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RESEARCH PAPER

Effects of Dietary Calcium Levels on Growth Performance, Blood Biochemistry and Whole Body Composition in Juvenile Bighead Carp (*Aristichthys nobilis*)

Hualiang Liang[1], Haifeng Mi[2], Ke Ji[1], Xianping Ge[1,2]*, Mingchun Ren[1,2]*, Jun Xie [1,2]

[1] Nanjing Agricultural University, Wuxi Fisheries College, Wuxi 214081, China.
[2] Chinese Academy of Fishery Sciences (CAFS), Freshwater Fisheries Research Center (FFRC), Key Laboratory for Genetic Breeding of Aquatic Animals and Aquaculture Biology, Wuxi 214081, China.

* Corresponding Author: Tel.:+86.510 85556566;
E-mail: gexp@ffrc.cn; renmc@ffrc.cn

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Abstract

An eight weeks feeding experiment was conducted to investigate the effects of dietary calcium (Ca) levels on growth performance, blood biochemistry and whole body composition in bighead carp (105.52 ±0.33g; 16.91±0.09cm). Six practical diets (31.0% crude protein, 6.0% crude lipid) were formulated to contain graded Ca levels ranging from 0.41% to 1.59% of dry diet. At the end of the feeding trial, results showed that survival rate (SR) was not significantly affected by dietary Ca level. Final weight (FW), weight gain (WG), specific growth rate (SGR) increased with the increasing dietary Ca level up to 1.26%, and thereafter showed a decreasing trend, while feed conversion ratio (FCR) showed a converse trend. Furthermore, high Ca level (1.59%) significantly increased the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and significantly decreased serum phosphorus (P) content ($P<0.05$). Alkaline phosphates (ALP) activity was significantly higher than other groups with Ca-deficient diets (0.41%) ($P<0.05$). High Ca level (1.59%) significantly decreased serum P levels. Serum Ca levels were not significantly affected by dietary Ca levels ($P>0.05$). Based on FCR and SGR, the optimal dietary Ca level was determined to be 1.26%, and the corresponding Ca/ P ratio was calculated to be 1.13:1.

Keywords: Bighead carp, calcium, growth performance, blood parameters, body composition.

Introduction

In mammal and aquatic animal, calcium (Ca) is accounted as one of the most important minerals and is essential for normal growth, skeletal development and several physiological processes in aquatic species (NRC, 2011; Lall, 2002). In aquatic animal, fish can absorb Ca from the surrounding water to fulfil part or the entire metabolic Ca requirement, because its concentration is high in water (Ichikawa and Oguri, 1961). Some studies have reported that dietary Ca may not be needed for some aquatic species such as black sea bream (*Sparus macrocephalus*) (Hossain and Furuichi, 1999). In contrast, some studies have reported that dietary Ca supplement was also necessary for fish. Sakamoto and Yone (1973) found the uptake of Ca from seawater was not sufficient to meet the Ca requirement of red sea bream, and it required 0.34% Ca in the diet. Hossain and Furuichi (1999, 2000a, b, c) also reported that dietary Ca supplement was necessary for redlip mullet (*Chelon haematocheilus*); Japanese flounder (*Paralichthys olivaceus*) and scorpion fish (*Sebastiscus marmoratus*).

Unlike Ca, there seems no doubt that dietary phosphorus (P) supplement was necessary for fish, because its concentration is low in both freshwater and seawater (Lall, 2002). The importance of P supplementation in diet has widely been reported in many species including freshwater species rainbow trout (*Oncorhynchus mykiss*) (Ogino & Takeda, 1978), common carp (*Cyprinus carpio*) (Ogino & Takeda, 1976; 1978) and marine species like gilthead sea bream (*Sparus aurata* L.) (Rodrigues & Teles, 2001), black sea bream (*Sparus macrocephalus*) (Shao *et al*., 2008), Atlantic salmon (*Salmo salar*) (Vielma & Lall, 1998) and juvenile haddock (*Melanogrammus aeglefinus* L.) (Roy & Lall, 2003).

Many studies suggested that the ratio of Ca to other minerals, particularly P, should be considered as well as individual dietary levels of minerals (Porn-Ngam, Satoh, Takeuchi, & Watanabe, 1993). An excess of Ca relative to P has an adverse impact on the growth and survival of some species including grouper (*Epinephelus coioides*) (Ye *et al*., 2006) and Pacific white shrimp (*Penaeus vannamei*) (Davis, Lawrence, & Gatlin, 1993). In brook trout (*Salvelinus fontinalis*), dietary Ca/P ratio is also accounted as most effective for utilization of the minerals (Phillips, 1959), which suggests the importance of the level of P

for the absorption of dietary Ca. The recommended levels of Ca/P ratio for fish are in range of 0.5:1 to 1:1.3 (Phillips, 1959; Nakamura & Yamada, 1980; Ye *et al*., 2006; Nose & Arai, 1979; Sanchez, Palacios, Perez, & Ross, 2000). Some studies have reported that whole-body protein and lipid contents were effect with dietary Ca supplementation in japanese seabass, *Lateolabrax japonicas.* However, dietary Ca supplementation in diet have no impact on whole body composition, such as fingerling scorpion fish (Hossain & Furuichi, 2000c), juvenile jade perch (*Scortum barcoo*) (Song, Mao, Wang, Zhu, & Han, 2009), Atlantic cod (*Gadus morhua*) (Kousoulaki, Fjelldal, Aksnes, & Albrektsen, 2010). Dietary supplements with an excess of Ca relative to P has an adverse impact on the growth and survival of fish, which is related to blood parameters and plasma biochemical parameters considered as convincible indicators for the physiological conditions and health status of fish in response to dietary supplements (Congleton & Wagner, 2006; Kader, Koshio, Ishikawa, Yokoyama & Bulbul, 2010; Kavitha, Ramesh, Kumaran, & Lakshmi, 2012; Davis, 2004). So there is necessary to investigate these parameters relates to growth and health status.

The bighead carp (*Aristichthys nobilis*) is one of the most important aquaculture species in China (Tong & Sun, 2015). Furthermore, it is also distributed in Asia, Europe and America (Kolar, Chapman, Courtenay, Williams, & Jennings, 2005). Commercial production of this fish species has been rapidly increased and reached approximately 3.36 million tons in 2015 in China (Ministry of Agriculture of the People's Republic of China, 2016). In recent years, studies about bighead carp nutrition have been conducted, such as requirement of protein (Santiago & Reyes, 1991), vitamins (Santiago & Gonzal, 2000). However, information about its mineral requirements is scarce, especially its Ca and Ca/P requirement. Therefore, the aim of this study was to determine the effect of dietary Ca level (Ca/P ratio) on the growth performance, blood biochemistry and whole body composition in bighead carp.

Materials and Methods

Diet Preparation

Formulation of the experimental diets are presented in Table 1. Six practical diets (31.0% crude protein, 6.0% crude lipid) were formulated to contain graded Ca levels (0.41 (control), 0.72, 0.93, 1.15, 1.26 and 1.59% of dry diet) by Calcium lactate. Dietary protein was supplied by fish meal, rapeseed meal, soybean meal, cottonseed meal, dietary lipid was supplied by fish oil. Ingredients were ground into powdered through form120 mash (aperture: 125 micron) sieve to make sinking pellet feed though a pelletizer (F-26 (II), South China University of Technology, China), and then dried at 45°C overnight and then stored at -20°C for further use.

Experimental Procedure

Bighead carp were obtained from the breeding farm of Freshwater Fisheries Research Centre (FFRC) of Chinese Academy of Fishery Sciences. Prior to the feeding trial, we selected the healthy bighead carp with similar sizes, reared in cages (length: 2m, width: 1m, height: 1m). To acclimatize with the experimental diet and conditions, bighead carp was fed with a commercial diet containing 31% protein and 6% lipid (Wuxi Tongwei feedstuffs Co. Ltd., Wuxi China) for two weeks. At the initiation of the experiment, the bighead carp (105.52±0.33g; 16.91±0.09cm) were fasted for 24h and weighed, then were randomly sorted into eighteen cages (length: 2m, width: 1m, height: 1m) with 15 fish in each cage for farm pond culture. Each diet was randomly assigned to triplicate cages for 8 weeks. Fish were hand-fed three times daily at 8:00, 12:00 and 16:00 until apparent satiation. During the experimental period, water temperature ranged from 26 to 28°C, pH from 7.1 to 7.7, dissolved oxygen from 6.3 to 7.6mg/L, ammonia nitrogen from 0.007 to 0.011mg/L, hydrogen sulfide from 0.004 to 0.009mg/L, and Ca from 43.17 to 52.12mg/L. Water temperature, dissolved oxygen, pH, ammonia nitrogen, hydrogen sulfide were tested by ProDSS Multiparameter Water Quality Meter (YSI, USA). Water Ca content was tested by EDTA titrimetric method (Jensen & Thursby, 1996).

Sample Collection

At the end of the experimental, six experimental bighead carp from each cage were collected and anesthetized with 100 mg L^{-1} 3-Aminobenzoic acid ethyl ester methanesulfonate (MS-222), then weighed and 1 milliliter blood samples were collected immediately from the caudal vein using disposable medical syringes. Then plasma was separated by centrifugation (3500×g, 10 min, 4°C) and then stored at -80°C until analysis. Another five bighead carp from each cage were randomly collected and stored at -20°C for whole fish body composition analysis. The initial weight and final weight of each cage fish was recorded to calculate specific growth rate (SGR), feed conversion ratio (FCR) and weight gain (WG).

Growth Parameters

Growth parameters were calculated as follows:

Specific growth rate (SGR) (%/d) =100 × [(In(final body weight (g)) -In (initial body weight (g)))/days]

Feed conversion ratio (FCR) = dry feed fed (g)/ wet weight gain (g)

Weight gain (WG) (%) = 100 × (final weight (g) -initial weight (g)) /initial weight (g)

Table 1. Formulation and proximate composition of experimental diets for feeding trial

ingredients	Diet Number					
	diet1	diet2	diet3	diet4	diet5	diet6
Fish meal[a]	5.00	5.00	5.00	5.00	5.00	5.00
Rapeseed meal[b]	27.00	27.00	27.00	27.00	27.00	27.00
Soybean meal[c]	26.00	26.00	26.00	26.00	26.00	26.00
Cottonseed meal[d]	7.55	7.55	7.55	7.55	7.55	7.55
Wheat meal[e]	24.15	22.95	21.72	20.61	19.29	18.02
Fish oil	5.00	5.00	5.00	5.00	5.00	5.00
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin C	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin and mineral premix[f]	1.00	1.00	1.00	1.00	1.00	1.00
Monopotassium phosphate	2.40	2.40	2.40	2.40	2.40	2.40
Calcium lactate	0.00	1.09	2.18	3.27	4.36	5.45
Bentonite	1.00	1.00	1.00	1.00	1.00	1.00
Glycine	0.70	0.81	0.95	0.97	1.20	1.38
Proximate analysis (% Dry basis)	100.00	100.00	100.00	100.00	100.00	100.00
Moisture	9.61	8.77	8.795	8.45	8.31	8.36
Protein	31.71	31.74	31.78	31.65	31.73	31.77
Lipid	6.52	6.56	6.51	6.53	6.55	6.51
Ash	8.75	8.92	9.62	9.27	10.22	10.42
Calcium (Ca)	0.41	0.72	0.93	1.15	1.26	1.59
Phosphorus (P)	1.13	1.12	1.13	1.11	1.12	1.16
Ca/P	0.41	0.72	0.93	1.15	1.26	1.59

[a]fish meal: crude protein 61.4%, crude lipid 9.3%; [b]Rapeseed meal: protein 37.5%, crude lipid 1.4%; [c]soybean meal: crude protein 44.2%, crude lipid 1.1%; [d]cottonseed meal: crude protein 49.3%, crude lipid 1.4%; [e]wheat meal: crude protein 11.8%, crude lipid 1.2%.
[f]Vitamin and mineral mix
Vitamin mix (IU / kg of diet or mg/ kg of diet): Vitamin A, 900 000 IU; Vitamin D, 250 000 IU; Vitamin E, 4500 mg; Vitamin K3, 220 mg; Vitamin B_1, 320 mg; Vitamin B_2, 1090 mg; Vitamin B_5 , 2000 mg; Vitamin B_6, 5000 mg; Vitamin B_{12} , 116 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60 000 mg; Biotin, 50 mg; Niacin acid, 2500 mg.

Survival rate (SR) (%) =100 × (survival fish number/total fish number)

Whole Body Composition Analysis

Moisture, crude protein, crude lipid and ash contents of diet and fish whole body were analyzed according to the established methods of AOAC (2003). The diet and fish whole body moisture were dried in an oven at 105°C until constant weight; crude protein (N×6.25) by Kjeldahl method after acid digestion; lipid by ether extraction using Soxhlet; ash by combustion at 550°C for 5h. Duplicate analyses were conducted for each sample.

Blood and Serum Chemical Analysis

The counts of red blood cell (RBC), white blood cell (WBC), hemoglobin (HGB) and hematocrit (HCT) were measured using an Auto Hematology Analyzer (BC-5300Vet, Mindray, PR China) with a test kit from Shenzhen Mindray Medical International Co. Ltd., PR China. Aspartate aminotransferase (AST) activity, alanine transaminase (ALT) activity, P, alkaline phosphates (ALP) activity, serum Ca and P levels were determined using an automatic biochemical analyzer (Mindray BS-400, Mindray Medical International Ltd., Shenzhen, China) as described in our previous studies (Ren *et al*., 2013; 2015, Liu *et al*., 2012).

Statistics Analysis

All data were subjected to one-way analysis of variance (ANOVA) using the software of the SPSS 16.0 for Windows. Significant differences between means were evaluated by Tukey's Multiple Range Test. Probabilities of $P<0.05$ were considered significant. Data are expressed as means with standard error of the mean (SEM).

Results

Growth Performance

Growth performance are presented in Table 2. Survival rate (SR) was not significantly affected by dietary Ca level. Final weight (FW), weight gain (WG), specific growth rate (SGR) increased with the increasing dietary Ca level up to 1.26%, and thereafter showed a decreasing trend, while feed conversion ratio (FCR) showed a converse trend. Based on FCR and SGR, the optimal dietary Ca/P ratio was determined to be 1.13:1, and the corresponding Ca and P level was calculated to be 1.26% and 1.12%, respectively.

Whole Body Composition

Whole body moisture, protein, lipid and ash content were not significantly affected by dietary Ca levels ($P>0.05$) (Table 3).

Blood Parameters

Red blood cell (RBC) counts, white blood cell (WBC) counts, hemoglobin (HGB) counts, haematocrit (HCT) were not significantly affected by dietary Ca levels (P>0.05) (Table 4). High Ca level (1.59%) significantly increased the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and significantly decreased serum P content (P<0.05) (Figure 1; 2; 5). Alkaline phosphates (ALP) activity was significantly higher than other groups with Ca-deficient diets (0.41%) (P<0.05) (Figure 3). Serum Ca levels were not significantly affected by dietary Ca levels (P>0.05). Serum Ca levels were not significantly affected by dietary Ca levels (P>0.05) (Figure 4).

Discussion

In aquatic animal, Ca is accounted as one of the most important minerals and is essential for normal growth (NRC, 2011). Studies were reported that the existence in tilapia of a compensatory Ca uptake mechanism vis-a-vis the availability of Ca in the water (Flik, Fenwick, Kolar, Mayer-Gostan, & Wendelaar Bonga, 1986; Hwang, Tung, & Chang, 1996; Vonck, Wendelaar Bonga, & Flik, 1998). In high-Ca water or sea water, fish could satisfy their requirements by uptake mechanism, while in low-Ca water or freshwater, fish could not satisfy their requirements and need to increase diets Ca levels to meet fish requirements. (Robinson, Rawles, Yette, Brown, & Greene, 1986; Vonck *et al.*, 1998). In the current study, fish fed with the Ca-deficient diet (0.41%) showed poor growth performance and the best growth performance were observed in 1.26% dietary Ca level, which indicated that Ca is necessary for bighead carp in the current breeding water. However, high Ca diet (1.59%) resulted in poor growth performance in bighead carp which indicated high dietary Ca might have adverse effects on the growth of bighead carp, which was similar to the report that higher dietary Ca levels has been shown to adversely affect the growth and survival in channel catfish (Gatlin & Phillips, 1989), rainbow trout (*Salmo gairdneri*) (Spinelli, Houle, & Wekell, 1983), Chinook salmon (*Oncorhynchus tshawytscha*) (Richardson, Higgs, Beams, & McBride, 1985), grouper (Ye *et al*., 2006) and Japanese seabass (*Lateolabrax japonicas*) (Song *et al*., 2016). Baldisserotto, Kamunde, Matsuo and Wood (2004) reported that low $CaCl_2$-supplemented (60 mg Ca^{2+} g^{-1} food) diet resulted in decreasing weight gain in rainbow trout. Based on FCR and SGR, a dietary Ca/P ratio for juvenile bighead carp were determined to be 1.13:1, which was similar to the ratios reported for some fish species, such as brook trout (1:1, Phillips, 1959); Mayan cichlid (*Cichlasoma urophthalmus*) (1.3:1, Sanchez *et al.*, 2000); common carp (1:1, Nakamura & Yamada, 1980); juvenile grouper (1:1, Ye *et al*., 2006); eel (*Anguilla japonica*) (0.93:1, Nose & Arai, 1979), higher than red sea bream (0.5:1, Sakamoto & Yone, 1973). Therefore, it is suggested that different fish species have different dietary Ca/P ratios to fulfil their growth potential.

In our study, dietary Ca supplementation had no

Table 2. Growth performance index of bighead carp fed experimental diets for 8 weeks

Diet NO.	Ca %	Ca/P	Initial Weight(g)	Final Weight(g)	WG(%)[1]	FCR[2]	SGR (% day^{-1})[3]	SR (%)[4]
Diet 1	0.41	0.36:1	105.33±0.33	170.42±1.13^{a}	61.80±1.04^{a}	1.63±0.03^{d}	0.86±0.03^{a}	100±0.00
Diet 2	0.72	0.64:1	106.33±0.33	182.35±1.09^{b}	71.50±1.42^{b}	1.41±0.02^{c}	0.96±0.02^{b}	100±0.00
Diet 3	0.93	0.82:1	105.33±0.33	183.33±1.20^{bc}	74.60±1.14^{bc}	1.36±0.02^{bc}	1.00±0.01^{bc}	100±0.00
Diet 4	1.15	1.04:1	105.33±0.33	188.33±1.45^{c}	78.81±1.86^{c}	1.29±0.03^{b}	1.04±0.04^{c}	100±0.00
Diet 5	1.26	1.13:1	105.33±0.33	196.33±0.88^{d}	86.40±1.34^{d}	1.18±0.02^{a}	1.11±0.03^{d}	100±0.00
Diet 6	1.59	1.43:1	105.33±0.33	187.33±1.20^{bc}	77.29±1.40^{bc}	1.31±0.04^{bc}	1.02±0.01^{bc}	100±0.00

[1]WG (%): weight gain; [2]FCR (%): feed conversion ratio; [3]SGR (% day^{-1}): specific growth rate; [4]SR (%): survival rate. Mean values and standard error (M±SE) are presented for each parameter. Significant differences with within the diets are indicated by different letters (P<0.05).

Table 3. Whole body composition (wet basis) of bighead carp fed experimental diets for 8 weeks

Diet NO.	Ca %	Ca/P	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Diet 1	0.41	0.36:1	82.17±0.61	11.33±0.28	2.49±0.06	4.15±0.07
Diet 2	0.72	0.64:1	82.13±0.36	11.23±0.12	2.47±0.08	4.07±0.05
Diet 3	0.93	0.82:1	82.23±0.61	11.17±0.21	2.36±0.05	4.03±0.09
Diet 4	1.15	1.04:1	82.56±0.53	11.44±0.28	2.31±0.04	4.10±0.04
Diet 5	1.26	1.13:1	82.61±0.46	11.56±0.11	2.26±0.06	3.91±0.06
Diet 6	1.59	1.43:1	82.47±0.75	11.49±0.21	2.39±0.07	4.06±0.08

Mean values and standard error (M±SE) are presented for each parameter. No Significant differences within the diets (P>0.05).

Table 4. Blood hematological parameters of bighead carp fed experimental diets for 8 weeks

Diet NO.	Ca %	Ca/P	WBC (10^9/L)[1]	RBC (10^{12}/L)[2]	HGB (g/L)[3]	HCT (%)[4]
Diet 1	0.41	0.36:1	111.17±3.71	1.57±0.06	76.25±3.84	24.49±2.51
Diet 2	0.72	0.64:1	115.73±3.02	1.64±0.05	81.44±1.92	25.94±3.24
Diet 3	0.93	0.82:1	117.62±6.39	1.66±0.08	82.86±5.14	23.43±3.22
Diet 4	1.15	1.04:1	114.91±2.29	1.64±0.05	80.86±1.82	28.49±2.23
Diet 5	1.26	1.13:1	102.85±5.34	1.46±0.07	72.00±3.18	24.09±2.64
Diet 6	1.59	1.43:1	111.54±3.38	1.57±0.06	76.00±2.59	27.78±1.54

[1]White blood cell (WBC), [2]red blood cell (RBC), [3]hemoglobin (HGB) and [4]hematocrit (HCT). Mean values and standard error (M±SE) are presented for each parameter. No Significant differences within the diets (P>0.05).

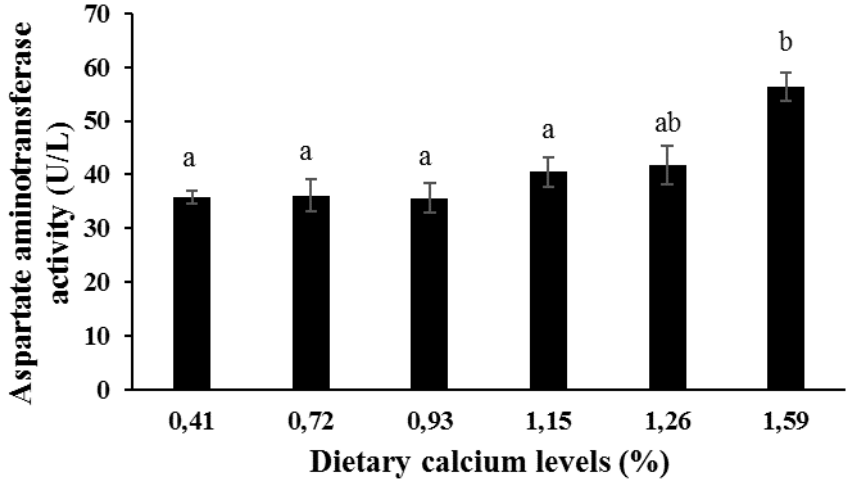

Figure 1. Aspartate aminotransferase (AST) activity of bighead carp fed diets with different calcium levels. Vertical bars represent mean ± SE values for triplicate samples. Value with different superscripts are significantly different (P<0.05).

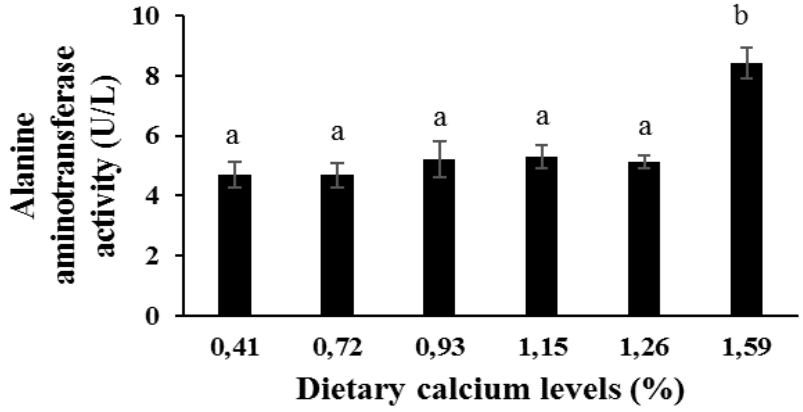

Figure 2. Alanine aminotransferase (ALT) activity of bighead carp fed diets with different calcium levels. Vertical bars represent mean ± SE values for triplicate samples. Value with different superscripts are significantly different (P<0.05).

significant effects on whole body ash content, which was similar with these results reported in fingerling scorpion fish (Hossain & Furuichi, 2000c), juvenile jade perch (*Scortum barcoo*) (Song *et al.*, 2009), Atlantic cod (Kousoulaki *et al.*, 2010) and juvenile grouper (Ye *et al.*, 2006). This finding suggested that dietary Ca supplementation did not change ash content or has no effect on internal deposition. A study showed that the Ca exchange rate of fish scales was three times that in bone (Berg, 1968).

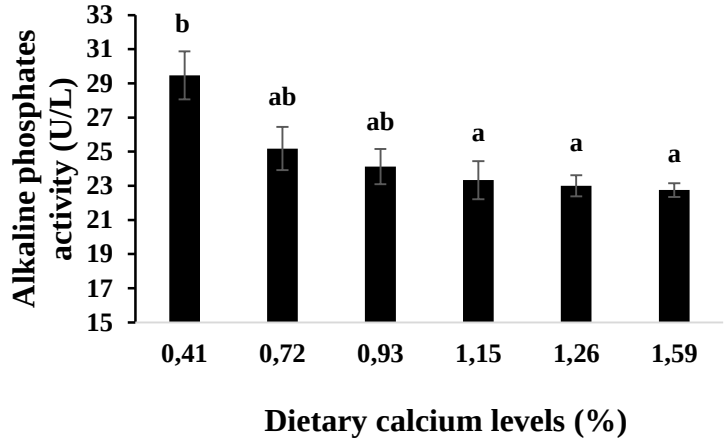

Figure 3. Alkaline phosphates (ALP) activity of bighead carp fed diets with different calcium levels. Vertical bars represent mean ± SE values for triplicate samples. Value with different superscripts are significantly different (P<0.05).

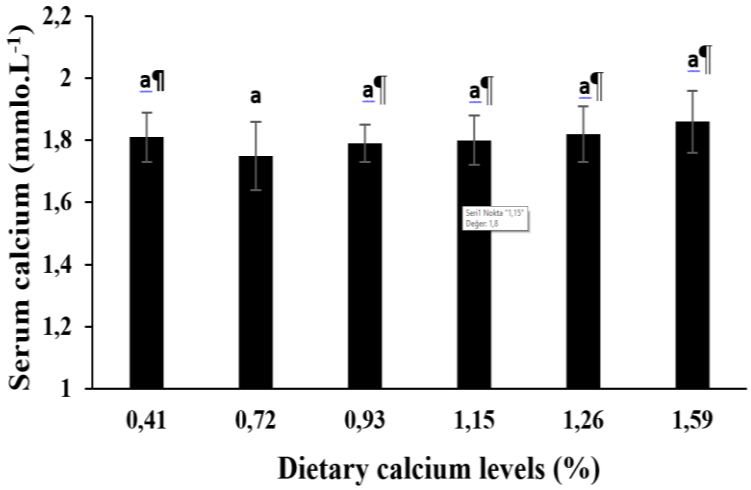

Figure 4. Serum calcium content of bighead carp fed diets with different calcium levels. Vertical bars represent mean ± SE values for triplicate samples. No Significant differences within the diets (P>0.05).

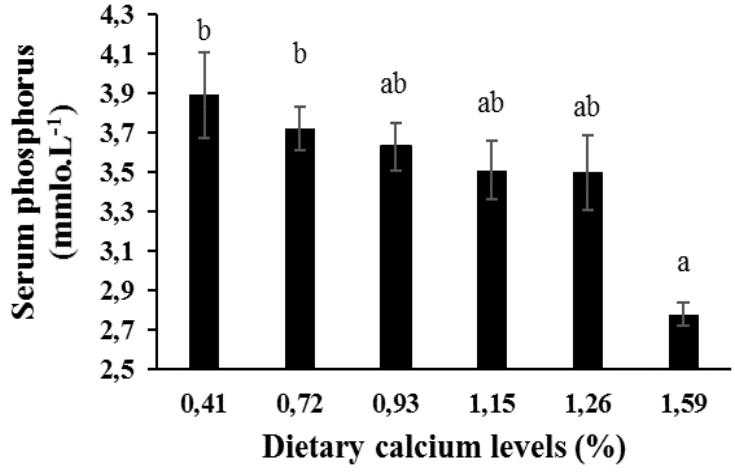

Figure 5. Serum phosphorus content of bighead carp fed diets with different calcium levels. Vertical bars represent mean ± SE values for triplicate samples. Value with different superscripts are significantly different (P<0.05).

Furthermore, dietary high Ca supplementation reduced Ca content in scales and not in bone (Berntssen, Waagbø, Toften, & Lundebye, 2003), which indicated the present study might be reasonable in that the ash. In our study, dietary Ca supplementation had no significant effects on whole body lipid, protein and moisture. Similar results were reported in juvenile jade perch (Song *et al.*, 2009), fingerling scorpion fish (Hossain & Furuichi, 2000c). Hossain and Yoshimatsu (2014) has reported that dietary increased Ca could also was used for building the hard tissues and other physiological functions at the developmental stage of fish, which indicated dietary Ca supplementation might be used for building hard tissues and other physiological functions in juvenile bighead carp. However, when japanese seabass were fed with the high Ca (3.1%; Ca/P, 2.73:1) group, whole-body protein and lipid contents were lower than other treatments (Song *et al*., 2016). The differences for various species of fish may be due to experimental environment conditions, diet composition and age. In our study, the highest Ca level is 1.59% far below 3.1%, which might affect trail result.

Blood parameters are considered as convincible indicators for the physiological conditions and health status of fish in response to dietary supplements (Congleton & Wagner, 2006; Kader *et al.*, 2010). In our study, dietary Ca supplementation had no significant effects on RBC counts, HGB counts and HCT, which indicated dietary Ca supplementation did not affect the capacity of carrying oxygen and collecting carbon dioxide. The number of WBC counts in the blood is related to inflammatory stimuli and immune system, which is often an indicator of disease. In our study, dietary Ca supplementation had no significant effects on WBC counts, which indicated dietary Ca supplementation did not affect the capacity of immune. In fish physiological diagnoses, we usually use plasma biochemical parameters to determine the general status of health (Kavitha *et al.*, 2012; Davis, 2004). Blood Ca, P levels are devoted to evaluate Ca and P nutritional status of animals, which can reflect the absorption of Ca and P in bone (Zhang *et al*., 2006). Plasma Ca levels were not affected with dietary Ca supplementation, suggesting Ca homeostasis in bighead carp. Similar results were reported in juvenile tilapia (*Oreochromis niloticus x O. aureus*); grass carp (*Ctenopharyngodon idella*) (Liang *et al*., 2012); Atlantic salmon (Berntssen *et al.*, 2003). However, serum Ca concentration increased significantly with dietary Ca levels, and reached a maximum (7.71 mmol L^{-1}) in Japanese seabass (Song *et al*., 2016). These results concluded that various species of fish could lead to varying plasma Ca levels significantly (Urasa & Wendelaar Bonga, 1987). Nakamura (1982) reported that Ca may interact with other essential dietary minerals, particularly P. In our study, plasma P levels showed decreasing trend with increasing dietary Ca levels. Nakamura (1982) observed a negative and linear relationship between the amount of P absorbed and the dietary Ca content in carp. When there is an excess of dietary Ca supplementation, the P is not absorbed by the intestine, which could explain the reason of the result (Andrews, Murai, & Campbell, 1973; Cowey & Sargent, 1979). High activities of blood AST and ALT generally indicates a weakening or damage of normal liver function in fish species (Kim & Lee, 2009). In the current study, high dietary Ca supplementation significantly increased serum AST and ALT activity and reached a maximum, which indicated that dietary Ca levels could weaken or damage of normal liver function in bighead carp. The results could explain the poor growth performance in bighead carp with dietary Ca excessive supplementation (1.59%). ALP activity affect the absorption of several minerals in particular Ca and P for bone mineralization (Coleman, 1992). In the present study, in bighead carp fed with Ca-deficient diets, ALP activity was significantly higher than other groups, demonstrating that low dietary Ca levels did not meet the bone mineralization requirement. ALP activity was also significantly higher than other groups with Ca-deficient diets in Japanese seabass (Song *et al*., 2016), which was in accordance with our trail result. Hurwitz and Griminger (1961) reported that lower Ca-containing diets also increased plasma ALP activity, which was a marker of insufficient bone mineralization in a fowl nutritional study.

The present study demonstrated that optimal dietary Ca level (1.26%) could improve growth performance and lowered FCR. However, higher Ca level (1.59%) damaged the liver function. Furthermore, dietary Ca supplementation had significant adverse effects on serum P absorbed. Thus, it is suggested that the optimal Ca level was determined to be 1.26%, and the corresponding Ca/ P ratio was calculated to be 1.13:1 based on this study.

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References

Andrews, J.W., Murai, T., & Campbell, C. (1973). Effects of dietary calcium and phosphorus on growth, food conversion, bone ash and hematocrit levels of catfish. *Journal of Nutrition, 103*, 766-771. https://doi.org/10.1093/jn/103.5.766

AOAC. (2003). Official methods of analysis of the association of official analytical chemists 15th ed. Association of Official Analytical Chemists Inc. Arlington VA.

Baldisserotto, B., Kamunde, C., Matsuo, A., & Wood, C.M. (2004). A protective effect of dietary calcium against acute waterborne cadmium uptake in rainbow trout. *Aquatic Toxicology, 67*, 57-73.

https://doi.org/10.1016/j.aquatox.2003.12.004

Berg, A. (1968). Studies on the metabolism of calcium and strontium in freshwater fish. 1. Relative contribution of direct and intestinal absorption. Mem. 1st. Ital. Idrobiol. Dott. Maroco Marchi, *23*, 161-196

Berntssen, M.H.G., Waagbø, R., Toften, H., & Lundebye, A.K. (2003). Effects of dietary cadmium on calcium homeostasis, Ca mobilization and bone deformities in Atlantic salmon (*Salmo salar* L.) parr. *Aquaculture Nutrition*, *9*(3), 175-183. https://doi.org/10.1046/j.1365-2095.2003.00245.x

Coleman, J.E. (1992). Structure and mechanism of alkaline phosphatase. *Annual Review of Biophysics & Biomolecular Structure*, *21*, 441-483. https://doi.org/10.1146/annurev.bb.21.060192.002301

Congleton, J.L., & Wagner, T. (2006). Blood-chemistry indicators of nutritional status in juvenile salmonids. *Journal of Fish Biology*, 69, 473-490. https://doi.org/10.1111/j.1095-8649.2006.01114.x

Cowey, C.B., & Sargent, J.R. (1979). Nutrition. In: W.S. Hoar and J. Randall, Eds. Fish Physiology. Academic Press, New York, Vol. III, 1-69. https://doi.org/10.1016/S1546-5098(08)60024-2

Davis, D.A., Lawrence, A.L., & Gatlin, D.M. (1993). Response of *Penaeus vannamei* to dietary calcium, phosphorus and calcium:phosphorus ratio. *Journal of the World Aquaculture Society*, *24*, 504-515. https://doi.org/10.1016/j.cbpb.2004.09.012

Davis, K.B. (2004). Temperature affects physiological stress responses to acute confinement in sunshine bass (*Morone chrysops* x *Morone saxatilis*). *Comparative Biochemistry & Physiology Part A Molecular & Integrative Physiology*, *139*, 433-440.

Flik, G., Fenwick, J.C., Kolar, Z., Mayer-Gostan, N. & Wendelaar Bonga, S.E. (1986). Effects of low ambient calcium levels on Ca2+ flux rates and internal calcium pools in the freshwater cichlid teleost, *Oreochromis mossambicus*. *Journal of Experimental Biology*, *120*, 249-264.

Gatlin, D.M., & Phillips, H.F. (1989). Dietary calcium, phytate and zinc interactions in channel catfish. *Aquaculture*, *79*, 259-266. https://doi.org/10.1016/0044-8486(89)90466-3

Hossain, M.A., & Furuichi, M. (2000a). Essentiality of dietary calcium supplement in redlip mullet *Liza haematocheila*. *Aquaculture Nutrition*, *6*, 33-38. https://doi.org/10.1046/j.1365-2095.2000.00123.x

Hossain, M.A. & Yoshimatsu, T. (2014). Dietary calcium requirement in fishes. *Aquaculture Nutrition*, *20*(1), 1-11. https://doi.org/10.1111/anu.12135

Hossain, M.A., & Furuichi, M. (1999). Necessity of dietary calcium supplement in black sea bream. *Fisheries Science*, *65*, 893-897. https://doi.org/10.2331/fishsci.65.893

Hossain, M.A., & Furuichi, M. (2000b). Necessity of calcium supplement to the diet of Japanese flounder. *Fisheries Science*, *66*, 660–664. https://doi.org/10.1046/j.1444-2906.2000.00130.x

Hossain, M.A., & Furuichi, M. (2000c). Essentiality of dietary calcium supplement in fingerling scorpion fish (*Sebastiscus marmoratus*). *Aquaculture*, *189*, 155-163. https://doi.org/10.1016/S0044-8486(00)00366-5

Hurwitz, S., & Griminger, P. (1961). The response of plasma alkaline phosphatase, parathyroids and blood and bone minerals to calcium intake in the fowl. *Journal of Nutrition*, *73*, 177-185. https://doi.org/10.1093/jn/73.2.177

Hwang, P.P., Tung, Y.C. & Chang, M.H. (1996). Effect of environmental calcium levels on calcium uptake in tilapia larvae (*Oreochromis mossambicus*). *Fish Physiology & Biochemistry*, *15*, 363-370. https://doi.org/10.1007/BF01875578

Ichikawa, R., & Oguri, M. (1961). Metabolism of radionucleotides in fish: I. Strontium-calcium discrimination in gill absorption. *Nippon Suisan Gakkaishi*, *27*, 351–357. https://doi.org/10.2331/suisan.27.351

Jensen, R., & Thursby, M. (1996). Water quality - determination of calcium content - edta titrimetric method. developed by technical committee iso/tc 147: water quality. 1. ed. *International Economic Review*, *37*(1), 21-49. https://doi.org/10.2307/2527244

Kader, M.A., Koshio, S., Ishikawa, M., Yokoyama, S., & Bulbul, M. (2010). Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. *Aquaculture*, *308*, 136-144. https://doi.org/10.1016/j.aquaculture.2010.07.037

Kavitha, C., Ramesh, M., Kumaran, S.S., & Lakshmi, S.A. (2012). Toxicity of Moringa oleifera seed extract on some hematological and biochemical profiles in a freshwater fish *Cyprinus carpio*. *Experimental & Toxicologic Pathology Official Journal of the Gesellschaft Fur Toxikologische Pathologie*, *64*, 681-687. https://dx.doi.org/ 10.1016/j.etp.2011.01.001.

Kim, S.S., & Lee, K.J. (2009). Dietary protein requirement of juvenile tiger puffer (*Takifugu rubripes*). *Aquaculture*, *287*, 219-222. https://doi.org/10.1016/j.aquaculture.2008.10.021

Kolar, C.S., Chapman, D.C., Courtenay, W.R., Williams, J.D., & Jennings, D.P. (2005). Asian carps of the genus hypophthalmichthys (*pisces, cyprinidae*). A biological synopsis and environmental risk assessment. *Molecular Vision*, *17*(12).

Kousoulaki, K., Fjelldal, P.G., Aksnes, A., & Albrektsen, S. (2010). Growth and tissue mineralisation of Atlantic cod (*Gadus morhua*) fed soluble P and Ca salts in the diet. *Aquaculture*, *309*, 181-192. https://doi.org/10.1016/j.aquaculture.2010.09.017

Lall, S.P. (2002). The minerals. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd ed. Academic Press, San Diego, CA, pp. 259-308.

Liang, J.J., Liu, Y.J., Yang, Z.N., Tian, L.X., Yang, H.J., & Liang, G.Y. (2012). Dietary calcium requirement and effects on growth and tissue calcium content of juvenile grass carp (*Ctenopharyngodon idella*). *Aquaculture Nutrition*, *18*(5), 544-550. https://doi.org/10.1111/j.1365-2095.2011.00916.x

Liu, B., Ge X.P., Xie J., Xu, P., He, Y.J., Cui, Y.T., Ming, J.H., Zhou, Q.L., & Pan, L.K. (2012). Effects of anthraquinone extract from Rheum officinale Bail on the physiological responses and HSP70 gene expression of *Megalobrama amblycephala* under *Aeromonas hydrophila* infection. *Fish & Shellfish Immunology*, *32*, 1-7. https://dx.doi.org/10.1016/j.fsi.2011.02.015.

Ministry of Agriculture of the People's Republic of China. (2016). Chinese fisheries yearbook. Chinese Agricultural Press, Beijing, China.

Nakamura, Y., & Yamada, J. (1980). Effects of dietary calcium levels, Ca/P ratios and calcium components on the calcium absorption rate in carp. Bulletin of the faculty of fisheries Hokkaido university, *31*, 277-282.

Nakamura, Y., (1982). Effects of dietary phosphorus and calcium contents on the absorption of phosphorus in the digestive tract of carp. *Nippon Suisan Gakkaishi*, *48*, 409-413. https://doi.org/10.2331/suisan.48.409

National Research Council (NRC). (2011). Nutrient requirement of fish and shrimp. National Academy Press, Washington, DC.

Nose, T., & Arai, S. (1979). Recent advances in studies on mineral nutrition in Japan. In: Advances in Aquaculture. Fishing News Books, Faraham, UK. pp. 584-590.

Ogino, C., & Takeda, H. (1976). Mineral requirements in fish. III. Calcium and phosphorus requirements in carp. *Nippon Suisan Gakkaishi*, *42*, 793-799. https://doi.org/10.2331/suisan.42.793

Ogino, C., & Takeda, H. (1978). Requirements of rainbow trout for dietary calcium and phosphorus. *Nippon Suisan Gakkaishi*, *44*, 1019-1022. https://doi.org/10.2331/suisan.44.1019

Phillips Jr, A.M. (1959). The known and possible role of minerals in trout nutrition and physiology. *Transactions of the American Fisheries Society*, *88*, 133-135. https://doi.org/10.1577/1548-8659(1959)88[133:TKAPRO]2.0.CO;2

Porn-Ngam, N., Satoh, S., Takeuchi, T., & Watanabe, T. (1993). Effect of the ratio of phosphorus to calcium on zinc availability to rainbow trout in high phosphorus diet. *Bulletin of the Japanese Society of Scientific Fisheries*, *59*, 2065-2070. https://doi.org/10.2331/suisan.59.2065

Ren, M.C., Habte-Tsion, H.M., Xie, J., Liu, B., Zhou, Q.L., Ge X.P., Pan, L.K., & Chen R.L. (2015). Effects of dietary carbohydrate source on growth performance, diet digestibility and liver glucose enzyme activity in blunt snout bream, *Megalobrama amblycephala*. *Aquaculture*, *438*, 75-81. https://doi.org/10.1016/j.aquaculture.2015.01.008

Ren, M.C., Liao, Y.J., Xie, J., Liu, B., Zhou, Q.L., Ge X.P., Cui, H.H., Pan, L.K., & Chen R.L. (2013). Dietary arginine requirement of juvenile blunt snout bream, *Megalobrama amblycephala*. *Aquaculture*, 414-415, 229-234. https://doi.org/10.1016/j.aquaculture.2013.08.021

Richardson, N.C., Higgs, D.A., Beams, R.A., & McBride, J.R. (1985). Influence of dietary calcium, phosphorus, zinc and sodium phytate in cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Nutrition*, *115*, 553-567. https://doi.org/10.1093/jn/115.5.553

Robinson, E.H., Rawles, S.D., Yette, H., Brown, P. & Greene, L.W. (1986) Calcium requirement of channel catfish reared in calcium-Free water. *Aquaculture*, *53*, 263-270. https://doi.org/10.1016/0044-8486(86)90356-X

Rodrigues, A.M.P., & Teles, A.O. (2001). Phosphorus requirements of gilthead sea bream (*Sparus aurata* L.) juveniles. *Aquaculture Research*, *32*, 157-161. https://doi.org/10.1046/j.1355-557x.2001.00013.x

Roy, P.K., & Lall, S.P. (2003). Dietary phosphorus requirement of juvenile haddock (*Melanogrammus aeglefinus* L.). *Aquaculture*, *221*, 451–468. https://doi.org/10.1016/S0044-8486(03)00065-6

Sakamoto, S., & Yone, Y. (1973). Effect of dietary calcium/phosphorus ratio upon growth, feed efficiency and blood serum Ca and P level in red sea bream. *Nippon Suisan Gakkaishi*, *39*, 343-348. https://doi.org/10.2331/suisan.39.343

Sanchez, C.C., Palacios, M.C.A., Perez, G.M., & Ross, L.G. (2000). Phosphorus and calcium requirements in the diet of the American cichlid *Cichlasoma urophthalmus* (Gunther). *Aquaculture Nutrition*, *6*, 1-9. https://doi.org/10.1046/j.1365-2095.2000.00118.x

Santiago, B.C.B., & Gonzal, A.C. (2000). Effect of prepared diet and vitamins A, E and C supplementation on the reproductive performance of cage-reared bighead carp *Aristichthys nobilis* (richardson). *Journal of Applied Ichthyology*, *16*(1), 8-13. https://doi.org/10.1046/j.1439-0426.2000.00137.x

Santiago, C.B., & Reyes, O.S. (1991). Optimum dietary protein level for growth of bighead carp (*Aristichthys nobilis*) fry in a static water system. *Aquaculture*, *93*(2), 155-165. https://doi.org/10.1016/0044-8486(91)90214-R

Shao, Q.J., Ma, J.J., Xu, Z.R., Hu, W.L., Xu, J.Z., & Xie, S.Q. (2008). Dietary phosphorus requirement of juvenile black sea bream, *Sparus macrocephalus*. *Aquaculture*, *277*, 92-100. https://doi.org/10.1016/j.aquaculture.2008.01.029

Song, J.Y., Zhang, C.X., Wang, L., Song, K., Hu, S.C., & Zhang, L. (2016). Effects of dietary calcium levels on growth and tissue mineralization in japanese seabass, *lateolabrax japonicus*. *Aquaculture Nutrition*, *23*(3): 637-648.

Song, L.P., Mao, S.Q., Wang, A.Y., Zhu, Y.A., & Han, B. (2009). Effects of dietary calcium and phosphorus on growth and body composition of juvenile *Scortum bacoo*. *Journal of Fisheries of China*, *33*, 488-495.

Spinelli, J., Houle, C.R., & Wekell, J.C. (1983). The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing various quantities of calcium and magnesium. *Aquaculture*, *30*, 71-83. https://doi.org/10.1016/0044-8486(83)90153-9

Tong, J., & Sun, X. (2015). Genetic and genomic analyses for economically important traits and their applications in molecular breeding of cultured fish. *Science China Life Science*, *58*(2), 178-186. https://doi.org/10.1007/s11427-015-4804-9

Urasa, F.M., & Wendelaar Bonga, S.E. (1987). Effects of calcium and phosphate on the corpuscles of Stannius of the teleost fish, *Oreochromis mossambicus*. *Cell & Tissue Research*, *249*, 681-690. https://doi.org/10.1007/BF00217340

Vielma, J., & Lall, S.P. (1998). Phosphorus utilization by Atlantic salmon, *Salmo salar*, reared in freshwater is not influenced by higher dietary calcium intake. *Aquaculture*, *16*, 117-128. https://doi.org/10.1016/S0044-8486(97)00300-1

Vonck, A.P.M.A., Wendelaar Bonga, S.E. & Flik, G. (1998). Sodium and calcium balance in Mozambique tilapia, *Oreochromis mossambicus*, raised at different salinities. *Comparative Biochemistry & Physiology*, *119*, 441-449. https://doi.org/10.1016/S1095-6433(97)00450-9

Ye, C.X., Li, Y.J., Tian, L.X., Mai, K.S., Du, Z.Y., Yang, H.J., & Niu, J. (2006). Effect of dietary calcium and phosphorus on growth, feed efficiency, mineral content and body composition of juvenile grouper, *Epinephelus coioides*. *Aquaculture*, 255, 263-271. https://doi.org/10.1016/j.aquaculture.2005.12.028

Zhang, C.X., Mai, K.S., Ai, Q.H., Zhang, W.B., Duan, Q.Y., Tan, B.P., Ma, H.M., Xu, W., Liufu, Z.G., & Wang, X.J. (2006). Dietary phosphorus requirement of juvenile Japanese seabass, *Lateolabrax japonicus*. *Aquaculture*, *255*, 201-209. https://doi.org/10.1016/j.aquaculture.2005.11.040