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Effect of dietary betaine on growth performance, antioxidant capacity and lipid metabolism in blunt snout bream fed a high-fat diet

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Abstract An 8-week feeding experiment was conducted to determine the effect of dietary betaine levels on the growth performance, antioxidant capacity, and lipid metabolism in high-fat diet-fed blunt snout bream (*Megalobrama amblycephala*) with initial body weight 4.3 ± 0.1 g [mean ± SEM]. Five practical diets were formulated to contain normal-fat diet (NFD), high-fat diet (HFD), and high-fat diet with betaine addition (HFB) at difference levels (0.6, 1.2, 1.8%), respectively. The results showed that the highest final body weight (FBW), weight gain ratio (WGR), specific growth rate (SGR), condition factor (CF), and feed intake (FI) ($P < 0.05$) were obtained in fish fed 1.2% betaine supplementation, whereas feed conversion ratio (FCR) was significantly lower in the same group compared to others. Hepatosomatic index (HSI) and abdominal fat rate (AFR) were significantly high in fat group compared to the lowest in NDF and 1.2% betaine supplementation, while VSI and survival rate (SR) were not affected by dietary betaine supplementation. Significantly higher ($P < 0.05$), plasma total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), aspartate transaminase (AST), alanine transaminase (ALT), cortisol, and lower high-density lipoprotein (HDL) content were observed in HFD but were improved when supplemented with 1.2% betaine. In addition, increase in superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in 1.2% betaine inclusion could reverse the increasing malondialdehyde (MDA) level induced by HFD. Based on the second-order polynomial analysis, the optimum growth of blunt snout bream was observed in fish fed HFD supplemented with 1.2% betaine. HFD upregulated fatty acid synthase messenger RNA (mRNA) expression and downregulated carnitine palmitoyltransferase 1, peroxisome proliferator-activated receptor α, and microsomal triglyceride transfer protein mRNA expression; nevertheless, 1.2% betaine supplementation significantly reversed these HFD-induced effects, implying suppression of fatty acid synthesis, β-oxidation, and lipid transport. This present study indicated that inclusion of betaine (1.2%) can significantly improve growth performance and antioxidant defenses, as well as reduce fatty acid synthesis and enhance mitochondrial β-oxidation and lipid transportation in high-fat diet-fed blunt snout bream, thus effectively alleviating fat accumulation in the liver by changing lipid metabolism.

Keywords Betaine · *Megalobrama amblycephala* · Growth performance · Lipid metabolism · Antioxidant capacity · Gene

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Introduction

In general, feed is regarded as the main cost of the aquaculture industry. This is due to the dependence on high-cost protein sources such as fish meal which come

from marine animals to meet the high dietary protein requirements of fish (Gui et al. 2010). As the demand for fish meal is increasing with the rapid development of the aquaculture industry, global yield of fish meal is failing to meet the production demand in the feeding industry. This situation has given rise to studies on finding alternative sources of high-quality additives to replace the high-cost fish meal. In recent years, several sources of additives such as plant protein, dietary lipid, and probiotic have been experimented on different fish species to either partially or completely replace the more expensive fish meal (Allen Davis and Arnold 2000; Aksnes et al. 2008; Ronnestad et al. 2003).

In fish nutrition, dietary lipids play a major role by supplying energy, essential fatty acids (EFAs), and phospholipids (Sargent et al. 1999; Watanabe 1982). As Li et al. (2012a) reports, increase in dietary lipid content can enhance feed efficiency and growth performance in fish. Thus, fat-rich diets have been extensively used in the intensive fish farming system. However, excess dietary lipid often leads to unwanted fat deposition in the liver (Du et al. 2006; Lu et al. 2013), resulting in high mortality rate, poor growth performance, and immune suppression of the fish (Bolla et al. 2011; Lu et al. 2014a). In order to prevent excessive lipid deposition, various ways have been researched by fish nutritionist. Some additives have been experimented and successfully used in controlling excess fat accumulation in the liver, and they have been shown to regulate the abnormal expression of key genes involved in lipid metabolism, i.e., berberine (Chen et al. 2016), choline (Zhou et al. 2015), zinc (Kang et al. 2009), and in grape seed proanthocyanidins (Quesada et al. 2009).

Betaine is a naturally occurring tertiary amine (trimethylglycine) present in animals, plants, and microorganisms, and it is a methyl derivative of the amino acid glycine with the chemical formula $(CH_3)_3N$ + CH_2COO- and a molecular weight of 117.2 (Patel and Mehta 2015). It is characterized as a methylamine because of its three chemically reactive methyl groups. The rich dietary sources include seafood, beets, broccoli, spinach, as well as grains such as wheat germ and bran. The main physiologic role of betaine is as an osmolyte and methyl donor (transmethylation), which, in turn, may be used for the synthesis of methionine, carnitine, phosphatidyl choline, and creatine, and plays a key role in protein and energy metabolism (Sheard and Zeisel 1989). As an osmolyte, betaine protects cells, proteins, and enzymes from environmental stress (e.g., low water, high salinity, or extreme temperature). Betaine has been found to protect the liver from the damaging effects of CCl_4 (Junnila et al. 2000). It has been revealed that betaine regulates erythrocyte (red blood cell) membrane ATPases through conformational changes, which results in cell volume control (Moeckel et al. 2002). In mammals, numerous studies have documented the protective effects of betaine on antioxidant status (Ganesan et al. 2010), mitochondrial function (Ganesan et al. 2007a), protein and glycoprotein metabolism, and lipid metabolism in isoprenaline-induced myocardial infarction in Wistar rats (Ganesan et al. 2007b). Whereas in livestock, betaine supplementation in diet reduces fat deposition in pigs (Huang et al. 2008), meat ducks (Wang et al. 2000, 2004), and laying hens (Zou and Jian-Jun 2002). While in broilers, both abdominal adipose and percent abdominal adipose were decreased in chicken fed with diets containing 0.06% betaine (Esteve-Garcia and Mack 2000; Mcdevitt et al. 2000). Betaine supplementation has also proven to decrease the activities of acetyl-CoA carboxylase, fat acid synthase, malic enzyme, and the messenger RNA (mRNA) levels of fatty acid synthase (FAS) gene in abdominal adipose tissue in finishing pigs (Huang et al. 2008).

In aquaculture, betaine can protect cells against dramatic changes in osmotic pressure, since it is related to osmoregulation and methyl donation. Moreover, studies have shown a positive effect of betaine as a flavor component, acting as a dietary feeding attractant in feed leading to improved growth in some fish species such as *Sparus auratus* (Kolkovski et al. 1997), *Morone saxatilis* (Papatryphon and Soares Jr. 2000), *Oreochromis niloticus* (Kasper et al. 2002), *Carassius auratus gibelio* (Xue and Cui 2001), red sea bream (Goh and Tamura 1980), and with the European eel (*Anguilla anguilla*) (Mackie and Mitchell 2006). Moreover, dietary betaine has also shown to have sparing effect on the dietary requirement for choline and methionine in rainbow trout (Rumsey 1991).

Blunt snout bream (*Megalobrama amblycephala*), commonly known as Wuchang bream, is an herbivorous freshwater fish species native to China. It has also been introduced to North America (north Canada to southern Mexico), Africa, and Eurasia (Habte-Tsion et al. 2013). This fish has been recognized as a main aquaculture species in the Chinese freshwater polyculture system with high economic value species. In 2012, its production level was approximately 0.7 million tons (Ministry

of Agriculture of the People's Republic of China 2013). Due to its fast growth rate, adaptability to local environment conditions, high larval survival rate, compatibility with native species, disease resistance, and tender flesh, blunt snout bream has been regarded as a good candidate species for aquaculture (Zhou et al. 2008). Nevertheless, compared to a number of other commercially produced fishes, its artificial rearing often suffers from liver steatosis, which correlates directly with a high rate of mortality or poor growth (Lu et al. 2013). To better understand the biological processes of excess fat accumulated in liver in this species, and identify a good additive for therapeutic intervention, this study was carried out to determine the effect of dietary betaine supplementation on growth performance, antioxidant capacity, and lipid metabolism in fingerling blunt snout bream fed high-fat diet.

Materials and methods

Fish and the feeding trial

Healthy blunt snout bream fingerlings were obtained from Yangzhou Fish Hatchery (Jiangsu, China). Prior to the experiment, fish were acclimatized for 2 weeks during which they were fed with a commercial diet. After the acclimation, fish of similar size (4.3 ± 0.1 g [mean ± SEM]) were stocked into 15 tanks at a stocking density of 25 fish per tank. The experimental diets were assigned to the tanks in a completely randomized design and each replicated three times. Fish were hand-fed to apparent satiation three times daily (08:30, 12:30, and 16:30 h) for 8 weeks. During the entire experimental period, all the water quality parameters were monitored and kept within the optimum ranges as follows: water temperature ranged from 26 to 28 °C, dissolved oxygen (DO) of ≥6 mg/l, pH of 7.2–7.6, total ammonia nitrogen of 0.02–0.04 mg/l, and photoperiod of 12 h (dark/light). To further maintain good water quality, water was changed three times a week.

Experimental design and diets

All feed ingredients were analyzed for proximate composition, and the data obtained were used as a basis for the feed formulation. Fish meal, soybean meal, cottonseed meal, and rapeseed meal were used as protein sources. In each diet, equal portion of fish oil and soybean oil was used as lipid sources (Table 1). Wheat flour was used as carbohydrate source. Five practical diets were formulated to contain normal-fat diet (NFD), high-fat diet (HFD), and high-fat diet with betaine addition (HFB) at difference levels (0.6, 1.2, 1.8%), respectively. Feed ingredients were ground into fine powder then completely mixed and blended oil and sufficient water to form soft dough. And then, dough was pelleted and air dried. The pellets were stored at −20 °C until used. The ingredients and composition of the diets are given in Table 1.

Sampling and analysis

At the end of the feeding trial, fish were starved for 24 h to evacuate the alimentary tract contents prior to sampling. Thereafter, 15 fish from each replicate were anesthetized in diluted MS-222 (tricaine methanesulfonate, Sigma, USA) at a concentration of 100 mg/l for sampling. Blood was quickly drawn from the caudal vein and then transferred immediately to heparinize capillary tubes and shaken gently in order to avoid hemolysis, and thereafter centrifuged at 2500 rpm at 4 °C for 10 min. The supernatant was then stored at −80 °C for subsequent analysis. It should be mentioned that blood sampling of fish was executed in the morning around 7:00 a.m., and each group was sampled at equally timed interval (about 10 min for each group). Also, individual liver sample was quickly removed and stored at −80 °C for subsequent assays.

Growth performance

At the end of the feeding trial, body weight and length of the fish were recorded and data collected were used in the following equations to calculate growth performance and feed utilization:

$$\textit{Weight growth rate}\ (\mathrm{WGR})\ (\%) = 100 \times (W_2 - W_1)/W_1$$

$$\textit{Specific growth rate}\ (\mathrm{SGR})\ (\%) = [\ln(W_2) - \ln(W_1)/t] \times 100$$

$$\textit{Hepatosomatic index}\ (\mathrm{HIS})\ (\%) = [\textit{weight of liver}\ (\mathrm{g})/W\ (\mathrm{g})] \times 100$$

$$\textit{Viscerosomatic index}\ (\mathrm{VSI})(\%) = [\textit{visceral weight}\ (\mathrm{g})/W\ (\mathrm{g})] \times 100$$

$$\textit{Condition factor}\ (\mathrm{CF}) = \left(W/L^3\right) \times 100$$

$$\textit{Feed conversion ratio}\ (\mathrm{FCR}) = \textit{consumed feed}\ (\mathrm{g})/\textit{weight gain}\ (\mathrm{g})$$

$$\textit{Abdominal}\ \mathrm{fat}\ \textit{rate}\ (\mathrm{AFR})\ (\%) = \textit{abdominal}\ \mathrm{fat}/W \times 100$$

$$\textit{Survival rate}\ (\mathrm{SR})\ (\%) = 100 \times (\textit{final number of fish}/\textit{initial number of fish})$$

where W is weight of body, W_2 is final weight, W_1 is initial weight, t is the period of the trial, and L is total length.

Proximate analysis

Experimental diets and whole fish were analyzed for proximate composition based on the standard AOAC method (AOAC and Chemists 1990). Moisture was determined by oven drying at 105 °C until constant weight. Crude protein (nitrogen × 6.25) was determined by the Kjeldahl method using an Auto Kjeldahl System (FOSS KT260, Switzerland), crude lipid by ether extraction using Soxtec System HT (Soxtec System HT6, Tecator, Sweden), and ash by combustion at 550 °C for 4 h.

Analysis of liver antioxidant status

Liver samples were homogenized on ice in 10 volumes (*v*/*w*) in a tissue homogenizer and centrifuged at 3000 rpm at 40 °C for 10 min. The supernatants were separated in aliquots and stored at −70 °C for subsequent analysis. Superoxide dismutase (SOD) activity was measured following the methods described by Wang and Chen (2005). Catalase (CAT) and reduced glutathione (GSH) were determined enzymatically with a commercial kit (Nanjing Jian Cheng Bioengineering Institute, China). The malondialdehyde (MDA) concentration was determined by the thiobarbituric acid test according to the published protocol by Zhang et al. (2008).

Determination of biochemical parameters

Plasma triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate transminase (AST), and alanine transaminase (ALT) contents were analyzed within 24 h of sampling using commercial assay kits produced by Jian Cheng Bioengineering Institute (Nanjing, China). Plasma cortisol level was measured using a validated and characterized radioimmunoassay.

Total RNA extraction, reverse transcription, and real-time PCR

Total RNA was extracted from the liver tissue using RNAiso Plus (Takara Co. Ltd., Dalian, China). RNA samples were treated by RQ1 RNase-Free DNase prior to RT-PCR (Takara Co. Ltd., Dalian, China) to avoid genomic DNA amplification. Complementary DNA (cDNA) was generated from 500 ng DNase-treated RNA using ExScript™ RT-PCR kit (Takara Co. Ltd., Dalian, China), and the mixture consisted of 500 ng RNA, 2 μl buffer (5×), 0.5 μl dNTP mixture (10 mM each), 0.25 μl RNase inhibitor (40 U/μl), 0.5 μl dT-AP primer (50 mM), 0.25 μl ExScript™ RTase (200 U/μl), and DEPC H_2O, with total volume up to 10 μl. The reaction conditions were as follows: 42 °C for 40 min, 90 °C for 2 min, and 4 °C thereafter. Real-time PCR was employed to determine mRNA levels based on the SYBR® Green I fluorescence kit. Specific primers were designed using Primer 5.0 version (Table 7). Primer characteristics used for real-time PCR are listed in the Supplementary Material. Real-time PCR was performed in a Mini Option real-time detector (Bio-Rad, USA). The RT-qPCR reactions were carried out in a final volume of 20 μl, containing 10 μl 1× SYBR Premix Ex Taq™, 0.4 μM of each primer, 0.4 μl ROX, 6.8 μl DEPC water, and 2 μl of cDNA template. The reactions were initially denatured at 95 °C for 10 min and then 40 cycles at 95 °C for 15 s, followed by annealing at 60 °C for 34 s. To assess the specificity of each amplicon, the melt curve analysis of 5 s per step from 65 to 95 °C was performed at the end of each PCR thermal profile. All amplicons were initially separated by agarose gel electrophoresis to ensure that they were

Table 1 Ingredients of the experimental diets fed to blunt snout bream

	Diets				
	NFD	HFD	HFB (0.6%)	HFB (1.2%)	HFB (1.8%)
Ingredients (DM basis %)					
Fish meal	6.50	6.50	6.50	6.50	6.50
Soybean meal	22.13	25.85	25.85	25.85	25.85
Rapeseed meal	10.68	10.68	10.68	10.68	10.68
Cottonseed meal	10.02	10.02	10.02	10.02	10.02
Wheat bran	16.00	5.87	5.27	4.67	4.07
Wheat flour	28.60	28.60	28.60	28.60	28.60
Fish oil	1.49	4.69	4.69	4.69	4.69
Soybean oil	1.49	4.69	4.69	4.69	4.69
Calcium biphosphate	1.80	1.80	1.80	1.80	1.80
Premix	1.00	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30	0.30
Betaine	0.00	0.00	0.60	1.20	1.80
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)					
Crude lipid	5.54	11.35	11.61	11.54	11.65
Crude protein	30.02	30.17	30.09	30.43	31.71
Ash	7.18	7.00	6.88	6.85	6.86
Moisture	8.33	7.65	7.96	8.56	7.17

Premix supplied the following minerals (g/kg) and vitamins (IU or mg/kg): $CuSO_4 \cdot 5H_2O$, 2.0 g; $FeSO_4$ $7H_2O$, 25 g; $ZnSO_4 \cdot 7H_2O$, 22 g; $MnSO_4 \cdot 4H_2O$, 7 g; Na_2SeO_3, 0.04 g; KI, 0.026 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; vitamin A, 900,000 IU; vitamin D, 200,000 IU; vitamin E, 4500 mg; vitamin K3, 220 mg; vitamin B1, 320 mg; vitamin B2, 1090 mg; niacin, 2800 mg; vitamin B5, 2000 mg; vitamin B6, 500 mg; vitamin B12, 1.6 mg; vitamin C, 5000 mg; pantothenate, 1000 mg; folic acid, 165 mg; choline, 60,000 mg

of correct size. A dissociation curve was determined during the PCR program to make sure that specific products were obtained in each run. At the end of the reaction, the fluorescent data were converted into C_t values. To calculate relative expression levels, blunt snout bream β-actin was used as internal control to normalize the C_t value in each sample, and the relative expression levels under different experimental diets were calculated by $2^{-\triangle\triangle Ct}$ method.

Statistical analysis

Data were compared by one-way analysis of variance (ANOVA) using the SPSS program version 16.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA) for Windows. If significant differences were found ($P < 0.05$), Duncan's multiple range tests was used to rank the means. The results were presented as mean ± SEM of three replicates.

Results

Effect of dietary supplementation on growth performance

In this present study, betaine supplementation at 1.2% affected final body weight (FBW), weight gain ratio (WGR), specific growth rate (SGR), feed intake (FI), condition factor (CF), feed conversion rate (FCR), hepatosomatic index (HSI), and abdominal fat rate (AFR). Conversely, VSI and SR were not affected by betaine supplementation among the groups (Tables 2 and 3). Significantly higher ($P < 0.05$) FI, FBW, WGR, and SGR were observed in fish fed dietary betaine supplementation at 1.2%, while FCR showed an opposite trend. The relationship between WG and dietary betaine supplementation levels was best expressed by the second-order polynomial: $Y = -29.472x^2 + 65.643x + 178.05$, and the optimum dietary betaine level suitable for maximum growth

Table 2 Growth performance and feed utilization of blunt snout bream fed high-fat diets with different inclusion levels of betaine

Parameter	NFD	HFD	HFB (0.6%)	HFB (1.2%)	HFB (1.8%)
IBW (g)	4.68 ± 0.16	4.73 ± 0.06	4.69 ± 0.31	4.62 ± 0.13	4.65 ± 0.16
FBW (g)	12.58 ± 0.66a	13.53 ± 0.06ab	13.97 ± 0.94ab	15.08 ± 0.41b	14.05 ± 0.41ab
WGR (%)	168.70 ± 1.08a	186.64 ± 5.20b	198.10 ± 1.46b	226.75 ± 2.19c	200 ± 2.68b
SGR (%)	1.76 ± 0.07a	1.88 ± 0.03b	1.95 ± 0.01b	2.11 ± 0.01c	1.96 ± 0.02b
FI (g)	489 ± 4.62a	501 ± 2.52ab	501.33 ± 4.91ab	505.33 ± 3.17b	502.33 ± 4.09ab
FCR	2.45 ± 0.44b	2.69 ± 0.13c	2.38 ± 0.04b	2.08 ± 0.01a	2.30 ± 0.02b
AFR (%)	2.03 ± 0.20a	3.69 ± 0.19c	2.86 ± 0.11b	2.47 ± 0.07ab	2.68 ± 0.16b
SR (%)	92 ± 4.62	90.67 ± 5.33	92 ± 0.00	93.33 ± 2.67	92 ± 2.31

Values are presented as means ± SEM; $n = 3$ per treatment. Means in the same row with different letters are significantly ($P < 0.05$) different

performance of blunt snout bream was estimated to be 1.2% (Fig. 1).

Whole body composition

The results presented in Table 4 demonstrate that dietary betaine supplementation did not affect moisture content among the groups ($P < 0.05$). Meanwhile, crude protein and ash content were significantly higher ($P < 0.05$), in 1.2% betaine supplementation groups, whereas crude lipid was significantly lower than those in the HFD group or HFD supplemented with 0.6% betaine groups.

Hepatic antioxidant activities

Hepatic oxidative status parameters affected by betaine supplementation in HFD are shown in Table 5. The results reveal that betaine-supplemented groups increased SOD, CAT, and GSH activities in the liver and reduced the content of hepatic MDA compared to HFD group. The activities of SOD, CAT, and GSH were significantly higher ($P < 0.05$) in fish fed 1.2% betaine supplementation, while MDA levels showed an opposite trend in the same group compared to others.

Effects of betaine on lipid metabolites

Blood biochemistry of blunt snout bream is shown in Table 6. The activities of TG, TC, LDL, HDL, AST, and ALT and the contents of cortisol were significantly ($P < 0.05$), affected by diet treatments. Higher TG, TC, AST, and ALT activities and higher cortisol levels in fish given HFD were significantly reduced ($P < 0.05$), with the inclusion of betaine from 1.2 to 1.8%. Higher LDL and lower HDL content observed in HFD compared to NFD were improved when HFD was supplemented with 1.2% betaine.

Effect of betaine on the expression of lipid metabolism-related genes

FAS mRNA expression was significantly elevated in HFD group compared to NFD group, and this increase was reserved by 1.2% betaine supplementation (Fig. 2a). Whereas carnitine palmitoyltransferase 1 (CPT1), peroxisome proliferator-activated receptor α (PPARα), and microsomal triglyceride transfer protein (MTTP) expression levels were significantly decreased in fish fed HFD in comparison with NFD, but were significantly upregulated with betaine supplementation at 1.2% (Fig. 2b–d).

Table 3 Morphometric parameters of blunt snout bream fed high-fat diets with different inclusion levels betaine

Parameter	NFD	HFD	HFB (0.6%)	HFB (1.2%)	HFB (1.8%)
CF	2.12 ± 0.04a	2.16 ± 0.04a	2.24 ± 0.09a	2.57 ± 0.13b	2.33 ± 0.12ab
VSI	10.59 ± 0.48	12.15 ± 0.38	12.40 ± 0.35	12.06 ± 0.85	11.85 ± 0.54
HSI	1.45 ± 0.09ab	1.61 ± 0.09b	1.39 ± 0.13ab	1.19 ± 0.09a	1.30 ± 0.07a

Values are presented as means ± SEM; $n = 3$ per treatment. Means in the same row with different letters are significantly ($P < 0.05$) different

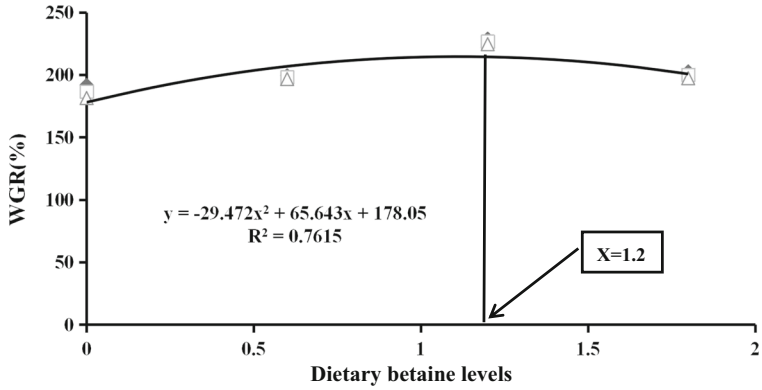

Fig. 1 Relationship between weight gain rate (WGR) and four dietary betaine levels based on second-order polynomial regression analysis, where *X* represents the optimal dietary betaine level for the maximum WGR of blunt snout bream

These results indicate that betaine supplementation reversed HFD-induced dysregulation of the mRNA expression levels of lipid metabolism-related genes (Table 7).

Discussion

The supplementation of betaine with a HFD enhanced feed intake and growth performance in blunt snout bream. In the present study, FBW, WGR, SGR, and FI were significantly improved at 1.2% betaine supplementation compared to other treatments, whereas FCR was significantly reduced. This clearly shows that betaine supplementation has beneficial effects when given at an optimal level, but beyond that level, the supplement may have negative impacts on catabolic activities. The improved growth of fish in 1.2% group could be attributed to improved feed intake and palatability through stimulation of the cephalic reflex induced by smell and taste of attractive substances in the diet (Fange and Grove 1979). Similarly, previous reports found improved growth performance and feed intake and reduced FCR in juvenile gibel carp (Xue and Cui 2001), African catfish (*Clarias gariepinus*) (Turan and Akyurt 2005), common carp (Akshayamanai and PrakashPatil 2016), and tilapia (And and Davis 2005). However, VSI and SR were not affected by betaine supplementation, but significantly increased the HSI and AFR in high-fat group compared to NFD and 1.2% betaine supplementation. The decrease in HSI and AFR in the present study suggests that betaine is a lipotropic agent which can prevent or reduce accumulation of fat in the liver. Our results concur with the previous findings on pigs (Huang et al. 2008), meat ducks (Wang et al. 2000), and laying hens (Zou and Jian-Jun 2002).

The relationship between WG and dietary betaine supplementation % levels was best expressed by the second-order polynomial: $Y = -29.472x^2 + 65.643x + 178.05$, and the optimum dietary betaine level suitable for maximum growth performance of blunt snout bream was estimated to be 1.2%. The whole body moisture content was not affected by dietary betaine levels among the groups in this study, but crude protein and ash contents were significantly higher, while crude lipid content was significantly lower in fish fed 1.2% of betaine supplementation than HFD group. Betaine has been reported to play a role in protein metabolism and also has the ability to reduce fat. The increased crude

Table 4 Whole fish proximate composition (live weight) (mean ± SEM) in blunt snout bream fed high-fat diet

Parameter	NFD	HFD	HFB (0.6%)	HFB (1.2%)	HFB (1.8%)
Moisture (%)	75.87 ± 3.31	74.31 ± 0.71	73.55 ± 3.90	74.71 ± 8.17	74.48 ± 4.87
Crude protein (%)	12.98 ± 0.08b	12.34 ± 0.08a	12.97 ± 0.06b	13.99 ± 0.30c	12.80 ± 0.14ab
Crude lipid (%)	7.18 ± 0.64a	10.47 ± 0.39c	10.40 ± 0.18c	8.25 ± 0.35ab	9.50 ± 0.46bc
Ash (%)	2.55 ± 0.02ab	2.46 ± 0.02a	2.44 ± 0.06a	2.70 ± 0.05b	2.49 ± 0.08a

Values are presented as means ± SEM; $n = 3$ per treatment. Means in the same row with different letters are significantly ($P < 0.05$) different

Table 5 Antioxidant capacity (liver) of blunt snout bream fed high-fat diets with different inclusion levels betaine

Parameter	NFD	HFD	HFB (0.6%)	HFB (1.2%)	HFB (1.8%)
SOD (U/mg prot)	45.44 ± 1.42a	41.75 ± 1.54a	50.47 ± 1.47b	51.00 ± 1.30b	50.35 ± 1.85b
CAT (U/mg prot)	89.16 ± 4.6b	71.82 ± 4.3a	93.61 ± 3.1bc	105.30 ± 10.7c	102.22 ± 10.7c
GSH (U/mg prot)	45.72 ± 2.37ab	43.16 ± 1.60a	49.04 ± 2.72b	50.05 ± 0.64b	49.75 ± 0.76b
MDA (nmol/mg prot)	9.05 ± 0.06a	17.97 ± 0.03d	15.40 ± 0.56c	11.77 ± 0.37b	12.29 ± 0.27b

Values are presented as means ± SEM; $n = 3$ per treatment. Means in the same row with different letters are significantly ($P < 0.05$) different

SOD superoxide dismutase, *CAT* catalase, *GSH* glutathione, *MAD* malondialdehyde

protein and reduced lipid content in the present study could have resulted due to betaine supplementation in the diet. Our results are similar to earlier findings obtained in *Oreochromis aureus* reared in fresh and seawater (Genc et al. 2006) and in rabbit (Hassan et al. 2011), but conflicting with the report obtained in GIFT tilapia (Luo et al. 2011). The dissimilarity of the results could be related to the change in the nutrient concentration, quality of the diet, ration size, feeding frequency, and other factors (Jobling et al. 2001). However, the effect of dietary betaine supplementation on body composition in high-fat diet has not been previously reported and needs further study.

Oxidative stress occurs when reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms. This may lead to a damage in biological macromolecules, disruption of normal metabolism and physiology of the affected fish (Trevisan et al. 2001; Sies 1992), eventually leading to the inception of health disorders (Miller et al. 1993). SOD is the first superoxide enzyme which has been shown to catalyze the dismutation of superoxide radical O_2^- into O_2 and hydrogen peroxide (H_2O_2) (Kohen and Nyska 2002). CAT plays a relatively minor role in the catabolism of H_2O_2 at low rates of H_2O_2 generation (Jones et al. 1981). It becomes indispensable when the rate of H_2O_2 production is enhanced during oxidative stress (Cañavate et al. 2007), and it has been observed that CAT and SOD are responsible for the elimination of peroxides, hence protecting tissues against oxidative damage (Ganesan et al. 2007b; Alirezaei et al. 2011). GSH plays an important role in detoxifying reactive oxygen species (ROS), and MDA is an indicator commonly used to evaluate lipid peroxidation (Parvez and Raisuddin 2005). In the present study, an improved level of SOD, CAT, and GSH activities was observed in 1.2 and 1.8% betaine supplementation. MDA content was significantly low in 1.2% supplementation compared to the HFD group. In this experiment, it was observed that an increase in SOD, CAT, and GSH activities could have decreased lipid peroxidation in blunt snout bream and subsequently improved fish health, an indication that

Table 6 Lipid metabolism (blood) of blunt snout bream fed high-fat diets with different inclusion levels betaine

Parameter	NFD	HFD	HFB (0.6%)	HFB (1.2%)	HFB (1.8%)
TG (mmol/l)	0.133 ± 0.005c	0.157 ± 0.010d	0.145 ± 0.003cd	0.094 ± 0.003a	0.114 ± 0.003b
TC (mmol/l)	0.026 ± 0.002ab	0.053 ± 0.005c	0.031 ± 0.002b	0.018 ± 0.003a	0.024 ± 0.002ab
Cortisol (μg/dl)	513.37 ± 5.64ab	589.54 ± 1.76c	534.10 ± 15.30b	502.58 ± 0.93a	509.63 ± 0.54a
HLD-C (μmol/l)	5.03 ± 0.05b	3.69 ± 0.53a	4.30 ± 0.01a	5.39 ± 0.05b	5.15 ± 0.07b
LDL-C (μmol/l)	3.20 ± 0.18a	5.36 ± 0.15c	3.75 ± 0.12b	3.69 ± 0.19ab	3.73 ± 0.20b
AST (u/l)	5.67 ± 0.19c	8.42 ± 0.12d	5.16 ± 0.20b	2.80 ± 0.10a	2.83 ± 0.21a
ALT (u/l)	23.46 ± 0.80 b	26.45 ± 0.82c	23.07 ± 0.44b	19.87 ± 0.82a	22.86 ± 0.58b

Values are presented as mean ± SME; $n = 3$ per treatment. Means in the same row with different letters are significantly ($P < 0.05$) different

NFD basal diet, *HFD* high-fat diet, *HFB* high-fat diet with betaine supplementation (6, 1.2, and 1.8%, respectively), *TG* triglycerides, *TC* total cholesterol, *HLD-C* lower high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *AST* aspartate transminase, *ALT* alanine transaminase

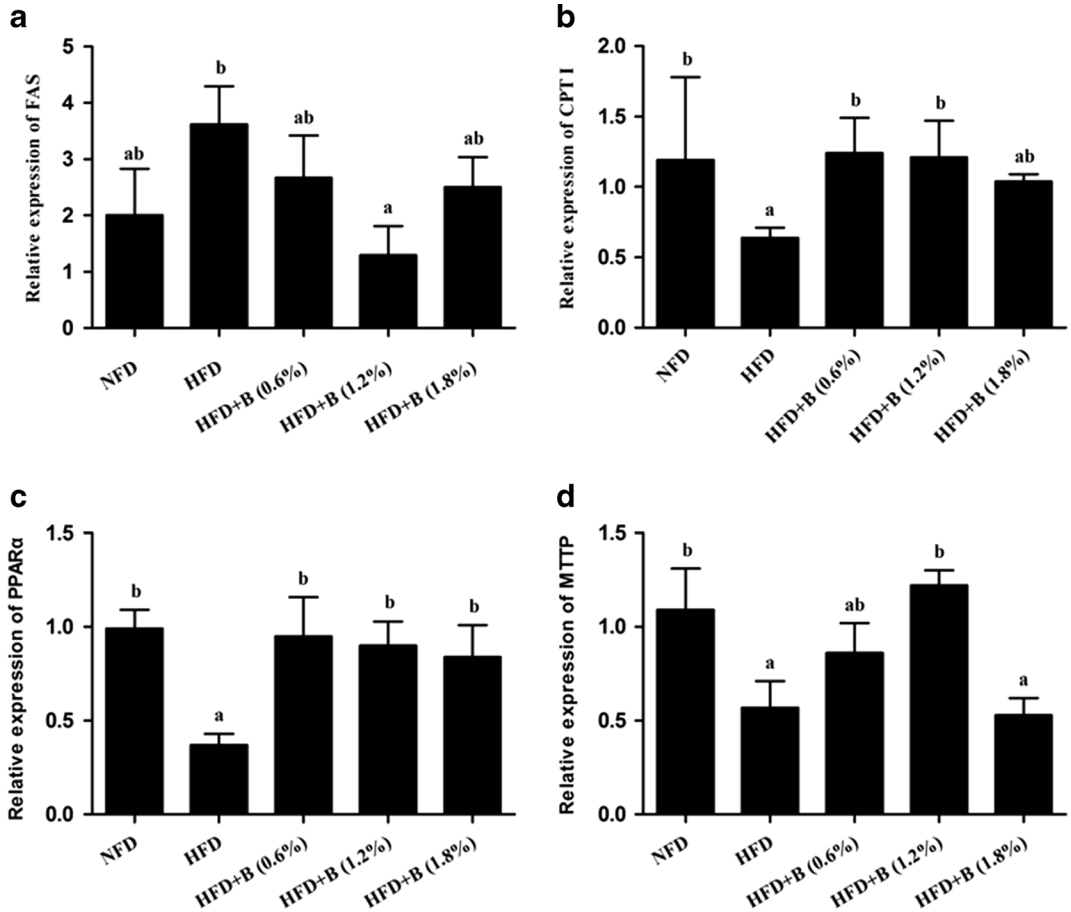

Fig. 2 Effect of dietary betaine on hepatic lipid metabolism-related gene expression. **a** Fatty acid synthase (*FAS*), **b** carnitine palmitoyltransferase 1 (*CPT*), **c** peroxisome proliferator-activated receptor α (*PPARα*), and **d** microsomal triglyceride transfer protein (*MTTP*). The expression level of each gene was normalized to that of the gene encoding β-actin. Data are represented as the mean ± SEM of $n = 3$ replicates. If significant differences were found ($P < 0.05$), Duncan's multiple range tests was used to rank the means

supplementation of betaine in HFD might decrease oxidative damage. It can be suggested that betaine might have antioxidant and nutrient effects against oxidative damage in the cells of blunt snout bream fed HFD. Furthermore, the protective effect of betaine against oxidative stress observed in this study may also support the idea that betaine is associated with antioxidant and methyl donor properties through its involvement in cell membrane stabilization and homocysteine remethylation (Alirezaei et al. 2011).

Betaine is regarded as a lipotropic agent and has been tested in many animal models (Song et al. 2007; Wang

Table 7 Primers used to detect mRNAs involved in lipid metabolism

Name	Target gene sequence	Sense primer (5′-3′)	Anti-sense primer (5′-3′)	Fragment length (bp)
FAS	KF918747	5′-GACCTGGAGGCTCGTGT-3′	5′-GGATGATGCCTGAGATGG-3′	319
CPT1	Lu et al. (2014b)	5′-TACTTCCAAAGCGGTGAG-3′	5′-AGAGGTATTGTCCGAGCC-3′	233
PPARα	KM980090.1	5′-ACCGAAACAAGTGCCAATA-3′	5′-TCAGTCACCGTCTCAACC-3′	415
MTTP	HM140628.2	5′-TACAAGGCTACCAAACA-3′	5′-AGACTTCCCACTGACG-3′	111

et al. 2010). Long-term feeding of HFD can induce liver dysfunction, which might lead to stress and eventually cause fish deaths. In this study, high plasma TG, TC, LDL, AST, ALT, cortisol, and low HDL contents were observed in fish given HFD. The increase in plasma TC, TG, and LDL in this study may show metabolic disorders of lipids and lipoproteins as well as liver damage (Mensinger et al. 2005; Takeuchi-Yorimoto et al. 2013), leading to high cortisol level resulting into poor health condition of the fish in HFD group. Our results indicated that inappropriate increase of HFD may cause stress response of fingerling blunt snout bream, which is in agreement with the findings documented by Li et al. (2012b). This suggests that high dietary lipid might lead to increased lipid peroxidation and cause oxidative stress of fish.

On the other hand, an improved plasma TC, TG, LDL, HDL, AST, ALT, and cortisol concentration were observed in 1.2 and 1.8% supplementation compared to the HFD and 0.6% supplementations of betaine. This improved plasma component that might be related to its lipolytic effects on adipose tissue which could positively influence body composition by reducing TG and TC synthesis, thereby improving LDL and HDL content and equally enhancing liver functioning enzyme activities such as AST and ALT of fish. The lipolytic effect of the betaine in this study concurs with the findings reported by Wang et al. (2014b), whereby betaine supplementation reduced the visceral fat accumulation and plasma TG level in the HFD-fed mice and in broiler when subjected to chronic heat stress (He et al. 2015).

The expressions of genes involved in lipid metabolism were detected. FAS is the key enzyme in de novo lipogenesis which is sensitive to both nutritional and hormonal modulation (Moustaïd et al. 1996). Fatty acid (FA) oxidation is essential in liver lipid metabolism, especially in animals fed HFD (Du et al. 2006). When dietary lipid intake exceeds the capacity of the hepatic cells to oxidize FAS, large amount of triglyceride is synthesized and deposited in vacuoles, leading to steatosis (Lu et al. 2014b). In the present study, FAS expression was significantly decreased in the 1.2% betaine-supplemented diet compared to others, an indication that betaine supplementation partly inhibits the HFD-induced excessive synthesis of fatty acids.

As a specific property, CPT1 is a key rate-limiting enzyme of β-oxidation (Korman et al. 2005), and it is also mentioned as a target of PPARα (Ferré 2004). The β-oxidation of fatty acids plays a key role in the production of energy and mostly occurs in the mitochondria. CPT1 located in outer membrane of the mitochondria mediates the uptake of long-chain fatty acids into the mitochondria. PPARα as a nuclear receptor is activated by fatty acids and regulates the transcription of numerous gene encoding enzymes in fatty acid oxidation, such as CPT1 in mitochondria and CYP2E1 in extra mitochondria (Yang et al. 2012). In this study, CPT1 was significantly downregulated in fish fed a HFD and significantly upregulated when fed HFD supplemented with 1.2 and 1.8% betaine, respectively. The results are in accordance with the previous report, which found that betaine supplementation increased CPT1 expression level and reduced the expression of FAS in mammals (Wang et al. 2012). In this present study, downregulations of PPARα and its target (CPT1) were obtained in the group treated with high-fat diet, which is similar with the previous study by Xu et al. (2015) who reported that high-fat diet decreased mRNA levels for PPARα as well as its downstream target CPT1. However, in this study, betaine supplementation in the high-fat diet of blunt snout bream increased the gene expression of PPARα and CPT1. Similar results were also found in a study by Wang et al. (2014a), which showed that betaine supplementation increased both PPARα and CPT1 expressions of apoE2/2 mice and reversed the inhibition of CPT1 induced by HFD. MTTP plays an essential role in lipoprotein (Wetterau et al. 1992) and assists in the assembly and secretion of apolipoprotein B (apoB)-containing lipoprotein, chylomicrons, and very low-density lipoprotein. In the present study, a downregulation of MTTP expression was observed in the HFD group compared to NFD group, and 1.2% betaine supplementation reversed HFD-induced inhibition of MTTP mRNA expression. According to the current results, it can be highlighted that hepatic MTTP is very crucial in regulating the assembly and secretion of triglyceride-rich lipoproteins, which further confirms that dietary betaine decreases fat accumulation in the liver.

In conclusion, the study confirms that supplementation of betaine at 1.2% can improve growth performance and antioxidant capacity, as well as reduce fatty acid synthesis and enhance mitochondrial β-oxidation and lipid transportation in high-fat diet-fed blunt snout bream, thus effectively alleviating fat accumulation in the liver by changing lipid metabolism.

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