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Effect of dietary taurine along with the different lipid levels on growth, antioxidant, innate immune responses, digestive, metabolic enzyme activity and health status of pangasius (*Pangasianodon hypophthalmus*)

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Abstract

High fat diets are provided to fish in order to supply additional energy and spare protein for better growth of fish. Taurine (2-aminoethanesulfonic acid) is an important nutrient which influences lipid utilization. Here, we investigated how taurine supplementation influences growth, digestive, metabolic enzyme activity, antioxidant, innate immune responses, and health status of pangasius fed with different levels of fat. Five experimental diets were formulated to contain 300 g/kg crude protein and varying level of crude lipid (6%, 8%, 10%, 12% and 14%) with 15 g/kg taurine and designated as T6%, T8%, T10%, T12% and T14%. Pangasius (*Pangasianodon hypophthalmus*) fingerlings were randomly stocked in twelve 150 L FRP tanks with 20 fingerlings (5.22 ± 0.04 g) in each and fed experimental diets for 56 days. The majority of the growth, nutrient utilization, and biological parameters in pangasius showed similarity between high lipid diet and low lipid diet in the presence of taurine. The whole-body composition and blood parameters of pangasius did not show any significant difference ($P > 0.05$) among high lipid diet and low lipid diet. The lipase activity showed significantly higher ($P < 0.05$) activity in T14% group when compared with T6% group. The significantly higher ($P < 0.05$) carnitine palmitoyl transferase were observed in fish fed with 12% and 14% lipid diet, whereas malic enzyme activity was significantly higher ($P < 0.05$) in T14% group than in T6% group. The antioxidant activity (superoxide dismutase, catalase, glutathione S-transferase, glutathione peroxidase) and innate immune parameters (respiratory burst, lysozyme and antiprotease activity) did not show significant difference ($P > 0.05$) among high lipid and low lipid diet. Interleukin 1β and transferrin also did not show any significant difference ($P > 0.05$) among the treatments. The study showed that taurine supplementation can help to counteract the deleterious effects of high lipid diets, such as lipid accumulation and elevated serum cholesterol and TG levels in pangasius. It was also found that both high-lipid and low-lipid diets resulted in similar growth and biometric outcomes in presence of dietary taurine.

Keywords Lipid, Growth, Taurine, Pangasius, Antioxidant enzymes, Metabolic enzyme

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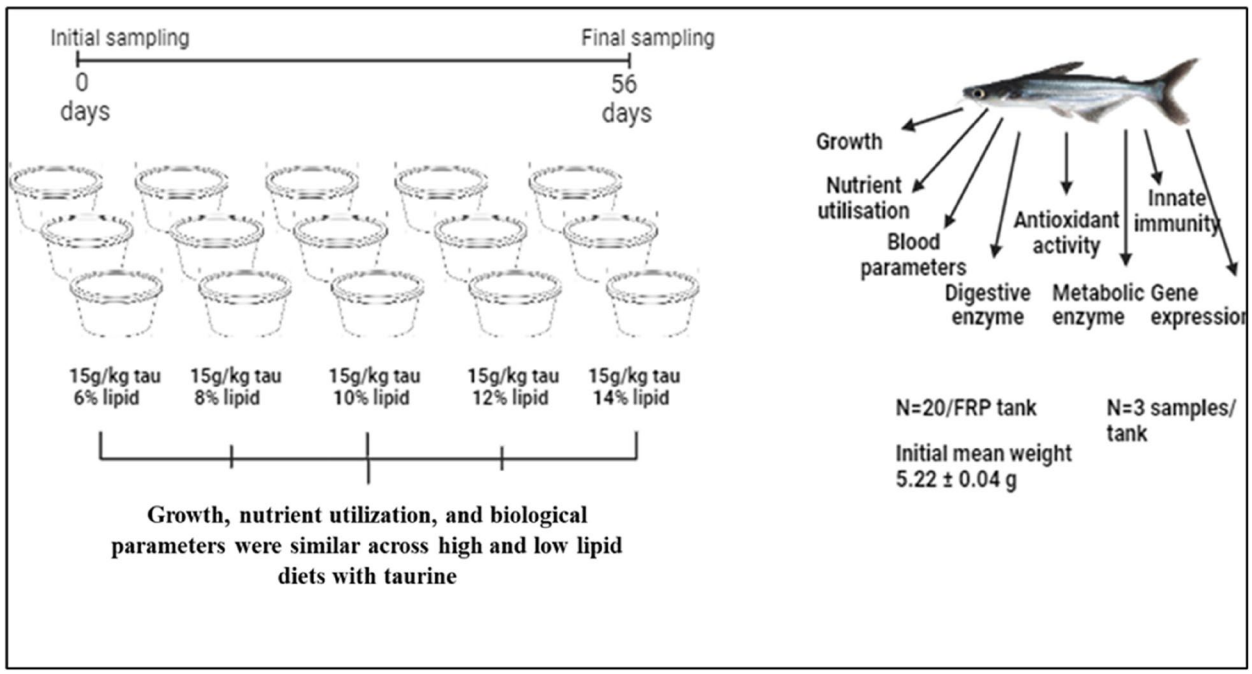
Graphical Abstract



Introduction

Dietary lipids are vital for aquatic species, offering energy for growth and development, as well as providing essential fatty acids and fat-soluble vitamins that support the animal's structure and biological functions [1, 2]. The appropriate level of dietary lipids plays a significant role in enhancing feed efficiency and facilitating the assimilation of other nutrients in aquatic animals [3]. In intensive aquaculture, raising dietary lipid levels to conserve protein is a commonly employed strategy to enhance fish growth, decrease nitrogen and phosphorus emissions, and safeguard the environment. However, excessive lipid consumption can result in negative outcomes, including the buildup of lipids in tissues, oxidative stress, and inflammation in the intestines. Previous studies have demonstrated that a high-fat diet results in severe lipid deposition in grass carp [4], oxidative stress and liver tissue damage in turbot [5] and hybrid grouper [6], reduced intestinal inflammation response in largemouth bass [7], and disturbance in the homeostasis of intestinal microbiota in rice field eel [8]. Thus, it is essential to explore and develop effective dietary strategies that can alleviate the harmful effects of a high-fat diet in aquatic species. Several studies have documented that adding taurine to high-fat diets can improve lipid metabolism, increase insulin sensitivity in skeletal muscles, and enhance glucose regulation in the liver of mice [9].

Taurine, an amino acid containing sulphur, is the most prevalent free amino acid in animals [10]. Taurine is regarded as a vital amino acid for many farmed fish species due to its key involvement in numerous physiological processes, including bile acid conjugation, immune function, osmoregulation, antioxidant defence, nervous system development, and tissue repair [11]. Supplementing with taurine in the diet has been found to lower cholesterol levels in peripheral tissues and reduce visceral fat in diabetic rats [12] and obese humans [13, 14]. Comparable effects in reducing lipid levels have been observed in several cultured fish species [15, 16]. For instance, taurine supplementation has led to decreased whole-body lipid content in European seabass [17], reduced serum total cholesterol (TC) and TG levels in yellow catfish [18], and diminished liver lipid content in California yellowtails [16] and rice field eels [19]. Research indicates that supplementing the diet with taurine can decrease peripheral cholesterol and visceral lipid accumulation by enhancing the activity of the rate-limiting enzyme cholesterol 7α-hydroxylase in the liver, promoting the conversion of cholesterol to cholic acid, and increasing the excretion of faecal cholesterol [20]. Taurine has been reported to act as an attractant, enhancing feed intake in fish [15, 21,

22]. Given taurine's demonstrated lipid-lowering effects, its supplementation in diets may effectively counteract the adverse impacts associated with high-lipid feeding in aquaculture. However, presently there is a dearth of published data regarding the potential of taurine to alleviate the detrimental consequences of a high-fat diet in aquatic animals.

Pangasius ((*Pangasianodon hypophthalmus*) farming is a popular aquaculture practice in many parts of the world, especially in Southeast Asia [23]. Pangasius, also known as basa or tra, is a type of freshwater catfish that is raised for its mild-tasting white flesh. These fish are versatile and can be raised in a variety of environments, including ponds, cages, and tanks. Fish that are fed a high lipid diet may have lower nutritional value, reduced flavour, and altered texture compared to fish that are fed a more balanced diet. This can affect consumer perception and demand for pangasius products. So, the present study is designed to determine the effect of taurine on different lipid levels on growth, lipid utilization, antioxidant, innate immune responses, digestive, metabolic enzyme activity and health status of pangasius.

Materials and methods

Feed preparation

Five experimental diets were formulated, each containing 300 g/kg of crude protein, 15 g/kg taurine with different levels of crude lipid. The diets included soy protein concentrate and corn gluten, with lipid percentages of 6%, 8%, 10%, 12%, and 14%. The optimal oil requirement for pangasius was found to be 10.1 g/kg [24]. The five experimental diets were labelled as T6%, T8%, T10%, T12%, and T14%. The feed was prepared as described by Peter et al. [25]. The pellets were subsequently dried in drier (Servo Enterprises drier, Chennai, India) to achieve a moisture content of less than 10% before being sealed in airtight bags for future use. Diet formulation and the proximate composition of feed are presented in Table 1.

Table 1 Ingredients (g/kg) and nutrient composition (g/kg DM) of the experimental diet of *Pangasianodon hypophthalmus*

Ingredients	T6%	T8%	T10%	T12%	T14%
Soy protein concentrate	80	80	80	80	80
Corn Gluten	180	180	180	180	180
Ground nut oil cake	160	160	160	160	160
Soyabean meal	200	200	200	200	200
Corn flour	180	160	140	120	100
Rice polish	110	110	110	110	110
Oil	25	45	65	85	105
Vitamin + mineral	10	10	10	10	10
Lysine	20	20	20	20	20
Methionine	10	10	10	10	10
Tricalcium phosphate	10	10	10	10	10
Taurine	15	15	15	15	15
Proximate composition					
Crude protein	298.8	297.6	291.8	296.4	297.4
Crude lipid	60.70	80.12	100.23	120.84	140.68
Ash	125.2	125.1	125.4	125.2	125.0

Vitamin (IU or g/kg premix): retinol palmitate, 50,000; thiamine, 5; riboflavin,5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalomin, 5; ascorbic acid, 10; cholecalciferol, 50,000; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotine, 0.25. 4. Minerals (g/kg) (supple vit M): $CaCO_3$,336; KH_2PO_4, 502; $MgSO_4 \cdot 7H_2O$, 162; NaCl, 49.8; Fe (II) gluconate, 10.9; $MnSO_4 \cdot H_2O$, 3.12; $ZnSO_4 \cdot 7H_2O$, 4.67; $CuSO_4 \cdot 5H_2O$, 0.62; KI, 0.16; $COCl_2 \cdot 6H2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02

Fish rearing and maintenance

A 56-day study was carried out at the wet laboratory of the Dept. of Aquaculture, Kerala University of Fisheries and Ocean Studies in Panangad, Kochi, India. Healthy pangasius fingerlings were obtained from Farmer's friend hatchery, transported to the laboratory, and placed in 500 L FRP tubs for a period of 2 weeks for acclimatization and were fed a commercial diet (320 g/kg crude protein and 60 g/kg crude lipid, CP Feeds, India) twice daily. A total of 15 FRP experimental tubs of 150 L capacity each were used, with three tubs assigned to each dietary treatment. Twenty pangasius fingerlings (average weight 5.22 ± 0.04 g) were randomly selected and stocked in each tub. The maintenance of fish in the experimental setup was carried according as mentioned in our previous experiment [25]. Water quality was evaluated following the APHA guidelines [26]. The water temperature varied between 23 °C and 26 °C. The dissolved oxygen, pH, and ammonia levels were recorded as 5.70 ± 1.18 mg/L, 7.9 ± 0.98, and 0.015 ± 0.04 mg/L, respectively.

Sampling procedure

After the 56 days feeding trial, the fish from each tank were weighed after one day of fasting and counted to calculate the individual final weight, length, total biomass and survivability after anaesthetizing with a solution of eugenol at 50 mg/L [27]. All the sampling procedures and calculations were performed according to our previous experiment [25].

Proximate analysis

Fish whole body was cut into small pieces and grounded in kitchen aid grinder (Sujata supermix Mixer Grinder, Mumbai, India). The feed sample was also ground into fine powder before analysis. The proximate analysis such as moisture, crude protein, crude lipid, ash and fibre was done according to AOAC method, with detailed procedures outlined in our previous work [26].

Serum biochemical assay

The hemoglobin was analysed from fresh whole blood following cyanmethemoglobin method [28]. The serum samples were analyzed for glucose, total protein, albumin, globulin, triglyceride (TG) and cholesterol spectrophotometrically (Thermo Scientific Evolution 201, Thermo Fisher Scientific, Waltham, MA) according to the method described in the analytical kits (Agapee, India). Globulin was calculated by deducting the albumin value from total protein value. The estimated VLDL cholesterol level is calculated by dividing the triglyceride value by 5 [29].

Digestive enzyme assays

The intestine samples were homogenized with 50 mM cold phosphate buffer (pH 7.0) at ratios of 1:10 (tissue: buffer) using mortar and pestle and the homogenate was centrifuged at 5000 ×g for 20 min. All the operations were carried in ice cold condition. The supernatant was used for amylase, protease and lipase analysis.

Detailed procedure for the determination of amylase activity, protease activity and lipase activity are described in our previous study [26].

Intermediary metabolic enzyme activity

Liver samples was homogenized with tris HCl buffer for the determination of carnitine palmyto transferase (CPT), malic enzyme (ME) and glucose 6 phosphate dehydrogenase (G6PD) activity. All samples were centrifuged at 10,000× g for 10 min at 4 °C to collect the supernatant for the analysis.

CPT activity was done following Singer et al. [30]. For detailed protocols for ME and G6PD, refer to Peter et al. [26]. The protein concentration of the supernatant of all the samples was measured at 600 nm following Lowry et al. [31]. Specific enzyme activities were expressed as units per mg of hepatic soluble protein.

Antioxidant enzyme assays

Liver and intestine samples were homogenized with phosphate buffer for superoxide dismutase (SOD) activity, catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx) and lipid peroxidation (LPO) activity. All samples were centrifuged at 10,000 ×g for 10 min at 4 °C to collect the supernatant for the analysis.

The procedure for SOD activity, CAT activity, GST activity, GPx activity and LPO activity analysis was followed as Peter et al. [26].

Innate immune parameter

Fresh blood from 2 fish was taken from each treatment for respiratory burst activity. A detailed procedure for oxidative burst activity, lysozyme and total antiprotease activity, refer to Peter et al. [26].

Gene expression

Liver samples from 3 fish were taken and preserved in TRI Reagent (SIGMA) to extract total RNA. The purity (260/280) of the isolated RNA was determined using a nano-drop (Thermo Scientific™ NanoDrop™ One/Onec Microvolume UV–Vis Spectrophotometer). Only RNA samples with ratios between 1.90 and 2.10 were used for expression quantification. Following the manufacturer's instructions, complementary DNA (cDNA) was synthesized from RNA using a cDNA synthesis kit (TAKARA).

For gene expression qRT-PCR analysis, we used the previously published primers for β-Actin, Interleukin 1β (IL- 1β), and Transferrin (Table 2). For detailed analytical protocols, refer to Peter et al. [26].

Data analysis

All the data were expressed as mean ± standard error. Normal distribution and homogeneity of variance of data were previously tested (Shapiro–Wilk). The data were compared using a one-way analysis of variance (ANOVA) and differences between means were tested for significance using Duncan multiple range test and T test. The significance level was set at $P < 0.05$. Statistical analyses were performed with the software package SPSS Version 16.0.

Results

Growth performance and feed utilization

Growth performance and morphometric parameters of pangasius fed with varying lipid diets with taurine (15 g/

Table 2 Primers used in the qPCR study of *Pangasianodon hypophthalmus*

Gene	Sequence (5′-3′)	Product size	Gene bank accession No.	References
β-Actin	F: CCACACAGTGCCCATCTACGA R: CCACGCTCTGTCAGGATCTTCA	133	100,534,412	[32]
[1]IL-1β	F: CAGAGGCTGAAGCACACTCA R: CCTTGTCCTGCCTGCTGTAA	148	100,304,696	[32]
Transferrin	F: CACCCCATAACCTTCACCCC R: CGCAGTTTTCCCCAAACCAG	149	100,335,020	[32]

[1] IL-1β Interleukin-1 beta

kg) are shown in Table 3. The fish receiving the high lipid diet (14%) and low lipid diet (6%) did not show significant ($P > 0.05$) difference in the final weight, weight gain, weight gain percentage, specific growth rate, hepatosomatic index, viscera somatic index and lipid retention ratio. FCR also did not show any significant difference ($P > 0.05$) among the treatments.

Body composition

As shown in Table 4, there were no significant differences in proximate composition of fish between treatment groups ($P > 0.05$). The body moisture, protein, lipid, and ash content of pangasius did not change when the oil incorporation levels were raised from 6 to 14%, rather it remained consistent.

Blood parameters

The varying lipid diets did not alter serum parameters such as TG, total protein, albumin, glucose, TC and LDL among the treatments (Table 5). Haemoglobin also did not show any significant difference among the treatments ($P > 0.05$).

Digestive enzyme activity

The intestinal digestive enzyme activities of pangasius fed diets with varying lipids with taurine addition are shown in Table 6. Amylase activity showed significantly higher ($P < 0.05$) activity in 12% and 14% diet group and were found to be similar. Protease activity did not show any significant difference ($P > 0.05$). Lipase activity showed significant difference ($P < 0.05$) and was highest in fish fed with 14% lipid diet.

Metabolic enzyme activity

Table 7 represent results of the analysis of the major liver enzymes involved in lipogenesis and β oxidation pathway. Significantly higher ($P < 0.05$) and alike CPT activity were observed in fish fed with 12% and 14% lipid diet. Whereas, ME activity was higher in 14% lipid diet group. No significant difference ($P > 0.05$) was found in G6PD activity among the treatments.

Antioxidant activity

As shown in Table 8, no obvious changes were seen in antioxidant enzymes (SOD, CAT, GST and GPx) of pangasius fed with varying levels of lipid ($P > 0.05$). The high lipid diet showed similar activity as that of low lipid diet.

Table 3 Growth performances and nutrient utilization of *Pangasianodon hypophthalmus* fed with varying lipid levels

Attributes	T6%	T8%	T10%	T12%	T14%	P value
[1]IW	5.22 ± 0.04	5.06 ± 0.05	5.23 ± 0.06	5.20 ± 0.07	5.12 ± 0.02	*P* > 0.05
[2]FW	25.12 ± 1.60[a]	24.37 ± 0.39[a]	25.52 ± 0.91[a]	25.60 ± 0.85[a]	24.45 ± 1.85[a]	*P* > 0.05
[3]WG	19.90 ± 1.48[a]	19.31 ± 0.27[a]	20.30 ± 0.7[a]	20.40 ± 0.15[a]	19.33 ± 0.64[a]	*P* > 0.05
[4]WG%	381.2 ± 29.2[a]	381.6 ± 7.9[a]	387.9 ± 22.3[a]	392.5 ± 22.1[a]	377.1 ± 38.4[a]	*P* > 0.05
[5]SGR	4.29 ± 0.180[a]	4.51 ± 0.03[a]	4.48 ± 0.07[a]	4.48 ± 0.01[a]	4.75 ± 0.06[a]	*P* > 0.05
[6]FCR	2.12 ± 0.25[a]	2.02 ± 0.47[a]	2.17 ± 0.58[a]	2.09 ± 0.69[a]	2.18 ± 0.78[a]	*P* > 0.05
[7]CF%	0.85 ± 0.26[a]	0.86 ± 0.07[a]	0.85 ± 0.09[a]	0.85 ± 0.02[a]	0.88 ± 0.02[a]	*P* > 0.05
[8]HSI%	2.54 ± 0.08[a]	2.50 ± 0.02[a]	2.83 ± 0.11[a]	2.52 ± 0.02[a]	2.38 ± 010[a]	*P* > 0.05
[9]VSI%	13.15 ± 0.89[a]	13.13 ± 0.86[a]	13.89 ± 0.34[a]	13.65 ± 1.67[a]	13.18 ± 0.54[a]	*P* > 0.05
[10]LRE%	57.26 ± 0.25[a]	57.21 ± 0.45[a]	57.01 ± 0.74[a]	57.39 ± 0.65[a]	57.41 ± 0.47[a]	*P* > 0.05

[1] *IW* Initial weight; [2]*FW* Final weight; [3]*WG* Weight gain (g) = Final weight − Initial weight. [4]*WG%* Weight gain percentage = (Weight gain/Initial weight) × 100. [5]*SGR* Specific growth rate = 100 × [(ln final weight − ln initial weight)]/days of experiment. [6]*FCR* Feed conversion ratio = Feed intake/wet weight gain. [7]*CF* Condition Factor = 100 × [Final body weight/Total length3 (cm)]. [8]*HSI* Hepato-somatic index % = 100 × (weight of liver [g]/weight of fish [g]). [9]*VSI* Viscera somatic index % = 100 × (weight of viscera [g])/(weight of fish [g]). [10]*LRE* Lipid retention efficiency = 100 × (lipid gain/Total crude lipid fed)

Values are means ± SEM. In the rows, different letters indicate statistical difference at $P < 0.05$

Table 4 Whole body proximate compositions (g/kg) of *Pangasianodon hypophthalmus* fed with varying lipid levels over 56 days

Attributes	T6%	T8%	T10%	T12%	T14%	P value
MOISTURE	734.1 ± 1.79[a]	735.7 ± 0.33[a]	734.3 ± 0.43[a]	73.5 ± 0.30[a]	735.8 ± 0.21[a]	*P* > 0.05
[1]CP	136.7 ± 1.09[a]	136.3 ± 0.38[a]	137.8 ± 0.70[a]	138.1 ± 0.85[a]	137.8 ± 0.31[a]	*P* > 0.05
[2]CL	71.26 ± 0.20[a]	71.70 ± 0.49[a]	70.73 ± 0.68[a]	70.83 ± 0.34[a]	70.87 ± 0.37[a]	*P* > 0.05
ASH	26.19 ± 0.08[a]	26.03 ± 0.41[a]	26.58 ± 0.55[a]	26.94 ± 0.25[a]	26.94 ± 0.41[a]	*P* > 0.05

[1] *CP* crude protein; [2]*CL* crude lipid

Values are means ± SEM. In the rows, different letters indicate statistical difference at $P < 0.05$

Table 5 Blood biochemistry (g/dl) of Pangasianodon hypophthalmus fed with varying lipid levels over 56 days

	T6%	T8%	T10%	T12%	T14%	P value
[1]HB	8.14 ± 0.01[a]	8.16 ± 0.01[a]	8.15 ± 0.6[a]	8.18 ± 0.01[a]	8.17 ± 0.03[a]	$P > 0.05$
[2]TG	102.6 ± 0.98[a]	101.43 ± 1.04[a]	101.97 ± 0.77[a]	102.73 ± 1.19[a]	101.97 ± 1.13[a]	$P > 0.05$
[3]TP	3.55 ± 0.07[a]	3.54 ± 0.02[a]	3.45 ± 0.07[a]	3.64 ± 0.02[a]	3.62 ± 0.02[a]	$P > 0.05$
[4]ALB	1.01 ± 0.03[a]	1.04 ± 0.03[a]	1.07 ± 0.01[a]	1.05 ± 0.05[a]	1.05 ± 0.05[a]	$P > 0.05$
[5]GLU	72.80 ± 0.60[a]	71.33 ± 0.32[a]	71.40 ± 0.32[a]	71.03 ± 0.45[a]	71.50 ± 0.06[a]	$P > 0.05$
[6]TC	143.90 ± 0.68[a]	144.88 ± 1.81[a]	144.90 ± 1.18[a]	144.95 ± 1.35[a]	145.44 ± 2.41[a]	$P > 0.05$
[7]VLDL	25.24 ± 0.59[a]	29.65 ± 0.93[a]	29.54 ± 0.30[a]	29.1 ± 0.35[a]	29.57 ± 0.28[a]	$P > 0.05$

[1] HB haemoglobin; [2]TG triglycerides; [3]TP total protein; [4]ALB albumin; [5]GLU glucose; [6]TC total cholesterol; [7]VLDL very low-density lipoprotein

Values are means ± SEM. In the rows, different letters indicate statistical difference at $P < 0.05$

Table 6 Digestive enzyme activity in intestine of *Pangasianodon hypophthalmus* fed with varying lipid levels over 56 days

	T6%	T8%	T10%	T12%	T14%	P value
AMYLASE (µg maltose released/min/mg protein)	10.42 ± 0.25[a]	10.45 ± 0.56[ab]	10.55 ± 0.14[ab]	12.39 ± 0.36[b]	12.42 ± 0.57[b]	*P < 0.05*
PROTEASE (µmole of tyrosine released/min/mg protein)	7.35 ± 0.15[a]	7.62 ± 0.24[a]	7.44 ± 0.08[a]	7.38 ± 0.52[a]	7.21 ± 0.61[a]	*P > 0.05*
LIPASE (Unit/mg protein)	1.89 ± 0.07[a]	2.32 ± 0.01[b]	2.42 ± 0.02[b]	3.89 ± 0.02[c]	4.24 ± 0.03[d]	*P < 0.05*

Values are means ± SEM. In the rows, different letters indicate statistical difference at *P < 0.05*

Table 7 Specific activity of metabolic enzymes (U/mg protein) in the liver of Pangasianodon hypophthalmus fed with varying lipid levels over 56 days

	T6%	T8%	T10%	T12%	T14%	P value
[1]CPT	1.05 ± 0.33[a]	1.32 ± 0.38[ab]	1.56 ± 0.11[b]	2.15 ± 0.21[c]	2.36 ± 0.55[c]	$P < 0.05$
[2]ME	0.41 ± 0.01[a]	0.43 ± 0.01[ab]	0.47 ± 0.03[ab]	0.49 ± 0.01[bc]	0.51 ± 0.01[c]	$P < 0.05$
[3]G6PD	9.0 ± 0.64[a]	9.03 ± 0.08[a]	8.97 ± 0.28[a]	9.28 ± 0.12[a]	9.23 ± 0.29[a]	$P > 0.05$

[1] CPT Carnitine palmitoyltransferase; [2]ME malic enzyme; [3]G6PD Glucose 6 phosphate dehydrogenase enzyme

Values are means ± SEM. In the rows, different letters indicate statistical difference at $P < 0.05$

Table 8 Antioxidant capacity (U/mg protein) in liver of Pangasianodon hypophthalmus fed with varying lipid levels over 56 days

	T6%	T8%	T10%	T12%	T14%	P value
[1]SOD	89.33 ± 0.59[a]	88.50 ± 0.49[a]	89.20 ± 0.08[a]	89.89 ± 0.80[a]	90.53 ± 0.12[a]	$P > 0.05$
[2]CAT	22.36 ± 0.11[a]	12.53 ± 0.13[a]	34.3 ± 0.92[a]	27.22 ± 0.07[a]	27.04 ± 0.79[a]	$P > 0.05$
[3]GST	1.11 ± 0.07[a]	1.35 ± 0.04[a]	1.68 ± 0.02[a]	1.54 ± 0.18[a]	1.62 ± 0.12[a]	$P > 0.05$
[4]GPx	14.12 ± 0.06[a]	15.28 ± 0.07[a]	15.31 ± 0.08[a]	14.28 ± 0.04[a]	14.33 ± 0.04[a]	$P > 0.05$
[5]LPO	2.22 ± 0.069[a]	2.39 ± 0.13[a]	2.45 ± 0.176[a]	2.47 ± 0.19[a]	2.52 ± 0.22[a]	$P > 0.05$

[1] SOD Superoxide dismutase, [2]CAT Catalase, [3]GST Glutathione S transferase, [4]GPx Glutathione peroxidase, [5]LPO Lipid peroxidation

Values are means ± SEM. In the rows, different letters indicate statistical difference at $P < 0.05$

The MDA activity also did not show significant difference ($P > 0.05$) and did not vary among treatments.

Innate immune response

Respiratory burst activity, Serum lysozyme activity, and serum antiprotease activity did not show significant difference ($P > 0.05$) among the treatments and were represented in Table 9. High lipid diet fed fish showed similar activity as that of low lipid fed fish.

Table 9 Serum immune parameters in the liver of Pangasianodon hypophthalmus fed with varying lipid levels over 56 days

	T6%	T8%	T10%	T12%	T14%	P value
[1]RB	0.99 ± 0.02[a]	0.96 ± 0.03[a]	0.98 ± 0.02[a]	0.96 ± 0.21[a]	0.99 ± 0.02[a]	$P > 0.05$
[2]LZY	19.45 ± 0.45a	19.65 ± 0.86[a]	20.01 ± 0.68[a]	19.86 ± 0.25[a]	19.90 ± 0.58[a]	$P > 0.05$
[3]SAP (% of trypsin inhibition)	90.78 ± 0.30[a]	90.25 ± 0.60[a]	90.06 ± 0.88[a]	90.51 ± 0.43[a]	90.84 ± 0.29[a]	$P > 0.05$

[1] RB respiratory burst activity; 2LZY lysozyme activity; [3]SAP (% of trypsin inhibition) serum antiprotease activity

Values are means ± SEM. In the rows, different letters indicate statistical difference at $P < 0.05$

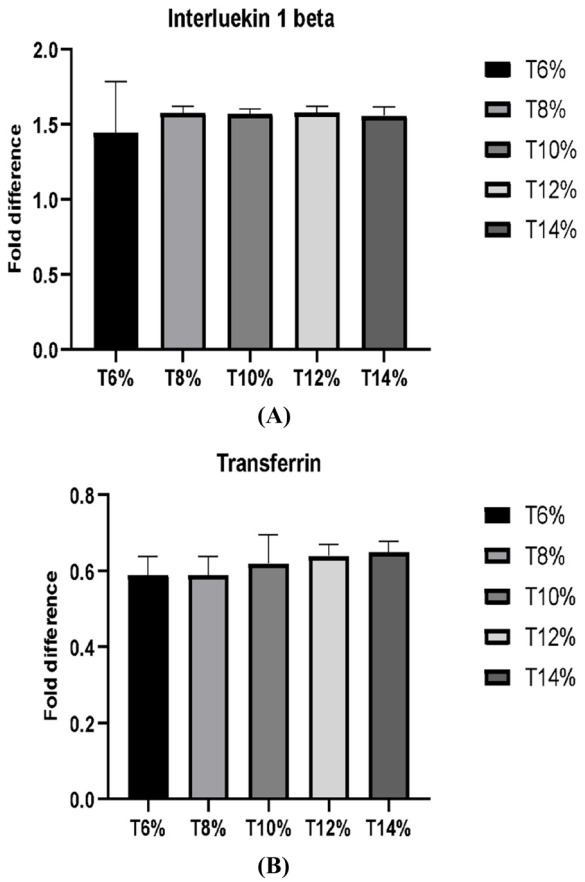


Fig. 1 Expression of different genes (**A**) hepatic IL- 1β (**B**) transferrin. Values are depicted as Mean ± SE

Gene expression

Relative expression of hepatic IL- 1β and transferrin did not show any significant difference ($P > 0.05$) among the treatments fed with varying lipid levels and is depicted in Fig. 1.

Discussion

The results of the current study showed that there was no significant difference in the growth and biometric indexes in pangasius among the feeding treatments, suggesting that this fish species displays a level of acceptance to higher fat diets in the presence of taurine. In a study by Sivaraman [24], it was found that pangasius requires 10% lipids in their diet. Furthermore, when the fish were fed diets with 12% and 15% lipids, there was a decrease in weight gain, along with lower body indices and digestive enzyme activity. Similar findings have been reported in juvenile grouper [33], silver barb [34], and black catfish [35], where weight gain increased with dietary lipid up to an optimal level and then decreased with further lipid increase. Excess lipid not only inhibits de novo fatty acid synthesis but also impairs the fish's ability to digest and absorb it, leading to a reduced growth rate [36]. In this study, higher lipid levels were effectively utilized, and no decrease in growth was observed when supplemented with taurine. In rice field eel, dietary taurine supplementation was found to improve weight gain, with the addition of 0.5% taurine showing no significant different from that of the high-fat group [37].

The process of lipid deposition is intricate and involves multiple steps, including lipid transport, uptake, synthesis, and catabolism. Results of our study indicates that there was no significant difference in whole body crude lipid among high fat diet and low-fat diet in the presence of taurine. Studies have also demonstrated that taurine, when fed at an optimal level, significantly decreased lipid deposition in tilapia [38], white seabream [39], Persian sturgeon [40] and European seabass [17]. To gain a deeper understanding of the impact of dietary taurine on lipid metabolism in pangasius fed with varying lipid diets, various serum lipid parameters were measured as earlier studies suggest taurine positively influences serum lipid levels in fish by generally lowering them [41]. Results of the present study indicated that the TG, and TC and VLDL contents in the serum of high lipid fed fish were similar to those of low lipid fed fish. Research has demonstrated that supplementing a high-cholesterol diet with taurine significantly reduced the rise in VLDL cholesterol levels in broiler chickens [42]. These findings suggest that dietary taurine specifically reduces the molecular form of VLDL when administered with a high-lipid diet. It has been postulated that taurine-conjugated bile acids have been found to effectively reduce cholesterol levels by

increasing serum high density lipoprotein and decreasing concentrations of LDL [43]. Taurine's hypolipidemic effects are potentially may be attributed from its ability to enhance bile acid synthesis and increase the activity of the cholesterol 7-alpha-hydroxylase enzyme, which is the key enzyme in cholesterol catabolism into bile acids [44]. This stimulatory influence of taurine on this enzyme has been demonstrated in rats [44] and guinea pigs [45]. Taurine is known to enhance the activity of hepatic lipase, an enzyme that plays a crucial role in reducing the levels of triglycerides (TG) in serum [46]. Hepatic lipase primarily functions by breaking down triglycerides in chylomicron remnants into glycerol and free fatty acids [47]. In the current study, the highest lipase activity was observed in the group fed a diet containing 14% lipid. Similar results have been reported, where taurine supplementation in broilers significantly increased hepatic lipase activity [48]. The aforementioned functions of taurine may contribute to the mitigation of adverse effects associated with a high lipid diet in the present study.

The CPT plays a crucial role in the decomposition breakdown of fatty acids [49], facilitating their conversion in to energy through metabolic pathways. Higher expression of CPT suggested that when the body use lipid metabolites to provide for the energy expenditure [42]. In the present study, higher CPT activity was shown by 12% and 14% group suggesting enhanced lipid metabolism in these groups. Previous investigations showed that the taurine supplementation enhanced the expression level of CPT in broiler chicken [42]. These results revealed that taurine may ameliorate hepatic lipid accumulation by increasing the expression of fatty acid β-oxidation gene such as CPT- 1. The liver ME activity helps in the production of NADPH necessary for the fatty acid synthesis in liver [50]. The present study showed the highest ME activity in 14% lipid group likely due to the increased demand for NADPH to support enhanced fatty acid synthesis associated with higher lipid availability in the diet. Similarly, G6PD is also involved in NADPH generation and is required for FAS-catalysed de novo fatty acid biosynthesis. In the present study, the activity of G6PD did not show any significant difference among the treatment groups. This suggested that fatty acid β-oxidation acceleration and lipogenesis inhibition may be the reason of the reduction in liver lipid deposition brought on by dietary taurine supplementation. Considering the above findings, we can assume that the harmful effects of a high lipid diet, such as lipid deposition, were not observed in this study due to the upregulation of lipid metabolic enzymes.

Long-term feeding with high fat diet can impair the lipid metabolism and can cause inflammation. Fish that are fed with a high-fat diet for an extended period may have increased mitochondrial membrane permeability, which could result in the excessive production of reactive oxygen species (ROS) and oxidative damage [3, 22]. However, the present study showed that there was no adverse effect in SOD, CAT, GST and GPx of fish fed with 14% lipid diet when compared with 6% lipid in the presence of taurine. Similar observations have seen in African catfish and yellowfin seabream [51, 52] when fed with 10% lipid with addition of taurine. The antioxidant capacity of taurine has been attributed to its role in Nrf2/Keap1 signalling pathway [53]. Taurine has direct antioxidant action by neutralising the harmful oxidative compounds and its metabolites also suppress ROS, mostly via the Keap- 1/Nrf2/HO- 1 pathway, to produce indirect antioxidant and even anti-inflammatory benefits [54]. This indicates that taurine can alleviate the oxidative damage caused by high fat diet in pangasius.

High dietary fat intake may impair the immune function of fish [55]. High fat diet feeding was shown to reduce the activity of lysozyme in blunt snout bream [56] and of lysozyme in turbot [4]. The present study showed that there was no significant difference in RB, lysozyme and antiprotease activity among the high lipid diet group and low lipid diet group. In aquatic species, such as Chinese mitten crabs and yellow catfish, taurine has been shown to be an efficient immunological booster [56, 57]. One of the reasons of enhancing immune function of taurine is that they can control the inflammatory responses and regulate the expression of anti-inflammatory cytokines [58]. So, we can assume that taurine may be the reason for mitigating the harmful effects of high lipid diet.

IL- 1β is considered to be one of the most powerful pro-inflammatory cytokines, playing a role in the body's reaction to microbial infections, tissue damage, and stress [59]. In the present study, there was no significant difference in IL- 1β among the high lipid diet and low lipid diet. In the present study, there was no significant difference in transferrin among the high lipid diet and low lipid diet fed fish. It is known that transferrin are multifunctional iron-binding proteins [60]. Another important function of transferrin in living organisms is in connection with innate immunity. In healthy conditions, iron levels are strictly regulated by several iron-binding proteins. However, in the present study transferrin did not differ among the low lipid diet and high lipid diet fed fish. After considering the overall findings, it is evident that there are no adverse effects of a high lipid diet when incorporating taurine supplementation in pangasius.

Conclusion

The study demonstrated that taurine supplementation in pangasius diets supports efficient lipid metabolism and growth across varying lipid levels. Key metabolic

enzymes, such as lipase, carnitine palmitoyl transferase, and malic enzyme, showed enhanced activity in fish fed higher lipid diets, reflecting improved lipid utilization. However, whole-body composition, blood parameters, antioxidant activity, and innate immune responses remained unaffected by the dietary lipid level. Furthermore, the study showed comparable effects on growth and biometric measurements between pangasius fish fed high lipid and low lipid diets. These findings indicate that dietary taurine inclusion ensures consistent growth performance and health outcomes, irrespective of dietary lipid content in aquafeed formulations.

Authors' contributions
N. P.: Writing original draft, Formal analysis, Investigation. C. P.: Writing – review ;amp editing, Supervision, Conceptualization. N. D.: Formal analysis, Investigation. V. V.P: Formal analysis. S. D.: Formal analysis. K.N.M.: Statistics.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was conducted with the approval of ethical committee of Kerala University of Fisheries and Ocean Studies in adherence with the current animal welfare laws in India.

Competing interests
The authors declare no competing interests.

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