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Comparative Evaluation of Dietary Raw and Solid-state Fermented *Sesbania* Leaf Meal in *Labeo rohita* (Hamilton, 1822)

Sudhanshu Raman, Ashutosh Dharmendra Deo, Md. Aklakur,
Narottam Prasad Sahu, Manish Jayant, Tincy Varghese

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ABSTRACT

Background: *Sesbania aculeata* leaf are rich in nutrients which makes it a potential ingredient in fish feed preparation. Solid-state fermentation (SSF) process of leaf enhanced nutrient content and reduced antinutritional factors. This experiment was conducted to optimize inclusion of raw and fermented *S. aculeata* leaf meal (RSLM and FSLM) in the diet of *Labeo rohita* at fingerling stage at different level.

Methods: Nine isonitrogenous (30% CP) diet for rohu fingerlings were prepared using RSLM and FSLM. SSF of the leaf meals were carried out using *Bacillus subtilis*. Feeding trail was conducted for 60 days at RSLM inclusion level of 10, 20, 30 and 40% (T1, T2, T3, T4) and FSLM (T5, T6, T7, T8) in triplicate treatments with 0% inclusion as control (C).

Result: The fish fed with FSLM based diet had higher weight gain (%), SGR (%) and lower FCR when compared to RSLM and Control. *S. aculeata* can be incorporated in rohu fingerling's diet upto 20% RSLM and 40% FSLM level without any adverse effect on the growth of the fish.

Key words: Anti-nutritional factor, Fish feed, *Labeo rohita,* Leaf meal, *Sesbania* leaf.

INTRODUCTION

The growing human population has accelerated demands for not only food security and poverty alleviation but most importantly, nutritional security. Fish has been considered a cheap and nutritious food that plays significant role in nutritional security (Maulu *et al.,* 2021). With increase in demand of fish, aquaculture has rapidly expanded, especially feed based vertical intensification, driving huge demand of fish feed. As feed attributed to about 60% to the total cost of production in commercial aquaculture (Silva *et al.,* 1995), the market demand catalyzed escalation in prices of feed and its ingredients. Thus, Scientists are now exploring for cheap, easily available and nutritionally rich feed ingredients for use in fish feed industry. Dorothy *et al.* (2018) suggested various nutrient rich plants leaves that has cost-effective potential for preparation of fish feed. *Sesbania aculeata* is one of the nutrient rich and fast-growing green fodder crop commonly cultivated for cattle fodders and green manure (Rani and Kumar, 2020). The leaves are rich in protein and essential fatty acids (Anand *et al.*, 2020). However, plant sourced ingredients often contain anti-nutritional factors (ANFs) and high fibers that requires certain processing procedures to reduce its negative impact on fish growth (Dorothy *et al.*, 2018; Meshram *et al.*, 2018). One of such processing method is SSF that is often used as an effective means to reduce ANFs and crude fibers and enhance protein contents (Anand *et al.*, 2020). For experimenting the inclusion of SLM in the diet of fish at various levels in two different forms (RSLM and FSLM), Rohu (*Labeo rohita*) was selected as the experimental fish, as it is one of the most commonly cultured carp in the freshwater inland water bodies of India. The fish is a fast-grower with omnivorous feeding nature and have potential to utilize plant-based inputs.

ICAR-Central Institute of Fisheries Education, Mumbai- 400 061, Maharashtra, India.

Corresponding Author: Ashutosh Dharmendra Deo, ICAR-Central Institute of Fisheries Education, Mumbai-400 061, Maharashtra, India. Email: ashutosh.cife19@rediffmail.com

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MATERIALS AND METHODS

Sixty days feeding trial was conducted at ICAR-Central Institute of Fisheries Education (CIFE), Mumbai, India with nine isonitrogenous (30% crude protein as per Renukaradhya and Varghese, 1986) experimental diets prepared (Table 1) with 10, 20, 30 and 40% inclusion level of RSLM (T1, T2, T3, T4), FSLM (T5, T6, T7, T8) and 0% inclusion of SLM as Control (C). The experiment, thus, comprises of 8 treatments (T1-T8) and a control(C), in triplicates, following a completely randomized design (CRD). Ten rohu fingerlings (4.43±0.12 g) were stocked in each FRP tank (300 L capacity) and were hand fed to apparent satiation with their respective experimental diets twice daily (6.00 h and 18.00 h). For sample analysis, two fish were randomly collected from each replicate of the experimental groups (n= 6), anesthetized by immersing in 10 L water having clove oil (50 µl L^{-1}) for 10 minutes prior to use (Anand *et al.,* 2020).

SSF of dried *S.aculeata* leaves were carried out using *B. subtilis* bacteria for 6 days with 100 g of leaf powder in

1000 ml conical flask. The total moisture content for fermentation mixture was maintained at 50% by adding 45 ml of distilled water. It was inoculated with 0.5 ml of spore suspension of *B. subtilis* (1×10^8 CFU), mixed and incubated at 37°C. The moisture and crude fiber content was determined by AOAC (1990), total nitrogen by automated nitrogen analyzer (KEL Plus-Classic DXVA, Pelican Equipment, India), ether extract by an automatic fat extraction system (SOCS PLUS-SCS 08 AS, Pelican Equipment, India). Total ash content was estimated in a muffle furnace (AI-7981, Expo Hi-Tech, Mumbai). The nitrogen-fee extract (NFE) content was calculated by subtracting the percentage of other nutrients content from 100. Phytic acid was estimated following Vaintraub and Lapteva (1988) while cyanid, tannin and saponin by AOAC (1990), mimosine by Megarrity (1978) and oxalate by Day and Underwood (1991).

Weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio of fish were evaluated at the end of feeding trial using the following equations;

$$\text{weight gain (\%)} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \times 100$$

Specific growth rate (SGR %) =

$$\frac{\text{ln (final weight)-ln (initial weight)}}{\text{No. of days}} \times 100$$

Feed conversion ratio (FCR) =

$$\frac{\text{Feed consumption (g in dry weight)}}{\text{Body weight gain (g on wet weight)}}$$

Protein efficiency ratio (PER) =

$$\frac{\text{Net weight gain (g in wet weight)}}{\text{Protein fed (g on dry weight)}}$$

The amino acid profiling was carried out at Aakar Biotechnologies Pvt. Ltd. Lucknow. Amylase activity was estimated by Rick and Stegbauer (1974) method while protease and lipase activity by Drapeau (1974) and Cherry and Crandall (1932) respectively, SOD assay by Misra and Fridovich (1972) method, Catalase activity by Takahara *et al*. (1960), lactate dehydrogenase (LDH) activity by Wroblewski and Ladue (1955) method, malate dehydrogenase MDH) activity by Ochoa (1955), aspartate aminotransferase (AST) and alanine aminotransferase(ALT) activity by Wooten (1964). Amino acid profile of the prepared diet (Table 4) was within the recommended level for carps (Zehra and Khan 2013).

The statistical software package 231 SPSS (ver. 22) was used to analyse the experimental data in which data were subjected to one-way ANOVA and Duncan's multiple range tests to determine the significant differences between the means.

RESULTS AND DISCUSSION

Solid-state fermentation

The average crude protein content in the RSLM was 25.37% (Table 2), which is lower than the value (27.1%) reported by Ayssiwede *et al*. (2011) which may be attributed to the differences in the growth stage and geographical parameters (Anand *et al*., 2020). SSF with *B. subtilis* increased protein and ash content in the SLM which might be due to increase in bacterial biomass in the fermentation system which is in line with Shi *et al,* (2021) on increased crude protein in drumstick's leaf meal after SSF with *B. subtilis*. The proximate composition of the experimental diet is given in Table 3 indicating SSF reduced NFE, fats and fiber content in SLM by 7.40%, 43.22% and 10.25% respectively suggesting that microbial fermentation consumed fat, digestible carbohydrate and fiber to synthesize essential amino acids and vitamins (Ramachandran *et al*., 2005). ANFs were decreased considerably after SSF (Table 2) which may be due to the secretion of tannase, phytase and other biological enzymes during fermentation

Tables 1: Feed formulation of the experimental diets (g kg^{-1} dry matter).

Ingredients	Control (0% SLM)	T1 (10%RSLM)	T2 (20%RSLM)	T3 (30%RSLM)	T4 (40%RSLM)	T5 (10%FSLM)	T6 (20%FSLM)	T7 (30%FSLM)	T8 (40%FSLM)
DSBM	260	255	230	215	200	243.9	230	200	189
GNOC	280	260	250	230	210	250	208	184	130
Wheat flour	73.8	98.8	83.8	68.8	53.8	120	126	140	114.7
DORB	300	200	150	100	50	200	150	90	80
RSLM	0	100	200	300	400	0	0	0	0
FSLM	0	0	0	0	0	100	200	300	400
Vit-mineral mix	20	20	20	20	20	20	20	20	20
Vit C	1	1	1	1	1	1	1	1	1
CMC	5	5	5	5	5	5	5	5	5
BHT	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.25
Oil	60	60	60	60	60	60	60	60	60
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000

SLM= Sesbania Leaf Meal, RSLM= Raw SLM, FSLM= Fermented SLM, DSBM= De-oiled Soyabean Meal, GNOC= Groundnut Oil Cake, DORB= De-oiled Rice Bran, Vit= Vitamin, CMC=Carboxy Methyl Cellulose, BHT-Butylated Hydroxy Toluene

Table 2: Effect of SSF on proximate composition and ANFs of the leaf meals.

Components	RSLM	FSLM	% Change
Crude protein (%)	25.37	32.20	26.92↑
Ether extract (%)	4.70	2.67	43.22↓
Crude fiber (%)	11.60	10.41	10.25↓
Total ash (%)	8.57	9.10	6.14↑
Nitrogen-free extract (%)	49.70	46.02	7.40↓
Tannin (mg/100 g)	0.20	0.08	60.14↓
Oxalate (%)	0.60	0.38	35.89↓
Saponin (%)	3.01	1.16	61.53↓
Cyanid (mg/100 g)	21.23	11.90	43.94↓
Phytate (mg/100 g)	15.26	6.57	56.90↓
Alkaloids (%)	24.06	10.93	54.58↓
TIA (mg/100 g)	0.44	0.22	49.18↓
Mimosin (%)	0.75	0.27	35.25↓

Arrow (↑ ↓) indicates % increase/decrease of nutrients.

process, resulting into breakdown of ANFs which is in line with the report of Meshram *et al*. (2018) that microorganisms reduce ANFs level during fermentation.

Growth performance

The growth performance of fish was significantly higher in T7 (30% FSLM) with 150.53±3.88 % weight gain (Table 5). The least growth was recorded in T4 (40% RLSM) followed by T3 (30% RSLM). Thus, upto 20% RSLM and 40% FSLM level can be incorporated in diet of rohu fingerlings without compromising the growth performances of the fish. High inclusion of RLSM was found to negatively impact on the growth, FCR and PER which might be due to high fiber and saponin content in RSLM (Table 2) which might have reduced the protein digestibility either by inhibiting the activity of chymotrypsin or by forming saponin-protein complexes (Anand *et al*., 2020). Fish fed with FSLM based diet performed better due to the low saponin content after SSF processing.

Table 3: Proximate composition of experimental diets (% dry matter basis).

Treatments	Moisture	Crude protein	Ether extract	NFE	Crude fibre	Ash
C	9.91±0.20	30.12±0.11	7.87±0.20	35.99±0.12	7.86±0.32	8.25±0.19
T1	9.89±0.33	31.30±0.17	7.35±0.13	34.88±0.25	8.35±0.75	8.23±0.14
T2	10.46±0.09	31.27±0.20	7.75±0.16	34.13±0.43	8.41±0.25	7.98±0.12
T3	10.58±0.21	30.08±0.22	7.45±0.15	35.74±0.33	8.47±0.18	7.68±0.24
T4	9.98±0.15	31.18±0.10	7.74±0.23	35.05±0.27	8.56±0.29	7.49±0.11
T5	9.82±0.25	31.16±0.14	7.83±0.09	34.65±0.11	8.10±0.34	8.44±0.27
T6	10.74±0.55	31.12±0.20	7.78±0.31	33.52±0.12	8.17±0.18	8.67±0.15
T7	9.99±0.21	30.20±0.10	7.87±0.16	35.72±0.22	8.25±0.43	7.97±0.11
T8	10.1±0.11	31.18±0.14	7.45±0.12	34.75±0.21	8.3±0.25	8.22±0.19

Values are Mean±SE (n=3).

Table 4: Amino acid compositions of experimental diets.

Parameters	C	T1	T2	T3	T4	T5	T6	T7	T8
Percentage of different amino acid in experimental diets									
Indispensable amino acid									
Histidine	1.34	1.31	1.29	1.26	1.24	1.32	1.30	1.28	1.25
Arginine	2.14	2.08	2.04	2.00	1.96	2.10	2.06	2.02	1.98
Threonine	2.27	2.21	2.17	2.13	2.08	2.23	2.19	2.15	2.11
Proline	1.18	1.15	1.13	1.11	1.09	1.16	1.14	1.12	1.10
Valine	1.21	1.18	1.15	1.13	1.11	1.19	1.16	1.14	1.12
Methionine	2.40	2.34	2.29	2.25	2.21	2.36	2.32	2.27	2.23
Cystine	2.18	2.12	2.08	2.04	2.00	2.14	2.10	2.06	2.02
Isoleucine	2.11	2.06	2.02	1.98	1.94	2.08	2.04	2.00	1.96
Leucine	2.22	2.16	2.12	2.08	2.04	2.18	2.14	2.10	2.06
Phenylalanine	1.81	1.76	1.73	1.70	1.66	1.78	1.75	1.71	1.68
Lysine	2.03	1.98	1.94	1.90	1.87	1.99	1.96	1.92	1.88
Dispensable amino acid									
Alanine	1.30	1.26	1.24	1.22	1.19	1.28	1.25	1.23	1.21
Aspartic acid	1.62	1.58	1.55	1.52	1.49	1.59	1.57	1.54	1.51
Glutamic acid	1.69	1.65	1.62	1.58	1.55	1.66	1.63	1.60	1.57
Serine	1.48	1.77	1.81	1.84	1.88	1.45	1.42	1.40	1.37
Glycine	1.57	1.53	1.50	1.47	1.45	1.54	1.52	1.49	1.46
Tyrosine	1.33	1.29	1.27	1.25	1.22	1.54	1.72	1.90	2.28

Values are mean (n=3).

Proximate composition of fish carcass

Crude protein level was reduced in the carcasses of fish (Table 6) when RSLM is included in the diet which might be due to poor digestibility, absorption or bioavailability of proteins owing to the presence of ANFs such as trypsin inhibitors, tannin and saponin in the raw leafs (Francis *et al*., 2001). Increased lipid content in fish carcass in the treatment groups (both RSLM and FSLM) might be due to the toxic effect of saponin that interfered with the digestion of the fat. Ash content was higher in treatment groups compared to control which might be due to the phytic acid in plant leafs that reduces the absorption of minerals (Ranjan *et al*., 2018).

Digestive and metabolic enzyme assay

Protease activity was significantly high in T7 group and lowest in T3 and T4 (Fig 1), as all experimental diets were isonitrogenous in nature, hence, improved protease activity might be due to the decrease in ANFs after SSF (Anand *et al*., 2020). Lipase activity was low in SLM diet which may attribute to the presence of saponin that binds with bile salt, forms insoluble complexes and creates large micelle (Førde-

Table 5: Growth performance of rohu fingerling in the different treatment units.

Treatment	Initial weight (g)	Final weight (g)	WG%	SGR	FCR	PER
C	4.36±0.11	10.25±0.36^{bc}	135.01±2.49^{c}	1.42±0.01^{c}	3.00±0.03^{ab}	1.11±0.01^{cde}
T1	4.55±0.20	10.58±0.54^{bc}	132.22±1.78^{c}	1.40±0.01^{c}	3.06±0.07^{ab}	1.09±0.02^{cd}
T2	4.49±0.21	10.74±0.50^{c}	139.26±1.14^{c}	1.45±0.00^{c}	2.91±0.04^{a}	1.15±0.01^{de}
T3	4.41±0.17	9.49±0.41^{ab}	115.16±2.20^{b}	1.28±0.01^{b}	3.38±0.04^{c}	0. 99±0.01^{b}
T4	4.44±0.16	8.77±0.31^{a}	97.40±1.80^{a}	1.13±0.01^{a}	3.74±0.07^{d}	0.89±0.01^{a}
T5	4.51±0.07	10.68±0.14^{c}	136.95±3.59^{c}	1.44±0.02^{c}	2.89±0.08^{a}	1.16±0.03^{de}
T6	4.42±0.05	10.61±0.23^{bc}	139.98±4.87^{c}	1.46±0.03^{c}	2.95±0.14^{ab}	1.13±0.05^{cde}
T7	4.22±0.05	10.57±0.13^{bc}	150.53±3.88^{d}	1.53±0.02^{d}	2.81±0.09^{a}	1.19±0.04^{e}
T8	4.48±0.06	10.59±0.10^{bc}	136.26±2.30^{c}	1.43±0.01^{c}	3.17±0.02^{bc}	1.05±0.01^{bc}
p-value	0.42	<0.001	<0.001	<0.001	0.01	0.02

Values are Mean±SE (n=3). Values in same columns with different superscript differs significantly (P<0.05). WG %= Weight gain %, SGR= Specific growth rate, FCR= Feed conversion ratio, PER= Protein efficiency ratio.

Table 6: Proximate composition of whole body of rohu fingerling (%wet weight basis).

Treatment	Moisture	Crude protein	Ether extract	NFE	Total ash
C	76.37±0.07^{bc}	14.79±0.012^{d}	4.12±0.02^{c}	1.75±0.09^{ab}	2.97±0.01^{d}
T1	76.86±0.07^{d}	14.64±0.013^{c}	4.11±0.04^{b}	1.51±0.08^{a}	2.87±0.01^{c}
T2	75.92±0.13^{a}	14.61±0.01^{c}	4.11±0.04^{b}	2.49±0.15^{c}	2.84±0.01^{b}
T3	76.86±0.07^{d}	14.47±0.02^{b}	4.10±0.01^{ab}	1.87±0.09^{b}	2.72±0.01^{a}
T4	75.94±0.07^{d}	14.25±0.01^{a}	4.09±0.00^{a}	2.93±0.09^{d}	2.74±0.01^{a}
T5	76.44±0.07^{bc}	14.77±0.01^{d}	4.12±0.00^{c}	1.80±0.07^{ab}	2.87±0.01^{c}
T6	76.44±0.07^{bc}	14.76±0.01^{d}	4.11±0.01^{b}	1.81±0.09^{ab}	2.86±0.01^{c}
T7	76.22±0.07^{b}	14.80±0.01^{d}	4.11±0.01^{b}	1.98±0.09^{b}	2.89±0.01^{c}
T8	76.49±0.07^{c}	14.76±0.01^{d}	4.10±0.06^{ab}	1.75±0.08^{ab}	2.88±0.01^{c}
p-value	0.01	<0.001	<0.001	0.01	<0.001

Values are Mean±SE (n=3). Values in the same column with different superscript differs significantly (p<0.05). NFE= Nitrogen-free extract.

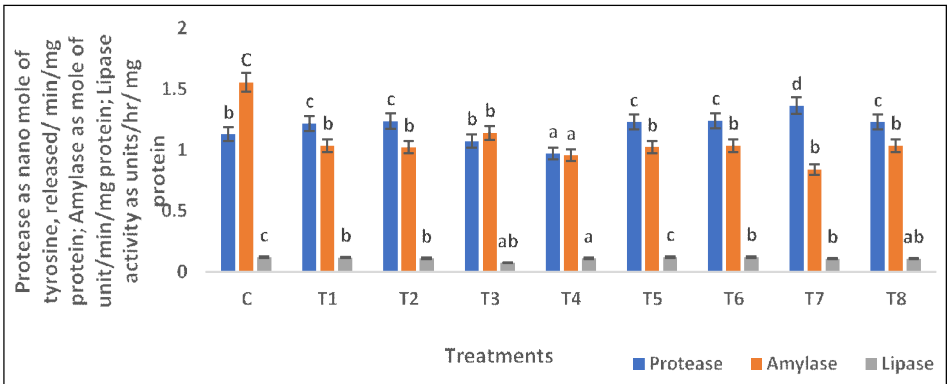

Fig 1: Effect of SLM on digestive enzymes activities of rohu fingerlings.

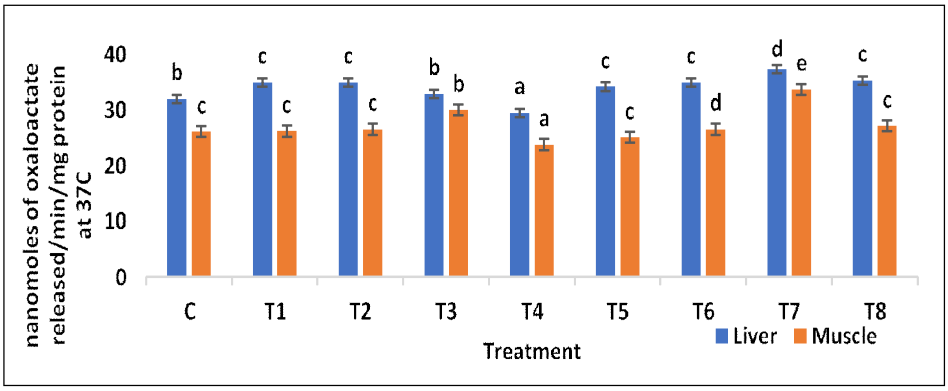

Fig 2: AST in muscle and liver of rohu fingerlings fed with SLM-based diet.

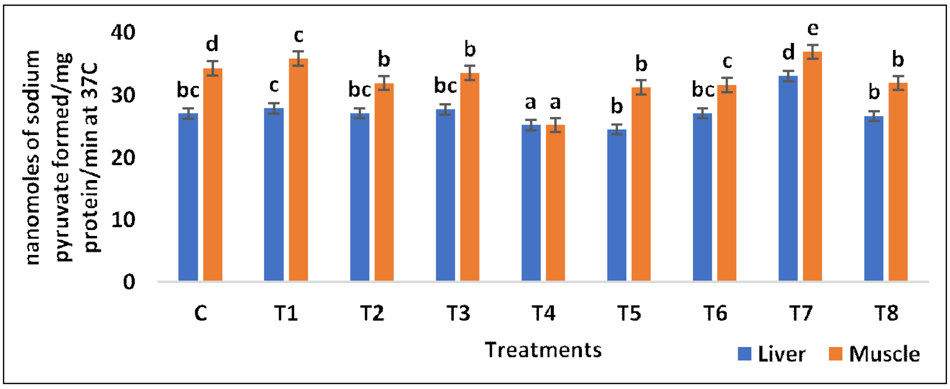

Fig 3: GPT(ALT) in muscle and liver of rohu fingerlings fed with SLM-based diet.

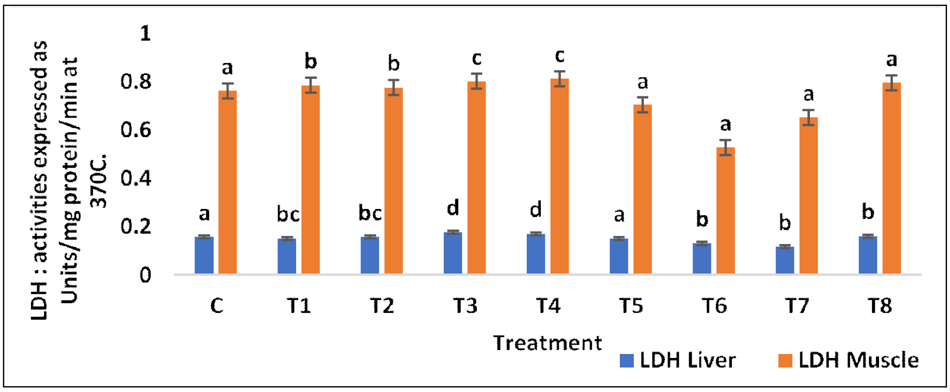

Fig 4: LDH in muscle and liver of rohu fingerlings fed with SLM-based diet.

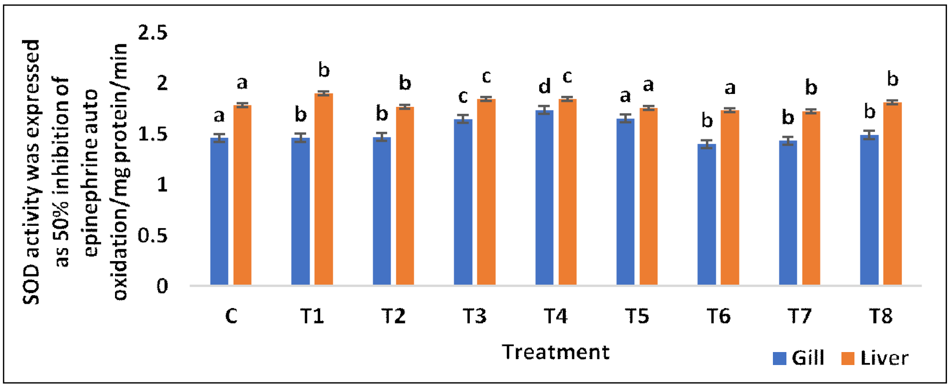

Fig 5: SOD in gill and liver of rohu fingerlings fed with SLM-based diet.

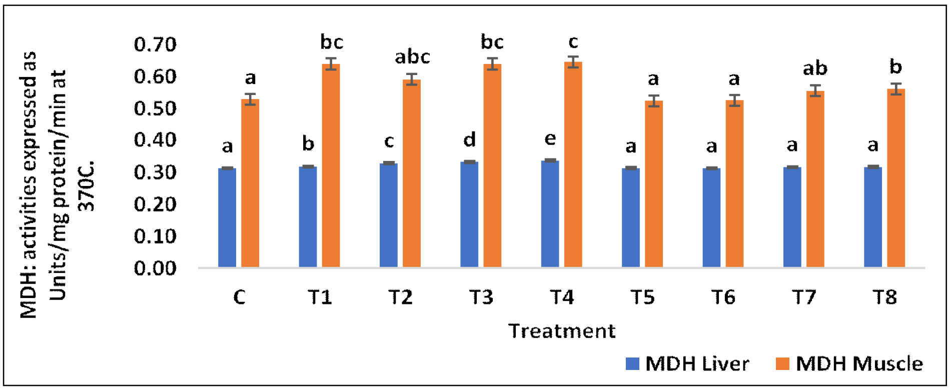

Fig 6: MDH in muscle and liver of rohu fingerlings fed with SLM-based diet.

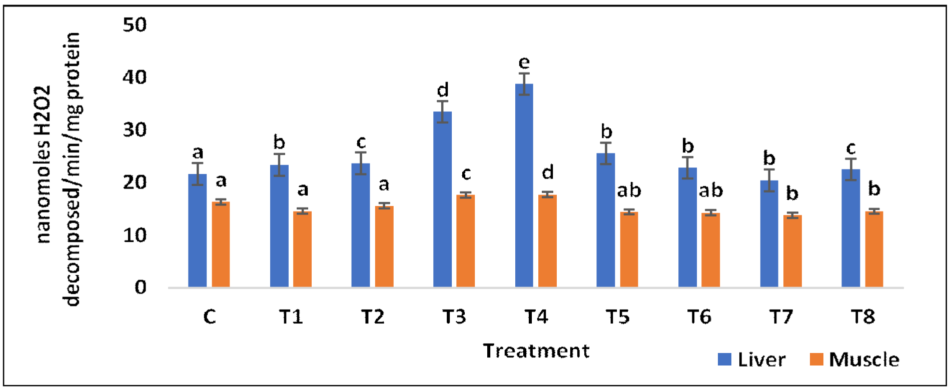

Fig 7: Catalase in muscle and liver of rohu fingerlings fed with SLM-based diet.

Skjærvik *et al*., 2006). In the present study, T7 showed higher activity of AST and ALT in muscle and liver (Fig 2 and 3) indicating the synthesis of non-essential amino acids that might attribute to good growth performances in the fish. Similarly, lower activity of AST and ALT in T4 group reflects negative effect of ANFs. The elevated LDH activity of T3 and T4 groups may be due to metabolic stress caused by ANFs (Sahoo *et al*., 2020). Similarly, higher MDH activities in the RSLM fed group shows high energy demand leading to the activation of TCA cycle (Fig 4 and 5) which are in line with Anand *et al*. (2020) that elevated LDH and MDH pattern indicates metabolic stress. SOD and catalase activities were enhanced in RSLM fed groups (Fig 6 and 7) and reduced in FSLM groups indicating adverse effects on antioxidant status of rohu. Thus, fish fed with FSLM has improved antioxidant status.

CONCLUSION

SSF of SLM with *B.subtilis* for six days enhanced nutritional value by increasing crude protein (to 26.92%) and reduced ANFs and fibre. RLSM can be included in the diet of rohu fingerlings upto 20% and FSLM upto 40% level without compromising the growth performance of the fish. Fish fed with 30% FSLM based diet exhibited best growth and physio-metabolic responses.

Conflict of interest: None.

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