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# Effects of Dietary L-carnitine and Betaine on Serum Biochemical Indices of Large Yellow Croaker (*Pseudosciaena crocea*) Cultured in Floating Net Cages

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**Abstract:** The objective of this study was to investigate the biochemical changes of large yellow croaker as affected by dietary supplements. Large yellow croaker (*Pseudosciaena crocea*) is a marine species that is widely cultured in China due to its high commercial value. However, the cage-cultured large yellow croakers were found to be less tasty compared with wild large yellow croakers due to high lipid in their body, which significantly impacts the commercial markets. Triglycerides, cholesterol and free fatty acids are main lipid ingredients in the animal body. The fish were fed with basal diet or basal diet supplemented with L-carnitine (0.08% of dry weight diet) or betaine (0.8% of dry weight diet) for 12 weeks in seawater floating net cages (3×2×1.5 m) each holding 60 fishes. Three net cages were assigned to each dietary treatment, as replications. The seawater temperature ranged from 18 to 31°C and salinity from 25 to 28 g/kg. Fish were hand-fed one of the experimental diets to apparent satiety twice daily (05:00 and 17:30) throughout the 12 week experimental period. The results indicate that L-carnitine or betaine in diets significantly reduced Serum Triglyceride (STG) and Serum Cholesterol (SCH) levels while increased Serum Free Fatty Acids (SFFA) content ($p<0.05$). The diets of L-carnitine or betaine supplements on serum biochemical indices of that fish species have positive effects. These results suggested that the supplementation with L-carnitine or betaine is one of the effective ways to improve the meat quality of large yellow croakers cultured in floating net cages.

**Keywords:** Cholesterol, free fatty acids, large yellow croaker, *Pseudosciaena crocea*, triglyceride

## INTRODUCTION

L-carnitine is a water-soluble quaternary amine and plays an important role in lipid β-oxidation to facilitate the importation of activated long-chain fatty acids into mitochondria and the accompanying intermediate compounds out of the mitochondrial matrix (Rebouche and Seim, 1998; Harpaz, 2005). Due to its role in lipid metabolism in fish, dietary carnitine supplementation has been found to enhance protein synthesis and promote growth performance (Ozorio *et al*., 2001). During the past two decades, the effects of L-carnitine supplementation on fish culture and nutrition have been studied in different cultured fish species. Some examples are European seabass (*Dicentrarchus labrax*) (Dias *et al*., 2001) and tilapia hybrids (*Oreochromis niloticus-Oreochromis aureus*) (Schlechtriem *et al*., 2004; Yang *et al*., 2009).

Betaine is an oxidized form of choline and a detectable flavor when added to diets for some species of fish. Betaine not only entices some aquatic animals to eat, but absorbed betaine accumulates in muscles cells and may be of benefit in fish exposed to changing salinities and is an important component in the sulfur amphetamine catabolic pathway (Clarke *et al*., 1994; Castro *et al*., 1998).

Large yellow croaker (*Pseudosciaena crocea*) is a marine species that is widely cultured in China due to its high commercial value. The annual quantity cultured in seawater net cages reached 66,000 metric ton in 2009 and 87,000 metric ton in 2010 (Fisheries Bureau of Ministry of Agriculture and China, 2011). However, the cage-cultured large yellow croakers were found to be less tasty compared with wild large yellow croakers, which significantly impacts the commercial markets. There have been a number of studies on large yellow croaker larvae (Yu *et al*., 2003; Ma *et al*., 2005; Liu *et al*., 2006; Mai *et al*., 2006a, b; Ai *et al*., 2008; Zhao *et al*., 2008; Wang *et al*., 2010). Most studies emphasized on growth rates and food conversion efficiencies, almost none have considered the potential effects of feeding supplements on the meat quality of fish. Furthermore, the contribution of dietary supplements with L-carnitine or betaine to change the fish lipid ingredients has not yet been reported. Therefore, the objective of the present study was to determine the effects of dietary supplementation with L-carnitine and betaine on serum biochemical indices of the cage-cultured large yellow croakers.

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Table 1: Formulation and proximate composition of experimental diets (% of Dry Matter, DM)

| Ingredients | Control | L-carnitine | Betaine |
|---|---|---|---|
| Fish meal[1] | 42 | 42 | 42 |
| Soybean meal | 16 | 16 | 16 |
| Wheat flour | 32. 75 | 32. 67 | 31.95 |
| Fish oil | 5.2 | 5.2 | 5.2 |
| L-carnitine | 0 | 0.08 | 0 |
| Betaine | 0 | 0 | 0.8 |
| Vitamin premix[2] | 2 | 2 | 2 |
| Mineral premix[3] | 2 | 2 | 2 |
| Ethoxyquin | 0.05 | 0.05 | 0.05 |
| Proximate composition (% of DM) | | | |
| Crude protein | 44.52±0.32 | 44.54±0.37 | 44.30±0.28 |
| Crude lipid | 9.87±0.087 | 9.43±0.030 | 9.46±0.120 |
| Ash | 10.37±0.03 | 10.37±0.05 | 10.44±0.02 |
| Moisture | 10.27±0.00 | 10.87±0.02 | 11.09±0.08 |

[1]: Fish meal: crude protein 65.5-69.5%, crude lipid 6.5-8.5%; [2]: Vitamin premix (mg or g/kg 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, niacin acid 200 mg, folic acid 20 mg, biotin 1.2 mg, retinalacetate 32 mg, cholecalciferol 5 mg, α-tocopherol 120 mg, ascorbic acid 2000 mg, choline chloride 2000 mg, ethoxyquin 150 mg, wheat middling 14.52 g; [3]: Mineral premix (mg or g/kg 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, $MnSO_4 \cdot H_2O$ 60 mg, $MgSO_4 \cdot 7H_2O$ 1200 mg, $Ca(H_2PO_4)_2 \cdot H_2O$ 3000 mg, NaCl 100 mg, zoelite 15.45 g

## MATERIALS AND METHODS

**Experimental diets:** The ingredients and proximate compositions of the experimental fish diets are presented in Table 1. Control (the basal diet) was formulated to contain a combination of fish meal, soybean meal, flour and fish oil, and then supplemented with L-carnitine (0.08% L-carnitine of dry weight diet) or Betaine (0.8% betaine of dry weight diet) respectively (Table 1). The grounded ingredients were thoroughly blended in a food mixer and then homogenized after fish oil was added. The mixture was further homogenized and extruded into proper pellets (ϕ5.0×5.0 mm) by a food extruder. The pellets were warm air dried until their water activity below 0.72 and then stored at -15 to -18°C until the time of use.

**Experimental procedures:** Large yellow croaker (*Pseudosciaena crocea*) obtained from a commercial farmer. The experiments were conducted at Xihu Bay of Ningbo, Zhejiang Province, China (located in 121° east longitude and 29° north latitude) during June to October, 2010. Before the feeding trial, fish with an average weight of about 300 g were collected and randomly grouped into seawater floating net cages (3×2×1.5 m) each holding 60 fishes. They were then conditioned with the basal diet for two weeks. Three net cage replicates were assigned to each dietary treatment. The seawater temperature ranged from 18 to 31°C and salinity from 25 to 28 g/kg. Fish were hand-fed one of the experimental diets to apparent satiety twice daily (05:00 and 17:30) throughout the 12 week experimental period. The net cages were washed once in the middle of the period to remove the plankton attached to the net.

**Sample collection and preparation:** At the initiation of sample collection, fish were fasted for 24 h. Eight fish from each net cage were randomly selected and anaesthetized with MS-222 (Sigma Chemical, St Louis, MO, USA). Fish weight and body length were individually measured and recorded, and a blood sample was drawn from the caudal vein with heparinized syringes. The whole blood was centrifuged at 3300 × g for 15 min and the supernatant was stored at -20°C until further analyses.

**Analytical methods:** Measurement of feed water activity on line using water activity dynamic detection device (developed in this study) and Ms1 water activity detector (Novasina Co., Switzerland).

Crude protein, ash, moisture and lipid contents of fish diets were determined according to the official methods of analysis of official analytical chemists (AOAC, 1995). Crude protein was calculated through converting the nitrogen content (6.25×N). Ash was determined by combustion at 550°C for 12 h. Moisture content was analyzed by oven drying at 105°C for 24 h. Lipids were extracted using a Soxhlet method.

Serum triacylglycerol, cholesterol and free fatty acids were analyzed using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) with diagnostic kits (Nanjing Biological Institute, Nanjing, China) according to the manufacturer's instructions.

**Calculations:** The variables were calculated with the following equations:

$$\text{SGR (specific growth rate, \% d}^{-1}) = (\text{Ln}W_2 - \text{Ln}W_1/t) \times 100 \quad (1)$$

$$\text{CF (condition factor, g cm}^{-3}) = W_2/(L_2)^3 \quad (2)$$

$$\text{HSI (hepatosomatic index, \%)} = (W_L/W_2) \times 100 \quad (3)$$

$$\text{VSI (viscerasomatic index, \%)} = (W_V/W_2)\ 100 \quad (4)$$

where,

$W_2$ & $W_1$ = Final and initial wet fish weight, g
t = Duration of experimental days, d
$L_2$ = Final fish body length, cm
$W_L$ & $W_V$ = Liver wet weight and viscera wet weight, g

**Statistical analysis:** Significant differences between groups were determined by a one-way Analysis of Variance (AVONA) followed by Duncan test. Statistical analysis was performed using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as means±standard deviation (S.D.). Mean values were considered significantly different at $p<0.05$.

## RESULTS AND DISCUSSION

Fish body weight measured as weight gain is of great importance to fish farmers. The growth promoting effects of L-carnitine supplementation in fish feeds have been attributed to the increase in utilization of energy as a result of the increase in fatty acid oxidation by the mitochondria. The phenomenon of growth promoting and fatty acid oxidation increasing has been demonstrated in isolated mitochondria of trout (Bilinski and Jonas, 1970). The results in present study also agree with the phenomenon (Table 2 and 3). As shown in Table 2, after 12 weeks of feeding trial, Specific Growth Rate (SGR) significantly increased from 1.63 to 1.69 and 1.67%, respectively in the diet group containing L-carnitine and betaine ($p<0.05$). Condition Factor (CF) decreased in L-carnitine group while it increased in betaine group, but the differences were also not significant ($p>0.05$). Diets with L-carnitine or betaine also reduced Hepatosomatic Index (HSI) and Viscerasomatic Index (VSI), although the differences were not significant ($p>0.05$). Table 3 shows the effect of diets on the triglyceride, cholesterol and free fatty acids levels in fish serum. The diets with L-carnitine and betaine significantly reduced Serum Triglyceride (STG) from 3.14 to 2.67 and 1.78 mmol/L, and reduced Serum Cholesterol (SCH) from 5.07 to 4.42 and 4.06 mmol/L, respectively ($p<0.05$). Both diet groups with L-carnitine and betaine significantly promoted the generation of Serum Free Fatty Acids (SFFA) from 407.51 to 514.39 and 486.48 μmol/L, respectively ($p<0.05$).

Serum triglyceride content changes with diets, which was mainly synthesized in the liver of a living organism and adipose tissue, but also by the intestinal absorption from food. Serum triglyceride mainly is existed in Very Low Density Lipoprotein (VLDL) and Chylomicron (CM). The clinical detection of serum triglyceride concentrations can be used for nutritional evaluation. Normal value of human serum triglyceride: 0.22-1.65 mmol/L (Baike, 2012a). Serum cholesterol refers to the total amount of cholesterol present in blood. Cholesterol is produced by the body and is found in the foods. Cholesterol is a waxy substance that circulates in the blood, and it is found in most of the body's cells. However, if the level of serum cholesterol gets too high, it may be harmful for a living organism. Normal value of human serum cholesterol: 2.9-6.0 mmol/L for adult; 3.1-5.2 mmol/L for children (Baike, 2012b). However there is no normal value of fish serum triglyceride or cholesterol. The result of present study indicates that L-carnitine and betaine in diets significantly ($p<0.05$) reduced serum triglyceride or cholesterol of large yellow croaker cultured in seawater floating net cages referencing the normal value of human serum triglyceride. That agrees with some findings reported by previous researchers. Tian *et al*. (2009) reported effect of dietary carnitine on body composition and lipid metabolism enzymes of grass carp (*Ctenopharyngodon idella*). The results showed that the addition of carnitine could improve the growth performance, decrease muscle and hepatopancreas fat content, promote lipid degradation. The proper dose of carnitine in grass carp diet was suggested to be 200 mg/kg. Lu *et al*. (2003) reported effects of betine hydrochloride on lipid metabolism of black pacu (*Colossoma brachypomum*). The results showed that adding betaine hydrochloride decreased moisture and crude fat contents of whole fish, decreased the moisture of fish muscle. Betaine hydrochloride decreased liver lipid significantly. The serum biochemical indexes indicated that the cholesterol and triglyceride concentration decreased significantly; lipase level of

Table 2: Effects of dietary L-carnitine or betaine on growth and biometric parameters in large yellow croaker

| Parameters | Control | L-carnitine | Betaine |
|---|---|---|---|
| $W_1$ (g)[1] | 302.24±10.45 | 302.67±9.78 | 300.98±6.35 |
| $W^2$ (g)[1] | 413.28±11.43 | 429.41±7.98 | 427.45±9.56 |
| SGR (%/d)[2] | 1.63±0.02[a] | 1.69±0.03[b] | 1.67±0.01[b] |
| CF (g/cm$^3$)[3] | 1.65±0.16 | 1.42±0.14 | 1.74±0.13 |
| HSI (%)[4] | 1.42±0.16 | 1.31±0.05 | 1.25±0.03 |
| VSI (%)[5] | 3.91±0.54 | 3.74±0.09 | 3.34±0.11 |

The values are mean±S.D. (n = 3); The different letters in the same row indicate significant differences ($p<0.05$); [1]:$W_2$ and $W_1$ were final and initial wet fish weight in gram respectively; [2]: SGR (Specific Growth Rate, %/d) = 100× ( $LnW_2$ - $LnW_1$) /t where, t = duration of experimental days; [3]: CF (Condition Factor, g/cm$^3$) = $W_2$/ $(L_2)^3$ where, L2 = final fish body length in cm; [4]: HSI (Hepatosomatic Index, %) = 100× liver wet weight (g) /$W_2$; [5]: VSI (Viscerasomatic Index, %) = 100× viscera wet weight (g) /$W_2$

Table 3: Effect of L-carnitine or betaine on serum parameters of large yellow croaker

| Parameters | Control | L-carnitine | Betaine |
|---|---|---|---|
| Triglyceride (mmol/L) | 3.14±0.34[c] | 2.67±0.25[b] | 1.78±0.21[a] |
| Cholesterol (mmol/L) | 5.07±0.54[b] | 4.42±0.46[a] | 4.06±0.59[a] |
| Free fatty acids (μmol/L) | 407.51±40.75[a] | 514.39±56.21[b] | 486.48±49.23[b] |

The values are mean±S.D. (n = 3); The different letters in the same row indicate significant differences ($p<0.05$)

0.8% betaine hydrochloride group increased. The result of mesenterium lipase activity showed that different levels of betaine hydrochloride had different effects on serum biochemical indexes.

Serum Free Fatty Acids (SFFA) levels provide an important measurement of the physiologic state. Most carnitine and betaine have an effect on lipolysis of large yellow croaker cultured in floating net cages.

L-carnitine is most concentrated in tissues that use fatty acids as their primary dietary fuel, such as skeletal and cardiac muscle. In this regard, L-carnitine plays an important role in energy production by chaperoning activated fatty acids (acyl-CoA) into the matrix for and accompanying intermediate compounds out of the mitochondrial matrix to prevent their accumulation. Carnitine-acylcarnitine translocase is responsible for the transport of carnitine and its esters across the inner mitochondrial membrane (Fritz and Yue, 1963). Carnitine is therefore a normal constituent of animal tissues and plasma, which is required for the transport of long-chain fatty acids to the site of oxidation. Carnitine also facilitates removal of short-chain organic acids from mitochondria, thereby freeing intramitochondrial coenzyme A to participate in the h-oxidation and tricarboxylic acid cycle pathways. It is a substrate for carnitine palmitoyltransferases I and II and carnitine acetyltransferase, enzymes that participate in and regulate fatty acid utilization (Borum, 1987).

## CONCLUSION

In conclusion, the results presented in present study show that dietary supplementation with L-carnitine or betaine can affect serum biochemical indices of large yellow croaker cultured in floating net cages. Supplementation with L-carnitine or betaine is one of the effective ways to improve the meat quality of cultured large yellow croakers.

For the future studies, sensory evaluation on fish meat will be carried out to confirm the taste quality as affected by dietary L-carnitine or betaine supplementation, and the combination of L-carnitine with betaine in one diet will also be conducted to investigate any synergistic effect on growth performance, body composition and meat quality of cultured large yellow croaker.

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of the FFA in serum is bound to albumin. The elevation of serum free fatty acid means lipolysis increasing. The normal value of human serum free fatty acids: 400-900 μmol/L (Wenku, 2012). In the present study, both diet groups with L-carnitine and betaine can increase Serum Free Fatty Acids (SFFA). These shows the diets of L-

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