

THE MODULATORY EFFECT OF DIETARY BETAINE ON INTESTINAL ABSORPTIVE CAPACITY, LIPOGENESIS AND EXPRESSION OF LIPID METABOLISM- AND GROWTH-RELATED GENES IN NILE TILAPIA FED ON SOYBEAN MEAL-BASED DIET

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Abstract: The current experiment was randomly designed in a 2×2 factorial design. Two dietary protein sources were utilized; fish meal and soybean meal; with betaine incorporation to both of them. This study aimed to determine the impact of incorporation of betaine into soybean meal-based (SBM) diets through its effects on growth performance, intestinal healthiness and expression of some lipid metabolism- and growth-related genes. Fish (19.84±0.20 g) were stocked in 12 aquaria and allotted into triplicate four groups (10 fish per aquarium). Four test diets were formulated to contain fish meal (FM) as a positive control, FM with betaine (FM + Betaine), SBM diet and SBM with betaine (SBM + Betaine), respectively. After 60 days, dietary betaine improved the growth performance of fish fed FM or SBM as revealed by higher final body weight, body weight gain and average daily gain and lower feed conversion ratio. Monitoring the whole-body composition revealed that addition of betaine to diet relatively augmented flesh protein content and reduced its fat content. In addition, betaine incorporation in diets significantly ($P<0.05$) increased the intestinal villi length especially in the jejunal portion as well as the numbers goblet cells. Furthermore, betaine had a downregulating effect on expression of lipid metabolism-related genes, fatty acid synthetase (*Fas*) and lipoprotein lipase (*Lpl*) and up-regulating effect on insulin growth like factor-1 (*Igf-1*) gene in liver. It could be concluded that dietary supplementation of betaine incorporation to soybean-based diets enable nutritionists to substitute FM in fish diet. Also, betaine could improve growth performance, carcass quality (through increasing protein and decreasing lipid in fish) and enhance intestinal functions capability.

Key words: Betaine; growth performance; insulin like growth hormone factor; lipid metabolism related genes; Nile tilapia; soybean meal

Introduction

Fish serve as a vital source of human dietary protein worldwide, particularly for African

people (1-3). Natural fisheries are progressively exhausted and the aquaculture end up basic means to compensate the need in this fundamental wellspring of dietary protein (4). Along these lines, to repay the insufficiency in animal protein resulted from the expanded interest for fish as human sustenance, fish farming is quickly extending everywhere throughout the world (5). Aquaculture contributes the greater part of the overall fish production all over the world. In Egypt, the aquaculture industry gives about 77% of the all national fish production providing around one million ton annually (6-8). Progressive enhancement of this industry must be correlated with corresponding improvement of fish feed quality representing 70 % of the total culturing costs (7, 9).

Nile tilapia (*Oreochromis niloticus*) represents the most prevalent freshwater fishes in Egypt, widely cultured because of its high growth rate, cheap price, palatability, ability to withstand stress and diseases, ability to spawn easily and the minimal requirements in regard to management and energy inputs (10). Heightening of aquaculture production frameworks exposes fish to various ecological stressors like; poor water quality, over-crowdness, improper handling and transport which may contrarily influence their growth and health, as well as limiting the outcome of aquaculture systems (11).

Nutrition assumes as a basic job in the growth, advancement and prosperity upkeep of fish (12). Years ago, fish meal was used as the basic protein source in tilapia diets (13). Due to the extending cost and unreliable supply of this ingredient, numerous attempts have been admitted to upgrade the growth performance and decrease the production costs of cultured tilapia through replacement of fish meal with another protein sources either from terrestrial animals or plants. This was done by utilization of probiotics or prebiotics as safe dietary supplements which have neither residue in the cultured fish nor harmful impacts for fish consumers (14-15). However, increasing amount of the fish meal alternatives in the diet may result in decreasing the diet palatability to fish; decreasing feed intake and subsequently decreasing fish growth rate (16).

Betaine (trimethylglycine) is synthesized from choline oxidation (17), present in most organisms and is fundamentally extracted from sugar beet processing (18). Interestingly, betaine was recorded to improve growth performance, fish health status, feed digestibility as well as flesh quality and immune status of fish species (19, 20). It was used as a feed attractant and appetizer through stimulating the olfactory bulb, increasing feed intake and minimizing the feed wastage and water pollution (21). In addition, betaine can play a role in the osmoregulation through increasing both water retention of perturbed cells and cytoplasmic osmotic pressure via raising the temperature and ionic tolerance of critical enzymes and cellular membranes that results in protection of intracellular enzymes against osmotically or temperature induced inactivation (22, 23). Moreover, betaine acts as a methyl donor so it plays a great vital role in energy metabolism and synthesis of methionine, carnitine, phosphatidyl choline, creatine and protein (24, 25). Betaine plays a fundamental role in lipid metabolism suggesting that it is a lipotropic factor having a hepatoprotective function (26-28). Dietary betaine additionally has the ability to reduce body lipid deposition (29, 30).

To the best of our knowledge, there is little published data on the supplemental effects of betaine on growth performance, intestinal absorptive capacity, intestinal morphometry, lipid metabolism and growth-related genes in Nile tilapia (*O. niloticus*). Thus, the current study was conducted to role out the effect of incorporation of betaine to soybean-based diets and to assess its effects on growth performance, intestinal absorptive capacity, and expression of lipid metabolism- and growth-related genes in Nile tilapia.

Material and methods

The feeding trials were carried out at the Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, for 10 weeks during 2018. Animal ethics committee, Faculty of Veterinary

Medicine, Kafrelsheikh University, Egypt; affirmed and approved the protocol and conducting of the study.

Preparation of the experimental diets

Two basal diets were formulated to be isonitrogenous and isocaloric in terms of crude protein (30%) and digestible energy (3.05 kcal g⁻¹). Each diet was based on either fish meal (FM) or soybean meal (SBM) as the main source of dietary protein. Then each diet was supplemented with 2 g betaine per kg diet (FM, FM+betaine, SBM and SBM+betaine) (Table 1) as suggested by Luo *et al.*, (31). Betaine was purchased from Biochem Ltd. Company, Egypt and incorporated to the diets after firstly mixed with dicalcium phosphate, limestone, salts and premix until the mixture became homogenous and finally added to the other finely ground concentrate with stirring. Proximate chemical analysis of diets was performed according to Association of Official Analytical Chemists methods as shown in (Table 2).

Experimental design

The experiment was randomly designed in a 2×2 factorial design. Two dietary protein sources were used with betaine incorporation to both of them. Thus, a total of four treatments run in triplicate were allotted into 12 glass aquaria (60×40×30 cm) provided with a constant aeration.

One hundred and twenty healthy Nile tilapia (*O. niloticus*) (initial body weight, 19.84 ± 0.20 g) were obtained from a private farm in Kafrelsheikh governorate, Egypt. Prior to the experiment, fish were acclimatized to basal diet for 2 weeks. At the beginning of the experiment, fish (n= 10) were stocked into each glass aquaria, and each diet was assigned to triplicate aquaria. Fish were fed till apparent satiation twice daily (at 9 a.m. and 5 p.m.) for 60 days and weighted every ten days in order to determine the growth and healthy status. Water parameters were monitored twice a week using water analysis device (Lamotte device, USA). Temperature ranged from (24-26°C), 6 mg L⁻¹ dissolved oxygen, 6.5-7 pH, ammonia adjusted to the normal permissible limits (< 0.1 mg total

ammonia) and 14 h of light and 10 h of dark photoperiod with 40 % daily water change.

Proximate chemical analysis

At the end of the feeding trial, fish were starved for 24 h, harvested and weighed individually then euthanized through over anesthetization using MS222 as a local anesthetic drug. 4 fish from each group were stored at (-20 °C) for proximate chemical analysis of fish tissue. Moisture content was carried out by drying the samples in a hot air oven at 60 °C for 48 h. Ash content was determined by samples incineration at 550 °C for 6 hrs. Crude protein (N×6.25) was assessed using the Kjeldahl method after acid digestion. Fat and fiber were determined using ANKOM Technology Method.

Morphometrical examination

Snippets of the different intestinal portions were sampled from another four fish and immediately fixed in a Bouin solution for histological observations. Fixed samples were subjected to dehydration through ascending grades of alcohol solution series (70 to 100%). After dehydration process, tissues were deparaffinized in xylene, embedded in paraffin, sectioned (5 µm) and stained with hematoxylin and eosin (H&E). The heights of intestinal villi in all parts were measured using ImageJ version 1.36 (National Institutes of Health). Mean villus height for each section was based on an average of 10 villus heights/section. Goblet cells No. was determined according to the equation: goblet cell number = goblet cell number in 8 fields of x 200 magnification power / total area examined per each fish, and was expressed as (No. of goblet cells/mm²)

Quantitative real-time PCR (qRT-PCR)

Liver samples were collected, quickly frozen in liquid nitrogen then kept at -80°C for gene expression analysis, then total RNA was extracted from 50 mg of liver tissue (n=3/group) using TRIzol reagent (easy-RED™, iNtRON Biotechnology). RNA quality was verified using formol gel electrophoresis examination. The cDNA was synthesized from each RNA sample (2 µg) using SensiFAST™ cDNA syn-

thesis kit (Bioline, United Kingdom). As presented in Table (3), gene specific primers of fatty acid synthase (*Fas*), lipoprotein lipase (*Lpl*), and insulin like growth factor-1 (*Igf-1*), were used to determine the expression in liver tissue and β actin was selected as a reference gene for normalization of gene expression. Real time PCR (qPCR) was carried out using SYBR[®] Premix and Stratagene MX3000 qPCR system. The reaction mix consisted of a 2 μ l cDNA sample, 12.5 μ l SYBR[®] Premix Ex TaqTM, 0.25 μ l PCR forward/reverse primers (10 μ M), and 10 μ l nuclease-free water. The thermo-cycling conditions for the reaction were as follows: 95 °C for 5 min, followed by 40 cycles consisting of 95 °C for 10 s, annealing for 15 s at primer specific annealing temperature. Dissociation curve analyses were performed beginning at 65°C and ending at 95°C, with incremental increases of 0.5°C every 5s to validate the specificity of the PCR products. All samples were run in duplicate. Relative gene expression levels were evaluated using $2^{-\Delta\Delta Ct}$ as previously described (32).

Calculations and statistical analysis

The following variables were calculated:

Total body weight gain (TWG)(g/fish) = final body weight (W2) – initial body weight (W1).

Average daily gain (ADG) = (W2 – W1) / t (experiment duration = 60 days)

Feed intake (FI, g fish⁻¹ 60 days⁻¹) = (offered feed – feed refusal recovered and dried)/number of fish

Feed conversion ratio (FCR) = feed intake (g)/weight gain (g).

The results were presented as mean \pm standard error (SE) of three replicates. All results were analyzed by one-way analysis of variance (ANOVA). When ANOVA identified differences between groups, multiple comparisons among means were made with Duncan's new multiple range test. Differences were considered significant at ($P < 0.05$). All statistical analyses were performed using the SPSS 24.

Results

Growth performance

As shown in table (4), addition of betaine to diets based on soybean meal protein significantly ($P < 0.05$) increased the feed intake. Fish fed on FM+betaine showed the highest FBW and BWG followed by that fed on SBM+betaine. Also, supplementation of betaine significantly ($P < 0.05$) increased FBW and BWG in fish fed SBM+ betaine as compared to fish fed SBM only. FCR was markedly decreased in fish fed on FM+ betaine or SBM+ betaine when compared to the other groups. Also, FI significantly differed according to the source of dietary protein either from FM or SBM-based diet.

Whole body chemical composition

Analysis of whole-body composition presented in table (5) showed that crude protein content was significantly ($P < 0.05$) increased by addition of betaine either with FM or SBM diets. FM+ betaine showed relatively higher CP content than other experimental treatments. Fat content was significantly ($P < 0.05$) decreased by addition of betaine to fish diets. There were no significant differences in moisture, ash, soluble carbohydrate and ADF content among all dietary treatments.

Assessment of intestinal absorptive capacity

Duodenal, jejunal and ileal villi morphometry were showed in (Table 6) and illustrated in (Fig. 1). Betaine significantly increased the intestinal absorptive capacity of fish supplemented with FM diet ($P < 0.05$), while, SBM markedly decreased intestinal villi parameters in comparison with fish supplemented with FM diet. Interestingly, supplementation of betaine improved the jejunal villi length in FM and SBM groups. Also, the number of goblet cells per area along the intestinal mucosal lining was increased in fish fed FM diets than fish fed with SBM as a protein source. Incorporation of betaine with SBM demonstrated marked restore of goblet cells like FM diet.

Expression of Fas, Lpl and Igf-I genes in liver tissue

Relative mRNA expression of *Fas*, *Lpl*, *Igf-I* genes in liver of Nile tilapia fed on different protein sources with and without addition of betaine was shown in Figure 3. Hepatic *Fas* and *Lpl* mRNA levels showed upregulation in fish fed on the diets deprived from betaine ($P<0.05$). Interestingly, addition of dietary betaine to either fish meal or soybean-based diets markedly

decreased *Fas* and *Lpl* mRNA levels ($P<0.05$). Fish fed on FM+ betaine had significantly ($P<0.05$) higher hepatic *Fas* mRNA level as compared to fish fed SBM+ betaine. *Igf-I* mRNA level in the liver of Nile tilapia fed on dietary betaine with SBM was significantly ($P<0.05$) upregulated as compared to fish fed SBM only. Fish fed diet with FM+ betaine showed significantly ($P<0.05$) higher hepatic *Igf-I* expression than other groups.

Table 1: Diets formulation and experimental design

Item (%)	FM	FM+ betaine	SBM	SBM+ betaine
FM	35.5	35.5	—	—
SBM	—	—	56.5	56.5
Wheat bran	3.8	3.7	6.7	6.6
Rice polishing	16	16	—	—
Corn grains	25	25	25	25
Wheat middling	18.9	18.8	4.96	4.86
Soya oil	—	—	1.2	1.2
Fish oil	—	—	1.8	1.8
Nacl	—	—	0.33	0.33
Minerals ¹	0.05	0.05	0.05	0.05
Vitamins ²	0.05	0.05	0.05	0.05
Vitamin C	0.1	0.1	0.1	0.1
Dicalcium phosphate	—	—	2.4	2.4
Limestone	—	—	—	—
Lysine hydrochloride	—	—	—	—
DL methionine	—	—	0.31	0.31
Antimycotoxin	0.1	0.1	0.1	0.1
CMC	0.5	0.5	0.5	0.5
Betaine	—	0.2	—	0.2

¹Minerals (Egypt pharma company) each 5 kgs of this product provide the following: 5000 mg Copper; 5 mg..Cobalt; 5000 mg..Iodine; 100 mg..Selenium; 30000 mg Iron; 40000 mg magnesium; 10000 mg manganese; 150000 mg Zinc; Calcium carbonate ad to 1000 gm.

²Vitamins (Egypt pharma company) each 5 kilograms of this product provide the following: 5000000 IU Vit.A; 1000000 IU Vit. D3; 50000 mg Vit. E; 10000 mg K3; 20000 mg B1; 20000 mg B2; 20000 mg B6; 20 mg B12; 100000 mg Niacin; 5000 mg Folic; 100 mg Biotin; 50000 mg Pantothenic Acid; Calcium carbonate ad to 1000 gm.

Table 2: Proximate chemical analysis of the basal diets

	FM	SBM
DE (Kcal/kg)	3045	3059
CP %	29.9	29.92
Fat %	6.25	3.6
ADF %	6.57	7.17
Ca %	1.9	0.7
Total P %	1.35	1.02
Available p %	0.71	0.46
Na %	0.15	0.15
Lysine %	1.98	1.67
Methionine %	0.75	0.73
Threonine %	1.18	0.99
Tryptophane %	0.31	0.24

ADF: Acid detergent fiber DE: digestible energy SBM: Soybean meal
 CMC: Carboxy-methyl cellulose FM: Fish meal.

Table 3: Primer sequences used for qPCR analysis

Gene	Primer sequence (5'-3')	Annealing Temperature	References
<i>Fas</i>	F: TGAAACTGAAGCCTTGTGTGCC	60°C	Tian <i>et al.</i> , (33)
	R: TCCCTGTGAGCGGAGGTGATTA		
<i>Lpl</i>	F: TGCTAATGTGATTGTGGTGGAC	59°C	Tian <i>et al.</i> , (33)
	R: GCTGATTTTGTGGTTGGTAAGG		
<i>Igf-1</i>	F: TCCTGTAGCCACACCCTCTC	60°C	Costa <i>et al.</i> , (34)
	R: ACAGCTTTGGAAGCAGCACT		
β -actin	F: CCACACAGTGCCCATCTACGA	63°C	Qiang <i>et al.</i> , (35)
	R: CCACGCTCTGTCAGGATCTTCA		

Fas: fatty acid synthase *Lpl*: lipoprotein lipase *Igf-1*: insulin like growth factor-1.

Table 4: Effect of dietary betaine on growth parameters of Nile tilapia fed on FM and soybean.

	IW (g)	FBW (g)	BWG (g)	ADG (g)	FI (g)	FCR
FM	19.95±0.21	50.07± 0.42 ^b	30.11±0.27 ^b	0.50±0.00 ^b	48.68±0.52 ^a	1.62± 0.03 ^b
FM+ betaine	19.62±0.13	54.61± 0.33 ^a	34.99± 0.20 ^a	0.58±0.00 ^a	50.24±0.25 ^a	1.44± 0.00 ^c
SBM	19.90±0.19	43.17± 0.54 ^c	23.27±0.73 ^c	0.39±0.01 ^c	42.67±0.77 ^b	1.84± 0.03 ^a
SBM+ betaine	20.00±0.08	51.16±0.30 ^b	31.16±0.34 ^b	0.52±0.01 ^b	49.29±0.55 ^a	1.59± 0.01 ^b

IW: Initial weight FBW: Final body weight BWG: Body weight gain
 ADG: Average daily gain FI: Feed Intake FCR: Feed Conversion Ratio.

**Values are means ± standard error (n = 10). Means with different small letter in the same column differ significantly ($P < 0.05$).

Table 5: Effect of dietary betaine on whole-body composition of Nile tilapia fed on FM and soybean

	Moisture%	CP%	Fat%	Ash%	Soluble carbohydrates%	ADF%
FM	57.83±0.30	18.78±0.17 ^{cb}	15.80±0.15 ^a	2.76±0.17	3.49±0.54	1.35 ± 0.17
FM+ betaine	57.92±0.29	19.85±0.15 ^a	14.82±0.36 ^{ab}	2.79±0.3	3.37±0.41	1.25 ± 0.10
SBM	58.2±0.31	18.52±0.4 ^b	15.33±0.21 ^b	2.79±0.31	3.61±1.07	1.48 ± 0.10
SBM+ betaine	57.62±0.28	19.35±0.34 ^{ab}	14.59±0.18 ^b	2.87±0.43	4.53±0.71	1.04 ± 0.08

CP: crude protein ADF: acid detergent fiber.

**Values are means ± standard error (n=4). Means with different small letter in the same column differ significantly ($P < 0.05$).

Table 6: Effect of dietary betaine on of intestinal villi length of Nile tilapia fed on plant and animal protein sources

Treatments	Villi Length (µm)		
	Duodenum	Jujenum	Ileum
FM	290.69±18.16	530.35±30.93 ^{bc}	355.98±30.05 ^a
FM+ betaine	351.20±32.03	684.92±26.96 ^a	414.96±37.16 ^a
SBM	278.73±23.06	465.48±24.04 ^c	220.44±19.76 ^b
SBM+ betaine	290.18±31.43	605.62±43.47 ^{ab}	255.62±30.66 ^b

Values are means±standard error (n = 4). Means with different small letter in the same column differ significantly ($P < 0.05$).

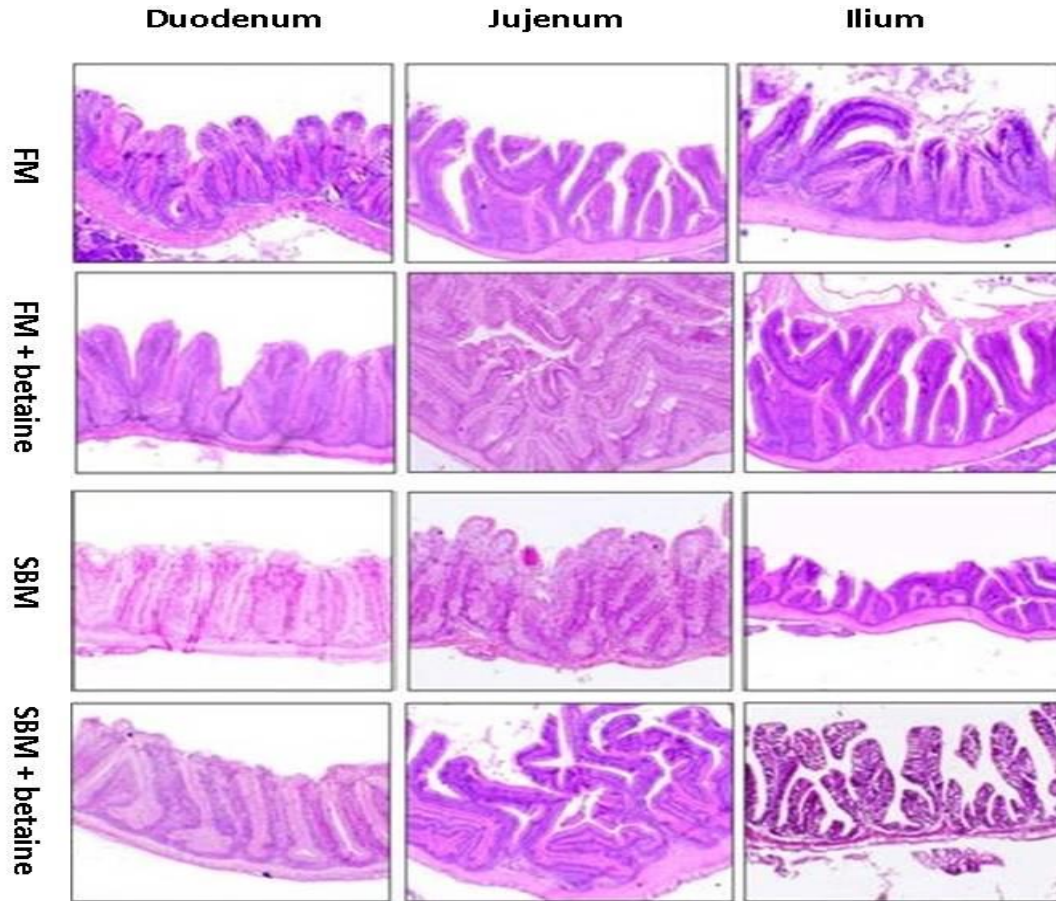


Figure 1: Haematoxylin & eosin stained photomicrograph, assessed the effect of dietary betaine on the duodenal, jujenal and ileal villi length (μm) of Nile tilapia fed on soybean meal and fish meal for 60 days

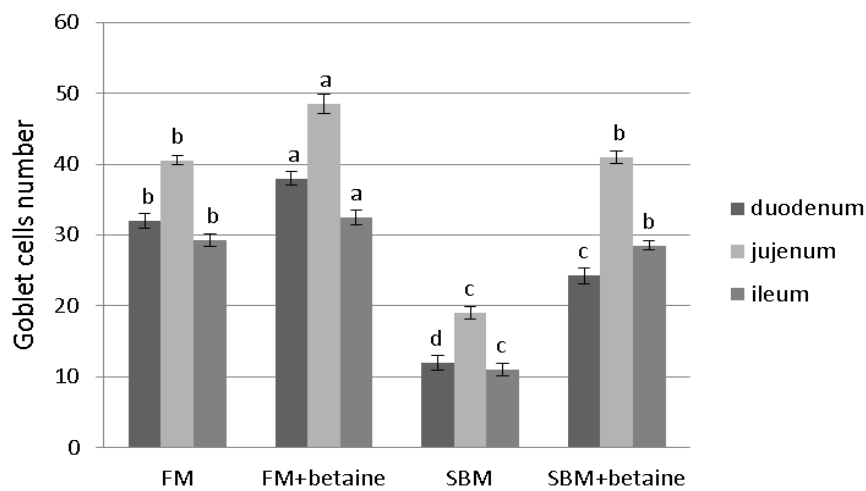


Figure 2: Effect of dietary betaine on intestinal goblet cells number of Nile tilapia fed on soybean meal and fish meal. Values are means \pm standard error ($n = 4$). Means with different small letters on columns for each organ differ significantly ($P < 0.05$)

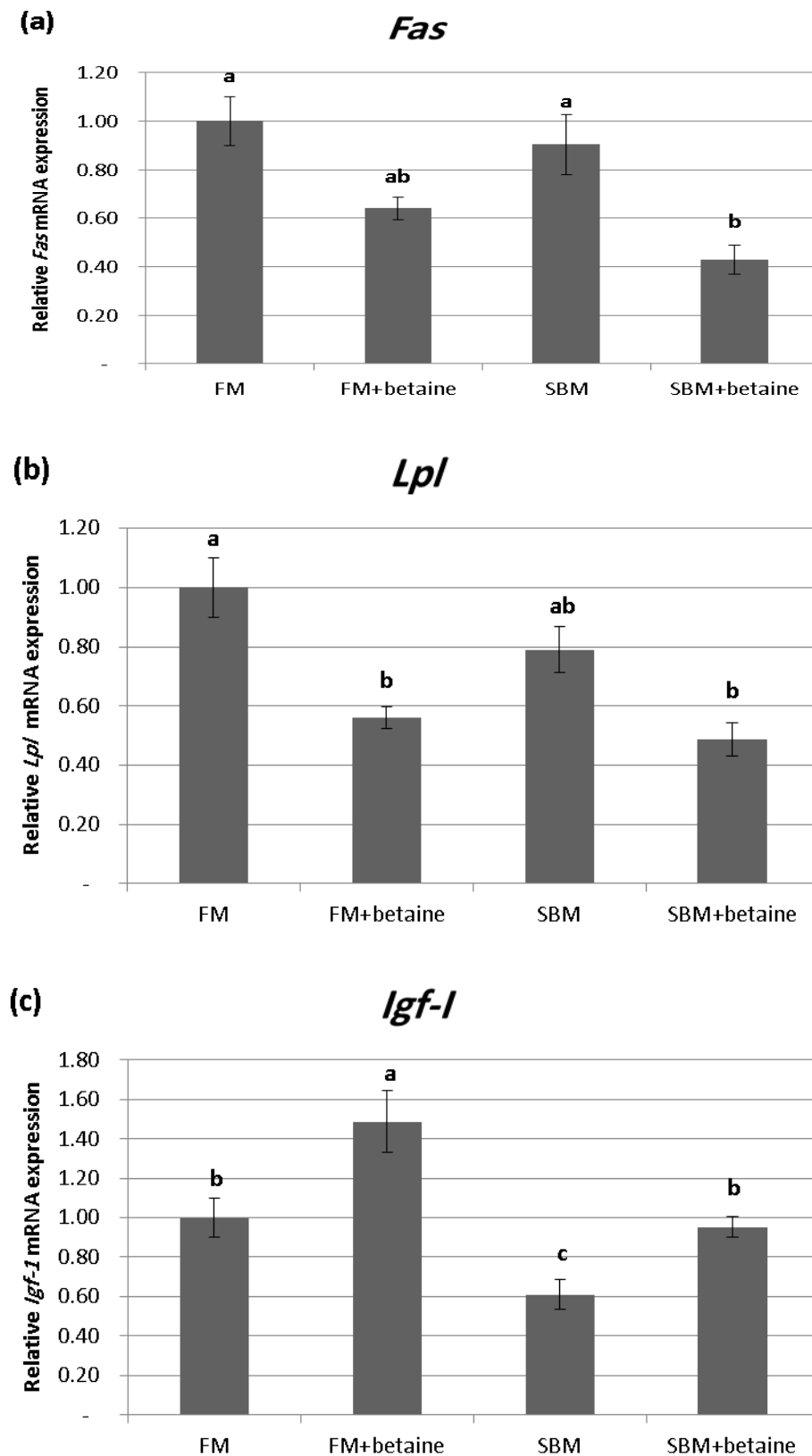


Figure 3: Effect of dietary betaine and different protein sources on mRNA level in liver (a) *Fas*: fatty acid synthetase; (b) *Lpl*: lipoprotein lipase; (c) *Igf-1*: insulin like growth factor-1 in Nile tilapia fed for 60 days. Values are expressed as the mean \pm SE ($n = 3$). Data with different letters on columns statistically differ ($P < 0.05$)

Discussion

Growth is the fundamental key of energy loss and/or gains in the aquatic environment; which can be measured mainly by determination of the weight gain. Because of the expanding cost and untrustworthy supply of fish meal, various trials have been applied to redesign the growth performance and decline the production expenses of refined tilapia through substitution of fish meal with another plant protein sources. This was performed by usage of probiotics or prebiotics as protected dietary enhancements which have neither buildup in the refined fish nor unsafe effects for the fish customers (14, 15).

In the current study, fish fed FM showed higher BWG and FBW than SBM-based diets. The result is coincided with Fontainhas-Fernandes *et al.*, (36) who reported that dietary FM was the main protein source showing the highest FBW and Khan *et al.* (37) who reported that inclusion of dietary FM up to 35% showed marked increase in the growth rate. This may be attributed to high protein quality of FM and its palatability. Betaine incorporation to SBM based diet improved feed intake relatively similar to FM. Similarly, betaine was used as a feeding stimulant in red sea bream (38), dover sole (39), European eel (40), Tilapia (31), juvenile grouper (41), and Chinese soft-shelled Turtle (42). This may indicate that betaine improved palatability of the diets which might be through stimulation of cephalic index induced by betaine smell and taste. Conversely, Hughes (43) suggested that betaine not affect the feed intake in Chinook Salmon Fry.

In addition, betaine incorporation in SBM-based diets not only increased FI but also growth performance compared to FM diet levels. Similar results were also reported in Indian major carp (25), rainbow trout (44), pike perch (45), and in tilapia fed 0.5% betaine (31). Kasper *et al.* (46) found that dietary betaine can spare the entire choline requirement in Nile tilapia in contrast with Vieira *et al.* (47). Regarding to the feed conversion ratio (FCR) which is considered as one of the most

economic indicators, addition of betaine to FM or SBM-based diets decreased FCR. Diet based on SBM+ betaine achieved the same effect FM on feed efficiency. This result is similar to that reported by (25) who used 0.25% of betaine in carp fish diet, (48) who used 0.5% betaine in juvenile fresh water prawn fish and (26) who got the best FCR in channel cat fish (26). Decreased FCR might be attributed to the improvement of growth performance (31). Recently, Zou *et al.* (49) reported that feeding Nile tilapia with 0.6% betaine in diet containing high level of plant protein significantly lowered FCR. The beneficial effect of betaine on growth performance may be attributed to its feeding stimulant effect together with increased intestinal amylase and protease activities (49, 50). The positive effect of betaine on growth performance might be also due to alternation in the metabolism which needs further examination. However, the improvement of FCR in this study was due to the rapid growth rate which saves maintenance requirements.

Interestingly, betaine supplementation to SBM-based diets increased the body protein and decreased the fat content. This may be attributed to betaine role as a methyl donor, sparing methionine amino acid needed for protein synthesis. In addition, betaine plays a key role in synthesis of phosphatidyl choline, carnitine and creatine. Moreover, it was suggested that betaine role as a methyl donor enhances lipid metabolism, stimulating the hepatoprotective function resulting in prevention of the fatty liver (26). Furthermore, the incorporation of betaine at 0.4% in *Allogynogenetic gibel* carp diet resulted in lowering in the hepatopancreas, muscle and the whole body lipid content (51). Additionally, Wang *et al.* (27) reported that supplementation of dietary betaine decreased lipogenesis and increased fatty acids oxidation. The above evidence suggests that betaine incorporation into Nile tilapia diets provides the priority for protein synthesis which consumes smaller energy amounts than fat synthesis.

Assessment of intestinal healthiness including absorptive capacity and digestive

functions through the height of intestinal villi, thickness of muscular layer and the number of goblet cells is an important morphometry indicator in case of evaluating different feed strategies in aquatic animals (52, 53). In the current study, betaine incorporation at 0.2% increased the intestinal villi length and number of goblet cells. Improving the intestinal absorptive and protective functions might be due to increased surface area available for absorption and/or due to secreted mucous from numerous goblet cells which has bactericidal effect through covering receptors of pathogens and keeping the integrity of intestinal epithelium (54, 55).

Lipid metabolism variations induced by betaine incorporation was monitored by changing in mRNA level of *Fas* which affects the synthesis of body fat and thereby affects the body fat accumulation (56). *Fas* and *Lpl* are considered key factors in lipid metabolism and fat deposition in the body (57). Current results revealed decreased *Fas* mRNA level associated with betaine incorporation in diet. Similar results were reported by Kim *et al.* (58) who revealed that protection of liver against steatosis could be occurred through supplementation of dietary betaine which decreased *Fas* and ACC (acetyl-CoA carboxylase) through affecting Forkhead Box O1 (FoxO1) binding peroxisome proliferator activated receptor gamma (PPAR γ). Current results are similar to that reported in *Allogynogenetic gibel* carp (51). Additionally, hepatic *Lpl* mRNA level markedly decreased with supplementation of dietary betaine. Oku *et al.* (59) reported that the expression of *Lpl* was tissue specific therefore, the effect of dietary betaine on *Lpl* expression needs further examination for Nile tilapia different organs other than liver.

Igf-1 considers the major anabolic factor needed for growth (60). *Igf-1* mRNA expression in the current study showed upregulation with addition of dietary betaine and down regulation when fish fed SBM-based diets without betaine which could subsequently reduce growth performance. Similar results were reported by Men *et al.* (61) who found that when fish fed on plant protein based diets at high

level (60%) showed lower hepatic *Igf-1* than that fed 30% in Japanese seabass.

Conclusion

From the obtained results, it could be concluded that betaine incorporation with 0.2% level to SBM-based diet improve palatability, growth performance, intestinal absorptive capacity, body protein on expense of lipid body accumulation, hepatic *Igf-1* gene expression and reduce the lipogenesis through markedly decrease *Fas* and *Lpl* mRNA level in liver.

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Conflict of interest

The authors declare that they have no conflict of interest.

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