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Effect of dietary *Citrus sinensis* peel extract on growth performance, digestive enzyme activity, muscle biochemical composition, and metabolic enzyme status of the freshwater fish, *Catla catla*

M. S. Shabana, M. Karthika and V. Ramasubramanian*

Abstract

Background: The present study was made to assess the effects of dietary *Citrus sinensis* peel extract on the growth performance, digestive enzyme activity, muscle biochemical compositions, profiles of fatty acid and amino acid, and metabolic enzyme status of the freshwater fish *Catla catla*. The methanolic extract of *C. sinensis* peel was supplemented with basal diets at 2, 6, and 10 g kg^{-1} and fed to *C. catla* for a 45-day experiment period.

Results: Fish fed with the different concentrations of *C. sinensis* peel-supplemented extract showed significant ($P < 0.05$) improvement in the survival, growth, muscle biochemical compositions, digestive enzyme activities, and profile of amino acids and fatty acids when compared to control. Among these different concentrations, 6 g kg^{-1} *C. sinensis*-supplemented diet produced a significantly better performance when compared to other concentrations. Similarly, the insignificant ($P > 0.05$) difference was observed in the metabolic enzyme activities (glutamic oxaloacetic transaminase and glutamic pyruvic transaminase) in all concentrations of *C. sinensis* peel-supplemented diet-fed fish. It indicates that the supplemented peel extract did not produce any adverse effect on *C. catla*.

Conclusion: The obtained results suggested that the 6 g kg^{-1} of *C. sinensis* can be supplemented in the diet of *C. catla* for regulating better survival and growth.

Keywords: *Citrus sinensis*, *Catla catla*, Amylase, Glutamic pyruvic transaminase, Glutamic oxaloacetic transaminase

Background

Aquaculture was continuously intensified due to the decrease of wild capture and increased demand for the protein food. Global production of food fish and other aquatic animals from aquaculture reached 170.9 million tons in 2016 (FAO year book, 2016). Among the cultivated fish species, *Catla c*atla is the most important farmed freshwater fish species with a high economic value due to its delicious taste and presence of rich protein and omega-3 fatty acids, which contain lower triglyceride levels which help to reduce inflammation throughout the body and support brain health. The fish production tended to increase during the first two quarters of 2017–2018, and it was estimated as 5.80 million tons (DAHDFF, 2017).

Nutrient composition of feed, such as protein, carbohydrate, lipid, vitamins, and minerals, is the most important factor affecting the health and growth of fish; hence, properly balanced supplemental feeds with a reliable feeding rate can be helpful to enhance survival and growth (Dawood & Koshio, 2016; Dawood, Koshio, & Esteban, 2017). In recent years, plant products (leaf, root, stem, bark, etc.) have been used as a natural immunostimulant instead of antibiotics in aquaculture feed formulations due to their eco-friendly and cost-effective properties compared to synthetic drugs. Fruit peels, such as *Musa sapientum*, *Citrus limon*, *Artocarpus heterophyllus*,

* Correspondence: vraman68@rediffmail.com
Aquatic Biotechnology and Live Feed Culture Laboratory, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu 641 046, India

Mangifera indica, *Hippophae rhamnoides*, and *Punica granatum*, exhibit anti-inflammatory, antitumor, antioxidant, and antimicrobial activities due to the presence of rich flavonoid glycosides, coumarins, β- and ɤ-sitosterols, vitamins, and volatile compounds (Chiba et al., 2003; Gao et al., 2006; Liu, Heying, & Tanumihardjo, 2012). The orange peel is a primary by-product produced by the fruit processing industries, and it accounts approximately 45% of the total bulk (Farhat et al., 2011). *Citrus sinensis* showed several medicinal properties like anticancer, antidiuretic, immunity enhancer, and tonic to digestion (Grosso et al., 2014). However, to the best of our knowledge, the information about the influence of dietary *C. sinensis* peel extract on fish is not yet reported so far. Thus, the present study was aimed to assess the effect of *C. sinensis* peel extract on the survival, growth, digestive enzyme activities, muscle biochemical compositions, profile of amino acids and fatty acids, and metabolic enzymes of the *C. catla*.

Materials and methods

Experimental fish

The freshwater fish *C. catla* were obtained from the Aliyar dam in Tamil Nadu fisheries development corporation, Pollachi, Coimbatore District, India. Fish were acclimatized to laboratory condition in a large cement tank (6″ × 4″ × 3″) with ground water with an optimal level of physico-chemical characteristics (temperature, 27.33 ± 0.57 °C; dissolved oxygen, 7.23 ± 0.58 mg/L; pH, 7.2 ± 0.1; total dissolved solids, 0.68 ± 0.06 g/L; biological oxygen demand, 18.63 ± 0.35 mg/L; chemical oxygen demand, 67.33 ± 5.03 mg/L; ammonia, 0.4 ± 0.1 mg/L) for 2 weeks. During the acclimatization period, fish was fed with commercial feed thrice (at 06:00 h, 12:00 h, and 18:00 h) per day. Feces and unfed feeds were cleared out daily while renewing the 80% of tank water to maintain the healthy environment.

Preparation of crude extracts of *C. sinensis* peel

The orange peels (*C. sinensis*) were collected from various fruits and juice stalls at Coimbatore. The collected peels were washed thrice in distilled water, chopped, and shade dried at room temperature for 2–3 weeks. The dried peels of *C. sinensis* were grounded into coarse powder for the ease of extraction of active compounds. The powdered plant material (150 g) was wrapped in a filter paper, placed in a Soxhlet apparatus, and extracted with absolute methanol. After extraction, the condensation process was carried out, which condensed the solvent into a liquid form. Finally, it was kept in a water bath for 1 h and a half to eliminate the solvent from the extract and then stored at 4 °C until used for experimentation (Anju, Arun, Sayeed, & Narasimhan, 2011). This process was repeated five times to get an adequate amount of extract for feed formulation.

Feed formulation

Feed preparation was made in the laboratory according to Table 1. The ingredients including fishmeal, soybean meal, wheat bran, tapioca flour, eggs, and cod liver oil and vitamin mix were purchased from the local markets. For this diet preparation, the fish meal and soybean meal were served as the protein source, the carbohydrate sources were wheat and tapioca flour, and lipid source was cod liver oil. Also, tapioca flour and egg albumin were taken as binding agents, and vitamin B complex with vitamin C was also added as an essential micronutrient. The above ingredients except egg albumin, cod liver oil, vitamins, and minerals were mixed thoroughly and steam cooked for 20 min at 105 °C. Different concentrations of *C. sinensis* peel extract (2 g kg^{-1}, 6 g kg^{-1}, and 10 g kg^{-1}) were added along with the heat-sensitive ingredients like vitamin, mineral premix, egg albumin, and cod liver oil to the steam cocked basal diet and mixed well to form a dough. Further, the dough was pelleted using indigenous hand pelletizer (Retro stainless steel, BM brand) and dried at room temperature until the constant weight was reached.

Experimental procedure

Four groups of *C. catla* were assigned for 45 days of the experiment in triplicate. Three groups were fed with 2, 6, and 10 g kg^{-1} *C. sinensis* peel-supplemented diets. The remaining one group was served as control (fed with "0" concentration of *C. sinensis* peel-supplemented diet). Each group consisted of 50 fish. The water medium was renewed every day by siphoning method. At the end of

Table 1 Composition of formulated feed along with the Citrus sinensis peel extract

Ingredients	Composition (g kg^{-1})			
	Control	2.00	6.00	10
Fish meal	100	100	100	100
Rice bran	200	200	200	200
Groundnut oil cake	200	200	200	200
Wheat bran	100	100	100	100
Soybean meal	250	250	250	250
Corn flour	50	50	50	50
Egg albumin	50	50	50	50
Cod liver oil	20	20	20	20
Minerals	5	5	5	5
Vitamins	25	25	25	25
Peel extract	0	2	6	10

Vitamins: Becadexamin (manufactured by Geltec), each capsule contains vitamin A 5000 IU, vitamin D_3 400 IU, tocopheryl acetate 15 mg, vitamin B 5 mg, nicotinamide IP 45 mg, D-panthenol IP 5 mg, folic acid IP 1000 μg, dibasic calcium phosphate IP 70 mg, copper sulfate pentahydrate 0.1 mg, manganese sulfate monohydrate BP 0.01 mg, zinc sulfate monohydrate IP 28.7 mg, potassium iodide IP 0.025 mg, light magnesium oxide IP 0.15 mg

the feeding experiment, fish from each treatment were sampled to analyze various parameters.

Assessment of survival, growth, and food index

Survival, growth, weight gain, length gain, specific growth rate, and food index parameters, such as feed intake, feed conversion ratio, and protein efficiency ratio were calculated according to the following equations (Tekinay & Davies, 2001)

$$\text{Survival}\ (\%) = \text{no.of live fish/no.of fish introduced} \times 100$$

$$\text{Length gain}\ (\text{cm}) = \text{final length}\ (\text{cm}) - \text{initial length}\ (\text{cm})$$

$$\text{Weight gain}\ (\text{g}) = \text{final weight}\ (\text{g}) - \text{initial weight}\ (\text{g})$$

$$\text{Feed intake}\ \left(\text{g day}^{-1}\right) = \text{feed intake}\ (\text{g})/\text{total number of days}$$

$$\text{Specific growth rate}\ (\%) = \log w_2 - \log w_1/t \times 100$$

$$(\text{where } w_1 \text{ and } w_2 = \text{initial and final weight}\ (\text{g}), \text{and}$$

$$t = \text{duration of an experiment in days})$$

$$\text{Feed conversion ratio} = \text{feed intake}\ (\text{g})/\text{weight gain}\ (\text{g})$$

$$\text{Protein efficiency ratio} = \text{weight gain}\ (\text{g})/\text{protein intake}\ (\text{g}).$$

Assay of digestive enzyme activity and muscle biochemical compositions

Activities of the digestive enzymes (protease, amylase, and lipase) were assayed on the initial and final days of the feeding experiment. Forty fish per treatment (10 fish per tank) were randomly selected; the whole digestive tract and the muscle were taken to analyze the digestive enzyme activity and biochemical compositions. The whole digestive tract was homogenized in ice-cold double-distilled water and centrifuged at 9300*g* under 4 °C for 20 min. The supernatant was used as crude enzyme source. The casein-hydrolysis method was used to determine the total protease activity (Furne et al., 2005). Amylase activity was determined by the starch-hydrolysis method (Bernfeld, 1955), and the lipase activity was analyzed by the method of Furne et al. (2005). The biochemical constituents of the fish muscle, such as protein (Lowery, Rosebrough, Farr, & Randall, 1951), carbohydrate (Roe, 1954), and total lipid (Folch, Less, & Sloane Stanley, 1956) were estimated by the following standard methods.

Assay of metabolic enzyme activity

The metabolic enzymes, such as glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GPT), were analyzed in the muscle of *C. catla* according to the method of Reitman and Frankel (1957). Five fish from each individual tank (20 fish per treatment) were collected, and the tissue (100 mg) was homogenized in 0.25 M sucrose and centrifuged at 3300 rpm for 20 min in a high-speed cooling centrifuge at 4 °C. The supernatant was used as the enzyme source. The optical density was taken using a spectrophotometer at 505 nm within 15 min. GOT and GPT activity was expressed as units per liter.

Amino acid profile analysis

High-performance thin-layer chromatographic (HPTLC) method (Hess & Sherma, 2004) was used to analyze the profile of amino acids in the muscle of *C. catla* (20 fish per treatment, 5 fish per tank) fed with formulated experimental feeds. Standard amino acids like proline, serine, asparagine, glutamine, methionine, aspartic acid, glutamic acid, alanine, valine, phenyl alanine, lysine, glycine, threonine, isoleucine, and tyrosine, arginine, cysteine, histidine, leucine, and tryptophan were also performed in parallel. The peak area of the sample was compared and quantified with standard amino acids. The obtained amino acids were expressed as grams per kilogram of dry weight.

Fatty acid profile analysis

Gas chromatographic and mass spectrometry (GC-MS) method of Martins et al. (2003) was used to analyze the profile of fatty acids using 5 fish per group. Fatty acids were obtained from lipids by saponification. Each fatty acid in the unknown sample was identified based on the retention time and peak area of the standard fatty acids and expressed as %/2 μL methylated fatty acid.

Statistical analysis

The data were expressed as mean ± S.D. and analyzed by one-way analysis of variance (ANOVA) using SPSS (21.0), followed by Duncan's multiple range test (DMRT) to compare the differences among treatments. Differences were considered significant at $P < 0.05$.

Results

Survival and nutritional index

Survival, growth, weight gain, feed intake, specific growth rate, and protein efficiency ratio were significantly increased ($P < 0.05$) in the fish fed with 6 g kg^{-1} *C. sinensis*-supplemented diets when compared to other concentrations of *C. sinensis* and control diet-fed fish. In context, the feed conversion ratio was found to be significantly decreased in the fish fed with 6 g kg^{-1} *C. sinensis*-supplemented diets when compared with control and other concentrations of *C. sinensis*-incorporated feed-fed fish group (Table 2).

Activity of digestive enzymes and muscle biochemical compositions

The digestive enzymes such as protease, amylase, and lipase were found to be significantly elevated ($P < 0.05$)

Table 2 Survival, growth, and food index evaluation of *Catla catla* fed with *C. sinensis*-supplemented diets

Parameters	*Citrus sinensis* concentration g kg^{-1}			
	Control	2.00 (diet 1)	6.00 (diet 2)	10 (diet 3)
SR (%)	76.30 ± 1.22^{d}	80.92 ± 1.16^{c}	92.21 ± 2.50^{a}	86.07 ± 2.35^{b}
Length (cm)	6.18 ± 1.99^{a}	7.15 ± 1.78^{a}	7.70 ± 1.53^{a}	7.37 ± 1.56^{a}
Weight (g)	1.67 ± 0.05^{c}	2.45 ± 0.34^{b}	3.43 ± 0.08^{a}	2.65 ± 0.11^{b}
LG (cm)	2.27 ± 0.08^{d}	3.24 ± 0.06^{c}	3.79 ± 0.14^{a}	3.46 ± 0.04^{b}
WG (g)	0.84 ± 0.40^{c}	1.62 ± 0.03^{b}	2.60 ± 0.04^{a}	1.82 ± 0.17^{b}
FI (g day^{-1})	0.33 ± 0.15^{b}	0.50 ± 0.17^{ab}	0.66 ± 0.05^{a}	0.53 ± 0.20^{ab}
SGR (% day^{-1})	1.86 ± 0.44^{c}	3.60 ± 0.05^{b}	5.77 ± 0.16^{a}	4.04 ± 0.27^{b}
FCR	0.39 ± 0.01^{a}	0.30 ± 0.06^{b}	0.25 ± 0.03^{b}	0.29 ± 0.00^{b}
PER	0.29 ± 0.09^{b}	0.53 ± 0.12^{b}	1.12 ± 0.55^{a}	0.64 ± 0.06^{ab}

Mean values within the same row sharing the same superscript are not significantly different ($P > 0.05$), *Citrus sinensis*-free diet; $n = 3$ (three samples from each treatment), mean ± SD
Initial length and weight were 3.91 ± 0.03 cm and 0.83 ± 0.06 respectively
SR survival rate, *LG* length gain, *WG* weight gain, *FI* feed intake, *SGR* specific growth rate, *FCR* feed conversion ratio, *PER* protein efficiency ratio

in the fish fed with 2–10 g kg^{-1} *C. sinensis*-supplemented diets when compared to control. However, the differences in these enzymes' activity between 2 and 10 g kg^{-1} *C. sinensis* were insignificant in the case of amylase and lipase activities (Table 3). In the present study, the concentrations of biochemical constituents, such as protein, carbohydrate, and lipid contents were significantly ($P < 0.05$) increased in fish fed with 6 g kg^{-1} of *C. sinensis* peel-supplemented diets when compared to other concentrations of *C. sinensis* and control, while lipid content showed insignificant ($P > 0.05$) difference between 2 and 6 g kg^{-1} of *C. sinensis*-supplemented diet-fed fish when compared to control diet-fed fish. In context, these biochemical compositions were significantly ($P < 0.05$) decreased in 10 g kg^{-1} of *C. sinensis* peel extract-supplemented diet-fed fish group when compared to 6 g kg^{-1} *C. sinensis* peel extract diet-fed fish group (Table 3).

Metabolic enzyme status

The metabolic enzymes (GOT and GPT) were insignificantly ($P > 0.05$) elevated in liver tissue of the fish fed with 2–6 g kg^{-1} *C. sinensis* peel-supplemented diets, whereas fish fed with 10 g kg^{-1} *C. sinensis* peel extracts showed significantly ($P < 0.05$) better elevation in the GOT and GPT activities (Table 4).

Amino acid profile

Seventeen amino acids were detected in the muscle of *C. catla* fed with different concentrations of *C. sinensis*-supplemented diets. Among these, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine were essential amino acids, and arginine, cysteine, glutamine, glycine, proline, tyrosine, alanine, and aspartic acid were non-essential amino acids. The essential amino acids such as lysine, phenylalanine, threonine, tryptophan, and valine were found to be significantly ($P < 0.05$) elevated in the fish fed with the different concentration of *C. sinensis* extract-supplemented diets compared to control, while the non-essential amino acids, such as glutamine, glycine, tyrosine, and aspartic acids, were significantly ($P < 0.05$) elevated in 2–6 g kg^{-1} *C. sinensis*-supplemented diet fish when compared to the control. However, the insignificant difference was observed between 10 g kg^{-1}

Table 3 Muscle biochemical composition of *Catla catla* fed with different concentrations of *Citrus sinensis*-supplemented diets during the experimental period

Parameters	*Citrus sinensis* concentration g kg^{-1}				
		Control	2.00 (diet 1)	6.00 (diet 2)	10 (diet 3)
Protein (mg/G)	Initial	27.68 ± 0.66	27.68 ± 0.66	27.68 ± 0.66	27.68 ± 0.66
	Final	33.93 ± 1.19^{d}	41.11 ± 1.31^{c}	63.61 ± 3.16^{a}	55.54 ± 2.21^{b}
Carbohydrate (mg/G)	Initial	14.13 ± 0.91	14.13 ± 0.91	14.13 ± 0.91	14.13 ± 0.91
	Final	21.74 ± 1.05^{b}	25.12 ± 1.93^{b}	44.48 ± 4.94^{a}	39.57 ± 1.64^{a}
Lipid (mg/G)	Initial	4.80 ± 0.58	4.80 ± 0.58	4.80 ± 0.58	4.80 ± 0.58
	Final	6.72 ± 1.09^{b}	7.22 ± 1.31^{ab}	9.70 ± 1.10^{a}	7.25 ± 1.52^{ab}

Mean values within the same row sharing the same superscript are not significantly different ($P > 0.05$), *Citrus sinensis*-free diet; $n = 3$ (three samples from each treatment), mean ± SD

Table 4 Activities of digestive and the metabolic enzymes (U/mg protein) in *Catla catla* fed with different concentration of *Citrus sinensis*-supplemented diets

Enzymes			*Citrus sinensis* concentration g kg^{-1}			
			Control	2.00 (diet 1)	6.00 (diet 2)	10 (diet 3)
Digestive enzymes (U/mg protein)	Protease	Initial	0.71 ± 0.22	0.71 ± 0.22	0.71 ± 0.22	0.71 ± 0.22
		Final	1.11 ± 0.36^{c}	1.69 ± 0.19^{bc}	3.21 ± 0.65^{a}	2.25 ± 0.48^{b}
	Amylase	Initial	0.30 ± 0.03	0.30 ± 0.03	0.30 ± 0.03	0.30 ± 0.03
		Final	0.58 ± 0.11^{c}	1.15 ± 0.40^{bc}	2.23 ± 0.55^{a}	1.56 ± 0.27^{ab}
	Lipase	Initial	0.28 ± 0.11	0.28 ± 0.11	0.28 ± 0.11	0.28 ± 0.11
		Final	0.37 ± 0.06^{b}	0.62 ± 0.10^{ab}	1.15 ± 0.49^{a}	0.76 ± 0.26^{ab}
Metabolic enzymes (U/L)	GOT	Initial	9.05 ± 0.04	9.05 ± 0.04	9.05 ± 0.04	9.05 ± 0.04
		Final	15.03 ± 0.89^{a}	16.12 ± 2.75^{a}	17.79 ± 2.64^{a}	16.39 ± 1.06^{a}
	GPT	Initial	6.64 ± 2.11	6.64 ± 2.11	6.64 ± 2.11	6.64 ± 2.11
		Final	10.03 ± 0.94^{a}	12.98 ± 1.18^{a}	13.80 ± 3.32^{a}	13.75 ± 2.41^{a}

Mean values within the same row sharing the same superscript are not significantly different ($P > 0.05$), *Citrus sinensis*-free diet; $n = 3$ (three samples from each treatment), mean ± SD

C. sinensis extract and control diet fed fish in the case of glutamine and tyrosine in (Table 5).

Fatty acid profile

In the present study, four saturated (myristic acid, palmitic acid, stearic acid, and heptadecanoic acid), two unsaturated (paullinic acid, fumaric acid), two mono-unsaturated (palmitoleic acid, oleic acid), and seven poly unsaturated (linoleic acid, arachidonic acid, eicosatetraenoic acid, docosahexaenoic acid, docosapentaenoic acid, docosatetraenoic acid, and eicosapentaenoic acid) fatty acids were detected in fish muscle through GC-MS analysis. All these fatty acids were found to be insignificantly elevated in the fish fed with the different concentration of *C. sinensis* when compared to control (Table 6).

Table 5 Amino acid (g kg^{-1}) profile of *Catla catla* fed with *C. sinensis*-supplemented diets

Amino acids		Dietary supplementary of *C. sinensis* g kg^{-1}			
		Control	2.00 (diet 1)	6.00 (diet 2)	10 (diet 3)
Essential	Histidine	1.40 ± 0.19^{a}	1.46 ± 0.39^{a}	1.52 ± 0.25^{a}	1.41 ± 0.26^{a}
	Isoleucine	1.35 ± 0.43^{b}	1.72 ± 0.09^{ab}	1.87 ± 0.11^{a}	1.66 ± 0.13^{ab}
	Leucine	3.14 ± 0.80^{a}	3.46 ± 0.11^{a}	3.64 ± 0.25^{a}	3.20 ± 0.61^{a}
	Lysine	0.48 ± 0.01^{c}	1.10 ± 0.02^{ab}	1.25 ± 0.16^{a}	1.07 ± 0.03^{b}
	Methionine	1.31 ± 0.31^{b}	1.67 ± 0.16^{ab}	1.82 ± 0.16^{a}	1.61 ± 0.20^{ab}
	Phenylalanine	1.56 ± 0.30^{b}	1.71 ± 0.04^{a}	1.81 ± 0.09^{a}	Trace
	Threonine	3.63 ± 0.29^{c}	4.62 ± 0.06^{ab}	5.01 ± 0.36^{a}	4.45 ± 0.12^{b}
	Tryptophan	5.02 ± 0.16^{c}	5.52 ± 0.01^{ab}	5.82 ± 0.16^{a}	5.11 ± 0.37^{bc}
	Valine	1.65 ± 0.17^{c}	1.91 ± 0.02^{ab}	1.97 ± 0.01^{a}	1.80 ± 0.01^{bc}
Conditionally essential	Arginine	4.36 ± 0.04^{c}	4.57 ± 0.18^{b}	4.81 ± 0.04^{a}	4.55 ± 0.04^{b}
	Cysteine	1.66 ± 0.29^{a}	1.91 ± 0.06^{a}	1.97 ± 0.01^{a}	1.81 ± 0.16^{a}
	Glutamine	1.63 ± 0.14^{b}	1.87 ± 0.12^{a}	1.93 ± 0.05^{a}	1.77 ± 0.11^{ab}
	Glycine	1.88 ± 0.10^{c}	4.31 ± 0.44^{ab}	4.88 ± 0.10^{a}	4.17 ± 0.40^{b}
	Proline	1.90 ± 0.22^{a}	1.99 ± 0.00^{a}	2.08 ± 0.76^{a}	1.91 ± 0.07^{a}
	Tyrosine	2.15 ± 0.74^{b}	2.74 ± 0.14^{ab}	2.97 ± 0.01^{a}	2.63 ± 0.21^{ab}
Non-essential	Alanine	1.47 ± 0.25^{a}	1.54 ± 0.08^{a}	1.62 ± 0.17^{a}	1.53 ± 0.04^{a}
	Aspartic acid	0.19 ± 0.01^{b}	1.84 ± 0.11^{a}	2.01 ± 0.50^{a}	1.69 ± 0.07^{a}

Mean values within the same row sharing the same superscript are not significantly different ($P > 0.05$), *Citrus sinensis*-free diet; $n = 3$ (three samples from each treatment), mean ± SD

Table 6 Fatty acid profile of *Catla catla* fed with *C. sinensis*-supplemented diets

Fatty acids (%/2 μL methylated fatty acid)		Dietary supplementary of *C. sinensis* g kg^{-1}			
		Control	2.00 (diet 1)	6.00 (diet 2)	10 (diet 3)
Saturated	Myristic acid	3.29 ± 0.99[a]	3.43 ± 0.89[a]	3.45 ± 1.21[a]	3.33 ± 0.87[a]
	Palmitic acid	4.89 ± 1.30[a]	5.67 ± 5.07[a]	5.77 ± 2.44[a]	5.12 ± 0.67[a]
	Stearic acid	2.37 ± 0.57[a]	2.87 ± 1.14	3.06 ± 0.80[a]	2.40 ± 1.31[a]
	Margaric acid	2.08 ± 0.74[a]	2.21 ± 0.45[a]	2.28 ± 0.29[a]	2.14 ± 0.42[a]
Unsaturated	Paullinic acid	3.53 ± 1.36[a]	3.70 ± 0.94[a]	3.98 ± 0.72[a]	3.63 ± 0.88[a]
	Fumaric acid	2.66 ± 0.47[a]	3.03 ± 1.39[a]	3.34 ± 0.37[a]	2.71 ± 0.12[a]
Monounsaturated	Palmitoleic acid	1.64 ± 0.09[a]	1.86 ± 0.73[a]	2.32 ± 0.70[a]	1.73 ± 0.74[a]
	Oleic acid	4.70 ± 2.02[a]	6.05 ± 3.42[a]	6.71 ± 0.58[a]	5.36 ± 2.81[a]
Polyunsaturated	Linoleic acid	4.32 ± 1.76[a]	4.50 ± 1.20[a]	5.19 ± 0.44[a]	4.39 ± 0.89[a]
	Arachidonic acid	2.93 ± 0.70[a]	3.07 ± 0.69[a]	3.37 ± 1.07[a]	2.99 ± 0.73[a]
	Eicosatetraenoic acid	2.05 ± 0.47[a]	2.18 ± 0.51[a]	2.47 ± 1.17[a]	2.13 ± 0.51[a]
	Docosahexaenoic acid	3.42 ± 0.60[a]	3.91 ± 0.95[a]	3.98 ± 0.76[a]	3.85 ± 0.66[a]
	Docosapentaenoic acid	2.29 ± 0.27[a]	2.85 ± 0.75[a]	3.21 ± 0.98[a]	2.74 ± 0.98[a]
	Docosatetraenoic acid	2.09 ± 0.71[a]	2.25 ± 0.32[a]	2.65 ± 0.31[a]	2.19 ± 0.04[a]
	Eicosapentaenoic acid	1.58 ± 0.41[a]	1.64 ± 0.65[a]	2.46 ± 0.44[a]	1.61 ± 0.31[a]

Mean values within the same row sharing the same superscript are not significantly different ($P > 0.05$), *Citrus sinensis*-free diet; $n = 3$ (three samples from each treatment), mean ± SD

Discussion

Citrus fruit contains a rich source of secondary metabolites like natural flavonoids, polyphenols, steroids, and saponins. Citrus has antimicrobial and antioxidant properties against various microbes like *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, and *Escherichia coli* (Mathur et al., 2011). Essential oil obtained from the citrus peel manifest antibacterial activity has also been reported (Upadhyay, Dwivedi, & Ahmad, 2010). Plant-based extracts with antimicrobial and immunostimulant properties have been used as therapeutic and prophylactic agents against fish pathogens in aquaculture industries to maintain an eco-friendly environment (Newaj-Fyzul & Austin, 2015). Further, dietary administration of plant extracts can stimulate the immune response by reducing the pathogen load which leads to better survival and growth of fish culture (Abdel–Tawwab, Ahmad, Seden, & Sakr, 2010; El-Desouky, El-Asely, Shaheen, & Abbass, 2012; Gabriel et al., 2015; Kaleeswaran, Ilavenil, & Ravikumar, 2011). In the present study, the significant improvement in survival, growth rate, length and weight gain, feed intake, specific growth rate, and protein efficiency ratio indicates that the supplementation of 2–6 g kg^{-1} *C. sinensis* peel extract has the ability to promote growth performance and feed intake of *C. catla*. Previously, Acar et al. (2015) reported that the dietary inclusion of essential oil extract from *C. sinensis* produced better survival and growth of *Oreochromis mossambicus*. Plant extract, such as *Citrus sinensis*, *Cynodon dactylon*, *Aloe vera*, *Camellia sinensis*, *Echinacea purpurea*, and *Allium sativum*, -supplemented diet-fed *C. catla*, *Penaeus monodon*, *Oreochromis niloticus*, *Carrasius auratus*, *Lates calcarifer*, and *Macrobrachium rosenbergii* which showed better survival, growth performance, feed intake, specific growth rate, and protein efficiency have been reported (Abdel–Tawwab et al., 2010; Aly & Mohamed, 2010; El-Desouky et al., 2012; Gabriel et al., 2015; Kaleeswaran et al., 2011; Kumar et al., 2013; Yogeeswaran et al., 2012). The significant decreases of survival, growth, feed intake, specific growth rate, and protein efficiency ratio in 10 g kg^{-1} *C. sinensis* feed-fed fish suggest that this concentration might be over dose, which led to the negative impact on the fish. Similar results have been reported in *Cyprinus carpio* when fed with ethanolic extract of *Ocimum basilicum*-supplemented diets (Amirkhani & Firouzbakhsh, 2013).

The biochemical compositions, such as protein, carbohydrate, and lipid, are the physiological indicators of fish health, and the nutritive value of fish depends upon their biochemical constituents. In the present study, the significant improvement in muscle biochemical composition (protein, carbohydrate, and lipid) suggests that the synthesis and the storage of the biochemical compositions in *C. catla* were promoted due to supplementation of *C. sinensis* extracts in the diet. Similar results have also been reported in tilapia (*Oreochromis niloticus*) fed on citrus essential oil-supplemented diet (Acar, Kesbic, Yilmaz, Gultepe, & Turker, 2015). Xiaohong et al. (2017) reported the significant increase in the muscle biochemical composition of golden pompanos (*Trachinotus auratus*) fed with dietary dandelion extracts.

The fish digest the nutrients in the feed with the help of the digestive enzymes, subsequently increasing the feed efficiency (Widanarni & Jusadi, 2015). In the present study, the digestive enzymes (protease, amylase, and lipase) were found to be significantly improved in the *C. sinensis*-supplemented diets; it indicates that the supplementation of *C. sinensis* promotes the secretion of these digestive enzymes, which in turn improves the digestion of nutrients, followed by growth of the *C. catla*. Similarly, administration of *Ricinus communis* in the diet of black tiger shrimp showed significant improvement in the activity of digestive enzymes. The administration of garlic, ginger, turmeric, and fenugreek into the diets of *M. rosenbergii* PL which showed an increase in the activities of protease, amylase, and lipase has been reported earlier (Poongodi, Saravana Bhavan, Muralisankar, & Radhakrishnan, 2012).

In the present study, the insignificant elevations in the GOT and GPT in 2–6 g kg^{-1} *C. sinensis* indicate the normal health of liver in fish. In context, the significant alterations of GOT and GPT in the 10 g kg^{-1} *C. sinensis*-supplemented diets suggest some damage in the liver of fish, which leads to poor survival and growth of fish. Previously, the administration of *Origanum vulgare* extract in the diet of Nile Tilapia (*O. niloticus*) showed significant alteration in GOT (glutamic oxaloacetic transaminase) and GPT (glutamic pyruvate transaminase) has been reported (El-Araby & EL-Arabey, 2016).

Amino acids are the building blocks of proteins and serve as body builders of an organism. Amino acids are utilized by various cell structures as key components (Anaya & Daniello, 2006). All animals need a constant source of amino acids for tissue protein synthesis and synthesis of other compounds associated with metabolism including hormones, neurotransmitters, purines, and metabolic enzymes (Halver & Hardy, 2002). In the current study, significant improvements in essential and non-essential amino acids in the fish fed with *C. sinensis* indicate that the supplementation of *C. sinensis* extract had influence on the synthesis of amino acids which led to better growth and survival of *C. catla*. Previously, administration of mango seed kernel, banana peel, and papaya peel in the diets of *M. rosenbergii* which influenced the synthesis of essential and non-essential amino acids have been reported (Aarumugam et al., 2013). Similar results have also been reported in *Acipenser ruthenus* (juvenile sterlet sturgeon) fed on garlic extracts in the supplemented diet (Lee, Seong, Chang, & Jeong, 2012). Poongodi (2011) reported that the significant increase of essential and non-essential amino acids in the diets of *M. rosenbergii* PL after the administration of garlic, ginger, turmeric, and fenugreek. Similar results have also been reported in *Acipenser ruthenus* (juvenile sturgeon) fed on garlic extracts in the supplemented diet (Lee et al., 2012).

Fatty acids play the crucial role in the maintenance of the metabolic and the physiological process which leads to the better growth, survival, and the reproduction of the aquatic organisms. In the present study, the insignificant elevation in the saturated and the unsaturated fatty acids in *C. catla* fed with *C. sinensis* extract-supplemented diets suggests that methanolic extracts of *C. sinensis* did not produce any negative impact on the fatty acid synthesis in the experimental fish *C. catla*. Similarly, the influence of dietary garlic extracts on fatty acid synthesis has been reported in *Acipenser ruthenus* and *M. rosenbergii* (Aarumugam et al., 2013; Lee et al., 2012). Previously, administration of spirulina (*Arthrospira platensis*) and/ or thyme (*Thymus vulgaris*) in the diets of *Oryctolagus cuniculus* (New Zealand white rabbit) showed significant changes in fatty acid contents (Mattioli et al., 2017). Similar results have also been reported in *M. rosenbergii* PL fed on garlic, ginger, turmeric, and fenugreek extracts in the supplemented diet (Poongodi, 2011).

Conclusion

The result of the present study revealed that the dietary incorporation of 2–6 g kg^{-1} methanolic extract of *C. sinensis* fruit peel significantly improved the survival, growth performance, activities of digestive enzyme, muscle biochemical constituents, and amino acids. The insignificant alteration in metabolic enzyme activity in 2–6 g kg^{-1} *C. sinensis* indicates good health status of the fish. Among these different concentrations of *C. sinensis*, 6 g kg^{-1} produced better performance. Therefore, the present study suggests that the methanolic extract of 6 g kg^{-1} *C. sinensis* peel can be supplemented with the basal diets of *C. catla* for regulating better culture practice in the aquaculture industry.

Abbreviations

DAHDFF: Department of Animal Husbandry, Dairying & Fisheries Ministry of Agriculture & Farmers Welfare; DMRT: Duncan's multiple range test; FAO: Food and Agriculture Organization; GC-MS: Gas chromatographic and mass spectrometry; GOT: Glutamic oxaloacetate transaminase; GPT: Glutamic pyruvate transaminase; HPTLC: High-performance thin-layer chromatographic

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Authors' contributions

MS participated in the design of this research work and performed the collection of samples. MK contributed in the analytical part. MS undertook the characterization studies. MS wrote the manuscript. VR supervised the findings of this work. All authors discussed the result and contributed to the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data supporting the conclusions of this work is included within the article. The authors can be contacted for any additional supporting data required by the journal.

Ethics approval and consent to participate
The authors declare that no animal was sacrificed for this study. The collected species was not in the IUCN red list. We declare that we do not need ethical clearance for the present work.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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