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Preliminary study to evaluate the effects of dietary bile acids on growth performance and lipid metabolism of juvenile genetically improved farmed tilapia (*Oreochromis niloticus*) fed plant ingredient-based diets

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Abstract

A 9-week feeding experiment was conducted to investigate the effects of dietary bile acids (BAs) on juvenile genetically improved farmed tilapia (GIFT) (*Oreochromis niloticus*) based on the evaluations of growth performance and parameters relevant to lipid metabolism. Each of five vegetable protein-based diets containing BAs at a level of 0, 0.05, 0.15, 0.45 or 1.35 g/kg diet was fed to three replicates with 40 fish (8.2 g per fish). The results showed that weight gain (WG) increased significantly with the increase in BAs from 0 to 0.15 g/kg diet and then decreased significantly at a higher BA supplementation. Dietary BAs significantly reduced the crude lipid content in the whole body, muscle and liver tissue of GIFT. Fish fed diet with 1.35 g BAs/kg diet developed serious nuclear migration and vacuolization in hepatocytes. Gall bladder appeared to contain white solid and has fragile capsules. Dietary BA supplementation had significant effects on serum biochemical indices and activities of lipid metabolism enzymes in liver and intestine. In conclusion, dietary bile acid supplementation (0.15 g/kg) can facilitate the lipid metabolism and therefore promote the growth of tilapia. However, overdosed dietary BAs induced gallstone development, disrupted lipid metabolism and depressed the growth performances of GIFT.

KEYWORDS
bile acids, gallstone, growth, lipid metabolism, tilapia (genetically improved farmed tilapia)

1 | INTRODUCTION

Bile acids (BAs) are formed in the liver from cholesterol, and then, they are conjugated with taurine or glycine before excretion from the hepatocyte (Hofmann, 1999). One of the most important functions of BAs is the removal of cholesterol in bile from the hepatocyte to the intestinal lumen for excretion (Hofmann, 1999). BAs also play important roles in the digestion of nutrients, absorption of lipid and fat-soluble vitamins, and shaping of the gut microflora (Hagey, Møller, Hofmann, & Krasowski, 2010; Ridlon, Kang, Hylemon, & Bajaj, 2014). Recent research indicates that BAs as liver-specific metabolic signals function in alleviating liver injury and promoting liver regeneration by activating their receptors (Fan, Wang, Xu, Yan, & Huang, 2015).

With the increased use of plant feed ingredients to replace fishmeal and fish oil in aquatic feed production, supplementation of BAs is attracting the attentions of the feed industry because some key compounds for BAs synthesis, such as cholesterol and taurine, are usually limited in plant feed ingredients. Recent studies on BAs or their conjugates as a feed additive have demonstrated their positive effects on promoting fish performance in some carnivorous fish. Adhami, Amirkolaie, Oraji, and Kenari (2017) observed that dietary BAs increased the digestibility of dietary protein and ash as well as

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lipase activity of juvenile rainbow trout, *Oncorhynchus myk*. Similarly, bile salt supplementation to a soybean meal-based diet was shown to improve the growth and nutrient utilization by normalizing digestive processes of rainbow trout (Iwashita et al., 2008; Yamamoto et al., 2007). Taurocholate (a conjugate of cholic acid and taurine) supplementation partially compensated the decreased growth and the occurrence of histological abnormalities in liver of turbot (*Scophthalmus maximus*) fed plant protein-based diets (Gu, Bai, & Kortner, 2017). The above findings indicated that exogenous BAs could benefit fish fed plant-based feed because their production of BAs might be disturbed.

GIFT (genetically improved farmed tilapia) strain of Nile tilapia (*Oreochromis niloticus*) was developed through conventional breeding methods. This strain of Nile tilapia has become one of the important freshwater species cultured in intensive aquaculture systems around the world due to their advantages of fast growth and high survival rate (Ponzoni et al., 2011). To reduce production cost and improve profitability, cost-effective feed has been manufactured for this fish using alternative ingredients including soybean meal, cottonseed meal and rapeseed meal, or non-conventional protein ingredients (meat and bonemeal, blood meal and feather meal), and plant seed oil. However, fishmeal-free feed has been found to have negative effects on the performance of this fish: depressed growth rate (Fontaínhas-Fernandes, Gomes, Reis-Henriques, & Coimbra, 1999; Zhao, Feng, Ning, Pan, & Zhao, 2011; Zhong et al., 2010), abnormal liver morphology (Gou et al., 2016) and poor tolerance to stress (Lin, Mao, & Guan, 2011). Thus, the improvement of growth performance and health of GIFT fed non-fishmeal feed remains to be a major challenge for the feed industry. Based on the aforementioned research on BA supplementation, we hypothesized that bile acid synthesis and circulation may be impaired in the GIFT fed a fishmeal-free diet, which is lacking cholesterol, taurine or other factors essential for their metabolic pathways. Exogenous BAs may be able to alleviate the deleterious influences on tilapia fed non-fishmeal feed. No study has been reported to investigate the beneficial effects of BAs supplementation and their optimal supplementation levels, which could vary among different species of fish fed fishmeal-free feed. Therefore, the objective of this study was to evaluate the effects of dietary BA supplementations on growth performance, body composition and parameters related to hepatic metabolism in juvenile GIFT strain.

2 | MATERIALS AND METHODS

2.1 | Experimental diet preparation

A basal diet without added BAs was formulated to contain 323 g/kg crude protein and 57 g/kg crude lipids (Table 1). Protein was provided by a combination of different plant ingredients including corn gluten, soybean meal, cottonseed meal and rapeseed meal. Additional lipid sources were obtained from soybean oil and corn oil. Five practical test diets were prepared by supplementing BAs to the basal diet at a level of 0, 0.05, 0.15, 0.45 or 1.35 g/kg diet at the expense of cellulose. The BAs (Shang dong Longchang Animal Health Product Co., Ltd, Jinan, China) mainly contained hyodesoxycholic acid, chenodeoxycholic acid and hyocholic acid at a level of 699.2, 189.2 and 77.5 g/kg BAs, respectively, estimated by HPLC.

TABLE 1 Formulation and proximate composition of the base experimental diet

Ingredients	g/kg	Proximate composition	g/kg diet as fed
Corn gluten meal	100	Protein	323
Soybean meal	220	Lipid	57
Cottonseed meal	80	Ash	77
Rapeseed meal	280	Moisture	103
Whole wheat flour	200		
Corn oil	15		
Soybean oil	15		
Choline chloride	1		
Vitamin premix[a]	10		
Mineral premix[b]	40		
Dl-methionine	2		
Bentonite	20		
Sodium methoxycellulose	10		
Microcrystalline cellulose	7		
Total	1,000		

[a]The premix provided the following per kg of diets: retinol acetate 5,000 IU; cholecalciferol 2,000 IU; α-tocopheryl acetate 60 mg; L-ascorbyl-2-monophosphate-Mg120 mg; menadione 5 mg; thiamine hydrochloride 5 mg; riboflavin 20 mg; pyridoxine hydrochloride 10 mg; nicotinic acid 120 mg; calcium pantothenate 10 mg; folic acid 1 mg; biotin 0.1 mg; inositol 400 mg.

[b]The premix provided the following per kg of diet: $Ca(H_2PO_4)_2$ 26,000 mg, $Ca(CH_3CHOHCOO)_2$ 6,540 mg, $FeSO_4$ 42.5 mg, $MgSO_4$ 1,340 mg, NaH_2PO_4 1,744 mg, NaCl 870 mg, $AlCl_3$ 3 mg, KIO_3 2.5 mg, KCl 1,500 mg, $CuCl_2$ 20 mg, $MnSO_4$ 16 mg, $CoCl_2$ 2 mg, $ZnSO_4$ 60 mg.

All dry ingredients were ground into <245 μm particle size before being weighed accurately (~0.1 g) and mixed into a homogenous mixture using a groove-type mixer (CH-50, Changzhou Golden Ball Drying Equipment Co., Ltd., China). Lipid and then water (about 30% of total dry mixture) were added and mixed thoroughly. The wet mash was extruded through a meat grinder (TY-432; Shang Hai Tai Yi Machinery, China). The resultant noodle-like product was dried at room temperature for about 24 hr with blowing air from an electrical fan until the dietary moisture content was <100 g/kg. The noodle-like diets were crumbled and sieved to generate approximate pellets (1.5 ~2.0 mm in diameter). The dry pellets were placed in plastic bags and stored at −20°C until used.

2.2 | Fish maintenance and feeding management

Juvenile tilapia were purchased from the Guangxi Tilapia National Breeding Station (Nanning, China). Prior to the experiment, the fish were reared in six aquaria of an indoor recirculating aquarium system (RAS) at the Yangtze River Fisheries Research Institute (Wuhan, China) and fed to apparent satiation three times daily.

The fish were fasted for 24 hr before they were distributed to 15 experimental tanks. Six hundred uniform-sized fish (average body weight: 8.2 ± 0.2 g, n = 30) were selected, and 40 fish for each tank were batch-weighed before they were randomly distributed to each tank. Each experimental diet was randomly assigned to triplicate tanks. Fish were hand-fed to apparent satiation three times daily (08:30, 12:30 and 16:40) for 9 weeks. Each feeding lasted for about 30 min when no fish was shown to actively approach feed pellets. The amount of feed being consumed by the fish from each aquarium was calculated weekly. The care, handling and sampling of fish were performed following animal care protocols approved by the Academic Committee of Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences.

The RAS consists of 18 tanks (500 L each; 400 L of water), which was supplied with urban tap water. Approximately 50% of the water in each tank was replenished once every 2 days. Dissolved oxygen was maintained at a level higher than 4 mg/L using a 750-w impeller aerator to provide continuous aeration to each tank. The experiment was conducted at ambient temperature and subjected to natural photoperiod. Water temperature and dissolved oxygen were continuously monitored by automatic sensors. Other water quality parameters were monitored in the morning once a week. During the experimental period, water temperature was 27.4 ± 1.5°C, pH 6.5~7.0 and total ammonia–nitrogen < 0.02 mg/L.

2.3 | Sample collection and analysis

At the end of 9-week feeding, all fish were fasted for 24 hr prior to final sampling. All of the fish were anesthetized with 0.05 g/L MS-222 (tricaine methane sulphonate) before the total survival and total weight of fish from each aquarium was recorded. Three fish from each replicate were randomly collected, killed by overdose MS-222 and stored at −20°C for final whole-body analysis. Another three fish per tank were randomly selected to measure body weight and body length to calculate the condition factor. Blood from each fish was obtained by puncturing the caudal vein using a 2-ml syringe. Samples were allowed to clot at 4°C for 2 hr and then centrifuged (960 *g*, 4°C) for 10 mins to collect serum samples.

Three fish from each aquarium were dissected on ice to obtain fillet and liver samples. The samples were then cryopreserved at −80°C prior to analysis. Parts of the livers were quickly fixed in 4% formaldehyde, dehydrated in a graded ethanol series and embedded in paraffin (Jiang et al., 2016). Section series of 4 μm were stained with haematoxylin and eosin (H&E).

Proximate analysis of experimental diets and fish samples followed the methods described by AOAC (1995). Protein content was determined by measuring total nitrogen (N × 6.25) levels using the Kjeldahl method following acid digestion with an auto Kjeldahl System (Kjelflex K-360; BUCHI Labortechnik AG, Flawil, Switzerland). Lipid content was detected by ether extraction using a Soxhlet method. Moisture content was measured by drying samples in an oven at 105°C for 24 hr, and ash levels were measured through combustion of samples at 550°C for 24 hr.

Activities of lipoprotein lipase, hepatic lipase and lipase were analysed by colorimetry using diagnostic reagent kits. The analytical kits for lipoprotein lipase and hepatic lipase (A067) and the lipase test kit (A054) were provided by Nanjing Jiancheng Bioengineering Institute (China). Activities of alanine aminotransferase (ALT) and aspartate transaminase (AST), along with the contents of serum triglyceride(TG), total cholesterol (TCHO), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), were analysed by an automatic biochemical analyser (Sysmex-800; Sysmex Corporation, Kobe, Japan) and commercial diagnostic reagent kits (Sysmex Wuxi Co., Lt., Wuxi, China).

2.4 | Statistical analysis

The results are presented as mean ± *SD* of three replicates. All data were subjected to one-way analysis of variance (ANOVA). When overall differences were significant ($p < .05$), Duncan's test was used to compare the mean values among the treatments. When the test of homogeneity of variances failed, the Games–Howell test was used. Statistical analyses were performed using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA).

3 | RESULTS

3.1 | Growth performance

The overall growth performance of juvenile GIFT was significantly affected by the dietary BAs supplementation (Table 2). The percent of weight gain (WG, %) increased significantly ($p < .05$) with the increasing dietary BAs supplementation from 0 to 0.15 g/kg, and then decreased significantly in the fish fed 0.45 or 1.35 g BAs/kg diet. Fish fed diet containing 1.35 g BAs/kg diet exhibited the lowest WG ($p < .05$). In contrast, feed efficiency was the highest in fish fed the

TABLE 2 Growth performance of juvenile genetically improved farmed tilapia fed the test diets containing different levels of bile acids for 9 weeks

Items	Dietary bile acids level (g/kg diet)				
	0	0.05	0.15	0.45	1.35
[a]IBW	8.2 ± 0.3	8.1 ± 0.3	8.1 ± 0.2	8.2 ± 0.2	8.3 ± 0.3
[b]FBW	74.0 ± 2.7^{ab}	76.6 ± 3.59^{abc}	82.5 ± 1.5^{c}	77.1 ± 3.1^{bc}	69.2 ± 2.6^{a}
[c]WG	805.9 ± 16.2^{b}	851.5 ± 10.1^{c}	915.7 ± 11.0^{d}	836.7 ± 13.4^{b}	740.9 ± 13.0^{a}
[d]FE	73.8 ± 2.2^{b}	75.5 ± 4.6^{b}	85.0 ± 5.8^{c}	74.6 ± 3.4^{b}	65.4 ± 1.0^{a}
[e]HSI	2.8 ± 0.2^{d}	1.9 ± 0.1^{b}	1.6 ± 0.1^{a}	1.9 ± 0.1^{b}	2.5 ± 0.1^{c}
[f]VSI	9.4 ± 0.3^{a}	10.2 ± 0.5^{b}	9.5 ± 0.2^{a}	10.6 ± 0.2^{b}	9.2 ± 0.5^{a}
[g]CF	4.0 ± 0.1^{b}	4.3 ± 0.1^{c}	4.2 ± 0.1^{c}	4.3 ± 0.1^{c}	3.8 ± 0.3^{a}
[h]SR	92.5 ± 2.5	95.5 ± 7.8	94.6 ± 2.9	93.0 ± 7.3	90.9 ± 8.9

Data were presented as mean ± *SD*, *n* = 3. Means in the same row sharing the same or none superscript letter are not significantly different, as determined by Duncan's test ($p > .05$).
[a]IBW (g) = initial mean weight.
[b]FBW (g) = final mean weight.
[c]WG (percentage of weight gain, %) = (FBW − IBW)/IBW × 100.
[d]FE (feed efficiency) = 100 × (total final fish weight g − total initial fish weight g + dead fish g)/feed intake.
[e]CF (condition factor, g/cm^3) = (body weight, g)/(body length, cm)^3 × 100.
[f]HSI (hepatosomatic index) = 100 × liver weight, g)/(body weight, g).
[g]VSI (viscerosomatic index) = 100 × (viscera weight, g)/(body weight, g).
[h]SR (serval rate) = 100 × (number of final fish/number of initial fish).

diet containing 0.15 g BAs/kg diet, and the lowest in fish fed the 1.35 g BAs/kg diet. Hepatosomatic index of fish fed different diets followed the same pattern as observed in WG. The changes in viscera somatic index did not correspond to the dietary BAs levels. Tilapia fed the diet with the highest level of BAs were found to have the lowest condition factor, followed by those fed the basal diet. Condition factor was similar for fish fed the other three diets ($p \geq .05$). Survival rate showed no significant differences among the treatments.

3.2 | Proximate composition of whole fish and liver

Dietary levels of BAs had significant effects on the levels of protein, lipid and ash content, but had no significant effects on the moisture content of whole fish (Table 3). Fish fed the 1.35 g BAs/kg diet had a higher whole fish crude protein level than that of the other four groups (Table 4). No significant differences were found in protein content of fish fed the other four diets. The content of BAs significantly

TABLE 3 Proximate composition of whole fish, muscle and liver tissues of juvenile genetically improved farmed tilapia fed test diets containing different levels of bile acids for 9 weeks

Nutrient component	Dietary bile acid level (g/kg diet)				
	0	0.05	0.15	0.45	1.35
Whole body (g/kg as wet)					
Moisture	732.0 ± 11.1	725.9 ± 2.6	736.3 ± 19.5	722.7 ± 4.8	718.6 ± 5.5
Crude protein	150.5 ± 3.6^{a}	151.4 ± 0.8^{a}	150.5 ± 0.09^{a}	152.2 ± 1.0^{a}	164.3 ± 3.7^{b}
Crude lipid	73.8 ± 3.0^{c}	58.7 ± 1.5^{a}	60.4 ± 3.1^{a}	68.7 ± 4.9^{b}	67.3 ± 2.2^{b}
Crude ash	30.0 ± 2.1^{a}	36.3 ± 1.5^{b}	36.7 ± 0.9^{bc}	38.7 ± 1.0^{cd}	39.3 ± 2.4^{d}
Muscle (g/kg as wet)					
Moisture	772.4 ± 8.7	782.3 ± 12.5	775.1 ± 12.1	778.1 ± 2.0	769.8 ± 7.0
Crude protein	191.7 ± 5.0^{b}	184.0 ± 2.1^{a}	183.7 ± 2.5^{a}	185.7 ± 3.5^{a}	195.8 ± 6.1^{b}
Crude lipid	12.7 ± 1.3^{d}	11.6 ± 0.6^{c}	10.0 ± 0.5^{b}	9.9 ± 1.2^{b}	8.0 ± 0.5^{a}
Liver (g/kg as wet)					
Moisture	689.3 ± 11.9^{a}	704.8 ± 3.9^{ab}	694.3 ± 15.0^{a}	718.6 ± 10.5^{bc}	736.0 ± 11.1^{c}
Crude protein	103.2 ± 7.8	105.1 ± 1.5	105.3 ± 2.6	103.0 ± 1.7	106.9 ± 2.4
Crude lipid	43.4 ± 2.0^{c}	40.7 ± 1.0^{bc}	39.9 ± 1.2^{b}	39.1 ± 2.5^{b}	29.8 ± 2.3^{a}

Data were presented as mean ± *SD*, *n* = 3. Means in the same line sharing the same or none superscript letter are not significantly different, as determined by Duncan's test ($p > .05$).

decreased the lipid content and increased the ash content of whole fish.

Fish fed the basal diet and the diet containing 1.35 g BAs/kg had significantly higher protein content in muscle than those fed the diets containing 0.05–0.45 g BAs/kg diet. The content of BAs significantly tended to increase the moisture content but decreased the lipid content of liver tissue, with the highest level of moisture and the lowest level of lipid in fish fed the diet containing 1.35 g BAs/kg. Liver protein content did not show any significant difference among fish fed the different dietary treatments.

3.3 | Activities of lipid metabolism enzymes in the liver and intestine

Activities of selected lipid metabolism enzymes in the liver and intestine were significantly induced by dietary BAs supplementation (Table 5). The activities of lipoprotein lipase (LPL) in the liver and intestine were both increased in response to the increased dietary BAs. Their value reached the highest level in the groups of fish fed 0.45 or 1.35 g BAs/kg diet. The activities of hepatic lipase (HL) also followed the similar patterns observed in LPL. Supplementation of BAs stimulated lipase activities in both liver and intestine tissues, with the highest level detected in fish fed the diet containing 1.35 g BA/kg.

3.4 | Liver histology and gall bladder morphology

Serious nuclear migration and vacuolization were observed in hepatocytes of fish fed the 1.35 g/kg bile acid diet (Figure 1), while the fish fed the other four diets showed normal liver cell morphology.

The appearance of gall bladders changed from a dark green to a light green colour as the supplemental level of BAs increased from zero to 0.15 or 0.45 g/kg diet (Figure 2). Most fish (except two fish) fed the diet with 1.35 g BAs/kg diet were found to have one or two white solids present in gall bladders with light yellow colour bile (Figure 2). The gall bladder capsule of these fish was extremely fragile and easy to rupture.

4 | DISCUSSION

Bile acids are known to function in facilitating the digestion of lipid by acting as emulsifying agents that render fats accessible to pancreatic

TABLE 4 Serum biochemical indices of juvenile genetically improved farmed tilapia fed test diets containing different levels of bile acids for 9 weeks

	Dietary bile acid level (g/kg diet)				
Items	0	0.05	0.15	0.45	1.35
TCHO	2.7 ± 0.03^{a}	2.68 ± 0.11^{a}	2.73 ± 0.09^{a}	3.22 ± 0.05^{c}	2.84 ± 0.04^{b}
HDL-C	1.13 ± 0.07^{a}	1.31 ± 0.04^{c}	1.30 ± 0.06^{c}	1.48 ± 0.05^{d}	1.17 ± 0.03^{b}
LDL-C	1.21 ± 0.03^{a}	1.76 ± 0.12^{b}	1.81 ± 0.10^{b}	1.92 ± 0.05^{c}	1.23 ± 0.06^{a}
TG	2.73 ± 0.06^{d}	2.56 ± 0.03^{c}	2.49 ± 0.05^{b}	2.51 ± 0.07^{bc}	2.18 ± 0.03^{a}
AST	34.3 ± 1.2^{a}	35.3 ± 1.6^{a}	33.8 ± 2.1^{a}	42.7 ± 2.0^{b}	54.3 ± 2.9^{c}
ALT	27.8 ± 2.3^{b}	26.7 ± 2.3^{b}	22.7 ± 2.0^{a}	29.0 ± 2.8^{b}	35.7 ± 1.6^{c}

TCHO, total cholesterol, mmol/L; HDL-C, high-density lipoprotein cholesterol, mmol/L; LDL-C, low-density lipoprotein cholesterol, mmol/L; TG, triglyceride, mmol/L; AST, aspartate transaminase, U/L; ALT, alanine aminotransferase, U/L.

Data were presented mean ± *SD*, *n* = 3. Means in the same row sharing the same superscript letter are not significantly different, as determined by Duncan's test ($p > .05$).

TABLE 5 Activities of lipid metabolism enzymes in liver and intestine of juvenile genetically improved farmed tilapia fed test diets containing different levels of bile acids for 9 weeks

	Dietary bile acid level (g/kg diet)				
Items	0	0.05	0.15	0.45	1.35
Liver					
Lipoprotein lipase, U/g protein	1,455 ± 85^{a}	1,673 ± 77^{b}	1,853 ± 131^{b}	2,178 ± 84^{c}	2,159 ± 146^{c}
Hepatic lipase, U/g protein	1,783 ± 90^{a}	2,123 ± 155^{b}	2,422 ± 85^{c}	2,248 ± 125^{bc}	2,140 ± 80^{b}
Lipase, U/g protein	112.0 ± 2.5^{a}	162.4 ± 4.6^{b}	192.2 ± 4.6^{c}	200.0 ± 2.4^{c}	218.3 ± 8.8^{d}
Intestine					
Lipoprotein lipase, U/g protein	1,504 ± 59^{a}	1,695 ± 69^{b}	1,832 ± 8^{bc}	1,930 ± 114^{cd}	2,075 ± 142^{d}
Lipase, U/g protein	13.9 ± 0.2^{a}	21.9 ± 0.8^{b}	30.6 ± 0.1^{c}	30.1 ± 0.8^{c}	33.1 ± 0.5^{d}

Data were presented mean ± *SD*, *n* = 3. Means in the same row sharing the same superscript letter are not significantly different, as determined by Duncan's test ($p > .05$).

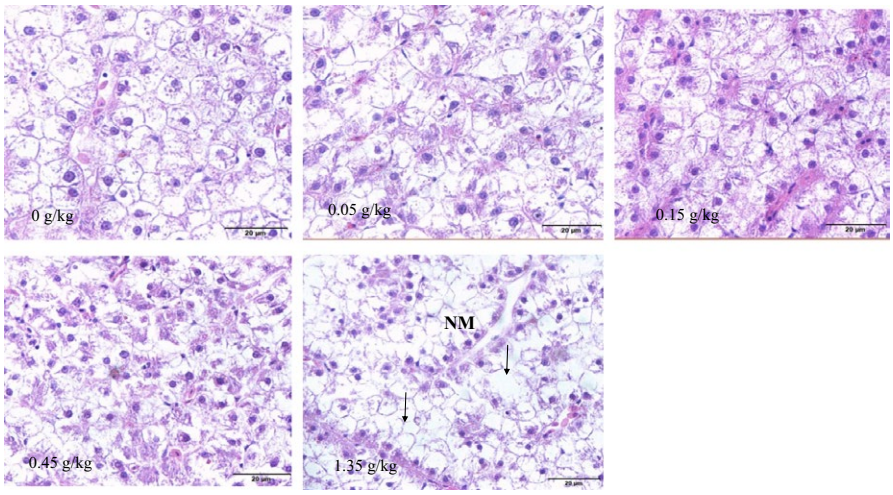

FIGURE 1 Histologically observed liver of genetically improved farmed tilapia tilapia fed with difference dietary bile acid supplementations (×400, scale bar = 20 μm; NM, nuclear migration; arrow, cellular vacuolization)

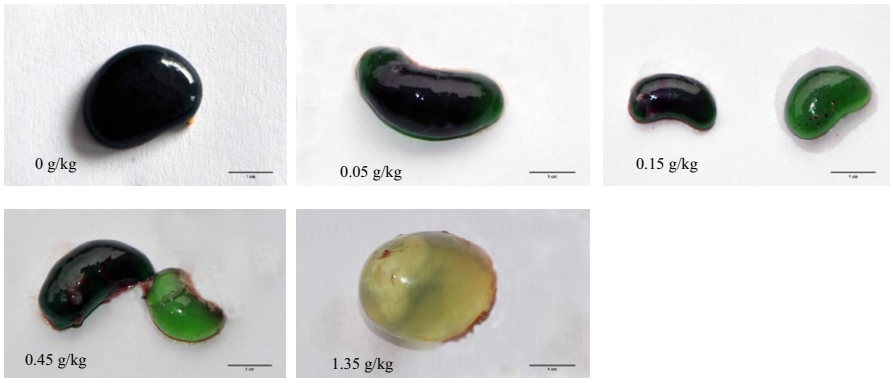

FIGURE 2 Gall bladder morphology of juvenile genetically improved farmed tilapia tilapia fed with difference dietary bile acid supplementations. Note: The substance around the gall bladder was the remnant of the liver

lipases and enhance the intestinal absorption of fat or fat-soluble vitamins (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011). Some critical compounds such as taurine and cholesterol for synthesis of bile acid or bile conjugate are low or missing in plant ingredient sources (NRC 2011). Thus, insufficient synthesis of BAs or bile salts may occur in fish fed plant-based feed and be responsible for poor utilization of lipid and depressed growth of fish. This can partially explain the beneficial effect of BAs on the growth performance of GIFT observed in this study. Similar to the current observation, BAs or bile salts were found to improve the growth performance of rainbow trout (Adhami et al., 2017; Iwashita et al., 2008; Yamamoto et al., 2007) and turbot (Gu et al., 2017) fed plant protein-based diets. Iwashita et al. (2008) also reported that increased bile salt levels were observed in rainbow trout when cholytaurine (a bile acid) was supplemented to a plant-based diet. The above-mentioned studies suggest that it may be critical to supplement BAs in plant-based feed to enhance growth of fish if this approach is cost-effective to the feed industry.

In this study, the weight gain increased from 740% to 950% in 9 weeks. In a similar study which was also conducted using the same RAS in our laboratory, the fish (initial weight ~10 g) fed the diets containing 6% fishmeal and the other ingredients were similar to the control diet in this experiment and had achieved 550%–630% (Yu et al., 2017) weight gain in 8 weeks. This evidence supports that GIFT can adapt to fishmeal-free diet, and BAs were able to promote the growth performance at an already high growth rate of tilapia.

In addition, a range of studies have demonstrated that bile acid supplementation promoted fish growth even though they were fed with diets containing fishmeal or other animal ingredients (Alam, Teshima, Ishikawa, & Koshio, 2001; Ji, Gu, Liu, Yang, & Li, 2017; Sun et al., 2014; Zeng et al., 2017). Moreover, the effective dose level of supplemental of BAs had been reported in different studies. Zheng et al. (2016) estimated that the optimum dietary BA supplementation was 0.22–0.27 g/kg based on WG and FCR of prenant's schizothoracin (*Schizothora xprenanti*) fed test diets containing 42% fishmeal. The optimum dietary bile acid (mixtures of hyodeoxycholic acid and lithocholic acid) supplementation was 0.17–0.19 g/kg for grass carp fed a diet containing 100 g/kg fishmeal and 40 g/kg fish oil based on the growth performance (Zeng et al., 2017). A much higher level up to 1.5 g/kg BAs was suggested for juvenile turbot even though the test diets contained about 400 g/kg fishmeal and 50-130 g/kg fish oil (Sun et al., 2014). These studies indicate that the required optimal level of supplemental BAs or the capacity of these compounds may vary depending on the type of bile acid used, species of fish, feed formulation and other culture conditions.

The GIFT fed 0.15 g BAs/kg diet also had the lowest value of hepatosomatic index and relatively low level of liver lipid content. These findings suggest that BA supplementation may have contributed to lipid

metabolism and prevented fatty liver, which is often seen in fish fed plant-based diets (Du, 2014). Similarly, dietary BA supplementation has been shown to reduce the accumulation of body fat (Sun et al., 2014) or promote whole-body lipid catabolism (Hu, Wang, Zhang, Song, & Li, 2015). The study by Gu et al. (2017) also indicates that dietary supplementation with taurocholate attenuated the negative effects of plant protein meal by enhancing lipid digestion and metabolism.

However, the beneficial role of BAs was dose dependent as shown in the present study. Tilapia fed the BAs at a level of 1.35 g/kg diet exhibited significant deleterious symptoms. Little information is available on the toxic effects of dietary BAs in fish. The mechanism for the deleterious effect of BAs remains unclear with the limited data presented in the current study. An overdose of exogenous BAs might impair the enterohepatic circulation of BAs. For example, previous studies have shown that administration of lithocholic acid (LCA) caused gallstone formation in rats because the addition of LCA increased the demand of taurine for conjugate production and resulted in taurine depletion (Hofmann & Hagey, 2008). Consequently, the administrated LCA and its metabolite were conjugated with glycine instead of taurine resulting in them being precipitated in bile as insoluble calcium salts (Hofmann & Mysels, 1992). In the current study, it is possible that the deficiency of dietary taurine led to formation of insoluble salts in the GIFT as discussed above. Analysis of gall bladder compositions may be able to confirm this hypothesis in a future study. Furthermore, brittle and fragile gall bladders seen in tilapia fed the highest dose of BAs may be the result of chronic inflammation and/or a calcification of the gall bladder wall, and a similar symptom called "porcelain gall bladder" has been reported in humans (Schnelldorfer, 2013). Porcelain gall bladder is rarely observed even in humans, and the underlying mechanism is still not well understood. This is the first study that has documented a similar condition in fish. In addition, less cholesterol might be needed for the synthesis of endogenous BAs and therefore an accumulation of cholesterol may be another reason responsible for gallstone formation in the tilapia fed the highest level of BAs. Thus, formation of gallstones in the GIFT might be caused by insoluble conjugated bile salts and/or retention of cholesterol in their gall bladders. This observation warrants for future investigation.

Bile acids are also known to be cytotoxic to hepatocytes (Malhi, Guicciardi, & Gores, 2010). Previous studies have shown that dietary LCA supplementation resulted in intrahepatic cholestasis and bile infarcts in mice (Woolbright et al., 2014) and accumulation of BAs was a major mediator of cholestatic human liver injury (Woolbright et al., 2015). In the present study, the liver histology showed that serious nuclear migration and vacuolization in hepatocytes of fish fed the 1.35 g BAs/kg diet. Meanwhile, fish fed the 1.35 g BAs/kg diet had the highest ALT and AST activities, which suggested liver cells were partly damaged. These results verified that high level of dietary bile acid supplementation is toxic to hepatocytes but the mechanism remained to be studied.

Results of this study also demonstrated that bile acid supplementation led to low lipid accumulation in whole body, tissues and serum in GIFT. Similar results have been observed in grass carp (Zeng et al., 2017), prenant's schizothoracin (Zheng et al., 2016) and turbot (Sun et al., 2014). The decreased lipid accumulation may be attributed to increased lipid metabolism for energy by enhancing lipolysis, a process for breaking down triglyceride. One of the major functions of BAs is known to be involved in lipid emulsification that helps to break down large lipid molecules into small globules, which provide lipase with increased suffice area for lipid digestion (NRC, 2011; Ogata et al., 2003). Therefore, increased lipase activity and decreased triglyceride levels were determined in serum of tilapia. Similar results were reported in juvenile rainbow trout (Adhami et al., 2017), grass carp (Zeng et al., 2017), turbot (Sun et al., 2014) and Japanese founder, *Paralichthys olivaceus* (Alam et al., 2001). In contrast, dietary BAs did not seem to affect the whole-body lipid content of turbot (Gu et al., 2017) or bullfrog, *Rana catabo* (Hu et al., 2015). These inconsistent results may be related to the different experimental conditions including species, nature of test the diets or other culture conditions used for different studies.

The influence of dietary BAs on fish body composition except lipid varies by species. Zeng et al. (2017) indicated that dietary BAs significantly increased crude protein content, but had no effect on moisture and ash content of the fish body. Gu et al. (2017) showed that dietary BAs had no significant effects on moisture, crude protein and ash content of juvenile turbot. Sun et al. (2014) reported that dietary BAs significantly decreased moisture, increased crude protein and ash content of whole body, muscle and liver. In this study, dietary BAs had no significant effects on moisture in whole fish, but had a significant effect on crude protein and ash content of whole body. While, nutritional components of muscles and liver showed a different trend.

Homeostasis of whole-body cholesterol is known to be influenced by exogenous cholesterol absorption, endogenous cholesterol biosynthesis, elimination of cholesterol through BAs in faeces (Van der Wulp, Verkade, & Groen, 2013). Also cholesterol is mainly transported in plasmas by being associated with lipoproteins. In the current study, no cholesterol was presented in the test diets. Thus, cholesterol in the fish came from endogenous biosynthesis. Supplementation of dietary BAs was shown to induce the overall levels of cholesterol and lipoprotein cholesterol in the serum of GIFT. Similarly, the activities of lipoprotein lipase and hepatic lipase were also increased. The increased cholesterol may have resulted from decreased de novo synthesis of BAs, which is one of the pathways for the elimination of cholesterol as observed in human and other animal models (Dietschy, Turley, & Spady, 1993; Kruit, Groen, van Berkel, & Kuipers, 2006; Lefebvre, Cariou, Lien, Kuipers, & Staels, 2009; Repa & Mangelsdorf, 1999). A recent report in turbot also observed an increased level of serum cholesterol in fish fed a taurocholate supplementation diet (Gu et al., 2017). On the other hand, it is unknown whether dietary BAs have the potential to directly increase the biosynthesis of cholesterol. This remains to be investigated in the future studies.

5 | CONCLUSION

In conclusion, the present study has demonstrated that dietary bile acid supplementation (0.15 g/kg diet) can promote the growth

performance of tilapia. Overdosed BAs (1.35 g/kg diet) resulted in toxic effects on the fish with impaired liver function, formation of gallstones and depressed growth. The BAs used in this study seem to improve lipid digestion and metabolism. However, the BAs used in the present study were a mixture of three different BAs and it would be of importance to investigate the effect of individual compounds as a feed additive. Future research is warranted to understand the mechanism of BAs as a feed additive in plant-based diets. Results of this study suggest that bile acid supplementation may be a potential feed additive to promote production efficiency of plant-based feeds. More research will be required to address their optimal levels due to the nature of feed formulation and difference species of targeted fish.

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