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Dietary supplementation of betaine in diets to improve the growth and feed utilization in hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) juvenile

Suplementasi betain pada pakan untuk meningkatkan pertumbuhan dan pemanfaatan pakan pada juvenil ikan kerapu cantang (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀)

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ABSTRACT

Betaine plays some important roles in feed utilization and fish metabolism. This study aimed to evaluate the effect of dietary betaine supplementation in diets on the growth performance and feed utilization in hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) A completely randomized experimental design with four dietary levels of betaine, i.e.0.0%, 0.5%, 1.0%, and 2.0% in quadruplicate was done. Hybrid grouper juvenile obtained from Fisheries Center Brackish Water Aquaculture, Situbondo, East Java with an initial body length and body weight of 5.89 ± 0.05 cm and 2.86 ± 0.09 g, respectively was used as the tested animal. The fish was maintained in a 60 cm × 40 cm × 40 cm aquaria with 75 L working capacity with an individual recirculating system with a fish density of 15 fish/aquarium for 50 days. An experimental diet was provided to apparent satiation twice a day. The results of this study demonstrated that dietary betaine supplementation at a level of 0.5% resulted in higher feed utilization efficiency (115.12 ± 3.34%), protein and methionine retentions (26.43 ± 0.75% and 33.22 ± 2.59%), growth performance (SGR; 3.52 ± 0.17 g/h) and lower ammonia excretion (1.40 ± 0.40 µg/L.g.h) than those of the control (P<0.05). Higher antioxidative status was indicated by the lower malondialdehyde (MDA) in the liver of fish fed with betaine supplemented diets at levels of 1–2%. In conclusion, betaine supplementation of 0.5% could increase feed utilization efficiency and growth performance of hybrid grouper.

Keywords: Betaine, hybrid grouper, growth performance, feed, antioxidative status

ABSTRAK

Betain memegang beberapa peranan penting dalam pemanfaatan pakan dan metabolisme pada ikan. Penelitian ini bertujuan untuk mengevaluasi pengaruh suplementasi betain dalam pakan terhadap kinerja pertumbuhan dan pemanfaatan pakan untuk juvenil ikan kerapu hybrid cantang (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀). Penelitian menggunakan rancangan acak lengkap yang terdiri atas empat perlakuan pakan pada tingkat suplementasi betain yang berbeda, yaitu 0.0%; 0,5%; 1,0%; dan 2.0% dan empat ulangan. Hewan uji yang digunakan dalam penelitian ini berupa juvenil ikan kerapu cantang yang berasal dari Balai Pengembangan Budidaya Laut Situbondo dengan panjang dan bobot awal masing-masing 5.89 ± 0.05 cm and 2.86 ± 0.09 g. Ikan dipelihara dalam akuarium berukuran 60 cm × 40 cm × 40 cm dengan kapasitas 75 L dilengkapi sistem resirkulasi individu dengan kepadatan 15 ekor per akuarium selama 50 hari. Ikan diberi pakan uji dengan frekuensi pemberian dua kali sehari sampai kenyang. Hasil penelitian menunjukkan suplementasi betain sebanyak 0.5% menghasilkan pemanfaatan pakan (115.12 ± 3.34%), retensi protein (26.43 ± 0.75%), dan metionin (33.22 ± 2.59%), kinerja pertumbuhan (3.52 ± 0.17%) dan ekskresi amonia (1.40 ± 0.40 µg/L.g.j) yang lebih baik daripada kontrol (P<0.05). Status antioksidasi yang juga lebih baik ditunjukkan dengan lebih rendahnya konsentrasi malondialdehid (MDA) pada hati ikan yang diberi pakan dengan suplementasi 1–2% betain. Berdasarkan hasil penelitian dapat disimpulkan bahwa suplementasi betain sebanyak 0.5% dapat meningkatkan efisiensi pemanfaatan pakan dan kinerja pertumbuhan ikan kerapu cantang.

Kata kunci: betain, kerapu cantang, pertumbuhan, pakan, status antioksidan

INTRODUCTION

Aquaculture production is predicted to increase steadily and is followed by the feed demand which is forecasted to reach up to 1.9 billion tons in 2027 (FAO, 2018). As a maritime country with a promising marine aquaculture prospect, Indonesia must rise its role as a global marine product supplier by developing various local marine species. One of the potential species to develop is the hybrid grouper that is a cross-breed between male *Epinephelus lanceolatus* and female *Epinephelus fuscoguttatus*. *Epinephelus lanceolatus* and *Epinephelus fuscoguttatus* have high economic values, high protein content, and a highly-demanded market. However, the rearing of this particular hybrid grouper has been facing several problems, i.e. long rearing period (Jiet & Musa, 2018), high feed conversion, fragile towards various environmental changes, easily exposed to stress, and disease threat (Dedi *et al*., 2018; Dahlia *et al*., 2017, Irawanto *et al*., 2018).

Feed quality affects growth significantly and is determined by the ingredients. The artificial feed is made through the various raw material combination to fulfill the nutrient profile required by a certain fish species. Besides the essential component, the feed quality is potentially increased by adding assorted additive compounds. Those additive compounds are usually aimed at a certain purpose, e.g. induce the immune system or prevent stress. One of the additive compounds that are frequently used as a supplement is betaine.

Betaine is a quarter ammonium zwitterionic substance that has quarter ammonium base cations, carboxylic acid anion, and salts (Tiihonen *et al*., 2014), which commonly known as *trimethyl-glycine*, *glycine, betaine*, *lysine*, and *oxyneurine*. These compounds function as an organic osmolyte to protect cells when exposed to stress or as a methyl group in the catabolism process through transmethylation for various biochemistry paths (Knight *et al.*, 2017). As an osmolyte, betaine can protect cells, proteins, and enzymes in an organism against numerous environmental stressors. On the other hand, as a methyl donor, betaine participates in the methionine cycle. As a result, it promotes greater efficiency of methionine utilization which is also known as limiting amino acids. By incorporating betaine, protein efficiency is well-controlled and its waste can be minimized. Betaine is also known as an attractant that increase fish interest towards feed so that it can boost feed consumption and decrease uneaten feed. Betaine supplementation in brown-marbled grouper acted as a feed enhancer (Lim *et al*., 2015) and promoted greater growth in *Labeo bata* (Ghosh *et al*., 2019). Luo *et al.* (2011) stated that Nile tilapia reached the best growth with 0.5% of betaine supplementation. Dietary supplementation with 1.69% of betaine and 28% of beetroot in feed decreased the total fatty acids in a rainbow trout muscle, but increased the PUFA-DHA content (Pinedo-Gil *et al*., 2019). Betaine supplementation along with the other attractants resulted in better feed efficiency and growth because of the higher nutrition absorption and adequate nutrition profiles (Jiang *et al*., 2019). A betaine supplementation of 0.4% in a 37% of protein diet without fish meal was able to improve the growth and lipid deposition in gourami fish (Dong *et al*., 2018). Adjoumani *et al*. (2019) reported that blunt snout bream had a higher growth performance and greater antioxidant capacity because of 1.2 or 0.8% of dietary betaine and 0.2 or 0.4% of dietary choline in a 32% of isoproteic feed. Betaine supplementation in grouper feed is still limited. Therefore, this study aimed to increase the growth and feed efficiency of hybrid grouper using betaine supplementation.

MATERIALS AND METHODS

Experimental design

This study was designed using completed randomized design with four treatments of betaine, i.e. 0.0% ($B_{0.0}$), 0.5% of betaine ($B_{0.5}$), 1.0% of betaine ($B_{1.0}$), and 2.0% of betaine ($B_{2.0}$). This study was conducted in IPB Fisheries and Marine Observation Station, Ancol, North Jakarta.

Experimental diets

The experimental diets were formulated in isonitrogenous and isoenergetic with different betaine supplementation levels was according to the treatment (Table 1). The ingredients were sieved on a 100 µm of mesh size, then mixed thoroughly. The mixture was pelleted and dried at 50°C for 12 hours. Thereafter, the experimental diets were stored in a fully closed container until further use.

Fish rearing

The juvenile of hybrid grouper was obtained from Brackish Water Aquaculture Development Centre, Situbondo, East Java. The average length and weight of the experimental fish were 5.89 ± 0.01 cm and 2.85 ± 0.05 g. respectively. The juvenile was reared in an aquarium sized in 60

Table 1. The composition of experimental diets with different betaine supplementation levels

	Treatment			
	$B_{0.0}$	$B_{0.5}$	$B_{1.0}$	$B_{2.0}$
Ingredients (%)				
Fish meal	50	50	50	50
Soybean meal	29	29	29	29
Wheat flour	4.85	4.35	3.85	2.85
Meat bone meal	6	6	6	6
Fish oil	4	4	4	4
Squid oil	4	4	4	4
Vitamin mix	0.5	0.5	0.5	0.5
Mineral mix	0.5	0.5	0.5	0.5
Betaine	0	0.5	1	2
$Ca(H_2PO_4)_2$	0.1	0.1	0.1	0.1
Ethoxyquin	0.05	0.05	0.05	0.05
Choline	0.5	0.5	0.5	0.5
Polimethylolcarbamide	0.5	0.5	0.5	0.5
Composition (%)				
Moisture	11.77	9.39	9.16	10.16
Crude protein	59.80	60.79	60.91	60.36
Crude fat	12.19	12.41	12.25	11.88
Crude ash	12.47	11.50	11.74	11.35
Crude fiber	3.55	3.65	4.34	1.28
NFE*	15.54	15.30	15.10	16.41
GE (kcal/kg)	453	460	462	443

*NFE: nitrogen-free extract was calculated according Jiang *et al.* (2015); NFE = 100 – (protein + fat + ash); and GE: gross energy.

cm ×40 cm × 40 cm previously filled with 70 L of seawater. An individual recirculation system was installed in each aquarium. After 7 days of acclimatization, the juvenile was distributed at a stocking density of 15 individuals per aquarium (209 individuals/m^3) and maintained for 50 days. Feeding was provided twice a day, 8.00 a.m. and 4 p.m. to apparent satiety. The juvenile growth was monitored using length and weight measurement on day 30. Fish survival, final weight and length were calculated at the end of the study. Three fish were collected from each treatment for proximate and antioxidant analysis. The water parameters consisted of salinity (refractometer), pH (pH meter PH-009(1)), and dissolved oxygen (PDO-519, Japan). All parameters were measured periodically during the study.

Experimental parameter

The tested parameters were feed efficiency, growth performance, and antioxidant status of the hybrid grouper juvenile. The feed efficiency test was analyzed by measuring feed conversion rate, feed efficiency, protein, methionine, and lipid retention, and ammonia excretion. Growth performance was measured through the following parameters, i.e. specific growth rate, condition factor, size variance, hepatosomatic index, and survival. Furthermore, superoxide dismutase (SOD) and malondialdehyde (MDA) were calculated to determine the antioxidant status.

Feed consumption was calculated from the margins amongst the initial feed quantity and the remaining feed during the rearing period (De *et al*., 2016). Feed efficiency was determined by the following formula (Ye *et al*., 2019);

$$EPP = \frac{(Wt + Wd - Wo)}{F} \times 100$$

EPP: Feed efficiency (%); Wt : final biomass (g); Wd : dead fish biomass (g); Wo : initial biomass (g); F: feed consumption (g). Nutrient retention (protein, methionine, lipid) was calculated according to Li *et al*. (2018).

$$NR = \frac{(Nt - No)}{Nf} \times 100$$

NR : Nutrient retention (%), protein (Pro), methionine (M), lipid (L); Nt: final nutrient content (g); No: initial nutrient content (g); Nf : total consumed nutrient (g). Ammonia excretion was measured using the following formula by Suprayudi *et al*. (2014). Ammonia measurement was done before feeding, an hour, two hours, and three hours after feeding with nonactive aeration. The specific growth rate is stated using the following formula (Wang *et al*., 2020);

$$SGR = \frac{(\ln Wt - \ln Wo)}{t} \times 100$$

SGR: Specific growth rate (%/day); W_t: final average body weight (g); W_o: initial average body weight (g); and t: rearing period (day). Size variance was determined based on the formula by Xie *et al*. (2011);

$$SV = \frac{S}{\bar{x}} \times 100$$

SV : size variance (%); S : standard deviation; $\bar{x}$: average size. Condition factor is the ratio between weight and length (Liang *et al*., 2020);

$$CF = \frac{W}{L^3} \times 100$$

CF : Condition factor; W : fish weight (g); L : fish length (cm). The survival rate was calculated using the following formula (Ye *et al*., 2020);

$$SR = \frac{N_t}{N_0} \times 100$$

SR : survival (%); Nt : final population (individual); No : initial population (individual). The hepatosomatic index is a ratio between liver weight and body weight. It is calculated using the formula below (Tan *et al*., 2018).

$$HSI = \frac{Wh}{W} \times 100$$

HSI : hepatosomatic index (%); Wh : liver weight (g); W : body weight (g).

SOD (superoxide dismutase) and MDA (malondialdehyde) analysis

The sample was weighed and added with phosphate buffer saline (PBS: 0.064 mol/L, pH 7.4), and homogenized in an ice bath. The homogenate was centrifuged at 3000 rpm for 15–20 minutes. It produced a supernatant that would be analyzed for SOD and MDA. SOD analysis was managed through WST-1 method (Ren *et al*., 2020). WST-1 is changed into formazan dye by the superoxide radical which is produced by the xanthine oxidase system. Furthermore, the formazan was quantified in 550 nm of absorbance. One unit SOD is a 50% reduction of WST-1 inhibiting enzyme. Meanwhile, the MDA was determined by the thiobarbituric acid reaction which is greatly absorbent in 532 nm (Fan *et al*., 2019).

Chemical analysis

The proximate analysis consisted of moisture, fat, protein, and ash analyses. Moisture was analyzed through the gravimetric method. Kjeldahl, Soxhlet, and Folch methods were applied to analyze protein, fat, and ash content, respectively. Proximate analysis was done based on the AOAC procedure (Xie *et al*., 2021). A bomb calorimeter was used to analyze the energy. Methionine and cystine were analyzed using HPLC method (Li *et al*., 2019). Ammonia analysis was conducted using the Phenate method (Zhou & Boyd, 2016). The absorbance used in ammonia analysis was 630 nm. The spectrophotometric method was not only used in ammonia analysis but also SOD and MDA (Sofian *et al*., 2016).

Data analysis

Statistical analysis was performed using a statistic software SPSS 16. Analysis of variance (ANOVA) was used to analyse the data and a post-hoc Duncan test was subsequently performed to determine the differences between the treatments at 95% of confidence level.

RESULTS AND DISCUSSION

Results

Growth

Table 2 showed that generally, betaine supplementation was able to boost the growth performance of hybrid grouper juveniles. Average final weight, final length, and specific growth rate indicated greater results in $B_{0.5}$, $B_{1.0}$, and $B_{2.0}$ treatment compared to control treatment. Especially, the $B_{0.5}$ treatment presented a significant result compared to other treatments. While the fish survival indicated no significant result ($P>0.05$).

Feed intake of hybrid grouper juvenile with betaine supplementation presented positive results. It was explained by the result of feed consumption, feed efficiency, protein, methionine, and fat retention, and also hepatosomatic index which are greater than those of the control treatment (Table 3). The ammonia excretion rate of hybrid grouper juvenile of betaine supplementation treatment showed better performance than the control treatment.

Even though the lipid content increased slightly in the betaine treatments, there was no significant difference amongst treatments (Table 4). Protein content was influenced by betaine supplementation with the highest was found in $B_{0.5}$ treatment. Water and methionine content was not different significantly amongst treatments ($P>0.05$).

Table 2. Initial weight, initial length, final weight, final length, specific growth rate (SGR), condition factor (CF), coefficient variation (CV), hepatosomatic index, and survival of hybrid grouper juvenile fed with a diet supplemented with different levels of betaine for 50 days.

	Treatment			
	$B_{0.0}$	$B_{0.5}$	$B_{1.0}$	$B_{2.0}$
Initial weight (g)	2.89 ± 0.04	2.88 ± 0.03	2.86 ± 0.04	2.78 ± 0.04
Initial length (cm)	5.92 ± 0.02	5.89 ± 0.03	5.89 ± 0.02	5.85 ± 0.01
Final weight (g)	14.19 ± 1.49^{a}	19.61 ± 1.13^{b}	15.85 ± 0.81^{a}	14.82 ± 0.70^{a}
Final length (cm)	9.21 ± 0.31^{a}	10.24 ± 0.12^{b}	9.58 ± 0.14^{a}	9.45 ± 0.11^{a}
SGR (%/day)	2.59 ± 0.15^{a}	3.52 ± 0.17^{b}	3.07 ± 0.20^{ab}	3.16 ± 0.19^{ab}
CF	1.78 ± 0.02^{ab}	1.82 ± 0.06^{b}	1.71 ± 0.04^{ab}	1.68 ± 0.04^{a}
CV (%)	8.26 ± 0.48	7.31 ± 1.10	10.96 ± 2.55	8.21 ± 0.37
HSI (%)	1.29 ± 0.12^{a}	1.33 ± 0.12^{a}	1.34 ± 0.13^{a}	1.27 ± 0.07^{a}
Survival (%)	78.33 ± 11.34	86.67 ± 7.20	85.00 ± 6.87	91.67 ± 5.00

Different superscript letters following mean values (± standard error) in the same row indicate significant differences ($P<0.05$).

Table 3. Feed intake, feed efficiency (FE), protein retention, methionine retention, lipid retention, and ammonia excretion rate of hybrid grouper juvenile fed with a diet supplemented with different levels of betaine for 50 days.

Parameter	Treatment			
	$B_{0.0}$	$B_{0.5}$	$B_{1.0}$	$B_{2.0}$
Feed intake (g)	163.69 ± 3.67^{a}	205.36 ± 6.71^{c}	179.38 ± 5.91^{ab}	196.69 ± 10.57^{bc}
FE (%)	86.93 ± 3.94^{a}	115.12 ± 3.34^{b}	101.77 ± 4.78^{ab}	89.29 ± 6.23^{a}
Protein retention (%)	14.68 ± 0.99^{a}	26.43 ± 0.75^{b}	20.92 ± 3.17^{ab}	17.92 ± 3.00^{a}
Methionine retention (%)	31.53 ± 4.11^{a}	33.22 ± 2.59^{a}	33.31 ± 4.94^{a}	34.76 ± 3.63^{a}
Lipid retention (%)	7.44 ± 1.97^{a}	29.40 ± 0.75^{b}	25.48 ± 4.06^{b}	23.73 ± 3.93^{b}
Ammonia excretion (µg/L.g.h)	6.5 ± 2.7^{a}	1.4 ± 0.4^{a}	2.3 ± 1.1^{a}	2.7 ± 1.6^{a}

Different superscript letters following mean values (± standard error) in the same row indicate significant differences ($P<0.05$).

The antioxidant status of hybrid grouper juvenile was shown by SOD and MDA concentration (Table 5). SOD concentration did not present any significant results. On the contrary, a lower MDA concentration showed in $B_{1.0}$ and $B_{2.0}$ of betaine supplementation.

Discussion

Dietary supplementation of betaine (trimethylglycine) has been reported to improve growth performance, health and immune status, feed digestibility, and flesh quality of fish (Abdelsattar *et al*., 2019; Ismail *et al*., 2020). The benefits of dietary supplementation of betaine in aquaculture including as a feed attractant that stimulate the attractability of feed (Lim *et al*., 2015), as an osmolyte that protects intracellular enzymes against osmotically or temperature-induced inactivation (Shankar *et al*., 2008), and as a methyl donor that facilitate the synthesis of methionine, carnitine, phosphatidylcholine, and creatine, which further improve protein, lipid, and energy metabolism (Wang *et al*., 2013; Figueroa-Soto & Valenzuela-Soto, 2018), and modulate gut microbiome (He *et al*., 2011). These beneficial effects of betaine were generally confirmed in the present study. The result of this study demonstrated that dietary betaine supplementation could improve the growth and feed utilization efficiency in hybrid grouper juveniles. The specific growth rate of hybrid grouper juvenile was within a range of 2.59–3.16 with the highest level was observed in treatment with 0.5% betaine supplementation. This range was slightly higher than that reported in a previous study for hybrid grouper at about 2.06 %/day (Othman *et al*., 2015; Shapawi *et al*., 2018).

The improved growth of hybrid grouper may be explained by the beneficial effects of betaine on feed utilization. The result of the present study also shows an increased feed intake in the fish fed with betaine supplemented diet, which confirms previous studies that demonstrated the positive effect of betaine as a feed attractant in some aquaculture species such as *Epinephelus fuscoguttatus* (Lim *et al*., 2015), hybrid tilapia (He *et al*., 2011), giant prawn (Felix & Sudharsan, 2004), rockfish (Kim & Cho, 2019), and turbot (Jiang *et al*., 2019). Feed efficiency significantly improved in the fish fed with betaine at 0.5% and 1.0%, which may be related to the increase in protein utilization efficiency, which was also higher in these treatments. Higher protein retention (29.43 ± 0.75%) was confirmed by the decrease in ammonia excretion rate at 1.4 ± 0.4 μg/L.g.h. The effect on protein utilization relates to the role of betaine in the methionine cycle. It has been reported that betaine could increase the expression of some genes related to the methionine cycle including betaine homocysteine methyltransferase, methionine adenosyl transferase-1, methyltetrahydrofolate

Table 4. Whole-body proximate composition and methionine level of hybrid grouper juvenile fed with a diet supplemented with different levels of betaine.

Parameter (%)	Treatment			
	$B_{0.0}$	$B_{0.5}$	$B_{1.0}$	$B_{2.0}$
Moisture	75.87 ± 0.27[a]	76.26 ± 0.55[a]	77.01 ± 0.66[a]	76.65 ± 0.56[a]
Crude ash	4.85 ± 0.18[a]	5.59 ± 0.25[b]	4.81 ± 0.23[a]	4.81 ± 0.23[a]
Crude lipid	1.55 ± 0.21[a]	3.27 ± 0.23[b]	3.02 ± 0.24[b]	2.94 ± 0.22[b]
Crude Protein	13.02 ± 0.42[a]	15.59 ± 0.38[b]	14.30 ± 0.53[ab]	13.22 ± 0.74[a]
Methionine	0.37 ± 0.02[a]	0.34 ± 0.03[a]	0.30 ± 0.02[a]	0.32 ± 0.02[a]

Different superscript letters following mean values (± standard error) in the same row indicate significant differences (P<0.05).

Table 5. Superoxide dismutase (SOD) and malondialdehyde (MDA) in hybrid grouper juveniles fed with a diet supplemented with different levels of betaine.

Parameter	Betaine treatment			
	$B_{0.0}$	$B_{0.5}$	$B_{1.0}$	$B_{2.0}$
SOD (μg/g pro)	11.44 ± 0.41[a]	11.70 ± 0.83[a]	10.42 ± 0.35[a]	11.90 ± 0.45[a]
MDA (μmol/L)	0.32 ± 0.02[b]	0.35 ± 0.04[b]	0.20 ± 0.01[a]	0.23 ± 0.02[a]

Different superscript letters following mean values (± standard error) in the same row indicate significant differences (P<0.05).

reductase, glycine N-methyltransferase, S-adenosyl homocysteine hydrolase, methionine synthase, cystathionine ß-synthase dan phosphatidylethanolamine methyltransferase (Kharbanda *et al*., 2009; Kwon *et al*., 2009; Oliva *et al*., 2009; Kim & Kim, 2005; Kharbanda *et al*., 2007; Juang *et al*., 2013). Interestingly, despite the increased protein retention, there was no significant difference in methionine retention. Lipid retention in the treatments with betaine supplementation was higher than that of the control. This result was different from previous reports, which generally report a decrease in lipid deposition in the fish when fed with betaine supplemented diets. Wang *et al*. (2013) dan Song *et al*. (2007) reported that betaine increased the mobilization of triacylglycerol by increasing the concentration of apolipoprotein B, reduced lipogenesis, and stimulate fatty acid oxidation and lipid catabolism. At the same time, betaine could also play a role in glucose and glycogen metabolism by increasing insulin receptor substrate 1 phosphorylation which increases glucose transportation, the decrease of gluconeogenesis, and hepatic lipolysis (Song *et al*., 2007), which may explain the increase lipid retention in the present study.

The present study showed that the highest fish growth performance was observed in the fish fed with a diet containing 0.5% betaine. It seems that the optimum supplementation level of betaine strongly depends on the fish species and the nutritional composition of the feed. Betaine 0.3% supplementation has been carried out on cantang hybrid grouper and resulted in growth that was not different from the substitution of 100% fish oil with wheat germ oil as the lipid source (Baoshan *et al*., 2019). Lim *et al*., 2015) reported that a betaine supplementation level of 1.2% could improve the feeding performance of tiger grouper fed with a diet containing soybean meal to substitute 50% of fishmeal as the protein source. Other studies in blunt snout bream showed that the supplementation of 1.2% betaine could improve the fish growth performance when fed with a high-fat diet (Adjoumani *et al*., 2019). On the other hand, a betaine supplementation level of 0.5% resulted in increased growth in giant prawns (Felix & Sudharsan, 2004). Likewise, a study in gibel carp showed that optimum growth was obtained when the fish fed a diet containing 0.4% betaine (Dong *et al*., 2018).

Betaine supplementation supports growth by optimizing nutrition intake, starts from inducing the fish appetite (El-Husseinny *et al*., 2008) until the nutrient utilization process in the digestion (Ratriyanto *et al*., 2009) to accomplish the protein requirement. The composition of amino acids in muscle tissue is affected by the amino acid profile because it represents the feed quality (Wu *et al*., 2017; Tan *et al*., 2019). Methionine plays a role as a donor in methyl group, transamination, and protein forming unit (Poppi *et al*., 2019; Xu *et al*., 2019). Betaine was reported to be able to increase the activity of *paraoxonase-1* which uses lipid peroxidation as the substrate, to reduce the activity of *cytochrome P-450 2E1* and *nitric oxide synthase 2*, and could reduce MDA concentration and increase the activity of SOD (Oliva *et al*., 2011). In the present study, there was no significant difference in SOD observed amongst the treatments. However, there was a significant reduction in MDA concentration in treatments $B_{1.0}$ and $B_{2.0}$, which indicate the increase in antioxidative capacity in these particular treatments. Meanwhile, the results of water quality measurements showed the optimum range for growth of hybrid grouper with a salinity range at 27–32 g/L, temperature 30–33°C, pH 6.5–8.0, and dissolved oxygen 4.2–6.1 mg/L.

CONCLUSION

The present study demonstrates that dietary supplementation of betaine at a level of 0.5% could improve the growth performance, feed utilization efficiency, and antioxidative status of hybrid grouper.

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