplemented with 60 mg curcumin/kg increased WGR and SGR of juvenile *M. amblycephalain* and reduced FCR. However, fish fed the basal diet supplemented with 120 mg curcumin/kg and 240 mg curcumin/kg had significantly lower WGR and SGR and significantly higher FCR than fish fed the basal diet supplemented with 60 mg curcumin/kg group. Curcumin overdose may lead to an imbalance of bacterial populations in the intestinal tract or affect palatability of feed (Xie et al., 2008; Liu et al., 2010). No significant differences in VSI, HIS, and CF were detected among all groups ($P > 0.05$), which is consistent with the curcumin toxicity study conducted by Wo et al. (2000a, b) and Ying et al. (2011). Our results and previous studies suggest that curcumin is an optimal dietary supplement with minimal adverse effects.

Hematological tests have been widely used for evaluation of fish health in both research, and commercial aquaculture (Blaxhall, 1972). The present study showed that the number of WBC was significantly enhanced in fish fed a diet supplemented with 60 mg curcumin/kg, suggesting that curcumin may be involved in inflammatory responses. Our results are consistent with the study reported by Nya and Austin (2009a) who observed increased WBC and other blood cells in rainbow trout (*Oncorhynchus mykiss*) fed a diet containing ginger. WBC are the first line of defense and play a critical role in innate immunity against microbial infections. Increased WBC numbers along with other immunological factors are considered indicators of the health status of fish (Kumar, et al., 2013). However in the present study, no significant differences in RBC count and HGB were observed between all the groups fed with curcumin supplemented diets, and the control group. This differs from previous studies. The number of RBC was significantly higher in rainbow trout fed with a ginger diet than the control (Nya and Austin (2009b) and Allah (2013). Studies have shown that immunostimulant herbal plants improved the immune responses of fish by increasing the number of RBC, WBC and HGB (Sahu, et al., 2007; Nya & Austin, 2009a, b; Talpur & Ikhwanuddin, 2012, 2013; Talpur, et al., 2013). The different results on blood cells between the present study and previous studies may be explained by the different species investigated.

Increases in total protein and albumin in serum are associated with strong innate immunity (Jha, et al., 2007; Nya & Austin, 2009b). In the present study, no significant changes in total protein were observed between curcumin fed groups and the control group, however, albumin in curcumin fed groups was significantly higher in the juvenile *M. amblycephalain* than in the control group. Changes of total protein in serum in our study are different from previous studies on tilapia, *Oreochromis niloticus*, (Cui et al., 2013) and Bloch, *Lates calcarifer*, (Talpur, et al., 2012), however changes of albumin in serum is consistent with the study conducted by Talpur (2012). This may be because the total protein and albumin of the juvenile *M. amblycephalain* is mainly involved in absorption and utilization of nutrients.

Fish fed diets supplemented with 60 mg curcumin/kg diet had a relatively lower alanine transaminase (ALT) and aspartate aminotransferase (AST) than fish fed other diets. ALT and AST in fish fed diets supplemented with 60 mg curcumin/kg were significantly lower than in the control group ($P < 0.05$). No significant difference of the lactate dehydrogenase (LDH) level were detected among all groups, LDH tended to decrease at first and then to increase. High aspartate aminotransferase (AST) activity generally indicates disorders of heart or muscle tissue, and damage to hepatocytes results in high alanine transaminase (ALT) and lactate dehydrogenase (LDH) activity (Liu & Li, 1996; Kim et al., 2014). The changes of ALT and LDH levels suggest that juvenile *M. amblycephalain* may be susceptible to the hepatotoxicity which may be dose-dependent with curcumin. The results of our study are consistent with previous studies on grass fish (*Ctenopharyngodon idellus*) which showed that appropriate amounts of curcumin protect the liver of juvenile *M. amblycephalain* from injury (Yu et al. 2013; Zhang et al. 2013).

Phagocytic cells are the most important components of the innate immune system in fish (Secombes, et al., 1990). Phagocytes produce toxic oxygen forms via respiratory burst (Neumann, et al., 2001). Given that superoxide anion is the first product released from respiratory burst, the measurement of O^{-2} has been commonly used to evaluate the function of respiratory burst (Harikrishnan, et al., 2011). Respiratory burst activity was first improved and then inhibited with increasing dietary emodin (*Emodin*) for juvenile *M. amblycephalain (*Zhang et al. 2014a). In the present study we found that the diet supplemented with 60 mg curcumin/kg significantly increased the phagocytic activity compared with the control group.

We observed that IL-1β activity significantly increased with supplementation of dietary curcumin at 60 and 240 mg/kg. As an important mediator of inflammatory responses, IL-1β is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis of fish. Curcumin inhibited the expression of IL-1β in cells (Srivastava, et al., 2011). It is not clear why our results differed from those reported by Srivastava et al.

Production of TNF-α is one of the earliest events in many types of liver injury. This triggers the expression of other cytokines to recruit inflammatory cells, kills damaged hepatocytes, and activates the healing response (Ramirez-Tortosa, et al., 2009). A number of studies in fish showed that TNF-α was an important macrophage-activating factor (MAF) produced by leukocytes (Whyte, 2007) by improving respiratory burst activity, phagocytosis, and nitric oxide production (Whyte, 2007). Different studies have confirmed that WBC, TNF-α activity and respiratory burst activity has been significantly affected in fish fed emodin diet or rabbits (*Disambiguation*) fed curcumin diet.(Ramirez-Tortosa, et al., 2009; Zhang, 2014a, b). However, the results of these studies and the present study were not consistent. In the present study, TNF-α activity which was associated with the WBC in serum, differed significantly between all curcumin-fed groups and the control group. Changes in TNF-α activity caused by curcumin in the present study suggest that it can protect liver of juvenile *M. amblycephalain* from injury.

Bactericidal activity of dietary components has been recognized as one of the key mechanisms for inhibiting and killing bacteria in fish and other animals (Ellis,

2001). We observed that ACH50 activity increased with the supplementation of curcumin at 60 mg curcumin/kg and then decreased with addition of 120 and 240 mg/kg curcumin. Results suggest that 60 mg curcumin/kg supplementation improved the immune response of juvenile *M. amblycephalain,* and insufficient or excessive, curcumin may not activate normal immune responses. Our results are consistent with the report in which dietary supplementation with probiotics and herbal mixtures inhibited the nonspecific immune response to *Streptococcus parauberis* and resulted in a significant increase of the ACH50 activity in olive flounder, *Paralichthys olivaceus*, (Harikrishnan et al. 2011).

In conclusion, this study has provided evidence that dietary supplementation of 60 mg curcumin/kg increased WGR, SGR, and WBC, reduced FCR, ALT, and AST, and improved non-specific immunity in *M. amblycephalain*. The optimal level of dietary curcumin to improve *M. amblycephalain* growth and immune responses is 60 mg curcumin/kg.

Acknowledgements

This study was supported by the Modern Agriculture Industrial Technology System special project, the National Technology System for Conventional Freshwater Fish Industries (CARS-46), the Special Fund for Agro-scientific Research in the Public Interest (20100302), the National Nonprofit Institute Research Grant of Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences (2014A08XK02), and the Three New Projects of Fishery in Jiangsu province (D2013-5).

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