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Research Article

Evaluation of Optimum Protein Requirement and Cost Effective Eco-friendly Source for *Labeo calbasu* (Hamilton, 1822)

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Abstract

Objective: The present study was conducted to evaluate the optimum protein level and eco-friendly, cost effective feed for significant growth performance of *Labeo calbasu*. **Materials and Methods:** Two experiments were conducted, in experiment 1, five experimental feeds (T_1 to T_5) containing 35, 38, 40, 42 and 45% protein were prepared. Processed soybean served as major protein source. Experiment 2 was to assess the cost effective protein source to replace soybean, five experimental feeds (D_1 to D_5) containing soybean, moong, cowpea, duckweed and water spinach were prepared. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. SPSS was used for analysis. **Results:** Weight gain, feed efficiency, intestinal enzymatic activities and proximate composition of carcass of fish fed on diet with 40% protein level were significantly ($p<0.05$) higher than the other groups. Significantly ($p<0.05$) higher growth performance, low feed conversion ratio (FCR) and lower excretion of ammonia and o-phosphate production in holding water was observed in fingerlings fed with diet D_4 of experiment. **Conclusion:** These results indicate that a dietary protein level of 40% and use of *Lemna* as protein source can be recommended for optimum growth, efficient protein utilization and cost effective culture of *L. calbasu*.

Key words: Ammonia excretion, cost effective protein, duckweed, *Labeo calbasu*, soybean

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Artificial feed plays an important role in culture system where it is required to maintain high densities of fish than the natural fertility of the water can support[1]. Dietary protein requirements of a fish species is of fundamental importance in aquaculture, because it significantly influences growth, survival and yield of fish as well as economics of a farming industry[2]. Moreover, accurate information on the protein requirement of fish is crucial for any aquaculture initiative owing to the high cost of protein ingredients that were usually required at high levels by most fishes[3]. Excessive dietary protein not only increases the cost of production but also affect an increase in ammonia excretion. Hence, the determination of protein requirement has become a priority for the optimum growth performance.

Increasing price and strict legislation has led to the use of plant protein source to partially or fully replace fish meal for providing essential nutrients to the fish. Soybean has gained much attention to replace the use of fish meal[4,5]. Research investigating cheaper alternative protein and energy feedstuffs for the development of low cost pelleted feeds suitable for use by the small scale farmers is also one of the important issues especially in the developing countries, where there was an acute shortage of animal protein in the diets. Cheaper ingredients like duckweed[6,7], water spinach[8-10] are considered suitable as major protein source for incorporation in pelleted feed of fish, however studies are required for each candidate species.

One of the commercially important indigenous fish species of Haryana, India is *Labeo calbasu* that can be considered as a candidate species for profitable aquaculture. The conservation status of the species was determined to be lower risk/near threatened[11], hitherto a few attempts have so far been made to study the food and feeding habits of this species, though information on similar aspects of related species are available. Due to high demand, this species fetches a high market price. The natural breeding of *L. calbasu* has become uncertain due to continuous habitat degradation and human interventions affecting feeding migration and spawning, decreasing its population size in India.

No significant study has so far been conducted on dietary requirements of this fish. Farmers take least interest in its culture owing to the unavailability of adequate feed. If proper feed for growth performance and feed efficiency are available in commercial aquaculture, then the costs of production are likely to be reduced. Lack of adequate knowledge on the effect of dietary protein on growth and physiological performance was hindrance for intriguing and enactment of appropriate feeding strategies for culture of this candidate species. Therefore, the present study was designed to appraise the optimum protein level and cheap plant protein source to replace soybean for significant growth performance of *L. calbasu*.

MATERIALS AND METHODS

Two experiments were conducted to evaluate the dietary protein level and eco-friendly, cost effective protein source for *Labeo calbasu* at Aquaculture Research Unit, Department of Zoology, Kurukshetra University, Kurukshetra (29°58′ N, 76°51′ E) during 2016.

Experiment 1: Evaluation of the optimum protein level in the diet of *Labeo calbasu*.

Five experimental feeds (T_1, T_2, T_3, T_4, T_5) containing 35, 38, 40, 42 and 45% protein were used in the study. Processed soybean (*Glycine max*) was used as major protein source. Soybean was hydrothermally treated at 121°C for 15 min to remove antinutritional factors (ANFs) such as trypsin inhibitors[5]. Ingredients were thoroughly grounded, sieved and mixed, preparing homogenous dough using distilled water. Pelleted feed was produced using pelletizer. Oven dried feeds were kept in air tight container. Casein was used to achieve higher protein percentage only in case of 45% protein containing diet T_5, which was stored in refrigerator at 4°C. The feeding trial was carried out for 90 days. The ingredient composition and the average proximate analysis of the formulated diets are represented in Table 1.

Experiment 2: Evaluation of the suitable plant protein source for the diet of *Labeo calbasu* for high growth performance and digestibility.

Five experimental feeds (D_1, D_2, D_3, D_4, D_5) containing soybean (*Glycine max*), moong (*Vigna radiata*), cowpea (*Vigna unguiculata*), duckweed (*Lemna minor*) and water spinach (*Ipomoea aquatica*) were used in the study. Soybean diet served as control. Ingredients were thoroughly grounded, sieved and mixed, preparing homogenous dough using distilled water. Pelleted feed was produced using pelletizer. Oven dried feed was kept in air tight container following which feeding trial was conducted. The ingredient composition and the average proximate analysis of the formulated diets containing varying plant protein source are represented in Table 2.

Table 1: Ingredient and proximate composition (% dry weight basis) of varying protein levels (Experiment 1)

Diets	T_1 (35%)	T_2 (38%)	T_3 (40%)	T_4 (42%)	T_5 (45%)
Ingredients composition					
Ground nut oil cake	650	650	650	650	570
Rice bran	109	58	32	6.5	6.5
Wheat flour	109	58	32	6.5	6.5
Processed soybean*	112	214	266	317	327
Casein	0	0	0	0	70
Mineral mixture**	10	10	10	10	10
Chromic oxide (Cr_2O_3)	10	10	10	10	10
Proximate composition					
Moisture (%)	3.07 ± 0.047^{D}	4.20 ± 0.023^{C}	4.56 ± 0.035^{B}	5.48 ± 0.046^{A}	2.47 ± 0.024^{D}
Crude protein (%)	34.96 ± 0.081^{E}	37.83 ± 0.156^{D}	39.71 ± 0.246^{C}	41.95 ± 0.056^{B}	44.73 ± 0.166^{A}
Crude fat (%)	9.08 ± 0.032^{C}	9.14 ± 0.038^{CB}	9.26 ± 0.061^{AB}	9.30 ± 0.061^{A}	9.40 ± 0.026^{A}
Crude fiber (%)	5.80 ± 0.021^{E}	6.11 ± 0.066^{D}	6.29 ± 0.018^{C}	6.52 ± 0.033^{B}	6.81 ± 0.034^{A}
Total ash (%)	7.14 ± 0.038^{D}	7.27 ± 0.077^{CD}	7.50 ± 0.187^{BC}	7.72 ± 0.095^{AB}	7.89 ± 0.035^{A}
Nitrogen free extract (%)	39.94 ± 0.117^{A}	35.45 ± 0.172^{B}	32.68 ± 0.498^{C}	29.03 ± 0.208^{D}	28.71 ± 0.251^{D}
Gross energy (kJ g^{-1})	18.71 ± 0.017^{B}	18.64 ± 0.026^{C}	18.65 ± 0.019^{BC}	18.57 ± 0.024^{D}	19.21 ± 0.007^{A}
Feed phosphorus (%)	1.30 ± 0.012^{C}	1.35 ± 0.009^{C}	1.41 ± 0.021^{B}	1.46 ± 0.019^{B}	1.52 ± 0.021^{A}

*Soybean was hydrothermally processed in an autoclave at 121°C (15 lbs for 15 min) to eliminate antinutrient factors (Garg *et al.*[5]). **Each kg has nutritional value: copper 312 mg, cobalt 35 mg, magnesium 2.114 g, iron 979 mg, zinc 2 mg, iodine 15 mg, L-methionine 1.920 g, L-lysine monohydrochloride 4.4 g, calcium 30%, phosphorous 8.25%, All values are Mean±SE of mean, Means with different letters in the same row are significantly ($p<0.05$) different, Data were analyzed by Duncan's multiple range test

Table 2: Ingredient and proximate composition (% dry weight basis) of diets with different protein sources (Experiment 2)

Diets	D_1 (Soybean)	D_2 (Moong)	D_3 (Cowpea)	D_4 (Duckweed)	D_5 (Water spinach)
Ingredients composition					
Ground nut oil cake	650	650	650	650	650
Rice bran	32	32	32	32	32
Wheat flour	32	32	32	32	32
Processed soybean*	266	-	-	-	-
Moong	-	266	-	-	-
Cowpea	-	-	266	-	-
Duckweed	-	-	-	266	-
Water spinach	-	-	-	-	266
Mineral mixture**	10	10	10	10	10
Chromic oxide (Cr_2O_3)	10	10	10	10	10
Proximate composition					
Moisture (%)	4.56 ± 0.035^{A}	3.31 ± 0.029^{C}	3.13 ± 0.067^{D}	2.94 ± 0.035^{E}	3.91 ± 0.024^{B}
Crude protein (%)	39.71 ± 0.246^{A}	39.31 ± 0.181^{A}	39.42 ± 0.238^{A}	40.81 ± 0.365^{A}	38.70 ± 0.297^{A}
Crude fat (%)	9.26 ± 0.061^{AB}	9.13 ± 0.046^{AB}	9.29 ± 0.061^{AB}	9.13 ± 0.043^{A}	9.11 ± 0.047^{B}
Crude fiber (%)	6.29 ± 0.018^{D}	6.66 ± 0.036^{B}	6.51 ± 0.021^{C}	6.40 ± 0.032^{CD}	6.98 ± 0.070^{A}
Total ash (%)	7.50 ± 0.187^{AB}	7.46 ± 0.038^{AB}	7.57 ± 0.075^{A}	7.19 ± 0.023^{B}	7.35 ± 0.038^{B}
Nitrogen free extract (%)	32.68 ± 0.498^{B}	34.13 ± 0.210^{A}	34.07 ± 0.437^{A}	33.52 ± 0.400^{AB}	33.95 ± 0.246^{A}
Gross energy (kJ g^{-1})	18.65 ± 0.019^{D}	18.76 ± 0.024^{C}	18.83 ± 0.007^{B}	19.01 ± 0.018^{A}	18.57 ± 0.031^{E}
Feed phosphorus (%)	1.41 ± 0.021^{B}	1.43 ± 0.026^{B}	1.51 ± 0.021^{A}	1.40 ± 0.019^{B}	1.51 ± 0.20^{A}

*Soybean was hydrothermally processed in an autoclave at 121°C (15 lbs for 15 min) to eliminate antinutrient factors (Garg *et al.*[5]). **Each kg has nutritional value: copper 312 mg, cobalt 35 mg, magnesium 2.114 g, iron 979 mg, zinc 2 mg, iodine 15 mg, L-methionine 1.920 g, L-lysine monohydrochloride 4.4 g, calcium 30%, phosphorous 8.25%, All values are Mean±SE of mean. Means with different letters in the same row are significantly ($p<0.05$) different, Data were analyzed by Duncan's multiple range test

Experimental design: The fingerlings of *L. calbasu* were procured from Fish Seed Production Centre, Pawarkheda, Hoshangabad (Madhya Pradesh, India) and acclimated in the Aquaculture Research Unit of Department of Zoology, Kurukshetra University, Kurukshetra (29°58′ N, 76°51′ E) for 15 days prior the experimental set up. The water in the aquaria was partially renewed daily with the previously equilibrated water to the desired temperature (25°C) and experiments were conducted in glass aquaria (60×30×30 cm)/plastic tubs (50 L). Each aquaria/tub was stocked with 10 fingerlings weighing approximately 1.95 g. Length-weight measurements were done fortnightly to adjust the feed quantity. The fingerlings were fed twice daily at 10:00 and 16:00 h at the rate 4% of their body weight. Any uneaten feed was collected 3 h after feeding and the dry matter content was determined for both supplied and uneaten diet[12] and the data were used for feed consumption calculation. Fecal matter was collected by siphoning from the bottom before the next feeding, dried and stored until analysis.

Sample collection and analysis: Fish were weighed at the end of the feeding trial (90 days) and various growth parameters were calculated as per standard procedures and formulae. Blood samples were obtained from the heart of randomly chosen two fish from each tank by syringe after they were starved for 24 h at the end of the feeding trial and pooled into vacutainers which were used for estimation of total erythrocyte count (TEC) and total leucocyte count (TLC). 10 fish at the initiation and three fish from each tank at the termination of the feeding trial were sampled and analyzed for the proximate analysis[12]. Analysis of percent moisture (by oven drying at 104°C for 24 h), crude protein (Kjeldahl apparatus), crude lipid (extraction with petroleum ether by Soxhlet apparatus), crude fibre (acid/alkali digestion) and ash (incineration in a muffle furnace at 600°C for 4 h) were done for both carcass and feed. Nitrogen-free extract (NFE) was calculated by subtracting the sum total for crude protein, lipid, ash, crude fibre and moisture from 100[13]. Gross energy in diets, faecal samples and fish body was calculated using the conversion factors of 20, 39.5 and 17.2 kJ g^{-1} for protein, lipid and carbohydrate, respectively[14]. Chromic oxide in diets and faeces was estimated spectrophotometrically[15] (T90+UV/VIS Spectrometer, PG Instruments Ltd, United Kingdom).

Intestinal enzymatic activity: At the termination of the experiment, from each treatment, fishes were obtained and kept on an ice tray. Intestine of fish were expirated and processed for determination of protease[16], amylase[17] and cellulase[18] enzymatic activity.

Water quality: Water samples from each aquarium/tub were analysed after every 15 days for dissolved oxygen, pH, conductivity, chloride, calcium and alkalinity following the standard methods[19].

Ammonia and phosphate excretion in holding water: At the end of feeding trials, fingerlings were offered same diet in same quantity. After 2 h, excess of feed was removed and fixed levels of water were maintained for experiments. Water samples from each aquaria/tub were collected at 2 h intervals to estimate the excretory levels of total ammonia (N-NH_4) and reactive orthophosphate (o-PO_4) following APHA[19] and were calculated[20]:

$$\text{Total ammonia excretion} = \frac{NH_4 - N\left(\text{mg L}^{-1}\right)\text{in aquarium water}}{\text{Fish weight}\left(\text{kg L}^{-1}\right)\text{ of water}}$$

$$\text{Reactive phosphate excretion} = \frac{o - PO_4\left(\text{mg L}^{-1}\right)\text{in aquarium water}}{\text{Fish weight}\left(\text{kg L}^{-1}\right)\text{ of water}}$$

Cost benefit analysis: The economic evaluations of the diets will be calculated from the method of New[21] as:

Incidence cost (Rs.) = Feed cost consumed to produce 1 kg weight gain in fish

$$\text{Profit index} = \frac{\text{Value of fish}}{\text{Cost of feed consumed}}$$

Statistical analysis: Significant differences among treatment groups were tested by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests[22] for experiments. To predict accurate dose dependent response to the dietary intake, a breakpoint for the optimum requirement was estimated using second degree polynomial regression analysis. Statistical significance were settled at a probability value of $p<0.05$ or $p<0.01$. All statistics were performed using suitable SPSS version 18.0.

RESULTS

Experiment 1: Evaluation of the optimum protein level in the diet of *Labeo calbasu*.

Growth, survival and digestibility: To predict accurate dose dependent response to the dietary intake, a breakpoint for optimum inclusion was estimated using second degree polynomial regression analysis. It showed optimum inclusion level for growth performance was somewhere near the diet containing 40% protein (T_3) (Fig. 1). Significant high growth performance in terms of live weight gain, growth percent gain in body weight (BW), growth per day, SGR, GCE, PER, APD ($p<0.05$) was observed in treatment T_3 (Table 3). Survival rate

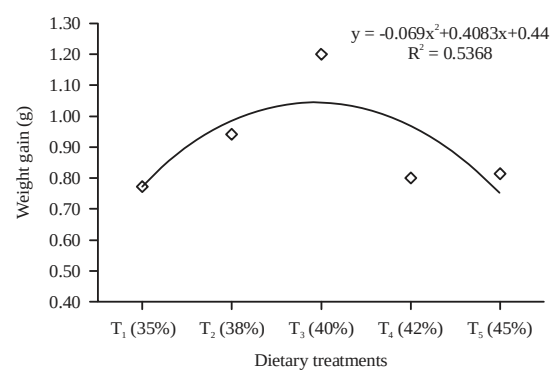

Fig. 1: Polynomial fit curve to show effect of varying protein percentage (T_1: 35%, T_2: 38%, T_3: 40%, T_4: 42%, T_5: 45%) fitting to the data of live weight gain in the fingerlings of *L. calbasu*

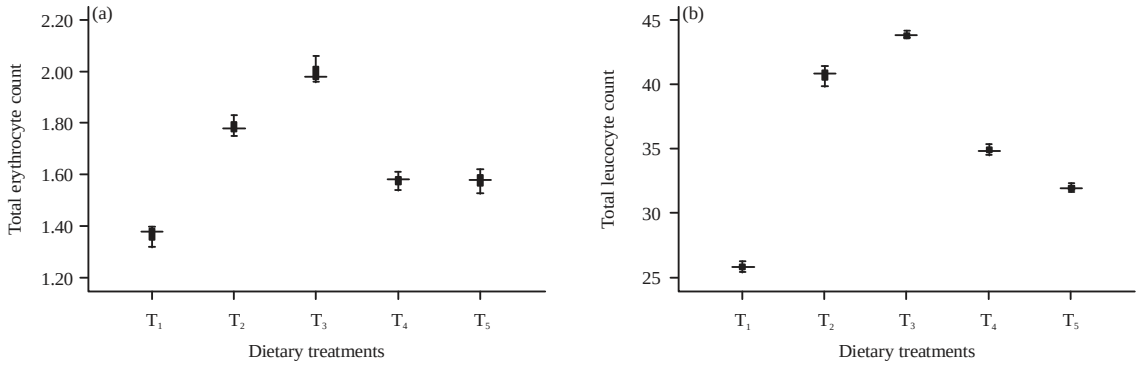

Fig. 2(a-b): Hematological values (a) Total erythrocyte count and (b) Total leucocyte count of *L. calbasu* fed on soybean based diets containing varying percentage of protein (T_1: 35%, T_2: 38%, T_3: 40%, T_4: 42%, T_5: 45%)
All values are Mean±SE of mean

Table 3: Growth performance and the intestinal enzyme activities of *Labeo calbasu* fed on the diets containing varying percentage of protein (Experiment 1)

Growth parameters	T_1 (35%)	T_2 (38%)	T_3 (40%)	T_4 (42%)	T_5 (45%)
Initial weight (g)	1.97 ± 0.033^{A}	1.93 ± 0.033^{A}	1.83 ± 0.033^{A}	1.90 ± 0.100^{A}	1.97 ± 0.033^{A}
Final weight (g)	2.74 ± 0.040^{B}	2.87 ± 0.024^{AB}	2.90 ± 0.058^{A}	2.70 ± 0.103^{B}	2.78 ± 0.031^{AB}
Live weight gain (g)	0.77 ± 0.006^{C}	0.94 ± 0.012^{B}	1.07 ± 0.067^{A}	0.80 ± 0.023^{C}	0.81 ± 0.007^{C}
Survival rate (%)	100	100	100	100	100
Growth (%) gain in BW	39.33 ± 0.333^{C}	48.67 ± 1.368^{B}	58.28 ± 4.275^{A}	42.33 ± 2.475^{C}	41.39 ± 0.932^{C}
Growth/day (%) in BW	0.37 ± 0.002^{C}	0.44 ± 0.010^{B}	0.50 ± 0.028^{A}	0.38 ± 0.020^{C}	0.37 ± 0.007^{C}
Specific growth rate (SGR) (% BW/day)	0.16 ± 0.001^{C}	0.19 ± 0.004^{B}	0.22 ± 0.013^{A}	0.17 ± 0.008^{C}	0.17 ± 0.003^{C}
Feed conversion ratio (FCR)	2.13 ± 0.048^{A}	1.74 ± 0.059^{A}	1.37 ± 0.048^{B}	1.93 ± 0.222^{A}	2.00 ± 0.071^{A}
Gross conversion ratio (GCE)	0.47 ± 0.011^{B}	0.57 ± 0.019^{B}	0.73 ± 0.026^{A}	0.53 ± 0.055^{B}	0.50 ± 0.018^{B}
Protein efficiency ratio (PER)	1.12 ± 0.009^{C}	1.28 ± 0.036^{B}	1.33 ± 0.083^{A}	1.01 ± 0.059^{D}	0.92 ± 0.021^{D}
Apparent protein digestibility (APD) (%)	74.43 ± 0.233^{D}	77.47 ± 0.176^{B}	79.27 ± 0.145^{A}	76.37 ± 0.233^{C}	75.97 ± 0.120^{C}
Specific protease activity*	1.35 ± 0.029^{D}	2.03 ± 0.017^{B}	2.37 ± 0.037^{A}	1.67 ± 0.018^{C}	1.66 ± 0.041^{C}
Specific amylase activity**	1.19 ± 0.013^{C}	1.29 ± 0.007^{B}	1.34 ± 0.031^{A}	1.27 ± 0.013^{B}	1.25 ± 0.013^{B}
Specific cellulase activity***	1.27 ± 0.018^{D}	1.59 ± 0.018^{B}	1.80 ± 0.058^{A}	1.40 ± 0.012^{C}	1.43 ± 0.055^{C}

All the values are Mean±SE of mean, Means with different letters in the same row are significant ($p<0.05$) different, Data were analyzed by Duncan's Multiple Range Test, *mg of tyrosine liberated/mg of protein/h, **mg of maltose liberated/mg of protein/h, ***mg of glucose liberated/mg of protein/h

was 100% for all dietary treatments. Specific growth rate was high in fingerlings fed with T_3 (40% protein). FCR was significantly low whereas, PER was maximum in T_3 fed fingerlings, clearly illustrating the best utilization of the available feed. Erythrocyte and leucocyte count increased significantly with increase in the protein level and after the optimum protein content, both TLC and TEC declined (Fig. 2).

Biochemical analysis: The body composition of fish was also affected by the experimental diets (Table 4). As dietary protein level increased, accumulation of protein increased, fat and ash content decreased from dietary treatment T_1 to T_3. Fish fed on T_3 also had lower moisture and nitrogen free extracts and higher gross energy and phosphorus than the others.

Intestinal enzymatic activity: Enzymatic activity increased with increasing protein level while decreased with higher protein, which showed when it exceeded the optimum level (40%), no further improvement in growth performance and nutritive physiology was observed, rather these parameters declined (Table 3).

Water quality: The water quality parameters (temperature, pH, dissolved oxygen, total dissolved solids, conductivity, alkalinity, chlorides and calcium) were within the optimum range for the experimental feeding trial. Significantly ($p<0.05$) low values in total ammonia excretion (866.73±12.242 mg kg^{-1} BW/day) and reactive phosphate production (565.67±5.933 mg kg^{-1} BW/day) were observed in the fingerlings fed on the diet containing 40% protein (T_3) (Fig. 3). Rhythmic pattern of excretion were observed with highest peak at 8 h and 14 h by group fed on the diet containing 45% protein (T_5), in case of ammonia excretion and at 6 h and 16 h for reactive phosphate production also in dietary treatment T_5.

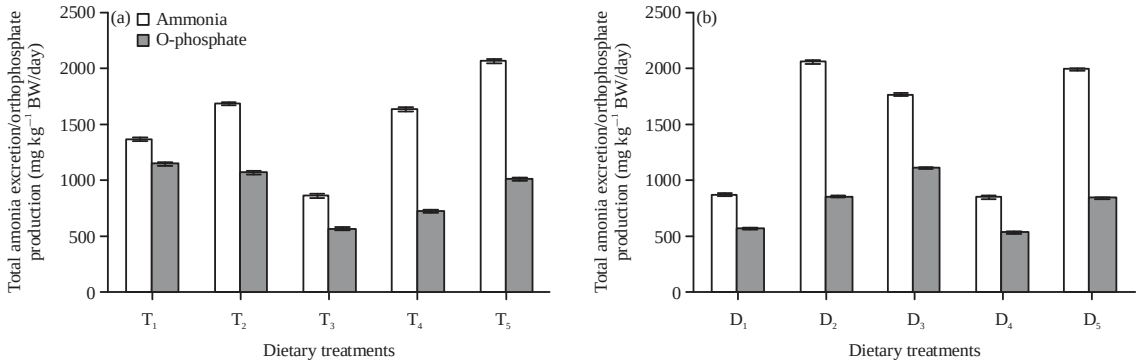

Fig. 3(a-b): Post prandial excretory patterns of total ammonia and total orthophosphate (mg kg^{-1} body weight per day of fish) in holding water for fish *L. calbasu* in (a) Treatments containing varying proportion of protein, T_1: 35%, T_2: 38%, T_3: 40%, T_4: 42%, T_5: 45% and (b) D_1: Soybean, D_2: Moong, D_3: Cowpea, D_4: Duckweed, D_5: Water spinach
All values are Mean±SE of mean

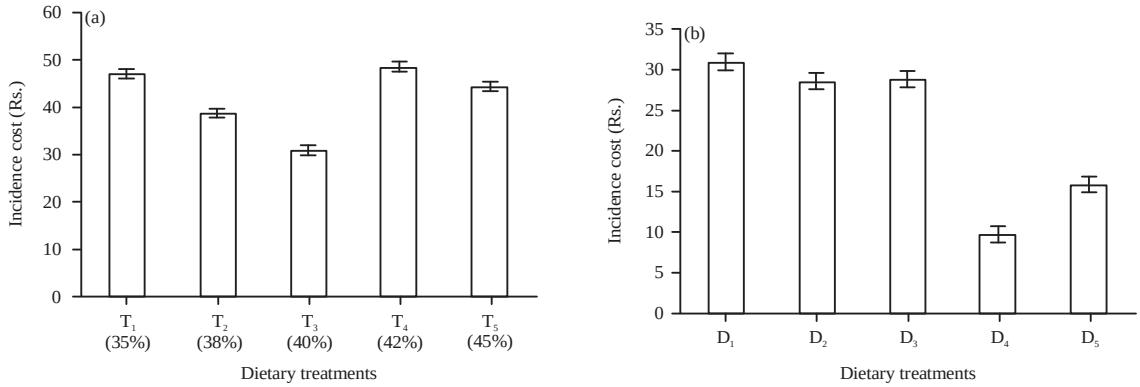

Fig. 4(a-b): Cost benefit analysis of *L. calbasu* fed (a) Treatments containing varying proportion of protein, T_1: 35%, T_2: 38%, T_3: 40%, T_4: 42%, T_5: 45% and (b) D_1: Soybean, D_2: Moong, D_3: Cowpea, D_4: Duckweed, D_5: Water spinach
All values are Mean±SE of mean

Table 4: Proximate carcass composition of *L. calbasu* fed on diets containing varying percentage of protein (Experiment 1)

Proximate composition	Initial	T_1 (35%)	T_2 (38%)	T_3 (40%)	T_4 (42%)	T_5 (45%)
Moisture (%)	70.71±1.202	68.11±0.380^{A}	65.10±1.087^{BC}	63.92±0.113^{C}	66.64±0.672^{B}	66.11±1.041^{BC}
Crude protein (%)	9.77±0.294	10.96±0.045^{C}	11.93±0.393^{B}	14.03±0.167^{A}	11.39±0.072^{BC}	11.83±0.285^{B}
Crude fat (%)	4.44±0.232	5.07±0.066^{D}	6.63±0.084^{B}	7.23±0.296^{A}	5.61±0.097^{C}	5.81±0.070^{C}
Total ash (%)	2.37±0.088	3.54±0.114^{AB}	4.19±0.222^{A}	2.92±0.240^{B}	4.25±0.308^{A}	3.78±0.425^{A}
Nitrogen free extract (%)	13.59±0.906	12.32±0.290^{A}	12.14±0.432^{A}	11.75±0.101^{A}	12.12±0.287^{A}	12.42±0.382^{A}
Gross energy (kJ g^{-1})	6.40±0.280	6.71±0.047^{D}	7.53±0.195^{B}	8.19±0.097^{A}	6.99±0.090^{C}	7.24±0.142^{BC}
Phosphorus (%)	0.52±0.009	0.48±0.009^{D}	0.55±0.019^{C}	0.66±0.010^{B}	0.68±0.009^{B}	0.72±0.003^{A}

All values are Mean±SE of mean, Means with different letters in the same row are significantly ($p<0.05$) different, Data were analyzed by Duncan's multiple range test

Cost benefit analysis: Incidence cost values of *L. calbasu* fingerlings offered diets for T_1, T_2, T_3, T_4, T_5 were observed to be 47.01, 38.72, 30.93, 48.50, 44.32 (INR) (Fig. 4). This corresponds to low cost of 40% protein based diet and higher growth performance was observed in fingerlings fed with 40% protein containing the feed (T_3) when compared with other groups. Profit index was the highest with this protein level, conferring that economic benefit raised up to 88%.

Experiment 2: Evaluation of the suitable plant protein source for the diet of *Labeo calbasu* for high growth performance and digestibility.

Growth, survival and digestibility: Survival rate was 100% for all dietary treatments. Significant high growth performance in terms of live weight gain, growth per cent gain in BW, growth per day, SGR, GCE, PER, APD ($p<0.05$)

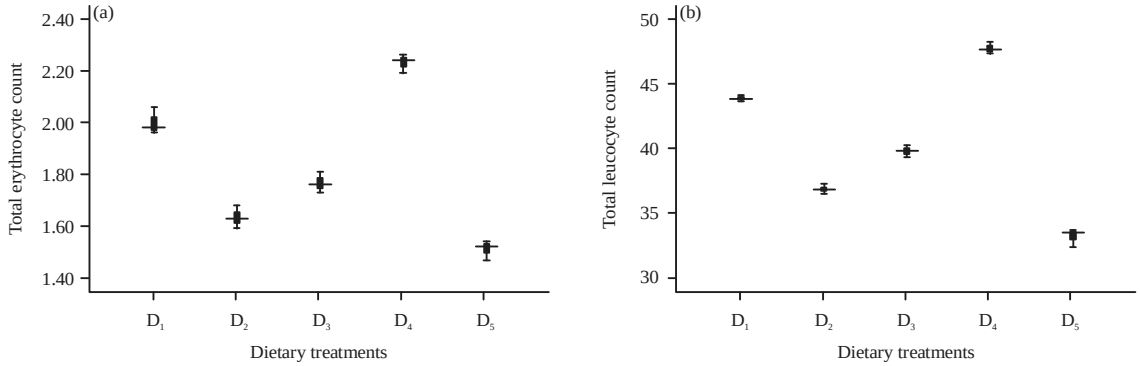

Fig. 5(a-b): Hematological values (a) Total erythrocyte count and (b) Total leucocyte count of *L. calbasu* fed on soybean based diets containing varying percentage of protein (D_1: Soybean, D_2: Moong, D_3: Cowpea, D_4: Duckweed, D_5: Water spinach)

All values are Mean±SE of mean

Table 5: Growth performance and the intestinal enzyme activities of *Labeo calbasu* fed on the diets containing varying plant sources (Experiment 2)

Growth parameters	D_1 (Soybean)	D_2 (Moong)	D_3 (Cowpea)	D_4 (Duckweed)	D_5 (Water spinach)
Initial weight (g)	1.83 ± 0.088^{A}	2.07 ± 0.033^{A}	1.87 ± 0.133^{A}	2.07 ± 0.088^{A}	1.97 ± 0.067^{A}
Final weight (g)	3.01 ± 0.124^{B}	3.00 ± 0.012^{B}	2.78 ± 0.160^{B}	3.43 ± 0.105^{A}	2.84 ± 0.072^{B}
Live weight gain (g)	1.18 ± 0.039^{B}	0.93 ± 0.024^{C}	0.91 ± 0.027^{C}	1.37 ± 0.018^{A}	0.87 ± 0.010^{C}
Survival rate (%)	100	100	100	100	100
Growth (%) gain in BW	64.29 ± 1.506^{A}	45.22 ± 1.909^{B}	49.25 ± 2.250^{B}	66.31 ± 2.134^{A}	44.32 ± 1.290^{B}
Growth/day (%) in BW	0.55 ± 0.004^{A}	0.41 ± 0.014^{B}	0.44 ± 0.016^{B}	0.56 ± 0.010^{A}	0.40 ± 0.010^{B}
Specific growth rate (SGR) (% BW/day)	0.24 ± 0.004^{A}	0.18 ± 0.006^{B}	0.19 ± 0.007^{B}	0.25 ± 0.006^{A}	0.18 ± 0.004^{B}
Feed conversion ratio (FCR)	1.36 ± 0.109^{B}	1.96 ± 0.102^{A}	1.69 ± 0.176^{AB}	1.49 ± 0.104^{B}	1.91 ± 0.109^{A}
Gross conversion ratio (GCE)	0.75 ± 0.056^{A}	0.51 ± 0.028^{B}	0.61 ± 0.071^{AB}	0.68 ± 0.048^{A}	0.53 ± 0.029^{B}
Protein efficiency ratio (PER)	1.61 ± 0.038^{A}	1.13 ± 0.048^{B}	1.23 ± 0.056^{B}	1.66 ± 0.053^{A}	1.11 ± 0.032^{B}
Apparent protein digestibility (APD) (%)	79.23 ± 0.176^{B}	75.67 ± 0.176^{C}	76.30 ± 0.153^{C}	82.23 ± 0.348^{A}	74.03 ± 0.088^{D}
Specific protease activity*	2.42 ± 0.015^{B}	1.75 ± 0.029^{D}	2.08 ± 0.044^{C}	2.79 ± 0.021^{A}	1.64 ± 0.023^{E}
Specific amylase activity**	1.37 ± 0.024^{B}	1.22 ± 0.012^{D}	1.30 ± 0.012^{C}	1.57 ± 0.007^{A}	1.19 ± 0.013^{D}
Specific cellulase activity***	1.86 ± 0.031^{B}	1.54 ± 0.012^{C}	1.60 ± 0.012^{C}	2.05 ± 0.024^{A}	1.45 ± 0.024^{D}

All the values are Mean±SE of mean. Means with different letters in the same row are significant ($p<0.05$) different, Data were analyzed by Duncan's multiple range test, *mg of tyrosine liberated/mg of protein/h, **mg of maltose liberated/mg of protein/h, ***mg of glucose liberated/mg of protein/h

Table 6: Proximate carcass composition of *L. calbasu* fed on diets containing varying plant source (Experiment 2)

Proximate composition	Initial	D_1 (Soybean)	D_2 (Moong)	D_3 (Cowpea)	D_4 (Duckweed)	D_5 (Water spinach)
Moisture (%)	70.71±1.202	63.68 ± 6.205^{C}	65.36 ± 0.305^{B}	66.00 ± 0.352^{AB}	62.85 ± 0.392^{C}	67.10 ± 0.729^{A}
Crude protein (%)	9.77±0.294	14.10 ± 0.208^{B}	11.82 ± 0.084^{C}	12.25 ± 0.187^{C}	14.82 ± 0.110^{A}	10.80 ± 0.146^{D}
Crude fat (%)	4.44±0.232	7.27 ± 0.273^{A}	5.75 ± 0.032^{BC}	6.21 ± 0.100^{B}	6.90 ± 0.153^{A}	5.29 ± 0.079^{C}
Total ash (%)	2.37±0.088	2.86 ± 0.183^{BC}	4.04 ± 0.147^{A}	2.67 ± 0.113^{C}	3.73 ± 0.193^{AB}	2.74 ± 0.546^{C}
Nitrogen free extract (%)	13.59±0.906	11.95 ± 0.159^{C}	13.03 ± 0.118^{AB}	12.65 ± 0.277^{B}	11.54 ± 0.092^{C}	13.26 ± 0.124^{A}
Gross energy (kJ g^{-1})	6.40±0.280	8.21 ± 0.059^{A}	7.30 ± 0.029^{C}	7.52 ± 0.033^{B}	8.26 ± 0.043^{A}	6.92 ± 0.032^{D}
Phosphorus (%)	0.52±0.009	0.64 ± 0.015^{A}	0.55 ± 0.009^{B}	0.62 ± 0.009^{A}	0.65 ± 0.012^{A}	0.57 ± 0.015^{B}

All values are Mean±SE of mean, Means with different letters in the same row are significantly ($p<0.05$) different, Data were analyzed by Duncan's multiple range test

was observed in fingerlings fed on duckweed diet D_4 (Table 5). Growth and FCR showed consistent improvement with the use of duckweed. Specific growth rate was high (0.25±0.006% BW/day) in fingerlings fed with D_4. FCR was significantly low (1.49±0.104) whereas, PER was maximum (1.66±0.053) in D_4 fed fingerlings, clearly illustrating the best utilization of the available feed. Significant higher TLC and TEC were also observed in D_4 fed fingerlings (Fig. 5).

Biochemical analysis: Different plant sources affected the body composition of fish significantly (Table 6). Protein accumulation was found to be significantly higher in D_4 fed fingerlings than the other groups including soybean diet D_1. Nitrogen free extracts were found to be low and high gross energy in D_4 fed fingerlings.

Intestinal enzymatic activity: Significantly high intestinal enzymatic activities were observed in the group of fish fed

with diet containing duckweed. This protein source increased the efficiency of digestion by increasing the enzymatic activity (Table 5).

Water quality: Water quality parameters were within the optimum limits for the experimental feeding trial. Rhythmic patterns at 6 and 14 h were observed for ammonia (D_5) and reactive phosphate excretion (D_3) in holding water (Fig. 3). Significant low values in total ammonia excretion (848.60±14.608 mg kg^{-1} BW/day) and reactive phosphate production (526.70±6.395 mg kg^{-1} BW/day) were observed in the fingerlings fed on D_4 (duckweed).

Cost benefit analysis: Incidence cost values of *L. calbasu* fingerlings offered diets for D_1, D_2, D_3, D_4, D_5 were observed to be 30.93, 28.60, 28.79, 9.80, 15.86 (INR) (Fig. 4). This corresponds to low cost of duckweed based diet and higher % increases in weight gain in fingerlings fed with duckweed containing feed (D_4) when compared with other groups. Profit index was improved with duckweed. Economic benefit rose up to 225% when soybean was replaced with dried duckweed as *L. calbasu* diets.

DISCUSSION

In the present study, significant variation in growth performance and biochemical parameters were found among the fish groups fed on diets with different proportion of protein and varying plant protein sources. The evaluation revealed high growth and high carcass protein accumulation in the fish fed on diets containing 40% protein level and duckweed as major plant protein source.

Increase in protein level resulted in increased growth performance up to the optimum level (40%), where after it decreased. However, other studies have shown that the growth response increased linearly up to the minimum required protein level, after which it plateaued[23,24]. Second degree polynomial regression analysis showed that the maximum weight gain response point in the present study occurred at the 40% dietary protein level which decreased at higher protein level. This decrease in weight gain at protein levels above the optimum is due to a reduction in available dietary energy for growth of fish because of insufficient non-protein energy necessary to eliminate and excrete the excess amino acids absorbed as also reported by Lee *et al.*[25].

Similar results were observed by Zeitoun *et al.*[26], revealing the growth maxima to be attained in a range of dietary protein concentration from 40-50% for *Salmo gairdneri* fingerlings. Labh *et al.*[27] found significant higher ($p<0.05$) average length-weight and specific growth rate in rohu fed with 40 and 50% protein diet, concluding that the incorporation of protein in the diet enhanced the growth of fish regardless of species weight group. 40% protein was found to be the most potent than any other levels of protein for Nile tilapia growth[28]. Similar results were observed by Renukaradhya and Varghese[29], in case of rohu fingerlings fed on 40% protein diet. Analysis of variance, multiple comparisons of treatments mean and second degree polynomial regression analysis led to similar conclusions concerning the dietary protein requirements. In the present studies, these different responses to excessive dietary protein levels might result from factors such as differences in fish species, dietary protein and energy levels. Contradictory results were investigated by Lee *et al.*[25], where 45% dietary protein level was found to be optimum for the growth of young Japanese flounder *Paralichthys olivaceus*. Singh *et al.*[30] found diet containing 30% protein level optimum for better growth performance of *L. rohita* in comparison with the fish fed on diets containing lower or higher protein level. The explanation behind this antithetical outcome to the present study may be the use of slaughter house waste used in the diets which is already a rich source of animal protein. These different responses to excessive dietary protein levels might result from factors such as differences in fish species, dietary protein and energy levels. Kim *et al.*[31] revealed the optimum dietary protein level for maximum growth of olive flounder, *Paralichthys olivaceus* to be 50-59%. However, this result was within the range of 40-70% protein level, which has been manifested for the protein requirement of some carnivorous fish species[3,32].

PER, APD tended to decrease with rising protein levels in this study reflecting that metabolic function of protein in fish was not in proportion to body protein deposition only. Excess dietary protein beyond optimum requirement seem to be catabolized likely for energy metabolism rather than utilizing for the synthesis of protein, which was in harmony with the earlier works with carp and several other fish species[33-36]. Positive relation with carcass protein and associated decrease in carcass moisture found in the present study was also demonstrated earlier[2]. The protein efficiency ratio (PER) linearly decreased when fish were fed on diets with increasing protein levels above the optimum requirement (40%), indicating that the excess of dietary protein would be deaminated, contributing to increased ammonia and reactive phosphate excretion. Similar results were demonstrated by Fernandes *et al.*[37].

When excessive protein was provided, the expensive dietary protein was metabolized as energy without being effectively utilized for growth. Plant products contain fewer

amounts of phosphate and nitrogen than animal protein therefore, the chances of eutrophication of pond could be minimized. Fish nutritionists have made determined attempts to reduce the feed cost as an approach to sustainable aquaculture. The attributes of protein source to be used in the diet affects the choice of dietary protein to be used in practical rations for pecuniary decision. The duckweeds (family Lemnaceae) are free-floating, self propagating plants with a worldwide distribution[38]. It was evident from the present study that the growth performance, PER and FCR of *L. calbasu* fingerlings were better with *Lemna* incorporated diet than with the soybean and other plant sources used. This may be attributed to the good nutritional quality of diet with duckweed as used in the present experiment in comparison with the diets with other plant ingredients. Thus, duckweed can be used as a feed ingredient in the diets for the *L. calbasu* fingerlings, effectively. Similarly, Hajra and Tripathi[39] reported high nutritive value of the duckweed *Spirodela polyrhiza* feed for grass carp in terms of protein-calorie supply and growth when fed in fresh condition. Das and Ray[40], determined the suitability of dried duckweed, *Lemna polyrhiza* as a feed ingredient for *Labeo rohita* fingerlings. Similar outcome was found by Mbagwu *et al.*[41], where the growth rate, mean weight gain, food conversion and survival of *Sarotherodon galilaeus* fingerlings fed test diet containing duckweed *Lemna paucicostata,* were observed to be higher than in fish fed on the control diet with 40% protein. The amino acid content of the duckweed compared favorably with that of the blood, soybean, cottonseed meal and greatly exceeded that of the groundnut meal. Ghosh and Ray[42], incorporated *Lemna* leaf meal which resulted in the best growth performance in *Cirrhinus* fingerlings. Kalita *et al.*[7] compared the efficacies of different aquatic weeds for *Catla* and *Cirrhinus* and their results were contradictory to the results of the present study, reporting water spinach *Ipomoea* to be efficient for fingerlings. This may be due to different dietary habits of different species. While, influence of duckweed on production of fish is positively significant indicating that duckweed might be used as preferable feed items for fishes in polyculture[43]. Kohinoor *et al.*[44] noted the potency of duckweed as low cost supplementary feed through 6 months production trial of Thai sharpunti. Fresh duckweed is highly suited to intensive fish farming systems with relatively rapid water exchange for waste removal[45] and duckweed is converted efficiently to live weight by certain fish including carp and tilapia. Low ammonia (848.60±14.608 mg kg^{-1} BW/day) and o-phosphate production (526.70±6.395 mg kg^{-1} BW/day) in holding water by D_4 clearly indicated the efficient utilization of available feed, resulting in reduction in water pollution. Bhatnagar *et al.*[46], Bhatnagar and Raparia[47], Raparia and Bhatnagar[48] and Bhatnagar and Lamba[49] have also reported that when the utilization of the available feed was efficient and optimum excretion of the metabolites in holding water is low, it supported high growth. The deamination of the excess of dietary protein in fish fed high protein diets would lead to increased ammonia excretion, which was highly toxic to fish and whose accumulation must be prevented in recirculating aquaculture systems. Low incidence cost and high profit index was also observed by Raparia[50].

The conclusion of study by Yilmaz *et al.*[51], also supported the use of duckweed in commercial grow out carp feeds as a dry ingredient. Duckweed meal should be appraised for both cold and warm water fish nutrition.

CONCLUSION

A diet containing 40% protein with duckweed as major plant protein source may be recommended for optimum growth and efficient protein utilization by *L. calbasu*, helpful in improving the economics of fish farming without deterioration of the water quality. This may be highly beneficial in conserving the candidate species and upsurge its population in natural waters.

SIGNIFICANCE STATEMENTS

The present study clearly unveils the fact that the incorporation of duckweed as major protein source in the feed of carp *L. calbasu* at optimum protein level can not only prevent the food wastage and minimize the ammonia excretion and phosphate production in the holding water but can also be a cost efficient and environment friendly plant protein source which is easily available at farmer's doorstep. This will be further beneficial to the researchers of similar field to conserve and promote the intensive culture of various fish species and a new commercially viable cost effective fish feed can be developed.

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