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Growth performance, nutrient digestibility, antioxidant status and metabolic enzyme activity in pearlspot (*Etroplus suratensis*), fed carbohydrates of different complexities

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Abstract

Carbohydrate utilization by fish is influenced by many factors and structural complexity is one among them. A 60-days feeding study was conducted to assess the capacity of pearlspot, Etroplus suratensis to utilize different complexity of carbohydrate in growth performance, nutrient digestibility, antioxidant status, metabolic and digestive enzyme activity and histology of liver. Five isonitrogenous (35%) and isolipidic (7%) diets were formulated to contain 35% of glucose (GLU-feed), sucrose (SUC-feed), dextrin (DEX-feed), starch (STA-feed) and cellulose (CEL-feed), respectively. The survival and condition factor were not affected ($p > 0.05$) by the diets. Fish fed with the STA-feed showed significantly ($p < 0.05$) higher weight gain and specific growth rate. The feed efficiency ratio and protein efficiency ratio were significantly higher ($p < 0.05$) and alike in STA-feed and Dex-feed groups. Except for protein, the other whole-body composition was affected significantly ($p < 0.05$) by the different carbohydrate sources. The activities of antioxidant enzymes were significantly ($p < 0.05$) higher in groups fed on complex carbohydrate diets. Simillar trend was observed in the protease enzyme activity. Significantly ($p < 0.05$) higher dry matter digestibility was recorded in starch fed group. STA-feed group increased the specific activity of malate dehydrogenase and hexokinase.The glucose 6 phosphate dehydrogenase (G6PDH) activity was significantly ($p < 0.05$) higher in the GLU-feed group, and gradually decreased with increase in the complexity of carbohydrates.The DEX-feed and CEL-feed groups showed heavy fatty change with numerous large lipid droplets as compared to other groups. Overall, these results indicate that dietary starch was more efficiently utilized than other carbohydrate sources by pearlspot.

Keywords Carbohydrate complexity, Pearlspot, Growth performance, Metabolic enzymes, Digestibility

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Introduction

In aquaculture nutrition, protein is essential for supporting fish growth and physiological maintenance. Studies indicate that incorporating non-protein energy sources, such as carbohydrates and lipids, can enable protein-sparing effects, directing protein utilization towards growth rather than energy production, thus reducing feed costs and minimizing environmental impact [33]. However, fish have a comparatively limited ability to metabolize carbohydrates, as their efficiency in carbohydrate digestion varies with dietary habits and specific metabolic adaptations [22]. Notably, carbohydrate utilization differs among fish species, with variations in digestive enzyme activity and metabolic pathways affecting how they process carbohydrate types, complexities, and amounts [30, 40].

The utilization of carbohydrate forms, including sugars and non-sugars, varies among different fish species [30]. Grass carp (*Ctenopharyngodon idella*) effectively utilizes glucose (monosaccharide) over starch [57]. Conversely, tilapia (*Oreochromis niloticus*) and Chinese longsnout catfish (*Leiocassis longirostris* Günther) excel at utilizing starch (homopolysaccharide) over glucose. Additionally, channel catfish (*Ictalurus punctatus*) [60] and mrigal (*Cirrhinus mrigala*) [50] preferentially utilize dextrin (oligosaccharide) compared to other carbohydrate types. Carbohydrate complexity impacts nutrient digestibility, with glucose exhibiting the highest apparent digestibility and cellulose, a complex polysaccharide, showing the lowest digestibility [26, 46]. Fish's ability to utilize dietary carbohydrates is closely tied to their amylase activity [37]. Interestingly, studies have found that fish tend to have higher amylase and protease activities when fed complex carbohydrates compared to simple carbohydrates.

Fish have traditionally been considered glucose intolerant however, they have active glycolytic and gluconeogenesis pathways [45]. The type of dietary carbohydrates affects fish digestion and nutrient metabolism differently [37, 39]. This suggests that metabolic enzymes could serve as indicators for selecting suitable carbohydrate sources and utilization efficiency by fish species. Additionally, there is a link between dietary carbohydrate sources and the antioxidant status of fish [21]. Some studies also suggest that the complexity of carbohydrates can influence fish antioxidant status [37]. Antioxidant enzyme activity associated with dietary carbohydrate complexity may contribute to improved fish health and immunity.

The pearl spot (*Etroplus suratensis*) is a versatile fish found along India's east and south-west coasts. It can thrive in both freshwater and brackish water, making it ideal for commercial culture. Previous research suggests crude protein 30% [35] and lipid 8.6% [2] is optimum for their growth. However, there is a lack of information regarding the ideal dietary carbohydrate level and carbohydrate source preferences for pearl spot. Investigating carbohydrate complexity's effects on various physiological aspects, such as digestive and metabolic enzymes, digestibility, antioxidant enzyme activities, and liver histology, can help determine suitable carbohydrate sources. Addressing these knowledge gaps is crucial for formulating a well-balanced feed to meet the pearl spot's nutritional needs in aquaculture.

Materials and methods

Feed preparation

Five isonitrogenous (35% crude protein) and isolipidic (7% crude lipid) diets were prepared. Each diet included one specific carbohydrate source such as glucose, sucrose, dextrin, starch, and cellulose constituting 35% of the total diet. These diets were designated as GLU-feed, SUC-feed, DEX-feed, STA-feed, and CEL-feed, respectively. The purified ingredients, including casein, glucose (dextrose monohydrate), sucrose, dextrin, corn starch, and α-cellulose, were sourced from Sisco Research Laboratories (SRL) Pvt. Ltd., India. Titanium oxide (TiO2) served as an inert marker for nutrient digestibility. Fishmeal and vegetable oil were obtained from the local market. All the purified ingredients were used in powdered form, with fish meal being sieved for debris removal and achieving even-sized powder. Sucrose was ground and sieved due to its coarse structure. The ingredients used in feed formulation and their respective quantities are detailed in Table 1.

In preparing the feed, dry ingredients were precisely weighed and thoroughly mixed. Vegetable oil and choline chloride were added and blended to ensure a uniform mixture. Water was then incorporated to form a smooth dough, which was pelletized using a semi-industrial pelletizer with a 2 mm diameter die. The pellets were dried overnight at 60°C until moisture content dropped below 10%. Once dried, the pellets were cooled, crumbled to an appropriate size for pearlspot fingerlings, and stored in airtight plastic containers for future use.

Fish and experimental setup

Four hundred number of healthy pearlspot fingerlings of similar size were procured from Rajiv Gandhi Centre for Aquaculture (RGCA) – Multispecies Aquaculture Complex (MAC), Vallarpadam, Kochi, Kerala. They were acclimatized in fibreglass-reinforced plastic (FRP)

Table 1 Ingredients and proximate composition of experimental diet for *Etroplus suratensis* with dietary carbohydrate of different complexities

INGREDIENTS (g/Kg)		GLU-Feed	SUC-Feed	DEX-Feed	STA-Feed	CEL-Feed
Fish meal		300	300	300	300	300
Casein		200	200	200	200	200
Carbohydrate source	Glucose	350	-	-	-	-
	Sucrose	-	350	-	-	-
	Dextrin	-	-	350	-	-
	Starch	-	-	-	350	-
	Cellulose	-	-		-	350
Vegetable oil		70	70	70	70	70
[a]CMC (binder)		15	15	15	15	15
[b]Vitamin&[c]Mineral premix		40	40	40	40	40
Yeast		10	10	10	10	10
Choline chloride		10	10	10	10	10
Titanium oxide (inert marker)		5	5	5	5	5
PROXIMATE COMPOSITION (g/Kg)						
Protein		337.8	340.7	340.8	338.4	338.3
Lipid		69.1	69.4	67.4	68.3	65.3
Ash		99.7	92.3	96.4	90.5	104.5

[a] Carboxymethyl cellulose.[b]Vitamin (IU or g kg^{-1} premix): retinol palmitate, 50,000 LU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamin, 5; ascorbic acid, 10; cholecalciferol, 50,000 IU; -tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotine, 0.25. [c]Minerals (g kg^{-1}): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4.7H_2O$, 162; NaCl, 49.8; Iron (II) gluconate. J 0.9; $MnSO_4.H_2O$, 3.12; $ZnSO_4.7H_2O$. 4.67; $CuSO_4.5H_2O$. 0.62; KI. 0.16; $CoCl_2.6H_2O$, 0.08; ammonium molybdate. 0.06; $NaSeO_3$, 0.02

tanks with a capacity of 1000 L for a period of two weeks. During the acclimatization period, fishes were fed with commercial feed (32% dietary protein, 6% crude lipid, ABIS Aqua Star, India) thrice a day up to satiation. Fish were gradually weaned onto experimental diets over the period of a week. From that, three hundred healthy and uniform sized fish (3.55 ± 0.01 g) were selected for the study. The selected fishes were sorted and randomly distributed into 15 numbers of 100 l capacity plastic tubs with 20 healthy fishes in each corresponding to triplicate tubs of the five dietary treatments. The experimental tanks were equipped with sponge filters and a gentle aeration system, carefully adjusted to avoid disturbing the fecal matter, thereby facilitating its collection for subsequent digestibility analysis. The fish were fed with their respective experimental diets to apparent satiation thrice a day at 9:00 am, 1:00 pm and 4:00 pm, respectively for 60 days. Intake of daily feed was recorded. Residual feed, if any, was siphoned and removed 20 min after feeding, dried and weighed, before feeding the next day. The faecal matter was siphoned out for first 10 days of the feeding trial and the rest 50 days, the fecal matter was carefully collected from the experimental tanks with help of a siphoning pipe placing underneath a layer of muslin cloth, transferred to the container and immediately stored at −20°C to prevent nutrient degradation. This collected fecal material was preserved for subsequent dry matter and nutrient digestibility analysis. All samples were pooled for each treatment group and dried before analysis to maintain consistency and accuracy in measuring nutrient retention and digestibility coefficients in the feed. Proper care was taken to maintain good water quality throughout the experimental period. During the course of experiment, 25% of water was exchanged once a day and half the quantity of water was replaced once every fortnight so as to avoid deterioration in water quality due to accumulated organic matter and metabolites.

During the experimental period, physicochemical parameters were maintained for the rearing of pearlspot.The experiment was conducted under natural photoperiod and the water temperature was recorded using mercury thermometer (Duvcon Instruments, India) daily in the morning.The pHwas measured using Cyberscan Eutech instruments (pH510, Eutech Instruments, Singapore), dissolved oxygen with oxygen probe (HACH, HQ40D,USA) and ammonia–nitrogen using spectrophotometer (Thermo scientific- Evolution^{TM}201 UV- Visible Spectrophotometer, USA). No adverse values were recorded for dissolved oxygen (5.3–6.4 mg/l) a pH (7.53–8.16), and temperature (26°C-31°C) [19, 29]. Ammonia – nitrogen value remained below the limits of safe level for culture of the fish [14]. At the end of 60 days, whole

fish weight and length was recorded for somatic indices, liver and intestine samples were collected from each tank and suitablt pooled treatment wise for the determination of proximate analysis, nutrient digestibility, antioxidant enzyme status, digestive and metabolic enzyme activity in pearlspot. Liver and viscera weights were recorded along with fish body weights for each group to calculate the hepatosomatic index (HSI) and viscerosomatic index (VSI).The study protocol was approved by ethics committee of Kerala University of Fisheries and Ocean Studies.

Proximate composition analysis

At the end of the study, 3 fish from each tank were sacrificed, pooled treatment wise and stored frozen for final whole body proximate analysis. The proximate composition of fish, feed and faecal matter was carried out by the standard methods [4].

Moisture content was analyzed by oven drying the samples at 105°C for 24 h until a constant weight was achieved. Crude protein was measured through concentrated acid digestion (nitrogen × 6.25) using a Kjelplus Classic-DX VATS (B) apparatus from Pelican Instruments, Chennai, India. Crude lipid content was determined via petroleum ether extraction with the Pelican Equipments SCS 06 R (E-TS) system, also from Chennai. Total ash content was obtained by incinerating the samples in a muffle furnace at 550°C for 6 h (Nabertherm, LE 2/11/R6, Lilienthal, Germany).

Digestive enzyme analysis

A 10 cm segment of the anterior intestine was collected from three fish per tub for digestive enzyme analysis. Intestinal contents were gently removed, and the tissue was homogenized with 50 mM cold phosphate buffer (pH 7.0) at a 1:10 tissue-to-buffer ratio using a mortar and pestle. The homogenate was then centrifuged at 5000 g for 20 min, with all procedures conducted at 4°C. The resulting supernatant was stored at −20°C for subsequent assays of protease, amylase, and lipase activities. Total protein in liver and intestinal tissue samples was determined using the Lowry et al. [38] method, with bovine serum albumin (BSA) as the standard. Absorbance readings were taken at 660 nm.The protease activity was measured using the casein digestion method with a spectroscopic stop rate assay, following Kuntiz [34] at 37°C (pH 7.5). A reaction mixture of casein solution and tissue sample was incubated, and after stopping with trichloro acetic acid (TCA), centrifuged. The supernatant, combined with sodium carbonate and Folin & Ciocalteu's reagent, was incubated at 37°C, and absorbance was measured at 660 nm to express protease activity as μmole tyrosine per mg protein per minute. Amylase activity followed Bergmeyer et al. [9], using starch in potassium phosphate buffer, boiled briefly, and stopped with sodium–potassium tartrate,activity was read at 575 nm as μg reducing sugars/min/ml. Lipase activity was determined by Winkler & Stuckmann [61] with p-NPP as substrate in Tris HCl buffer containing gum Arabic and Triton X-100, with absorbance recorded at 410 nm to define one unit as 1 nmol p-nitrophenol per ml per minute.

Antioxidant enzyme activities

Liver samples was collected from 3 fish in each tub for the analysis. The analysis of liver antioxidant enzyme activity was performed by homogenising the liver samples in 50 mM phosphate buffer for catalase and glutathione peroxidase activity. The tissue sample was homogenised with carbonate buffer for superoxide dismutase activity. All the samples were centrifuged (Sorvall ST 8R,Thermo Fisher Scientific, Germany) at 10,000 × g for 5 min at 4 °C to get the supernatant.

Superoxide dismutase (SOD) activity was measured following Das et al. [13] by combining sodium carbonate buffer, Nitroblue Tetrazolium (NBT), Triton X-100, hydroxylamine hydrochloride, and the sample in a cuvette, with absorbance read at 560 nm. One unit of SOD activity was defined as the amount needed to inhibit 50% of nitrite formation under assay conditions. Catalase activity in liver tissue was assayed using Aebi [1], by measuring H_2O_2 decomposition at 240 nm in potassium phosphate buffer with tissue homogenate and hydrogen peroxide. One unit of catalase decomposes 1.0 μmole of H_2O_2 per minute at pH 7.0 and 25°C. Glutathione peroxidase was estimated using Wendel [59] by incubating sodium phosphate buffer, Ethylenediaminetetraacetic acid (EDTA), sodium azide, reduced glutathione, and sample, then adding hydrogen peroxide and TCA, followed by 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) for absorbance at 412 nm. One unit oxidizes 1.0 μmole of reduced glutathione per minute at pH 7.0 and 25°C.

Metabolic enzyme activities

Liver metabolic enzyme activities were assayed using 3 liver samples from each tub.

Malate dehydrogenase activity was estimated following Bergmeyer & Gawehn [8]. The reaction mixture, containing β-NADH and oxaloacetic acid, was combined with the sample, and the decrease in absorbance at 340 nm was recorded over 5 min. One unit of malate dehydrogenase converts 1.0 μmole of oxaloacetate and β-NADH to L-malate and β-NAD + per minute at 25 °C, pH 7.5.

Hexokinase (HK) activity, measured by Bergmeyer et al. [9], involved a reaction mixture with Triethanolamine buffer, D-glucose, ATP, magnesium chloride, NADP, glucose 6-phosphate dehydrogenase, and sample, with absorbance recorded at 340 nm. One unit phosphorylates 1.0 µmole of D-glucose per minute at pH 7.6. Glucose 6-phosphate dehydrogenase (G6PDH) was assayed using Smith et al. [51], with a reaction of sodium phosphate buffer, D-glucose, NADP, and sample, and absorbance recorded at 340 nm. One G6PDH unit oxidizes 1.0 µmole of D-glucose or D-galactose per minute at pH 7.0. For glucose 6-phosphatase, BIS–TRIS buffer, glucose 6-phosphate, TCA, and the sample were centrifuged, and color developed with 2,4,6-Tris(2-sulfonatophenyl) methanesulfonic acid (TSCR) was read at 660 nm, with activity calculated using a phosphate standard curve [43].

Nutrient digestibility analysis

The digestibility studies was done using titanium oxide (TiO_2) as inert marker by following the method of Short et al. [49],Vandenberg and De La Noüe [58]. Standard curve was plotted using standard titanium oxide (TiO_2) solution (0.5 mg/ml) added with 30% H_2O_2. Minimum 0.1 g of sample was taken and ashed in muffle furnace at 580° C for 6h. In that ashed sample, 10 ml of 7.4 M H_2SO_4 was added and boiled for 3 h until dissolve and taken in the 100 ml volumetric flask. 20 ml of 30% H_2O_2 was added into the flask and made upto 100 ml using distilled water. The OD value was taken at 410 nm at 0 h, 24 h and 48 h time period. The apparent digestibility coefficients were calculated by the following formula:

$$Dry\ matter\ digestibility\ (\%) = 100 - 100\ (\frac{\%\ marker\ in\ feed}{\%\ marker\ in\ faeces})$$

$$Apparant\ digestibility\ of\ Protein\ (\%) = 100 - 100(\frac{\%\ marker\ in\ feed}{\%\ marker\ in\ faeces} \times \frac{\%\ protein\ in\ faeces}{\%\ protein\ in\ feed})$$

$$Apparant\ digestibility\ of\ Lipid\ (\%) = 100 - 100(\frac{\%\ marker\ in\ feed}{\%\ marker\ in\ faeces} \times \frac{\%\ lipid\ in\ faeces}{\%\ lipid\ in\ feed})$$

$$Apparant\ digestibility\ of\ Nitrogen free\ extract\ (NFE)\ (\%) = 100 - 100(\frac{\%\ marker\ in\ feed}{\%\ marker\ in\ faeces} \times \frac{\%\ NFE\ in\ faeces}{\%\ NFE\ in\ feed})$$

Tissue histology

The liver tissues were first prepared by cutting them into blocks of size less than or equal to 1 cm^3 and kept in Bouin's fixative at least twenty times of its volume. After the several steps of i.e., hydration, clearing, impregnation, blocking, trimming, and sectioning the sections were stained with hematoxylene and eosin [7]. The photograph of the sections were taken using Olympus CX21i with camera Magnus DC 14 microphotography system.

Data analysis and calculations

Data collected on various parameters were presented as mean ± SEM (Standard error of mean). The data were analysed by one-way variance analysis (ANOVA). The level of significance had been set at $p < 0.05$. Statistical differences among treatments were determined using Duncan multiple-range test at 5% probability level. The statistical analysis was done with the SPSS Version 26.0 for Windows software (SPSS, Chicago, IL, USA).

All growth, survival and feed utilisation parameters were calculated following standard methods as give below.

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}.$$

$$\text{Weight gain\%} = [(\text{Weight gain (g)}) \times (\text{Initial weight (g)} - 1)] \times 100.$$

$$\text{SGR} = 100 \times [\ln(\text{final weight (g)}) - \ln(\text{initial weight (g)})] \times \text{days of experiment}^{-1}.$$

Condition Factor (CF) $= 100 \times$ [Final body weight (g) $\times$ Total length $- 3$ (cm)].

Hepato−somatic index (HSI) $= 100\times$ [weight of liver (g)] $\times$ [weight of fish (g)] $^{-1}$.

Viscera somatic index (VSI) $= 100 \times$ [weight of viscera (g)] $\times$ [weight of fish (g)]$^{-1}$. Survival% $=$ (Number of fish on final harvest $\times$ Number of fish at initial stocking $- 1$) $\times$ 100. Feed conversion ratio (FCR) $=$ Feed intake (g) $\times$ wet weight gain (g)$^{-1}$.

Protein efficiency ratio (PER) $=$ Weight gain (kg) $\times$ Crude protein fed (kg)$^{-1}$.

Protein gain $=$ (Final weight $\times$ Final crude protein content) $-$ (Initial weight$\times$Initial crude protein content).

Lipid gain $=$ (Final weight $\times$ Final crude lipid content) $-$ (Initial weight $\times$ Initial crude lipid content).

Protein retention efficiency (PRE) $= 100\times$ [(Protein gain (g)] $\times$ [Total crude protein fed (g)] $^{-1}$.

Lipid retention efficiency (LRE) $= 100\times$[lipid gain(kg)]$\times$[Total crude lipid fed (kg)] $^{-1}$

Table 2 Growth performance and nutrient utilization of *E. suratensis* fed different classes of carbohydrates for 60 days

ATTRIBUTES	GLU-feed	SUC-feed	DEX-feed	STA-feed	CEL-feed	*P* value	F value
Initial body weight (g/fish)	3.55 ± 0.02	3.53 ± 0.01	3.56 ± 0.02	3.60 ± 0.02	3.53 ± 0.02	0.101	2.59
Final body weight (g/fish)	4.57 ± 0.13^{a}	5.31 ± 0.12^{b}	5.84 ± 0.12^{b}	6.71 ± 0.19^{c}	5.60 ± 0.21^{b}	0.000	23.07
Weight Gain (g/fish)	1.02 ± 0.13^{a}	1.79 ± 0.12^{b}	2.28 ± 0.09^{b}	3.11 ± 0.20^{c}	2.07 ± 0.21^{b}	0.000	22.06
Weight Gain Percentage (%)	28.75 ± 3.65^{a}	50.61 ± 3.44^{b}	64.02 ± 2.43^{b}	86.46 ± 5.92^{c}	58.45 ± 10.7^{b}	0.000	21.02
Survival %	98.00 ± 1.66	97.67 ± 1.66	97.00 ± 2.90	100.00 ± 0.00	98.00 ± 2.00	0.620	0.68
Specific Growth Rate (SGR%)	0.42 ± 0.04^{a}	0.69 ± 0.03^{b}	0.82 ± 0.02^{b}	1.04 ± 0.05^{c}	0.76 ± 0.06^{b}	0.000	22.27
Condition Factor	2.04 ± 0.06	2.04 ± 0.05	2.08 ± 0.05	2.25 ± 0.10	2.33 ± 0.05	0.060	2.45
Hepato-Somatic Index (HSI)	2.30 ± 0.18^{ab}	2.74 ± 0.19^{b}	2.05 ± 0.10^{a}	2.33 ± 0.04^{ab}	2.50 ± 0.07^{b}	0.007	4.11
Viscero-Somatic Index (VSI)	7.09 ± 0.27^{a}	8.87 ± 0.53^{b}	6.57 ± 0.27^{a}	6.48 ± 0.36^{a}	7.83 ± 0.74^{a}	0.000	6.25
Total feed intake (FI (g)/ fish)	3.96 ± 0.05^{ab}	3.93 ± 0.27^{ab}	3.44 ± 0.14^{a}	4.36 ± 0.18^{bc}	4.89 ± 0.16^{c}	0.002	9.13
Feed Conversion Ratio (FCR)	3.99 ± 0.42^{c}	2.20 ± 0.08^{b}	1.51 ± 0.02^{a}	1.41 ± 0.05^{a}	2.40 ± 0.15^{b}	0.000	23.68
Feed Efficiency ratio (FER)	0.26 ± 0.02^{a}	0.46 ± 0.01^{b}	0.66 ± 0.02^{c}	0.71 ± 0.02^{c}	0.42 ± 0.02^{b}	0.000	63.61
Protein Gain (g/fish)	0.13 ± 0.01^{a}	0.26 ± 0.01^{b}	0.29 ± 0.01^{b}	0.39 ± 0.02^{c}	0.29 ± 0.02^{b}	0.000	21.96
Protein Efficiency Ratio (PER)	0.74 ± 0.08^{a}	1.31 ± 0.04^{b}	1.90 ± 0.00^{c}	2.06 ± 0.07^{c}	1.22 ± 0.08^{b}	0.000	61.99
Lipid Efficiency Ratio (LER)	2.30 ± 0.13^{a}	6.41 ± 0.12^{b}	10.78 ± 0.09^{c}	10.22 ± 0.20^{c}	6.33 ± 0.21^{b}	0.000	78.68
Protein Retention Efficiency (PRE)	9.31 ± 1.06^{a}	18.70 ± 0.66^{b}	24.26 ± 0.01^{c}	26.08 ± 1.03^{c}	17.08 ± 0.95^{b}	0.000	62.94
Lipid Gain (g/fish)	0.20 ± 0.01^{b}	0.07 ± 0.00^{a}	0.18 ± 0.00^{b}	0.19 ± 0.01^{b}	0.23 ± 0.02^{c}	0.000	45.33
Lipid Retention Efficiency (LRE)	70.85 ± 2.47^{c}	23.58 ± 0.88^{a}	74.90 ± 0.77^{d}	61.09 ± 1.72^{b}	70.82 ± 1.84^{c}	0.000	190.41

Results are showed as means ± SEM (Standard error of mean). In the rows, different letters indicate statistical difference at $p < 0.05$

Results

Growth and nutrient utilization

Growth and nutrient utilization parameters data were depicted in Table 2. The growth of pearlspot was recorded by taking the weight of whole biomass at each 10-day interval of the study. In all the treatments, pearlspot showed an increasing growth trend over the culture period. At the end of the growth trial, condition factor (CF) and survivability were not affected significantly (P>0.05) by the different dietary carbohydrate complexities. The final body weight and SGR of pearlspot fed with SUC-feed, DEX-feed and CEL-feed were similar. However, signifinaly higher (P<0.05) final body weight (6.71g), weight gain (3.11 g) and percentage weight gain (86.46%) was recorded in the starch fed group.The significantly higher (P<0.05) feed intake was recorded in the groups fed with cellulose followed by starch. The FCR of fish fed with STA-feed (1.41) and DEX-feed (1.51) were significantly (P<0.05) lower and alike, whereas, GLU-feed (3.99) group had the highest FCR. Similarly, PER and PRE were significantly (P<0.05) higher in fish fed with STA-feed and DEX-feed. The LRE (74.90) of pearlspot was significantly (P<0.05) higher in DEX-feed group. In somatic indices, fish fed with sucrose and dextrin as carbohydrate source showed significantly (P<0.05) higher VSI and significantly lower (P<0.05) HSI, respectively.

Whole body carcass composition

The whole-body carcass composition of pearlspot fingerlings was influenced by the dietary carbohydrate complexities (Table 3). The group fed with glucose (72.31%) exhibited significantly ($P<0.05$) lower body moisture content. The rest three groups exhibited almost similar ($P>0.05$) body moisture content. The whole body lipid content of pearlspot was significantly ($P<0.05$) higher in the glucose fed group. There was no significant difference ($P>0.05$) in body protein content among the treatments.

Table 3 Body composition (g/100g wet weight) of *Etroplus suratensis* fingerlings at the beginning and after 60 days of feeding dietary carbohydrate of different complexities

Attributes	Initial	Final					*P* value	F value
		GLU-feed	SUC-feed	DEX-feed	STA-feed	CEL-feed		
Moisture	77.16 ± 0.06	72.31 ± 0.28^{a}	74.89 ± 0.35^{b}	74.43 ± 0.37^{b}	73.50 ± 0.25^{ab}	73.53 ± 0.45^{ab}	0.013	5.47
Crude protein	13.10 ± 0.68	13.33 ± 0.16	13.82 ± 0.07	13.38 ± 0.12	13.35 ± 0.09	13.68 ± 0.22	0.224	1.71
Crude lipid	3.79 ± 0.06	7.32 ± 0.16^{d}	3.75 ± 0.11^{a}	5.37 ± 0.27^{b}	5.12 ± 0.25^{b}	6.51 ± 0.20^{c}	0.000	44.61
Ash	3.83 ± 0.00	5.16 ± 0.06^{b}	5.19 ± 0.06^{b}	4.71 ± 0.03^{a}	4.76 ± 0.03^{a}	4.44 ± 0.02^{a}	0.000	104.32

Results are showed as means ± SEM (Standard error of mean).In the rows, different letters indicate statistical difference at $p<0.05$

In the rows, different letters indicate statistical difference at $p<0.05$

Table 4 Apparent digestibility coefficient of *Etroplus suratensis* fed different classes of carbohydrates over a period of 60 days

Attributes	GLU-feed	SUC-feed	DEX-feed	STA-feed	CEL-feed	*P* value	F value
ADC_d	92.06 ± 0.76^{b}	92.87 ± 0.55^{b}	89.06 ± 1.09^{a}	92.77 ± 0.93^{b}	85.05 ± 1.52^{a}	0.020	271.56
ADC_p	92.04 ± 0.25^{b}	93.14 ± 0.44^{b}	89.20 ± 0.43^{a}	93.01 ± 0.28^{b}	94.96 ± 0.40^{bc}	0.000	21.718
ADC_l	89.68 ± 0.51^{d}	86.42 ± 0.57^{c}	82.67 ± 0.53^{b}	93.37 ± 0.74^{e}	60.29 ± 0.54^{a}	0.000	330.514
ADC_c	98.75 ± 0.78^{e}	91.72 ± 0.81^{d}	87.56 ± 1.17^{c}	82.77 ± 1.68^{b}	24.79 ± 2.87^{a}	0.000	2326.32

Results are shown as mean ± SEM. Abbreviations: ADC_d – Apparent digestibility coefficient of dry matter, ADC_p—Apparent digestibility coefficient of protein, ADC_l Apparent digestibility coefficient of lipid, ADC_c Apparent digestibility coefficient of carbohydrate. In the rows, different letters indicate statistical difference at $p<0.05$

Table 5 Digestive enzyme activity in intestine of *Etroplus suraatensis* fed different classes of carbohydrates diet over a period of 60 days

Attributes	GLU-feed	SUC-feed	DEX-feed	STA-feed	CEL-feed	*P* value	F value
Protease(μmole of tyrosine released/min/mg protein)	64.62 ± 0.87^{a}	63.85 ± 1.48^{a}	66.07 ± 1.45^{a}	87.36 ± 2.07^{b}	84.62 ± 1.65^{b}	0.000	37.468
Amylase(μmole of maltose released/min/mg protein)	0.80 ± 0.18^{a}	0.64 ± 0.09^{a}	0.73 ± 0.22^{a}	4.14 ± 0.91^{b}	0.57 ± 0.09^{a}	0.000	2.77
Lipase (nmol of p-nitro phenol released/ml/min/mg protein)	7.02 ± 0.13^{e}	6.81 ± 0.11^{d}	6.57 ± 0.09^{c}	6.37 ± 0.07^{b}	5.25 ± 0.1^{a}	0.000	2.76

Results are shown as mean ± SEM.In the rows, different letters indicate statistical difference at $p<0.05$

Table 6 Antioxidant enzyme activity in the liver of *Etroplus suratensis* fed different classes of carbohydrates over a period of 60 days

Attributes	GLU-feed	SUC-feed	DEX-feed	STA-feed	CEL-feed	*P* value	F value
SOD (U/mg protein)	$359.38 \pm 22.23b^{c}$	177.04 ± 18.77^{a}	329.50 ± 23.49^{b}	527.99 ± 9.24^{d}	402.59 ± 18.99^{c}	0.000	4.12
CAT (U/ mg protein)	43.74 ± 1.92^{a}	88.85 ± 5.15^{b}	91.03 ± 8.2^{b}	81.79 ± 6.1^{b}	39.38 ± 5.91^{a}	0.000	3.48
GPx (U /mg protein)	2.64 ± 0.45^{bc}	1.57 ± 0.09^{ab}	0.64 ± 0.09^{a}	2.54 ± 0.37^{bc}	2.26 ± 0.32^{b}	0.000	2.96

Results are shown as mean ± SEM. Abbreviations: *CAT* Catalase, *SOD* Superoxide dismutase, *GPx* Glutathione peroxidase. In the rows, different letters indicate statistical difference at $p < 0.05$

The ash content of pearlspot fed with polysaccharides was lower than the mono- and disaccharide group.

Nutrient digestibility

The nutrient digestibility of the experimental diets is depicted in Table 4. Apparent digestibility coefficient (ADC_d, %) of dry matter was similar ($P > 0.05$) and significantly higher ($P < 0.05$) in pearlspot fed with GLU-feed, SUC-feed and STA-feed groups, whereas, CEL-feed group showed around 7% lower ADC_d(%) than the above groups. The apparent protein digestibility ($ADC_{p,}$ %) was significantly ($P < 0.05$) lower in dextrin fed group, however, cellulose fed group showed the higher ADCp (%). The CEL-feed and STA-feed groups depicted lower and higher Apparent digestibility coefficient of lipid ($ADC_{l,}$ %), respectively. The ADC of carbohydrate ($ADC_{c,}$ %) was increased with decrease in the complexity of carbohydrates. The GLU-feed showed the highest ADC_c (%) compared to other dietary treatments.

Digestive enzymes

The activity of intestinal digestive enzymes like protease, amylase and lipase were examined to find the effect of different carbohydrate complexities on the digestion and absorption of proteins, carbohydrates and lipids in pearlspot fingerlings. Significant difference ($P < 0.05$) was found in all the enzyme activities across the treatments (Table 5). The significantly ($P < 0.05$) higher protease activity was recorded in STA-feed (84.36 µmole of tyrosine released/min/mg protein) and CEL-feed (84.67µmole of tyrosine released/min/mg protein) groups as compared to other dietary carbohydrate groups, but, it was non-significant ($P > 0.05$) between STA- and CEL-fed groups. However, amylase activity (4.14µmole of maltose released/min/mg protein) was significantly ($P < 0.05$) higher in starch fed group. Lipase enzyme activity in pearlspotwas significantly reduced ($P < 0.05$) as the complexity of dietary carbohydrate increased.

Antioxidant enzymes

The antioxidant enzyme activities ofthe liver are presented in Table 6. The dietary treatments significantly affected ($P < 0.05$)superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities. The liver SOD activity of pearlspot was significantly ($P < 0.05$) higher in STA-feed (527.99 U/mg protein) group. Among all the dietary carbohydrate (source) groups, the CAT enzyme activity was significantly lower ($P < 0.05$) in GLU-feed (43.74 U/mg protein) and CEL-feed (39.38 U/mg protein) groups although it was found to be similar ($P > 0.05$) between these two groups. Pearlspot fed with dextrin demonstrated significantly ($P < 0.05$) lower liver GPx activities (0.64 U/mg protein) among the feeding groups.

Metabolic enzymes

The dietary carobohydrate sources significantly ($P < 0.05$) influenced the activity of malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PDH), hexokinase (HK) and glucose-6-phosphatase (G6Pase) (Table 7). The MDH activity (2.33 U/mg protein) was significantly higher ($P < 0.05$) in the fish fed with STA-feed group.Whereas, GLU-feed and SUC-feed groups

Table 7 Metabolic enzyme activity of *Etroplus suratensis* fed different classes of carbohydrates over a period of 60 days

Attributes	GLU-feed	SUC-feed	DEX-feed	STA-feed	CEL-feed	P value	F value
MDH (U/mg protein)	0.89 ± 0.14^{a}	0.58 ± 0.16^{a}	1.43 ± 0.23^{b}	2.33 ± 0.12^{c}	1.44 ± 0.62^{b}	0.000	3.48
G-6-PDH (U/mg protein)	0.16 ± 0.01^{cd}	0.16 ± 0.02^{cd}	0.11 ± 0.01^{ab}	0.13 ± 0.02^{bc}	0.092 ± 0.00^{a}	0.000	4.12
HK (U/mg protein)	0.09 ± 0.00^{ab}	0.07 ± 0.00^{a}	0.12 ± 0.00^{b}	0.24 ± 0.04^{c}	0.26 ± 0.02^{c}	0.000	2.96
G-6 Pase (U/mg S)	0.79 ± 0.04^{bc}	0.30 ± 0.04^{a}	0.92 ± 0.02^{c}	0.72 ± 0.08^{b}	0.32 ± 0.02^{a}	0.000	43.52

Results are shown as mean ± SEM

Abbreviations: *MDH* Malate dehydrogenase, *G-6-PDH* Glucose 6 phosphate dehydrogenase, *HK* Hexokinase, *G6Pase* Glucose 6 phosphatase. In the rows, different letters indicate statistical difference at $p < 0.05$

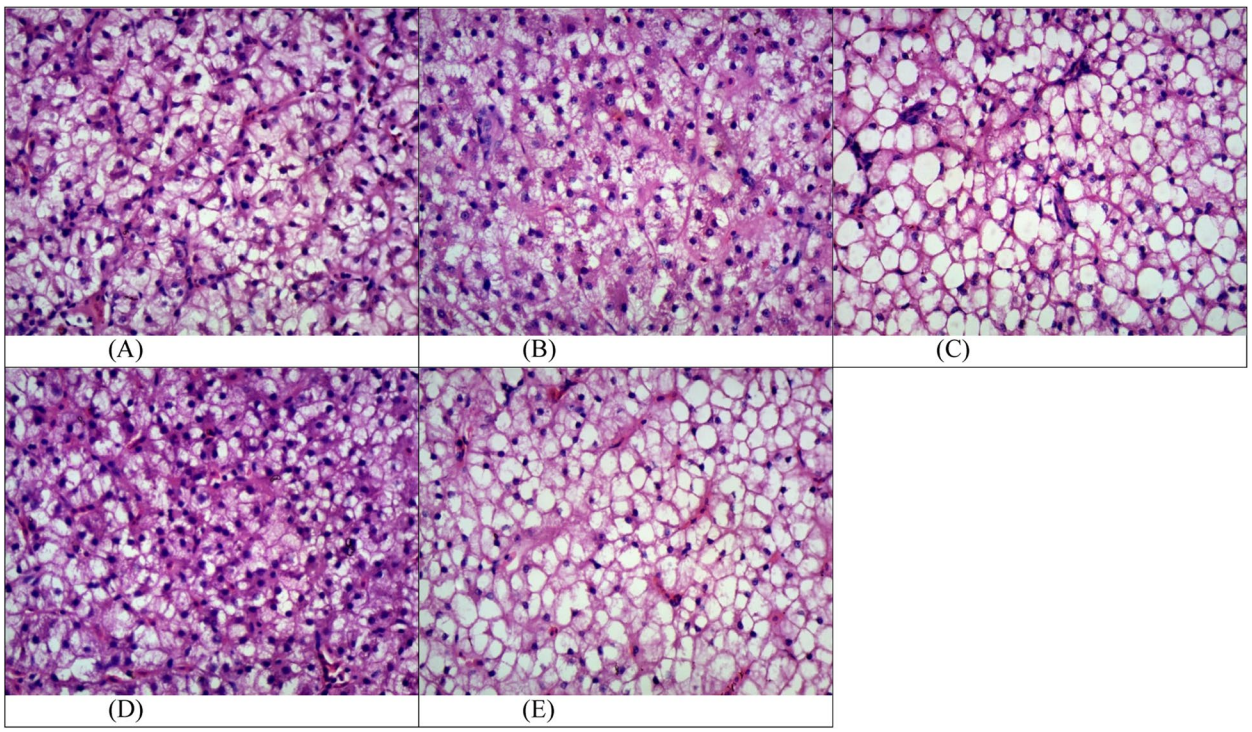

Fig. 1 Representative paraffin sections of liver from *Etroplus suratensis* fed with different compexities of carbohydrate for 60 days; The sections were stained in H & E to enhance the contrast (40x); **A** GLU-feed; **B** SUC-feed; **C** DEX-feed; D STA-feed; **e** CEL-feed

exhibited lower activity ($P<0.05$) although it was similar ($P>0.05$) between these two groups. The G6PDH activity wassignificantly ($P<0.05$) higher in the GLU-feed group, and gradually decreased ($P<0.05$) with increase in the complexity of carbohydrates. However, the starch (0.24 U/mg protein) and cellulose (0.26 U/mg protein) fed groups showed significantly ($P<0.05$) higher HK activity among all carbohydrate (source) fed groups, but was similar ($P>0.05$) between these two groups. No specific trend in G6Pase activity was observed in pearlspot fed with differernt complexities of carbohydrate. The groups fed with sucrose and cellulose showed significantly lower ($P<0.05$) G6Pase activity among all dietary carbohydrate (complexities) groups. However, no significant difference ($P<0.05$) in G6Pase activity found between sucrose and cellulose fed groups.

Tissue histology

After 60 days of feeding, liver of fish fed on GLU-feed, STA-feed and CEL-feed group showed focal to diffuse vacuolar degeneration of hepatocytes, whereas SUC-feed group showed degeneration represented by pyknotic nuclei (Fig. 1). But in the case of DEX-feed group mild degeneration was found. In DEX-feed and CEL-feed groups showed heavy fatty change with numerous large lipid droplets compared to other groups.

Discussion

This study assessed dietary carbohydrates with different complexities in pearlspot diets. Overall, pearlspot exhibited increased growth throughout the culture period. The highest growth and feed utilization efficiency were observed in the starch-containing diet, indicating starch as a major energy substrate compared to other carbohydrate sources (glucose, hexose, dextrin, and cellulose). Starch preference is observed in various fish species like rainbow trout [10], common carp [48] and hybrid tilapia [23]. Gelatinized starch preference has been reported in common carp [55], while some species like white sturgeon [24] and grass carp [57] favor monosaccharides and disaccharides over starch.

In this study, the group that received glucose (GLU-feed) exhibited the lowest growth performance and feed conversion efficiency. The rapid assimilation of glucose in the intestinal tract elevates blood glucose levels, which may interfere with metabolic processes and hinder growth, potentially explaining the reduced growth observed in this group [6, 16]. In fact, the fish fed the glucose diet had lower feed conversion ratio and protein utilization values compared to those fed with other

diets. The less protein efficiency ratio in the glucose fed groups clearly indicated that the maximum amount of protein was catabolized and used for energy purposes, rather than utilized for somatic growth as reported by Azaza et al. [6]. The higher feed intake is always resulted in higher growth in fish because of the association with IGF-1 (Insulin like growth factor), a mitogenic polypeptide which mediates growth hormone for somatic growth of vertebrates [41]. In the present study, higher feed intake was observed in cellulose fed group followed by starch fed group. However, starch fed group showed higher growth performance is the indication of higher utilisation efficiency of starch than cellulose in their feed. Generally, cellulose is not properly digested and utilised by fish [53]. Interestingly, in this study the cellulose fed group exhibited similar growth trend as that of dextrin and sucrose fed group. This result suggests that pearlspot may have considerable cellulase activity or cellulolytic microflora responsible for cellulose utilisation. The somatic indices, HSI and VSI was significantly higher in sucrose fed group and this could be due to the unutilised sucrose stored as glycogen and fat in the liver of fish [5].

Consistent with the current findings, studies on various dietary carbohydrate sources have shown that groups fed glucose exhibited the highest levels of dry matter, crude lipid, and ash in species such as gibel carp [56], cobia [12], and southern catfish [20]. In the present study, the significantly higher body lipid content in the glucose-fed group may result from the conversion of excess glucose into lipids, which is part of glucose homeostasis and responsible for de novo lipogenesis [54]. In contrast, body protein content did not vary among treatments, which aligns with findings in amur sturgeon [27], blunt snout bream [46], and Nile tilapia [3], where different carbohydrate complexities did not affect protein levels. Conversely, increases in body protein were observed in white sturgeon [24], channel catfish [18], and rohu [60] when subjected to varying dietary carbohydrate complexities. These discrepancies in body composition may be attributed to differences in fish species, environmental conditions, feeding habits, and the nutrient composition of feeds used in various studies.

Carbohydrate digestibility in fish generally decreases with an increase in structural complexity [44]. In this study, the cellulose-fed group exhibited low dry matter digestibility, which may explain the increased feed intake observed in this group, as the fish sought to meet their energy requirements, consistent with findings in Nile tilapia [5, 6], seabass [31], and gilthead seabream [17]. Notably, despite the low dry matter digestibility, the apparent protein digestibility was significantly higher in the cellulose-fed group. The combination of higher feed intake and enhanced protein digestibility may compensate for growth in this group. Cellulose acts as a dietary bulk agent, facilitating slower movement of digesta through the intestine and promoting the activity of digestive enzymes.

In this study, the activity of protease in the groups fed with cellulose and starch was significantly higher. These findings are consistent with results observed in black sea bream [54], Songpu mirror carp [36], and sturgeon [11], where protease activity was elevated in fish fed complex carbohydrates compared to those fed glucose, indicating that complex carbohydrates stimulate protease secretion in the intestine. Furthermore, the ability of fish to utilize dietary carbohydrates is directly influenced by amylase activity [47]. In the present study, the STA-feed group exhibited the highest amylase activity, while other treatments showed similar and non-significant levels. The hydrolysis of complex carbohydrates into glucose by amylase, followed by ingestion by fish, likely explains the higher amylase activity observed in the starch-fed group, given that complex carbohydrates are digested and absorbed more slowly than glucose. This result aligns with findings in mirror carp [36] and sturgeon [11].

Several studies have demonstrated that the activity of digestive enzymes is significantly influenced by substrate concentration. In the present study, an increase in carbohydrate complexity resulted in reduced lipase activity, indicating that the complexity of carbohydrates affects their inclusion levels in the diet in relation to lipase activity.There is very less information on how the nutrient composition and constituents of feed affect the endogenous antioxidant enzymes and oxidative capacity of fish. It has been seen in preceding studies that when fish was fed with high quantity of corn starch showed stress symptoms and reduced activity of antioxidant enzymes whereas, the antioxidant values were higher at medium and less corn starch diets [21, 52, 62]. In the present study, the SOD activity was significantly higher in the starch fed group indicated suitability of starch as carbohydrate source in the diet of pearlspot than other sources. Similarly, catalase activity was significantly lower and similar in glucose and cellulose fed groups than other carbohydrate sources. Catalase imparts antioxidant effect by converting build-up of H_2O_2 in to molecular oxygen and water. The more utilisation of catalase could be the reason for less activity in these groups. Similarly, less activity of glutathione peroxidase (GPx) was observed in dextrin fed group. It showed that the dextrin may increase the lipid peroxidation, thus GPx enzyme activity tend to decrease to reduce the free radicals.

In the present study, the fish fed with starch and cellulose showed significantly higher activities of hexokinase, which implies that the complex carbohydrates promoted glycolysis and utilised for energy production.

Similar results were reported in giant grouper larvae and large yellow croaker [36, 39] when fed with carbohydrate of different complexities. Contradictory to our results, glucose fed group had the highest hexokinase activity in black sea bream [54]. Whereas, it is reported that the diet composition had no effect on hexokinase activity in gilthead sea bream [15] and in mirror carp [36]. G6PDH is an important enzyme in the production of NADPH during the first phase of pentose phosphate pathway and provides energy for cellular growth [44]. G6PD in liver of fish is also involved in lipogenesis [28]. In this study, dietary glucose significantly enhanced G6PDH activity in the pearlspot and elevetated body lipid indicates excess glucose has been converted into lipid. Malate dehydrogenase (MDH) is a periportal enzyme that catalyses the final step of the citric acid cycle (TCA), where it reversibly oxidizes malate to oxaloacetate by reducing NAD^+ to NADH [42]. In this study, the MDH activity showed higher in the starch fed group, whereas, lower in the glucose and sucrose fed groups. The increased activity of MDH in the liver tissue suggest the ability of complex carbohydrates to enhance energy production, that may be utilized for maintenance of homeostasis, and protein sparing. G6Pase activity was higher in the dextrin fed group in this study may be due to the lower feed intake which leads to the gluconeogenesis in the fish, whereas, lower activity was recorded in sucrose and cellulose fed groups.

The histological changes were observed in the liver of the dietary treatments were alleviated to variable degrees in the groups fed dietary carbohydrate complexities. DEX-feed and CEL-feed groups had higher lipid vacuolation, these vacuoles may have represented cytosolic fluid spaces which can occur in the residual spaces after the glycogen has been metabolized [32]. These vacuoles may also have been the result of lipid accumulation which has also been observed in preceding studies [25]. However, the lipid accumulation related vacuolation in liver was not observed in glucose fed group. This could be due to deposition of lipid in adipose tissues other than liver.

Conclusion

The results of the present study indicate that juvenile pearlspot can efficiently utilize starch as an energy source in their diet. The polysaccharides induced glycolysis and effectively catabolized for energy production by pearlspot. The utilisation of monosaccharide, glucose was poor and therefore, it reduced the growth performance and feed utilization in pearlspot. However, the potentiality towards cellulose utilisation was evident in the growth and nutrients parameters of the fish. The role of carbohydrate complexity also influenced the antioxidant status of fish and starch fed group exhbited better antioxidant potential among the dietary treatments.These findings may help fish nutritionists to conduct further studies regarding carbohydrate nutrition and ingredient selection, thus lead to the commercial feed formulation for this species in near future.

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Authors' contributions
1. C.P. (1st author): Designed the study, methodology,biochemical analysis, Statistical, analysed the results and drafted the paper. 2. R. R. (2nd author): Feeding, nourishing the fish for the culture period and biochemical analysis. 3. R. K. (3rd author): Feed preparation, feeding the fish, biochemical analysis. 4. A. S. (4th author): Data curation, biochemical analysis. 5. K. N. M. (5th author): Reviewing and editing.

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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.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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