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Growth evaluation of *Oreochromis niloticus* fed different concentrations of choline and methionine

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ABSTRACT

Choline and methionine are playing a vital role in fish metabolic and growth. The objective of the present study was to determine the effects of choline and methionine supplementation on the growth performance of Nile tilapia Oreochromis niloticus. In total eight treatments diet, namely: P2-P4 with 0.04; 0.08; 0.12 % of choline; P6-P8 with same levels choline plus methionine (0.15 %); a diet with only 0.15 % methionine (P5); and control without any supplementation (P1) were fed to tilapia (initial weight 21.05±1.6 g) for 12 weeks. Final weight (FW), Body Weight Gain (BWG), Average Daily Gain (ADG), Specific Growth Rate (SGR), and Protein Efficiency Ratio (PER), Feed Efficiency (FE), Feed Intake (FI), Feed Conversion Rate (FCR), Survival Rate (SR), crude protein and lipid of fish fed various levels of choline and methionine were also measured. The results showed that tilapia fed 0.04 % choline (P2) had significantly better growth parameters and feed efficiency than other groups. Meanwhile, SR of tilapia was not affected by any supplementation of choline and methionine. The tilapia fed 0.08% choline (P3) showed the highest crude protein (52.50 ± 0.98 %) in the carcass proximate composition but low lipid (19.03 ± 0.10 %). It is concluded that the study demonstrated the benefits of choline (0.04-0.08 %) supplementation in the tilapia diet in term of growth and carcass proximate composition.

Introduction

Choline is a pivotal nutrient and has an important responsible in cell structure, cell maintenance, and specific metabolic functions (Baldissera *et al.*, 2019; Khosravi *et al.*, 2015; NRC, 1993). It is also a part of acetylcholine, a neurotransmitter in the synapsis of neuron, which acts in the impuls transmission across synapses (Topal *et al.*, 2016; Wauben and Wainwright, 1999). One of the essential functions of choline metabolism is a source of methyl groups for reaction transmethylation formation of methionine from homocysteine (Combs, 2008; Kim *et al.*, 2019). Choline is a methyl group donor via its degradation products which is known as betaine (Day and Kempson, 2016; Workel, 2005). Some animals can produce choline if the methyl donor (Example: Methionine) is presented in the feed. Choline (example: phosphatidylcholine) can be synthesized through methylation of phosphatidylethanolamine assisted by S-adenosyl methionine (SAM) as a methyl donor (Abbasi *et al.*, 2018; Combs, 2008).

Choline, also known as a vitamin-like nutrient, is identified as an important feed component especially in fish (NRC, 1993). Dietary choline supplementation enhanced growth indices, boosted protein and lipid deposition, increased hepatopancreatic and intestinal enzyme activities in Jian carp *Cyprinus carpio* var. Jian (Wu *et al.*, 2011). Dietary choline requirement for several fish species have been predicted and reported to be at the range of 400 to 1,000 mg kg^{-1} diet (Fernandes *et al.*, 2016; Luo *et al.*, 2016; Qin *et al.*, 2017). Previous study by Shiau and Lo (1999) stated that the optimum growth of tilapia can be achieved by addition 1,000 mg choline per kg diet. A previous study also confirmed that choline level about 230 mg kg^{-1} diet sufficient

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for juvenile parrot fish (*Oplegnathus fasciatus*) requirement (Khosravi *et al.*, 2015).

Meanwhile, methionine is a pivotal amino acid metabolite that uses for protein synthesis and the formation of SAM (Ratriyanto *et al.*, 2009). However, the rate of choline synthesis is not enough to achieve either the physiological or metabolic requirement in several animals (Wilson and Poe, 1988). This is due to the function of methionine for protein synthesis and the formation of mutual competence SAM to methionine available. Therefore, to meet the needs of choline and methyl donor for the methionine synthesis, choline should be supplemented in the diet.

Niu *et al.* (2016) found that the substitution of fish meal using soybean meal and methionine supplementation resulted in significantly better growth in juvenile golden pompano (*Trachinotus ovatus*). In addition, Wang *et al.*, (2013) stated that the methionine supplementation in for juvenile *Pseudobagrus ussuriensis* culture to obtain optimum growth and feed utilization is approximately 14 g kg^{-1} dry diet, respectively, in addition of 2.5 g kg^{-1} dry diets cysteine. However, the studies on nutrient requirement of the choline and methionine on the growth of Nile Tilapia (*Oreochromis niloticus*) are limited. Thus, the purpose of this research was to determine the effects of choline and methionine addition on the growth indices and carcass proximate composition of tilapia.

Tilapia is an omnivorous species and considered a valuable species because of high demand, easily maintained and cultivated ponds by utilizing natural feed and supplementary feeding in the form of artificial feed (Kordi, 2010). Tilapia is also considered to be the second most valuable fish culture in the globe, after carps (Moniruzzaman *et al.*, 2015; Ridha, 2006), and its cultivation is practiced in most of the tropical, subtropical and temperate regions, including Indonesia (Phillips *et al.*, 2015). In Indonesia, the biggest production of tilapia is still in West Java, reaching 8.524 ton in 2013 while East Kalimantan has 5.117 ton production of tilapia in the same year (Widiarti, 2015). The success and significantly improve in tilapia culture outcome have received attention among farmers, investors and especially researchers who attempts to boost productivity by optimizing their growth. Therefore, the objective of the present study was to determine the effects of choline and methionine supplementation on the growth performance of Nile tilapia *Oreochromis niloticus*.

Materials and Methods

Experimental diets preparation

Composition of the test diets formula is provided in Table 1. The test diets that were formulated and consisted of three diets (P2-P4) were prepared by supplementing 0.04; 0.08; 0.12 % choline, three diets (P6-P8) with same levels choline supplementation plus methionine (0.15 %), a diet with methionine (P5) and control diet without any supplementation (P1). The proximate analysis of test diet fish carcass was analysed using AOAC (1990).

Fish and feeding trial

Twenty-four cages (1 m x 1 m) were set up in a semi-intensive pond (17 m x 8 m) at a density of 50 fish per cage. The fish were fed a commercial diet (Hi Pro Vite FF-888, containing 36–38% protein, 2% lipid, 2% crude fibre, 10% ash and 12% moisture) for three days to become acclimated to the research condition and equipment. At final day of acclimation period, the fish (Initial body weight, 21.05±1.6 g) were randomly assigned to pond cage. The study was conducted in three replicates and fish were fed by hand to apparent satiation five times a day (08:00, 10:00, 11:30, 14:00 and 16:00 h) for 12 weeks. Two pumps ventury were used as aeration to maintain sufficient dissolved oxygen. Dissolved Oxygen (DO), temperature, and pH of pool water were measured twice a day (morning and afternoon). The levels of nitrite and TAN (total ammonia nitrogen) of pool water were checked once a week. Sampled-fish growth was noted every four weeks, and feeding rate was calculated following the change of the weight of the fish. The fish were then fasted for 24 h before weight and the carcass of fish sampling.

Sample collection and analyses

At the final of week 12, the sampled-fish in each cage were weighed and counted to calculate the growth performance such as Final weight (FW), Body Weight Gain (BWG), Average Daily Gain (ADG), Specific Growth Rate (SGR), Feed Efficiency (FE), Feed Intake (FI), Feed Conversion Rate (FCR), Protein Efficiency Ratio (PER), and Survival Rate (SR). The growth performance was determined by using the below equation as previously used by Manjappa *et al.* (2016); Nur *et al.* (2017), Yusup and Nugroho (2017); and Cruz *et al.*, (2018):

BWG (*Body Weight Gain*) = Wt –Wo

$$\text{ADG (Average daily weight Gain)} = \frac{[\text{Wt-Wo}]}{\text{number of day}}$$

$$\text{SGR (\% day}^{-1}\text{)} = \frac{[\text{Ln Wt -Ln Wo}]}{t} \times 100$$

Where, W_0 is the initial fish weight (g) at time T_1 (day), and W_t is the final fish weight (g) at time T_2 (day).

Table 1. Formulation and proximate composition of the diets (% dry matter)

Ingredients	P1	P2	P3	P4	P5	P6	P7	P8
Corn	6.40	6.40	6.40	6.40	6.40	6.40	6.40	6.40
Wheat	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soya Bean Meal	31.94	31.90	31.86	31.82	31.78	31.75	31.71	31.67
Cassava starch	17.80	17.80	17.80	17.80	17.80	17.80	17.80	17.80
Animal protein	18.90	18.90	18.90	18.90	18.90	18.90	18.90	18.90
Fish + Vegetable oil	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Vitamin mix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral mix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline	0.00	0.04	0.08	0.12	0.00	0.04	0.08	0.12
DL-Methionine	0.00	0.00	0.00	0.00	0.15	0.15	0.15	0.15
Proximate composition								
Crude Protein	31.85	31.79	32.06	32.69	31.86	32.38	32.42	32.41
Crude Lipid	3.79	3.88	3.88	3.90	3.79	3.72	3.95	2.67
Ash	9.23	9.17	9.06	8.89	8.94	8.87	8.80	8.95

Note: P1= control, P2= choline (0.04 %), P3= choline (0.08 %), P4= choline (0.12 %), P5= methionine (0.15 %), P6= choline (0.04%) and methionine (0.15 %), P7= choline (0.08 %) and methionine (0.15 %), P8= choline (0.12%) and methionine (0.15 %). Vitamin mix: vitamin A 1200000 UI, vitamin D3200000 UI, vitamin E 1200 mg, vitamin K3 2400 mg, vitamin B14800 mg, vitamin B24800 mg, vitamin B124800 mg, vitamin B6 4800 mg, vitamin C 48 mg, calcium panthotenate 12000mg, niacin 24000 mg, biotin 48 mg, choline 108 g, mineral mix: cobalt 10 mg, copper 3000 mg, iron 50000 mg, iodine 100 mg, manganese 20000 mg, Se 100 mg, zinc 30000 mg, carrier 1000 g, antioxidant 25 g.

Table 2. Growth indices and feed utilities of Tilapia (*Oreochromis niloticus*) fed different combination ratio of choline and methionine diets for 12 weeks.

Parameters	Groups							
	P1	P2	P3	P4	P5	P6	P7	P8
IW (g)	21.00 ± 0.11^{a}	21.00 ± 0.66^{a}	21.06 ± 0.66^{a}	21.13 ± 0.66^{a}	21.06 ± 0.66^{a}	21.00 ± 0.00^{a}	21.00 ± 0.31^{a}	21.13 ± 0.11^{a}
FW (g)	85.62 ± 0.81^{ab}	103.15 ± 0.91^{c}	86.08 ± 1.58^{b}	87.33 ± 2.07^{b}	85.26 ± 0.54^{ab}	81.92 ± 0.87^{a}	84.85 ± 1.43^{ab}	86.09 ± 0.98^{b}
BWG (g)	64.62 ± 0.81^{ab}	82.15 ± 0.91^{c}	65.02 ± 1.64^{b}	66.19 ± 2.01^{b}	64.19 ± 0.52^{ab}	60.92 ± 0.87^{a}	63.85 ± 1.43^{ab}	64.96 ± 0.91^{b}
ADG	0.76 ± 0.009^{ab}	0.97 ± 0.01^{c}	0.77 ± 0.01^{b}	0.78 ± 0.02^{b}	0.76 ± 0.006^{ab}	0.72 ± 0.01^{a}	0.76 ± 0.01^{ab}	0.77 ± 0.01^{b}
SGR	1.67 ± 0.01^{ab}	1.89 ± 0.01^{c}	1.67 ± 0.02^{b}	1.68 ± 0.02^{b}	1.66 ± 0.007^{ab}	1.62 ± 0.01^{a}	1.66 ± 0.02^{ab}	1.67 ± 0.009^{ab}
FE	0.71 ± 0.02^{a}	0.88 ± 0.04^{b}	0.69 ± 0.01^{b}	0.74 ± 0.03^{a}	0.75 ± 0.04^{a}	0.65 ± 0.07^{b}	0.76 ± 0.02^{ab}	0.72 ± 0.02^{a}
FI	90.07 ± 2.36^{ab}	93.42 ± 4.52^{b}	93.60 ± 2.32^{b}	88.82 ± 5.51^{ab}	85.96 ± 4.79^{ab}	95.43 ± 1.77^{b}	84.09 ± 3.37^{ab}	89.41 ± 3.40^{ab}
FCR	1.39 ± 0.04^{a}	1.13 ± 0.05^{b}	1.44 ± 0.04^{a}	1.34 ± 0.07^{a}	1.33 ± 0.07^{a}	1.56 ± 0.16^{b}	1.31 ± 0.04^{a}	1.37 ± 0.04^{a}

Different alphabets (a<b<c) show significantly different average for different treatments at $P < 0.05$. Note: P1= control, P2= choline (0.04 %), P3= choline (0.08 %), P4= choline (0.12 %), P5= methionine (0.15 %), P6= choline (0.04%) and methionine (0.15 %), P7= choline (0.08 %) and methionine (0.15 %), P8= choline (0.12%) and methionine (0.15 %); IW = Initial weight; FW = Final Weight, BWG = Body Weight Gain, ADG= Average Daily Weight Gain (g/fish/day), SGR= specific growth rate (%/d). FE = Feed efficiency, FI = Feed intake (g/fish/day); FCR= Feed conversion ratio.

Feed efficiency (FE)

FE = [(Final fish weight (g) + dead fish weight (g)) – initial weight / Feed consumed (g dry weight] x 100

Feed Intake = feed consumption/(days×(final body weight+initial body weight) / 2).

Feed conversion ratio (FCR)

FCR is stated as the feed consumed in dry weight per unit live weight gain.

$$FCR = \frac{\text{Feed consumed (g dry weight)}}{\text{Body Weight Gain}}$$

Survival rate (SR)

$$SR(\%) = \frac{(No - Nt)}{No} \times 100$$

Where N_0 is the number of fish at initial day and N_t is the number of fish dead during of experiment (Muchlisin *et al.*, 2016).

Further, 1.2 kg to 1.5 kg of fish per cage was randomly selected samples for the measurement of the proximate fish carcass (crude protein and lipid). The proximate fish carcass was analysed using AOAC (1990) standard methods in laboratories feed mill PT. Suri Tani Pemuka unit Gresik, East Java, Indonesia.

Statistical analysis

Resulted data were expressed as mean ± standard error (SE). Growth data and proximate analysis were analyzed by means of a one-way analysis of variance (ANOVA) using SPSS Statistics software, version 22 (SPSS, Inc., USA). Comparisons between treatment media were carried out using the Duncan test. Percentage data was arcsine transformed before ANOVA and then reversed. All significant tests were at levels $P < 0.05$.

Results

Growth, feed utilization and survival

Growth performance and feed utilization of tilapia fed different ratio combination of choline and methionine for 12 weeks are presented in Table 2 and

PER in Figure 1. After 12 weeks of the feeding trial, tilapia fed 0.04 % choline (P2) in the diet showed the highest final weight, ADG, SGR, and PER. In feed utilization, there was also significantly better (*P<0.05*) in the FI, FE, and FCR of tilapia fed 0.04 % choline (P2) in the diet. Meanwhile, the SR of tilapia was not significantly affected by dietary any combination ratio of choline and methionine (Figure 2).

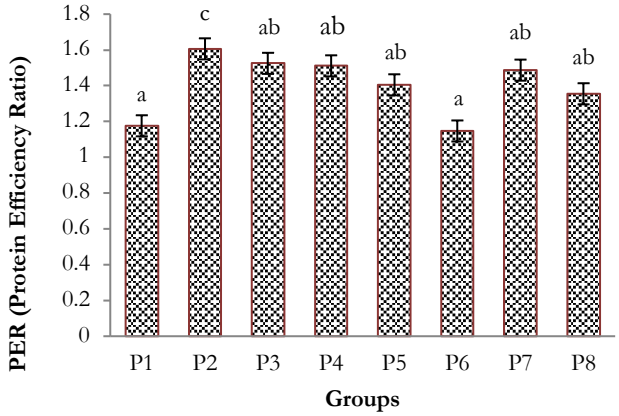

Figure 1. Protein efficiency ratio (PER) of Tilapia (*Oreochromis niloticus*) fed different ratio combination of choline and methionine diets at week 12. Different alphabets (a,b,c) indicate significantly different means for different treatments at $P < 0.05$. Note: P1= control, P2= choline (0.04 %), P3= choline (0.08 %), P4= choline (0.12 %), P5= methionine (0.15 %), P6= choline (0.04 %) and methionine (0.15 %), P7= choline (0.08 %) and methionine (0.15 %), P8= choline (0.12 %) and methionine (0.15 %).

Carcass proximate composition

Besides the growth performance, dietary choline supplementation improved the percentage of crude protein in the carcass of tilapia. The proximate composition of the fish carcass from the experiment is shown in Table 3. Crude protein (52.50 ± 0.98 %) was significantly (*P<0.05*) higher in the tilapia fed choline 0.08 % (P3) in the diet compared to other groups. However, dietary 0.08% choline supplementation in the diet resulted the highest crude lipid (22.34 ± 2.41%) was found in the carcass of tilapia fed control diet, followed by fish fed 0.04 % choline in the diet. However, the administration choline and methionine in the diet may reduce lipid content in the carcass. This result is in agreement with past research stated that the addition of choline and methionine in the diet can reduce fat deposition in carp (*Cyprinus carpio*) (Wu *et al*., 2011).

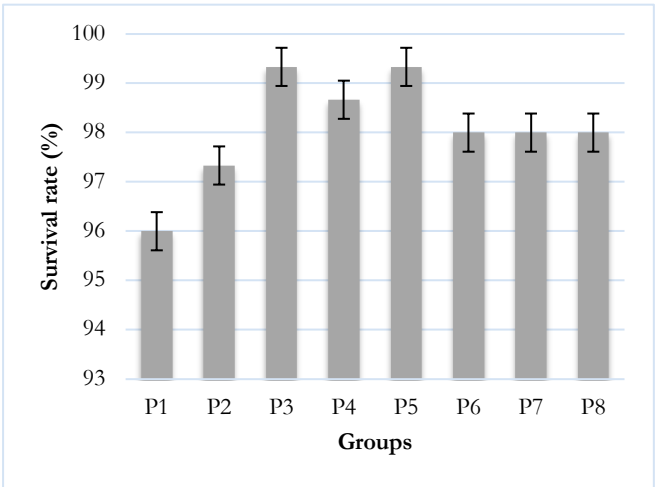

Figure 2. Survival rate of Tilapia (*Oreochromis niloticus*) fed different ratio combination of choline and methionine diets for 12 weeks. Note: P1= control, P2= choline (0.04%), P3= choline (0.08 %), P4= choline (0.12 %), P5= methionine (0.15%), P6= choline (0.04 %) and methionine (0.15%), P7= choline (0.08 %) and methionine (0.15 %), P8= choline (0.12%) and methionine (0.15 %). No significant different among groups.

Table 3. Carcass crude protein and lipid of Tilapia (*Oreochromis niloticus*) fed different ratio combination of choline and methionine diets for 12 weeks

Diet	Crude Protein (%)	Crude Lipid (%)
P1	50.89 ± 1.78^{ab}	22.34 ± 2.41^{c}
P2	49.21 ± 0.59^{a}	21.46 ± 1.48^{bc}
P3	52.50 ± 0.98^{c}	19.03 ± 0.10^{ab}
P4	50.92 ± 0.98^{ab}	21.34 ± 0.74^{bc}
P5	51.98 ± 1.11^{b}	20.59 ± 1.81^{abc}
P6	51.19 ± 0.86^{ab}	19.65 ± 1.94^{abc}
P7	50.74 ± 1.60^{ab}	20.32 ± 2.22^{abc}
P8	51.09 ± 1.42^{ab}	17.85 ± 1.24^{a}

Different alphabets (a<b,<c) indicate significantly different means for different treatments at $P < 0.05$.Note: P1= control, P2= choline (0.04 %), P3= choline (0.08 %), P4= choline (0.12 %), P5 = methionine (0.15 %), P6= choline (0.04%) and methionine (0.15 %), P7= choline (0.08 %) and methionine (0.15 %), P8= choline (0.12 %) and methionine (0.15 %).

Discussion

Choline plays a pivotal role in many metabolic functions such as transmission nerve impulse across synapsis (Wauben and Wainwright, 1999) and methyl donor in the anabolism of various methylated metabolites (Combs Jr and McClung, 2016). Similar to choline, methionine is an essential amino acid which benefit to fish (Niu *et al*., 2016) and acts as methyl donor (Bender, 2003; Furuya, 2017). Current research stated that tilapia fed 0.04 % choline in the diet had highest FW, ADG, SGR, PER and FE

significantly. This finding was similar to previous study revealed that dietary choline in the diet increased growth performance of juvenile yellow catfish (*Pelteobagrus fulvidraco*) (Luo *et al.*, 2016); blunt snout bream (*Megalobrama amblycephal*) (Li *et al.*, 2016) and grouper (*Epinephelus coioides*) (Qin *et al.*, 2017). In addition, the choline supplementation without methyl methionine in the diet might not optimal to increase crude protein level in the carcass as shown in the P2 group in present findings. This result is supported by the statement that methionine is the first important amino acid in the catabolic pathway and has several potential interactions with choline and other dietary constituents (Kasper *et al.*, 2000).

According to NRC (1993) choline supplementation should be added to the diet of fish, although most of the animals could synthesize choline in the liver. That is because the choline production in the body does not meet the metabolic and physiological needs of the animal. In addition, choline has been identified as an important intermediate in the catabolism of methionine (Vemury *et al.*, 1980). As reported by Luo *et al.* (2016), choline involves in lipid homeostatic in muscle and liver by influencing metabolism in mRNA levels. Thus, the addition of adequate choline in the diet may benefit to improve growth performance of fish by increasing the transport of lipids from the liver to muscle for storage and enhancing muscle lipid content. In contrast, deficiency choline in the diet may result in poor growth performance and poor feed intake in hybrid tilapia (Shiau and Lo, 2000).

Furthermore, PER value of the fish shows a protein digestibility of the ratio between the number of acidic amino acids that can be absorbed by the small intestine in the body and the amount of protein consumed by the formation of body tissues. In the process of digestion and absorption of food, intestinal enzymes have a pivotal role in the digestive activity and growth of the fish. Yang *et al.* (2006) stated that choline might increase the activity of the hepatopancreas in producing trypsin that plays a pivotal role in changing the trypsin peptides of proteins into amino acids. Thus, the increase in this enzyme activity can increase the uptake of amino acids by the small intestine.

In the current research, feed intake and FCR of tilapia fed 0.04 % in the diet was significantly better than other groups. It is stated by And and David (2005) that there is an interrelationship between choline and methionine in the portion of choline and methionine requirement. Choline can be a spare of the portion of methionine requirement if no sufficient methionine. However, methionine cannot be a spare of choline requirement in the presence of adequate choline. Therefore, the addition of choline in the diet of fish is compulsory to increase growth performance, including feed intake and FCR. Choline can also replace the role of methionine as a donor of methyl groups via betaine homocysteine methyltransferase (BHMT) (Zeisel, 2017). It is further explained that dietary adequate choline 0.04% can be added in the diet of fish without methionine supplementation.

On the other hand, a study in Pangasius catfish (*Pangasius bocourti*) fed diet 0.71 g 100 g^{-1} DL-methionine revealed a significantly increased weight gain and FCR in test group after 10 weeks of the feeding study (Yuangsoi *et al.*, 2016). Bakke *et al.* (2010) revealed that fish growth is affected by the activity and capacity of the fish digestive tract. The digestive tract enzymes activity in the brush border such as amylase, trypsin, Chymotrypsin, lipase, Na+/K+-ATPase, and γ-GT improved in trial fish which fed the methionine supplementation in the diet and enhanced in fish fed optimal value of methionine (Wu *et al.*, 2017; Xiao *et al.*, 2011).

Current study has also shown that dietary choline and methionine in any ratio combination had no effects on the survival rate (Figure 2). This was in agreement with the previous study on Pangasius catfish that dietary either methionine (Yuangsoi *et al.*, 2016) or choline (Yeh *et al.*, 2015) had no effect on the survival rate. The reason for this may be attributable to the occurrence of choline and/or methionine that plays an important role in lipid metabolism and stress tolerance (Yeh *et al.*, 2015).

The deposition of liver fat in low dietary choline animals shows not only impaired liver lipoprotein secretion but also subsequent accumulation of triglycerides. According to Machlin (1991), and Twibell and Brown (2000) fat deposition in the liver of fish can be a tool of choline status in a variety of animals, though is not consistent in several of fish. For example: inclusion choline in the diet at or above the requirement resulted in significantly lower liver fat concentrations in channel catfish (*Ictalurus punctatus*) (Wilson and Poe, 1988), lake trout (*Salvelinus namaycush*) (Ketola, 1976), and hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) (Griffin *et al.*, 1994), while higher liver fat deposition has been found in red drum (*Sciaenops ocellatus*) (Craig and Gatlin III, 1996) and liver fat deposition of rainbow trout (*Oncorhynchus mykiss*) (Rumsey, 1991) and yellow perch (*Perca flavescens*) (Twibell and Brown, 2000) were not affected by dietary choline in the diet.

Conclusions

Based on the data results, it seems clear that juvenile tilapia requires choline supplementation in certain dietary formulations. Furthermore, it is suggested that dietary supplementation of 0.04-0.08% choline in the diet is useful to improve growth indices and protein carcass proximate composition of tilapia. Meanwhile, dietary supplementation methionine in tilapia feed is optional.

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Declarations of interest

The author(s) declare that there is no conflict of interest with regards to the research, authorship and/ or publication of this article.

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