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Research Article

Dietary Incorporation of Ractopamine Hydrochloride Improves Body Composition and Metabolic Enzyme Activity in Calbasu, *Labeo calbasu* Fingerlings, despite High- and Low-Protein-Supplemented Diet

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A 60-day feeding trial was conducted to examine the effect and interaction of dietary ractopamine hydrochloride (Rac) supplementation in diets with varied levels of protein and lipid for *L. calbasu* fingerlings. To attain this, the fish body compositions, digestive and metabolic enzyme activity were assessed. In total, nine diets were fed in triplicates to four hundred and five healthy Calbasu, *Labeo calbasu* fingerlings, based on ractopamine supplementation, viz., control (0 mg/kg), T1 (10 mg/kg), and T2 (20 mg/kg). The diets were further assigned in different levels of protein and lipid, viz., 35% protein and 6% lipid, 30% protein and 9% lipid, and 25% protein and 12% lipid. At the end of the trial, it has been observed that the inclusion of ractopamine hydrochloride at 20 mg/kg^{-1} of basal diet significantly ($p < 0.05$) improved the protein content in fed fish compared to the control. In addition, metabolic enzyme activity for hexokinase, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase along with aspartate amino transferase and alanine amino transferase activity were significantly ($p < 0.05$) improved with the inclusion of 20 mg/kg ractopamine hydrochloride. However, the supplementation of ractopamine hydrochloride does not improve ($p > 0.05$) digestive enzyme activity, viz., amylase, lipase, and protease activity in the fed fish. Conclusively, it implies that adding ractopamine hydrochloride at 20 mg/kg of basal diet (high and low protein content) enhances the whole-body protein content and improves the metabolic enzyme activity in *L. calbasu* fingerlings. Hence, ractopamine hydrochloride has a beneficial effect in *L. calbasu* fingerlings and would be a desirable feed supplement for this new species culture system.

1. Introduction

Fish feed is an important component for semi-intensive or intensive aquaculture systems, and feed supplements with synergistic or additive effects are relevant aspects of research in fish nutrition and fish physiology. Likewise, ractopamine hydrochloride (Rac) is a β-adrenergic agonist that alters nutrient metabolism, which helps to improve the production

indices and meat quality to the animals [1–3]. In addition, ractopamine hydrochloride is a phenylethanolamine, a repartitioning agent which can redirect nutrients from deposition in the adipose tissue towards muscle accretion. In general, feeding phenylethanolamine to animals can increase the weight gain, improve feed utilization efficiency, and increase carcass leanness and dressing percentage [1]. The mechanism of this effect involves altering the rates of protein degradation and synthesis [1] and enhancing lipolysis in adipocytes by activating the hormone-sensitive lipase. Fatty acids are produced, and a large extent was exported from the adipocytes to be used as oxidative fuels by other tissues [4]. To date, studies have been carried out in different fish species while administration of β-adrenergic agonists has been conducted on selected fish species, viz., rohu, *Labeo rohita* [5], channel catfish, *Ictalurus punctatus* [6], and blue catfish, *Ictalurus furcatus* [7]; rainbow trout, *Oncorhynchus mykiss* [2, 3, 8–10]; pacu, *Piaractus brachypomus* [11, 12]; and Hungarian carp [13]. However, so far, there is very scanty information available regarding the effect of ractopamine (β-adrenergic agonist) with varied levels of protein and lipid rich diets in freshwater fish species specifically in Indian medium or minor carps. With this backdrop, the present study has been designed and executed to determine the effect of incorporation of ractopamine hydrochloride with varied levels of protein and lipid-rich diet in *L. calbasu* fingerlings.

L. calbasu, commonly known as Calbasu, is a freshwater fish species belonging to the family Cyprinidae which has high consumer preference, wide distribution in major riverine systems and reservoirs in India. It is a popular food fish having good taste, less intramuscular bones, and high protein content and is also admired as a good sport fish [14–16]. Eventually, this fish species is promising to be new candidate food fish in tropical and subtropical climates. Hence, the present investigation would deliver the possible nutritional and enzymatic effects of dietary incorporation of ractopamine hydrochloride with varying levels of protein and lipid diets in terms of body composition, digestive, and metabolic enzyme activity of *L. calbasu* fingerlings after 60 days of feeding trial.

2. Materials and Methods

2.1. Experimental Animal and Feeding. Healthy *L. calbasu* fingerlings are free from any kind of diseases or infections, procured from CAU, Agartala, Tripura, and transported to the wet laboratory at ICAR-CIFE, Mumbai, by air in a tightly sealed plastic bag of 20 L capacity inflated with oxygen and enclosed in a carton box. Upon arrival, fish were acclimatized for 15 days during that time fish were fed with normal feed and periodically sampled to examine the status of body and gill of the fish. A total of four hundred and five (405) fingerlings of *L. calbasu* with average weight of 1.25 g to 1.34 g were randomly distributed in nine distinct experimental groups with three replicates. The daily ration was divided into two equal parts and was fed up to satiation at 9.00 am and 5.00 pm.

Feeding trial was conducted at the wet laboratory of ICAR-Central Institute of Fisheries Education, Mumbai, India, with continuous aeration facility. The acclimatized *L. calbasu* fingerlings were distributed into twelve rectangular tanks ($80 \times 57 \times 42$ cm) containing bore-well water with 15 fish in each tank. Experimental feeds were fed at 3% ABW basis for 60 days. Each alternate day, the experimental tanks were cleaned manually by siphoning out of the water (20%) along with the faecal matter and uneaten feed. The water quality parameters of the tank water were maintained in optimal ranges like pH 7.6–8.3, dissolved oxygen 6.6–7.8 mg L^{-1}, total hardness 238–245 mg L^{-1}, ammonia 0.08–0.15 mg L^{-1}, nitrite 0.001–0.004 mg L^{-1}, and nitrate 0.02–0.06 mg L^{-1}, respectively, for the entire period.

2.2. Feed Preparation. The experimental diets were prepared at the feed laboratory of ICAR-CIFE, Mumbai, by a mechanical pelletizer using locally available feed ingredients such as fish meal, soya meal, groundnut oil cake, rice bran, wheat bran, fish oil, and soya oil. All the ingredients were procured and dried suitably and aseptically processed. The ingredients were mixed properly with distilled water and autoclaved to form a dough. Once the dough become cool enough, then vitamin-mineral mixture and binders were added along with desired dose of Rac (ractopamine hydrochloride, Sigma-Aldrich, code-90274-24). In brief, two levels of ractopamine (at 10 and 20 mg kg^{-1} of basal diet) were added with varied levels of protein and lipid rich diets, viz., 35% protein and 6% lipid, 30% protein and 9% lipid, and 25% protein and 12% lipid, respectively, to conduct the experiment. The composition of the experimental feeds is presented in Table 1.

2.3. Whole-Body Composition of Labeo calbasu. The moisture content of the animal tissue was determined by taking a known weight of the sample in a moisture cup and drying it in a hot air oven at 100-105°C till a constant weight is achieved. The difference in weight of the sample indicated the moisture content, which was calculated by using the following formula:

$$\text{Moisture}\ (\%) = \frac{(\text{Wet weight of sample} - \text{Dried weight of sample}) \times 100}{\text{Wet weight of a sample}}. \tag{1}$$

The nitrogen content of the sample was estimated quantitatively by Kjeltec automated system (Kelplus, Classic-DX Vats (D). The crude protein percentage is obtained directly with an automatic titration machine.

The ether extract was estimated by a Soxhlet apparatus using petroleum ether (Boiling point 40-60°C) as the solvent. The calculation was made as follows:

$$\text{Ether extract}\ (\%) = \frac{\text{weight of the ether extract} \times 100}{\text{weight of the sample}}. \tag{2}$$

Ash content was estimated by taking a known weight of the sample in the silica crucible and placing it in a muffle

Table 1: Feed formulation of different experimental diets (g 100 g-1).

Ingredients (g 100 g^{-1})	Treatments								
	$CP_{35}L_6$	$T_1P_{35}L_6$	$T_2P_{35}L_6$	$CP_{30}L_9$	$T_1P_{30}L_9$	$T_2P_{30}L_9$	$CP_{25}L_{12}$	$T_1P_{25}L_{12}$	$T_2P_{25}L_{12}$
Fish meal	6	6	6	6	6	6	6	6	6
Soya meal	30	29.999	29.998	25	24.999	24.998	20	19.999	19.998
GNOC	20	20	20	21	21	21	20	20	20
Rice bran	19	19	19	20	20	20	19	19	19
Wheat bran	20	20	20	21	21	21	25	25	25
Fish oil	1	1	1	2	2	2	3.5	3.5	3.5
Soya oil	1	1	1	2	2	2	3.5	3.5	3.5
Vit & Min mix	2	2	2	2	2	2	2	2	2
Binder	1	1	1	1	1	1	1	1	1
Rac	0	0.001	0.002	0	0.001	0.002	0	0.001	0.002

furnace at 6000C for 6 hrs. The calculation is done as follows:

$$\text{Ash}\ (\%) = \frac{\text{weight of ash} \times 100}{\text{weight of sample}}. \quad (3)$$

The total carbohydrate (TC) was calculated by subtracting the percentage of other nutrients from 100 [17]:

$$\text{TC}\ (\%) = 100 - \text{crude protein}\ (\%) - \text{ether extract}\ (\%) - \text{Ash}\ (\%). \quad (4)$$

The digestible energy value of experimental diets and tissue was calculated as described by Halver [18] as per the following formula:

$$\text{Digestible energy (kcal/100 g)} = \text{protein}\ (\%) \times 4 + \text{lipid}\ (\%) \times 9 + \text{carbohydrate}\ (\%) \times 4. \quad (5)$$

2.4. Sampling. Fish were randomly collected at the end of the experiment from each treatment and control tanks, followed by dissection. Dissected tissues (liver and kidney) were kept in 0.25 M sucrose solution. The tissue samples were homogenized using TissueLyser (Qiagen, Hilden, Germany) and centrifuged at 10,000 rpm for 10 minutes at 4°C, and the supernatant was collected and stored at -80°C for further crude whole body composition indices including protein and selected digestive and metabolic enzyme analysis.

2.5. Digestive Enzyme Assay. The amylase (E.C. 3.2.1.1) activity was assayed with 2% (*w*/*v*) starch solution as substrate [19]. In brief, starch solution (2%) was prepared in phosphate buffer (pH 7) followed by the reaction mixture prepared which is consists of 2% (*w*/*v*) starch solution, phosphate buffer (pH 7) and the tissue homogenate. Further, the reaction mixtures were incubated at 37^0 C for 30 min. DNS was added after incubation and kept in a boiling water bath for 5 min. After being sufficiently cooled, the reaction mixture was diluted with distilled water and absorbance was measured at 540 nm. Maltose was used as the standard. Amylase activity expressed as mmole of maltose released from starch per min at 37°C temperature.

The protease (E.C.3.4.21.4) activity was determined by the casein digestion method as Drapeau [20]. Here, the enzyme reaction mixture consisted of 1% casein in 0.05 M Tris PO4 buffer (pH 7.8) and incubated for 5 min at 37°C followed by addition of tissue homogenate done. After 10 minutes of incubation, the reaction was stopped by adding 10% TCA and the whole content was filtered. The reagent blank was made by adding tissue homogenate just before stop the reaction and without incubation. Result of the study was expressed as one unit of enzyme activity which infers the amount of enzyme needed to release acid soluble fragments equivalent to Δ0.001A280 per minute at 37°C and pH 7.8.

The lipase (E.C. 3.1.1.3) activity assayed by the method of Cherry and Crandell [21]. In brief, two test tubes were labeled as test (T) and control (C); 3 ml of distilled water and 1 ml of homogenate were added into them. One of the tubes (C) was placed in boiling water for 5 min at 100°C and then cooled. This served to inactivate the lipase in control. Then, 0.5 ml of phosphate-buffered solution (pH 7) and 2 ml of olive oil emulsion was added to both the tubes, shaken well, and incubated at 37°C for 24 hrs. After that, 3 ml of 95% alcohol and two drops of phenolphthalein solution were mixed. Each of the tubes was titrated with 0.05 N NaOH up to the appearance of permanent pink color.

2.6. Metabolic Enzyme Assays. The hexokinase (EC 2.7.1.1) activity was measured by the method of Easterby and O'Brien [22]. In brief, a reaction cocktail was prepared by mixing Tris-HCl buffer (50 mM), ATP (30 mM), and MgCl2 (200 mM). After that, the pH was adjusted at 30°C with 0.1 N HCl or NaOH. The final reaction mixture contained 2.7 ml cocktail, 0.3 ml β-NADP (1 mM), and 0.01 ml G6PDH (500 U/ml). The reaction started after the addition of enzyme sample, and the increase in absorbance was measured at 340 nm and recorded at every 15 sec intervals for 3 min. Similarly, a blank was run with distilled water instead of addition of the sample. The result was expressed as one unit of enzyme which infers the amount of enzyme activity that phosphorylase 1.0 μmole of D-glucose/min at pH at 30°C.

The D-glucose-6-phosphate phosphohydrolase (E.C. 3.1.3.9) activity in the tissues (liver and kidney) estimated

by the method of Marjorie [23]. To perform this, an assay mixture was prepared which consisted of 0.3 ml of malate buffer (pH 6.5), 0.1 ml of 0.1 M glucose-6-phosphate solutions, and 0.1 ml of tissue homogenate and incubated for 15 min at 37°C. Finally, the reaction was terminated by addition of 1 ml of 10% TCA solution. One milliliter of the aliquot of the supernatant used for phosphate (Pi) estimation by a method of Fiske and Subbarow [24]. The result was expressed as one unit of enzyme activity which infers the amount that released one μg of phosphorus per min.

The fructose-1,6-bisphosphatase (EC 3.1.3.11) activity was measured based on the method of Freeland and Harper [25]. In brief, the reaction mixture was comprised of 0.05 M FDP (pH 7-7.3). 0.5 M $MgSO_4$ tissue homogenate and borate buffer (pH 9.5). Then, the mixture was incubated at 37° C for 30 min and stopped by adding TCA (10%). For that, the tubes were centrifuged at 5000 rpm for 10 min, and an aliquot of the supernatant was taken for phosphorus (Pi) estimation [24], and enzyme activity was expressed as μg phosphorus released/min/mg protein at 37° C.

The G6PDH (alpha-D-glucose-6-phosphates: NADP-oxidoreductase, E.C.1.1.1.49) activity in different tissues was assayed by the method of De Moss [26]. To perform such, total 3 ml of the reaction mixture was made, comprised of 1.5 ml of 0.1 M Tris buffer (pH 7.8), 0.2 ml of 2.7 mM NADP, 0.1 ml of tissue homogenate, 1.05 ml of distilled water, and 0.1 ml of 0.02 M glucose-6-phosphate (G6P). The reaction was started by adding G6P as substrate. The OD was recorded at 340 nm at a 15-second interval against distilled water. The G6PDH activity was expressed as unit mg $protein^{-1}$ min^{-1}. One unit was equal to 0.01OD min^{-1} ml^{-1} at 25°C.

The AST (L- aspartate: 2oxaloglutarate aminotransferase, E.C.2.6.1.1) activity was assayed in different tissue homogenates as described by Wooten [27]. In brief, the substrate comprised of 0.2 M D, L-aspartic acid and 2 mM a-ketoglutarate in 0.05 M phosphate buffer (pH 7.4). In the experimental and control tubes, 0.5 ml of a substrate was added. The reaction was started by adding 0.1 ml of tissue homogenate. The assay mixture was incubated at 37°C for 60 minutes. The reaction was terminated by adding 0.5 ml of 1 mM 2,4 dinitrophenylhydrazine (DNPH). In the control tubes, the enzyme source was added after the DNPH solution. The tubes were held at room temperature for 20 minutes with occasional shaking. Then 5 ml of 0.4 ml NaOH solution was added; the contents were thoroughly mixed. After 10 minutes, the OD was recorded at 540 nm against blank.

The ALT (L-alanine: 2 oxoglutarate aminotransferase; E.C.2.6.1.2) activity was determined using the same method as AST except that the substrate is comprised of 0.2 M DL-alanine instead of aspartic acid. The enzyme activity was expressed as nanomole pyruvate formed/min/mg protein at 37°C.

2.7. Data Analysis. The data was tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). To determine the significant effect of ractopamine supplementation in varied levels of protein diets or interaction in *L. calbasu* fingerlings, a two-way ANOVA was performed. All analyses were done by using statistical package SPSS version 16.0 (SPSS Inc. Chicago, III., USA). Each of the values was expressed as the mean ± SD. p values of <0.05 were considered significant.

3. Results

3.1. Body Composition. Protein and Rac showed significant ($p < 0.05$) effect of on crude protein content (Table 2). The highest (13.96 ± 0.75) crude protein was recorded in $T_{1.2}$ and the lowest (12.64 ± 0.36) in $T_{1.1.}$ Protein, Rac, and interaction (Rac × protein) showed significant ($p < 0.05$) effect on the crude lipid among the different experimental diets. The highest (5.47 ± 0.07) crude lipid was recorded in C_3 and the lowest (3.13 ± 0.11) in $T_{2.2}$.

3.2. Digestive Enzymes. Protein, Rac, and interaction (Rac × protein) did not show the significant effect on the amylase activity among different experimental fish (Table 3). The amylase activity was found to be the highest (0.242 ± 0.13) in the $T_{2.2}$ and the lowest (0.225 ± 0.10) in the C_3.

Protein and lipid level showed significant ($p < 0.05$) effect on the activity of lipase, but Rac and interaction (Rac × Protein) did not show the significant influence on lipase activity among the experimental fish (Table 3). The lipase activity was found to be the highest (1.69 ± 0.08) in the C_3 and the lowest (1.43 ± 0.12) in the C_1.

Protein showed significant ($p < 0.05$) effect on the protease activity, but Rac and interaction (Rac × protein) did not show the significant effect on protease activity among the experimental fish (Table 3). The protease activity was found to be the highest (1.67 ± 0.04) in $T_{2.2}$ and the lowest (1.45 ± 0.09) in $T_{2.1}$.

3.3. Metabolic Enzymes. Rac showed significant ($p < 0.05$) effect on hexokinase activity of liver; however, Rac and interaction (Rac × protein) did not show significant effect on hexokinase activity in the liver among the different experimental groups (Table 4). Fish fed with Rac and protein showed significant ($p < 0.05$) effect on the hexokinase activity of muscle, but interaction (Rac × protein) did not show the significant effect on muscle hexokinase activity among the different treatment groups (Table 4). In the liver, the highest (0.403 ± 0.11) hexokinase activity was found in C_3, and the lowest activity (0.240 ± 0.05) was found in $T_{2.2}$. In the muscle, the highest (0.170 ± 0.05) hexokinase activity was recorded in C_3, and the lowest (0.107 ± 0.04) activity was found in $T_{2.2}$.

Protein and Rac showed significant ($p < 0.05$) effect on the activity of G-6-Pase, but interaction (Rac × protein) did not show the significant effect on the activity of G-6-Pase among the different experimental fish (Table 5). The highest (54.98 ± 3.21) G-6-Pase value of liver recorded in the C_3 and the lowest (37.46 ± 2.32) recorded in $T_{3.2}$.

Protein showed significant ($p < 0.05$) effect on the activity of F-1,6-BPase but Rac and interaction (Rac × protein) did not show significant difference among the treatment fish (Table 5). The highest (115.48 ± 0.62) F-1,6-BPase value of

Table 2: Proximate composition of whole body of *Labeo calbasu* at the end of experiment.

Proximate composition (%)	Inclusion of ractopamine level (mg/kg)	Treatments	$P_{35}L_6$	$P_{30}L_9$	$P_{25}L_{12}$	p value
Moisture	0	C	75.48 ± 0.48	75.59 ± 1.11	75.26 ± 1.83	$p_{P\&L} = 0.548$ (NS)
	10	T_1	76.26 ± 1.19	75.53 ± 1.36	75.07 ± 1.06	$p_R = 0.428$ (NS)
	20	T_2	76.47 ± 0.92	76.00 ± 1.80	76.01 ± 1.74	$p_I = 0.950$ (NS)
Dry matter	0	C	24.51 ± 0.48	24.41 ± 0.48	24.74 ± 1.83	$p_{P\&L} = 0.548$ (NS)
	10	T_1	23.74 ± 1.19	24.46 ± 1.36	24.93 ± 1.06	$p_R = 0.428$ (NS)
	20	T_2	23.52 ± 0.92	23.99 ± 0.80	23.98 ± 1.74	$p_I = 0.950$ (NS)
Crude protein	0	C	$13.89^{bA} \pm 0.34$	$12.97^{abA} \pm 0.47$	$12.66^{aA} \pm 0.49$	$p_{P\&L} = 0.010$
	10	T_1	$13.70^{bA} \pm 0.84$	$13.19^{abA} \pm 0.67$	$12.64^{aA} \pm 0.36$	$p_R = 0.006$
	20	T_2	$13.96^{bB} \pm 0.75$	$14.69^{abB} \pm 0.73$	$13.50^{aB} \pm 0.22$	$p_I = 0.207$ (NS)
Crude lipid	0	C	$4.05^{aC} \pm 0.43$	$5.28^{bC} \pm 0.30$	$5.47^{cC} \pm 0.07$	$p_{P\&L} = 0.001$
	10	T_1	$3.42^{aB} \pm 0.20$	$4.51^{bB} \pm 0.42$	$4.81^{cB} \pm 0.15$	$p_R = 0.001$
	20	T_2	$3.24^{aA} \pm 0.08$	$3.13^{bA} \pm 0.11$	$4.11^{cA} \pm 0.29$	$p_I = 0.001$
Total ash	0	C	$3.55^{a} \pm 0.29$	$4.21^{b} \pm 0.47$	$4.58^{c} \pm 0.25$	$p_{P\&L} = 0.001$
	10	T_1	$3.43^{a} \pm 0.24$	$4.20^{b} \pm 0.25$	$4.36^{c} \pm 0.20$	$p_R = 0.979$ (NS)
	20	T_2	$3.35^{a} \pm 0.10$	$4.16^{b} \pm 0.09$	$4.43^{c} \pm 0.37$	$p_I = 0.053$ (NS)
Total carbohydrate	0	C_1	3.95 ± 0.92	3.03 ± 1.26	2.12 ± 0.70	$p_{P\&L} = 0.505$ (NS)
	10	T_1	3.59 ± 0.65	3.22 ± 1.14	3.99 ± 0.68	$p_R = 0.692$ (NS)
	20	T_2	3.50 ± 1.67	3.85 ± 1.08	2.98 ± 1.07	$p_I = 0.747$ (NS)

The values expressed as the mean ± SD ($n = 3$) were analyzed by two-way ANOVA at significance level $p \le 0.05$. Different lowercase letters in a row indicates difference due to different protein levels at $p \le 0.05$. Different uppercase letters in a column indicates significant difference due to different Rac levels at $p \le 0.05$. p_P = significance for protein levels, p_R = significance for Rac levels, and p_I = significance for interaction of protein and Rac levels. P%: protein percentage; L%: lipid percentage. C_1: control 1 (35% protein, 6% lipid, and 0 mg kg^{-1} Rac); $T_{1.1}$: treatment 1.1 (35% protein, 6% lipid, and 10 mg kg^{-1} Rac); $T_{1.2}$: treatment 1.2 (35% protein, 6% lipid, and 20 mg kg^{-1} Rac); C_2: control 2 (30% protein, 9% lipid, and 0 mg kg^{-1} Rac); $T_{2.1}$: treatment 2.1 (30% protein, 9% lipid, and 10 mg kg^{-1} Rac); $T_{2.2}$: treatment 2.2 (30% protein, 9% lipid, and 20 mg kg^{-1} Rac); C_3: control 3 (25% protein, 12% lipid, and 0 mg kg^{-1} Rac); $T_{3.1}$: treatment 3.1 (25% protein, 12% lipid, and 10 mg kg^{-1} Rac); $T_{3.2}$: treatment 3.2 (25% protein, 12% lipid, and 20 mg kg^{-1} Rac).

Table 3: Digestive enzyme activity of different experimental groups at the end of the experiment.

Digestive enzymes	Inclusion ractopamine level (mg/kg)	Treatments	$P_{35}L_6$	$P_{30}L_9$	$P_{25}L_{12}$	p value
Amylase	0	C	0.225 ± 0.10	0.240 ± 0.09	0.240 ± 0.11	$p_{P\&L} = 0.231$ (NS)
	10	T_1	0.228 ± 0.14	0.240 ± 0.10	0.239 ± 0.09	$p_R = 0.905$ (NS)
	20	T_2	0.231 ± 0.11	0.242 ± 0.13	0.241 ± 0.13	$p_I = 0.283$ (NS)
Lipase	0	C	$1.43^{a} \pm 0.12$	$1.56^{b} \pm 0.13$	$1.69^{c} \pm 0.08$	$p_{P\&L} = 0.032$
	10	T_1	$1.45^{a} \pm 0.12$	$1.61^{b} \pm 0.22$	$1.64^{c} \pm 0.15$	$p_R = 0.978$ (NS)
	20	T_2	$1.46^{a} \pm 0.13$	$1.57^{b} \pm 0.09$	$1.67^{c} \pm 0.14$	$p_I = 0.998$ (NS)
Protease	0	C	$1.64^{c} \pm 0.03$	$1.58^{b} \pm 0.11$	$1.46^{a} \pm 0.07$	$p_{P\&L} = 0.001$
	10	T_1	$1.66^{c} \pm 0.05$	$1.56^{b} \pm 0.05$	$1.45^{a} \pm 0.09$	$p_R = 0.986$ (NS)
	20	T_2	$1.67^{c} \pm 0.04$	$1.56^{b} \pm 0.06$	$1.49^{a} \pm 0.05$	$p_I = 0.996$ (NS)

The values expressed as the mean ± SD ($n = 3$) were analyzed by two-way ANOVA at significance level $p \le 0.05$. Different lowercase letters in a row indicates difference due to different protein levels at $p \le 0.05$. Different uppercase letters in a column indicates significant difference due to different Rac levels at $p \le 0.05$. p_P = significance for protein levels, p_R = significance for Rac levels, and p_I = significance for interaction of protein and Rac levels. P%: protein percentage; L%: lipid percentage. C_1: control 1 (35% protein, 6% lipid, and 0 mg kg^{-1} Rac); $T_{1.1}$: treatment 1.1 (35% protein, 6% lipid, and 10 mg kg^{-1} Rac); $T_{1.2}$: treatment 1.2 (35% protein, 6% lipid, and 20 mg kg^{-1} Rac); C_2: control 2 (30% protein, 9% lipid, and 0 mg kg^{-1} Rac); $T_{2.1}$: treatment 2.1 (30% protein, 9% lipid, and 10 mg kg^{-1} Rac); $T_{2.2}$: treatment 2.2 (30% protein, 9% lipid, and 20 mg kg^{-1} Rac); C_3: control 3 (25% protein, 12% lipid, and 0 mg kg^{-1} Rac); $T_{3.1}$: treatment 3.1 (25% protein, 12% lipid, and 10 mg kg^{-1} Rac); $T_{3.2}$: treatment 3.2 (25% protein, 12% lipid, and 20 mg kg^{-1} Rac).

TABLE 4: Hexokinase activity of different experimental groups at the end of the experiment.

Hexokinase activity	Inclusion ractopamine level (mg/kg)	Treatments	$P_{35}L_6$	$P_{30}L_9$	$P_{25}L_{12}$	p value
Liver	0	C	$0.381^{C} \pm 0.03$	$0.383^{C} \pm 0.03$	$0.403^{C} \pm 0.11$	$p_{P\&L} = 0.188$ (NS)
	10	T_1	$0.263^{B} \pm 0.04$	$0.353^{B} \pm 0.03$	$0.300^{B} \pm 0.02$	$p_R = 0.047$
	20	T_2	$0.240^{A} \pm 0.05$	$0.340^{A} \pm 0.13$	$0.290^{A} \pm 0.05$	$p_I = 0.824$ (NS)
Muscle	0	C	$0.143^{aC} \pm 0.03$	$0.163^{bC} \pm 0.04$	$0.170^{cC} \pm 0.05$	$p_{P\&L} = 0.05$
	10	T_1	$0.130^{aB} \pm 0.02$	$0.147^{bB} \pm 0.03$	$0.160^{cB} \pm 0.02$	$p_R = 0.007$
	20	T_2	$0.107^{aA} \pm 0.04$	$0.137^{bA} \pm 0.05$	$0.156^{cA} \pm 0.02$	$p_I = 0.800$ (NS)

The values expressed as the mean ± SD ($n = 3$) were analyzed by two-way ANOVA at significance level $p \leq 0.05$. Different lowercase letters in a row indicates difference due to different protein levels at $p \leq 0.05$. Different uppercase letters in a column indicates significant difference due to different Rac levels at $p \leq 0.05$. p_P = significance for protein levels, p_R = significance for Rac levels, and p_I = significance for interaction of protein and Rac levels. P%: protein percentage; L%: lipid percentage. C_1: control 1 (35% protein, 6% lipid, and 0 mg kg^{-1} Rac); $T_{1.1}$: treatment 1.1 (35% protein, 6% lipid, and 10 mg kg^{-1} Rac); $T_{1.2}$: treatment 1.2 (35% protein, 6% lipid, and 20 mg kg^{-1} Rac); C_2: control 2 (30% protein, 9% lipid, and 0 mg kg^{-1} Rac); $T_{2.1}$: treatment 2.1 (30% protein, 9% lipid, and 10 mg kg^{-1} Rac); $T_{2.2}$: treatment 2.2 (30% protein, 9% lipid, and 20 mg kg^{-1} Rac); C_3: control 3 (25% protein, 12% lipid, and 0 mg kg^{-1} Rac); $T_{3.1}$: treatment 3.1 (25% protein, 12% lipid, and 10 mg kg^{-1} Rac); $T_{3.2}$: treatment 3.2 (25% protein, 12% lipid, and 20 mg kg^{-1} Rac).

TABLE 5: Glucose-6-phosphate and fructose-1,6-bisphosphatase activity of different experimental groups at the end of experiment.

Gluconeogenesis enzymes	Inclusion ractopamine level (mg/kg)	Treatments	$P_{35}L_6$	$P_{30}L_9$	$P_{25}L_{12}$	p value
Glucose-6-phosphatse	0	C	$43.45^{cA} \pm 0.05$	$40.62^{bA} \pm 1.63$	$37.46^{aA} \pm 2.32$	$p_{P\&L} = 0.001$
	10	T_1	$47.54^{cB} \pm 1.96$	$42.99^{bB} \pm 0.29$	$40.41^{aB} \pm 0.90$	$p_R = 0.001$
	20	T_2	$54.98^{cC} \pm 3.21$	$48.51^{aC} \pm 2.72$	$44.61^{aC} \pm 5.35$	$p_I = 0.089$ (NS)
Fructose-1,6-bisphosphatase	0	C	$103.80^{b} \pm 5.60$	$109.97^{c} \pm 3.95$	$97.95^{a} \pm 2.04$	$p_{P\&L} = 0.001$
	10	T_1	$105.47^{b} \pm 4.19$	$113.14^{c} \pm 5.15$	$98.98^{a} \pm 3.87$	$p_R = 0.102$ (NS)
	20	T_2	$107.71^{b} \pm 2.74$	$115.48^{c} \pm 0.62$	$100.50^{a} \pm 2.50$	$p_I = 0.957$ (NS)

The values expressed as the mean ± SD ($n = 3$) were analyzed by two-way ANOVA at significance level $p \leq 0.05$. Different lowercase letters in a row indicates difference due to different protein levels at $p \leq 0.05$. Different uppercase letters in a column indicates significant difference due to different Rac levels at $p \leq 0.05$. p_P = significance for protein levels, p_R = significance for Rac levels, and p_I = significance for interaction of protein and Rac levels. P%: protein percentage; L%: lipid percentage. C_1: control 1 (35% protein, 6% lipid, and 0 mg kg^{-1} Rac); $T_{1.1}$: treatment 1.1 (35% protein, 6% lipid, and 10 mg kg^{-1} Rac); $T_{1.2}$: treatment 1.2 (35% protein, 6% lipid, and 20 mg kg^{-1} Rac); C_2: control 2 (30% protein, 9% lipid, and 0 mg kg^{-1} Rac); $T_{2.1}$: treatment 2.1 (30% protein, 9% lipid, and 10 mg kg^{-1} Rac); $T_{2.2}$: treatment 2.2 (30% protein, 9% lipid, and 20 mg kg^{-1} Rac); C_3: control 3 (25% protein, 12% lipid, and 0 mg kg^{-1} Rac); $T_{3.1}$: treatment 3.1 (25% protein, 12% lipid, and 10 mg kg^{-1} Rac); $T_{3.2}$: treatment 3.2 (25% protein, 12% lipid, and 20 mg kg^{-1} Rac).

the liver was recorded in the $T_{2.2}$ and the lowest (97.95 ± 2.04) recorded in C_3.

Protein and Rac showed significant ($p < 0.05$) effect on the activity of liver G-6-PDH, but interaction (Rac × protein) did not show a significant effect among the different experimental fish (Table 6). The highest (71.29 ± 2.53) G-6-PDH value of liver recorded in the C_3 and lowest (41.19 ± 4.42) recorded in $T_{1.2}$.

Rac showed significant ($p < 0.05$) effect on the activity of AST in liver, but Rac and interaction (Rac × protein) did not show significant ($p < 0.05$) effect among different experimental groups (Table 7). In the liver, the highest (3.96 ± 0.24) activity was found in C_2, and the lowest (2.71 ± 0.18) activity was recorded in C_3.

In the muscle, protein and Rac showed significant ($p < 0.05$) effect on the activity of AST and interaction (Rac × protein) did not show any significant change on AST activity of the different experimental fish (Table 7). In the muscle, the highest (12.75 ± 3.54) activity was found in C_1, and the lowest (6.17 ± 1.19) activity was recorded in $T_{2.2}$.

Protein and Rac showed significant ($p < 0.05$) effect on AST activity in the liver, but interaction (Rac × protein) did not show any significant effect on the AST liver among the different experimental fish (Table 8). In the liver, the highest (6.22 ± 1.62) activity found in the $T_{1.2}$ and the lowest (2.66 ± 1.26) activity was recorded in C_3.

In the muscle, significant ($p < 0.05$) effect of protein, Rac, and interaction (Rac × protein) has been observed in the ALP activity (Table 8). The highest (15.96 ± 1.29) activity was found in $T_{1.2}$, and the lowest (7.00 ± 1.29) activity was recorded in C_3.

4. Discussion

Feed supplement plays an important role to upkeep health and desirable growth of cultured fish in the semi-intensive

Table 6: Glucose-6-phosphate dehydrogenase activity of different experimental groups at the end of experiment.

Lipogenic enzymes	Inclusion ractopamine level (mg/kg)	Treatments	$P_{35}L_6$	$P_{30}L_9$	$P_{25}L_{12}$	p value
Glucose-6-phosphatse dehydrogenase	0	C	$54.96^{aC} \pm 9.38$	$62.08^{bC} \pm 11.97$	$71.29^{cC} \pm 2.53$	$p_{P\&L} = 0.005$
	10	T_1	$51.44^{aB} \pm 3.97$	$61.16^{bB} \pm 8.66$	$63.05^{cB} \pm 13.98$	$p_R = 0.006$
	20	T_2	$41.19^{aA} \pm 4.42$	$54.33^{bA} \pm 10.44$	$57.28^{cA} \pm 7.21$	$p_I = 0.657$ (NS)

The values expressed as the mean ± SD ($n = 3$) were analyzed by two-way ANOVA at significance level $p \leq 0.05$. Different lowercase letters in a row indicates difference due to different protein levels at $p \leq 0.05$. Different uppercase letters in a column indicates significant difference due to different Rac levels at $p \leq 0.05$. p_P = significance for protein levels, p_R = significance for Rac levels, and p_I = significance for interaction of protein and Rac levels. P%: protein percentage; L%: lipid percentage. C_1: control 1 (35% protein, 6% lipid, and 0 mg kg^{-1} Rac); $T_{1.1}$: treatment 1.1 (35% protein, 6% lipid, and 10 mg kg^{-1} Rac); $T_{1.2}$: treatment 1.2 (35% protein, 6% lipid, and 20 mg kg^{-1} Rac); C_2: control 2 (30% protein, 9% lipid, and 0 mg kg^{-1} Rac); $T_{2.1}$: treatment 2.1 (30% protein, 9% lipid, and 10 mg kg^{-1} Rac); $T_{2.2}$: treatment 2.2 (30% protein, 9% lipid, and 20 mg kg^{-1} Rac); C_3: control 3 (25% protein, 12% lipid, and 0 mg kg^{-1} Rac); $T_{3.1}$: treatment 3.1 (25% protein, 12% lipid, and 10 mg kg^{-1} Rac); $T_{3.2}$: treatment 3.2 (25% protein, 12% lipid, and 20 mg kg^{-1} Rac).

Table 7: Aspartate amino transferase activity of different experimental groups at the end of the experiment.

Aspartate amino transferase	Inclusion ractopamine level (mg/kg)	Treatments	$P_{35}L_6$	$P_{30}L_9$	$P_{25}L_{12}$	p value
Liver	0	C	$2.75^{A} \pm 0.23$	$2.79^{A} \pm 0.22$	$2.71^{A} \pm 0.18$	$p_{P\&L} = 0.163$
	10	T_1	$3.22^{aB} \pm 0.06$	$3.39^{aB} \pm 0.16$	$3.35^{aB} \pm 0.22$	$p_R = 0.001$
	20	T_2	$3.59^{aC} \pm 0.13$	$3.96^{aC} \pm 0.24$	$3.75^{aC} \pm 0.28$	$p_I = 0.098$ (NS)
Muscle	0	C	$9.00^{cA} \pm 2.01$	$6.71^{bA} \pm 1.74$	$6.17^{aA} \pm 1.19$	$p_{P\&L} = 0.001$
	10	T_1	$10.41^{cB} \pm 0.89$	$7.04^{bB} \pm 1.89$	$6.60^{aB} \pm 1.27$	$p_R = 0.024$
	20	T_2	$12.75^{cC} \pm 3.54$	$9.11^{bC} \pm 1.22$	$7.78^{aC} \pm 1.59$	$p_I = 0.890$ (NS)

The values expressed as the mean ± SD ($n = 3$) were analyzed by two-way ANOVA at significance level $p \leq 0.05$. Different lowercase letters in a row indicates difference due to different protein levels at $p \leq 0.05$. Different uppercase letters in a column indicates significant difference due to different Rac levels at $p \leq 0.05$. p_P = significance for protein levels, p_R = significance for Rac levels, and p_I = significance for interaction of protein and Rac levels. P%: protein percentage; L%: lipid percentage. C_1: control 1 (35% protein, 6% lipid, and 0 mg kg^{-1} Rac); $T_{1.1}$: treatment 1.1 (35% protein, 6% lipid, and 10 mg kg^{-1} Rac); $T_{1.2}$: treatment 1.2 (35% protein, 6% lipid, and 20 mg kg^{-1} Rac); C_2: control 2 (30% protein, 9% lipid, and 0 mg kg^{-1} Rac); $T_{2.1}$: treatment 2.1 (30% protein, 9% lipid, and 10 mg kg^{-1} Rac); $T_{2.2}$: treatment 2.2 (30% protein, 9% lipid, and 20 mg kg^{-1} Rac); C_3: control 3 (25% protein, 12% lipid, and 0 mg kg^{-1} Rac); $T_{3.1}$: treatment 3.1 (25% protein, 12% lipid, and 10 mg kg^{-1} Rac); $T_{3.2}$: treatment 3.2 (25% protein, 12% lipid, and 20 mg kg^{-1} Rac).

Table 8: Alanine amino transferase activity of different experimental groups at the end of the experiment.

Alanine amino transferase	Inclusion Ractopamine level (mg/kg)	Treatments	$P_{35}L_6$	$P_{30}L_9$	$P_{25}L_{12}$	p value
Liver	0	C	$4.70^{cB} \pm 1.28$	$3.90^{bB} \pm 0.91$	$2.66^{aA} \pm 1.26$	$p_{P\&L} = 0.001$
	10	T_1	$5.01^{cB} \pm 1.95$	$4.71^{bB} \pm 2.42$	$2.98^{aB} \pm 1.13$	$p_R = 0.045$
	20	T_2	$6.22^{cB} \pm 1.62$	$5.78^{bB} \pm 0.47$	$4.58^{aC} \pm 0.72$	$p_I = 0.070$ (NS)
Muscle	0	C	$11.73^{cA} \pm 0.28$	$8.92^{bA} \pm 1.75$	$7.00^{aA} \pm 1.29$	$p_{P\&L} = 0.001$
	10	T_1	$13.00^{cB} \pm 1.32$	$11.01^{bB} \pm 0.97$	$8.39^{aB} \pm 1.65$	$p_R = 0.001$
	20	T_2	$15.96^{cC} \pm 1.29$	$14.84^{bC} \pm 1.05$	$9.10^{aC} \pm 2.70$	$p_I = 0.020$

The values expressed as the mean ± SD ($n = 3$) were analyzed by two-way ANOVA at significance level $p \leq 0.05$. Different lowercase letters in a row indicates difference due to different protein levels at $p \leq 0.05$. Different uppercase letters in a column indicates significant difference due to different Rac levels at $p \leq 0.05$. P%: protein percentage; L%: lipid percentage. C_1: control 1 (35% protein, 6% lipid, and 0 mg kg^{-1} Rac); $T_{1.1}$: treatment 1.1 (35% protein, 6% lipid, and 10 mg kg^{-1} Rac); $T_{1.2}$: treatment 1.2 (35% protein, 6% lipid, and 20 mg kg^{-1} Rac); C_2: control 2 (30% protein, 9% lipid, and 0 mg kg^{-1} Rac); $T_{2.1}$: treatment 2.1 (30% protein, 9% lipid, and 10 mg kg^{-1} Rac); $T_{2.2}$: treatment 2.2 (30% protein, 9% lipid, and 20 mg kg^{-1} Rac); C_3: control 3 (25% protein, 12% lipid, and 0 mg kg^{-1} Rac); $T_{3.1}$: treatment 3.1 (25% protein, 12% lipid, and 10 mg kg^{-1} Rac); $T_{3.2}$: treatment 3.2 (25% protein, 12% lipid, and 20 mg kg^{-1} Rac).

or intensive aquaculture systems. Furthermore, in recent days, nutritional indices and health of farmed food fish-related research are on the top priority. In the present study, the whole-body composition of experimental fish highlights that dietary inclusion of ractopamine hydrochloride increases the crude protein and reduces the crude lipid

content in the experimental *L. calbasu* fingerlings after 60 days of feeding trial, and this finding was in accordance with Vandenberg & Moccia [3] where the elevated carcass protein fed with 5 mg/kg of ractopamine for four weeks and lower carcass fat fed with 10 mg/kg of ractopamine for eight weeks in *Oncorhynchus mykiss* were reported. In addition, the present finding was also supported by Haji-Abadi et al. [2] where they observed that dietary inclusion of Rac in rainbow trout diet enhances protein and reduces fat in the fillets. Mustin and Lovell [6] also reported that dietary supplementation of ractopamine in channel catfish reduces carcass fat and improves carcass protein in channel catfish (*Ictalurus punctatus*). Satpathy et al. [5] found that dietary supplementation of beta-adrenergic agonist salbutamol (repartitioning agent) results in lower carcass lipid and improves carcass protein in *Labeo rohita*; Neto *et al.* [28] reported that lower lipid percentage in a muscle of Nile tilapia supplementation with ractopamine supplementation. Further, Mustin and Lovell [6] also studied the effect of different levels of protein (240 g kg^{-1}, 300 g kg^{-1}, or 360 g kg^{-1}) and ractopamine (0 or 20 mg kg^{-1}) with restricted and satiation feeding observed that experimental fish muscle improved protein and improved weight in *Ictalurus punctatus* and reduced fat when fed to satiation. Another study suggests that dietary repartitioning agent has the beneficial effect on *Ictalurus furcatus* to improve the higher carcass protein and lower lipid [7] which is in line with the present findings. Dietary ractopamine supplementation of 10 mg/kg was found to be beneficial in lowering body lipid and raising body protein in all-female prawns [29] which supports our findings. Further, it has been previously reported for blue catfish *I. furcatus* [7] and channel catfish *I. punctatus* [6]. Satpathy *et al.,* [5] found that the dietary incorporation of βAA, salbutamol, was effective in producing growth enhancement, improved body composition (higher protein and lower lipid accretion), and efficient nutrient utilization in the rohu at 3 mg kg^{-1} dietary incorporation level and has a potential for application in formulating feeds for *L. rohita* under culture conditions which supports our findings too. The inclusion of β_2-adrenergic agonists (βAAs) in the rainbow trout diet acts as repartitioning agents and plays the significant role in inducing rainbow trout muscle accretion by altering both protein synthesis and degradation [30]. Bicudo *et al.* [11] studied the effects of four levels of dietary Rac on growth, body composition, and hematology of pacu, *Piaractus mesopotamicus* juveniles. They also concluded that inclusion of up to 40 mg Rac kg^{-1} of diet influenced some hematological and biochemical parameters of juvenile pacu.

Digestive enzymes play a significant role in the utilization of diets with the activities of enzymes affecting the efficiency of nutrient absorption, and so their characterization provides key information on the digestive ability of fish to hydrolyze protein, lipid, and carbohydrate in diets [31, 32]. Amylase activity did not vary significantly among the experimental groups, suggesting that dietary Rac does not have any effect on the amylase activity. A possible explanation for such results might be the experimental diets contain an almost the same amount of carbohydrate, so the amylase activity remains unchanged in all groups. Lipase activity varies significantly among the various treatment groups. Lipase activity increases with higher lipid levels in *L. calbasu* fingerlings. The possible explanation of increased lipase activity is that high-fat diet increases lipase activity. In animals, diets most likely modify the enzyme activity by changing the biosynthesis rate of individual enzymes in pancreatic juice accordingly to the diet composition. This result was supported by Li et al. [33]; they also reported that decreasing protein level and increasing lipid level in the diet for blunt snout bream (*Megalobrama amblycephala*) fingerlings lead to the increase of lipase activities. In our study, dietary supplementation of Rac at any level did not change lipase activity in *L. calbasu*; this result was supported by Araujo et al. [34] as they reported that Rac does not affect the lipase activity in animal model (pigs). Furthermore, protease activity varies significantly among the various treatment groups. Protease activity decreases with lower protein levels in *L. calbasu* fingerlings. The possible explanation of decreased protease activity is the lower protein content in the experimental diet, which was also supported by the findings of Le Moullac et al. [35] and Debnath *et al.* [36].

Hexokinase is the key enzyme of carbohydrate metabolism; it catalyzes the first reaction of glycolysis, which is phosphorylation of glucose into glucose-6-phosphate. In the present study, hexokinase activity significantly decreases in the liver but significantly increases in the muscles by dietary inclusion of Rac. Hence, it could be assumed that dietary supplementation of Rac enhances the fat hydrolysis into fatty acid and glycerol in muscle and the resulting fatty acids transported to the mitochondria for β-oxidation for energy production and metabolic energy demand of fish meets or fulfill from fat rather than carbohydrate and protein.

The hepatic G6Pase activity in *L. calbasu* fingerlings decreases significantly with reducing dietary protein level but increases with Rac level in the diet. This might be due to the fact that *L. calbasu* is an omnivorous fish, so it produces glucose mainly through gluconeogenesis. The possible explanation is that Rac might enhance gluconeogenesis in *L. calbasu*. The hepatic F16BPase activity also increases with protein level and did not change with dietary Rac supplementation in *L. calbasu* fingerlings.

The G6DPH activity in the liver of *L. calbasu* significantly decreases with Rac supplementation and increases with high lipids. The possible explanation is that Rac does enhance the lipolysis and inhibit the NADPH generation which is necessary for the lipogenesis. Result supported by findings of [37, 38] reported the supplementation of beta-adrenergic agonist Rac inhibiting the lipogenesis in animals (pigs). Furthermore, the presence of higher dietary lipid level enhances lipogenesis. A similar finding was reported by Regost et al. [39]; G6DPH activity is higher at 15% dietary lipid level as compared to the fed with the low-fat diet in turbot.

Aspartate aminotransferase is one of the two most important protein or amino acid metabolizing enzymes. Aspartate aminotransferase helps in the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate. Alanine aminotransferase catalyzes the reversible transamination of alanine to pyruvate. In the present study, AST and ALT activity of

the liver and muscles increases with Rac supplementation. This result is supported by Guimarães et al. [40], and it was found that activities of AST and ALT increased in the liver of tambaqui fed diets containing graded levels of ractopamine for 30 days; and Chikhou et al. [41] also observed elevated concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in Friesian steers fed with βAA.

5. Conclusion

We may conclude that the dietary supplementation of ractopamine hydrochloride (Rac) at 20 mg kg^{-1} through feed improves *L. calbasu* body composition particularly crude protein and enhances the metabolic enzyme activity in the liver and muscle despite of high and/or low crude protein and lipid levels in the basal diets. Hence, it may be suggested that the incorporation of ractopamine hydrochloride is suitable for *L. calbasu* farming system for better body composition and optimized metabolic or physiological health status. Further studies may be conducted to screen the intestinal microbiome and understanding the gut health after fed with ractopamine hydrochloride in the farmed food fishes for better explanation and suitability of such feed supplement.

Data Availability

All the data which supports the findings have been presented in the manuscript. Furthermore, data can be accessed from the corresponding author upon reasonable request.

Ethical Approval

All applicable ethical guidelines were followed to perform the experiment. Furthermore, all the procedures performed in the studies involving animals (fish) were in accordance with the ethical standards of the institution (CIFE; Mumbai) and ICAR, Govt. of India. In addition, the study has been approved by the institutional ethical committee as well as by research advisory group, ICAR-CIFE, Mumbai, India.

Conflicts of Interest

All the authors declare that they have no conflict of interest.

Authors' Contributions

RS did the original draft preparation, conducted experiment and data compilations. RD reviewed and edited the draft manuscript constructively and is responsible for the result interpretation. AS did the formal analysis and is involved in the review of the manuscript. KKJ is assigned to the conceptualization and design of experiment and feed data analysis. PPS and ADD did the supervision and review. All the authors have given consent to submit the manuscript.

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