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Effect of different protein levels on feed utilization, growth and body composition on fry of snowtrout *Schizothorax richardsonii*

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Abstract
Schizothorax richardsonii, locally called "asala" and also named as snowtrout is a popular coldwater species of temperate zone of trans-himalayan region. Slow growth of this species is the major constraints for its culture. For this purpose an outdoor experiment was carried out to determine the effect of different dietary protein levels on growth performance of snowtrout for 77 days. Fry of *S. richardsonii* with an initial mean weight of 2.8±0.3 g were fed with five experimental diets, 25%, 35%, 40%, 45% and 50% crude protein by dry weight. The experimental diets also differed in the ingredient composition of shrimp meal, soybean cake and corn gluten meal. Weight gain and specific growth rate were significantly ($P<0.05$) higher in fish fed the diet containing 45% protein than in those fed on the lower and higher protein diets. Protein efficiency ratio was not significant ($P>0.05$) among protein levels. Protein productive value was significantly ($P<0.05$) higher in fish fed the 45% protein diet. No significant differences ($P>0.05$) in carcass protein composition were found among the fish fed the different protein level diets. The present investigation revealed that dietary protein level of 45% had a positive impact on the growth performance of fry of *S. richardsonii*.

Keywords: Dietary protein, growth, protein efficiency, *Schizothorax richardsonii*, specific growth

Introduction
Schizothorax richardsonii Gray, 1832 is a coldwater cyprinid fish, commonly known as asala or snowtrout in Nepal. It has sustained a substantial natural fishery in the major rivers of Nepal. Its distribution in Nepal is confined to the rivers, streams and lakes of Himalayan foot hills. Besides Nepal, it is distributed in India, Bhutan, Pakistan and Afghanistan [1]. Snowtrout are commercially important because of its ornamental and food value. This species has been overfished in the hills and Himalayas where there is a shortage of good agricultural land and of economic opportunities [2]. *Schizothorax* fishes are characterized by slow growth, low fecundity, and late sexual maturation as adaptations to their rigorous environment [3]. These life-history characteristics further make them particularly sensitive to intense-exploitation. In-situ conservation of their natural populations has therefore become a primary concern. For the livelihood security of fisheries-dependent ethnic hill communities requires aquaculture practices of several cold water fish species including *S. richardsonii*. Aquaculture development of *S. richardsonii* is just beginning. Reproduction for seed and balanced feed for survival and growth are the major constraints hindering its exploitation. Concerted efforts have been made on the reproduction of this species in controlled environment and the success has been achieved in varying degree [4-7].

For any successful aquaculture program, one of the major problems is to formulate a relatively cheap diet that will give rapid growth and high survival. Dietary protein plays an important role in determining the rate of fish growth. Accurate information on the protein requirement of fish is crucial for any aquacultures because of the cost of protein ingredients are usually high [8]. Information on the effects of dietary protein requirements of *S. richardsonii* is scarce and available reports mostly described on ingredients suitability [9-12]. Studies on feed formulation for *S. richardsonii* have shown that the addition of supplemental vitamins (A, C and E) in the diets has very limited impact on the growth or survival [9]. Graded levels of dietary protein level had better feed conversion ratios, protein efficiency ratios and survival in *Schizothorax* fry fed fishmeal-based diet having 40% protein in diet [10]. Studies on diet development with plant protein sources (soybean, corn gluten, etc.) emphasizing dietary protein level for *S. richardsonii* inhabitating in southeast Asia is important to enhance growth and decrease mortality during early stage of development. Very little information is currently available on plant protein based diets that satisfy the nutrient requirement of *S. richardsonii*. The present study was to assess the best protein level in diet that incorporated shrimp powder, soybean cake and corn gluten on growth of *S. richardsonii* at fry stage.

Materials and Methods

Advanced fry of *S. richardsonii* (2.8±0.3 g) were obtained from the Fisheries Research Division, Godawari, Lalitpur, Nepal, the place where this feeding experiment was conducted. These fry were produced from captive stock of second generation descendent of wild caught fish from Melamchi River, Nepal located at 27°38'27" N, 85°42'23" E and 630 masl. Fish were acclimatized to the experimental rearing conditions for one week. Fry were fed with 25% crude protein (CP) diet during acclimation,

Five experimental diets (3582±234 kcal kg^{-1}) were prepared with graded protein levels (25% control, 35%, 40%, 45% and 50%). The major ingredients comprised mostly of shrimp powder and soybean cake in 30% and 35% crude protein (CP), corn gluten in 40% CP, maize gluten and soybean cake with 45% CP, and soybean cake in 50% CP as major source of protein in the experimental diets (Table 1). After acclimation the fry were randomly distributed in triplicate concrete tanks (0.425 m^2/tank volume) per diet group at a density of 280 fry.m^{-2}, following a completely randomized design. Groups were fed four times a day for 77 days, starting from 8:00 h to 17:00 h at 3 hour interval, with their respective diets to ad libitum determined by visual observation at each feeding. Respective feed containing buckets allotted for experimental units were weighed at the beginning and end of feeding day to estimate the daily feed intake. Fecal matter was removed by siphoning on alternate day. The flow-through system had a water flow of 4-5 L.min^{-1} through an inlet at one and an outlet at the opposite end of each tank. Fortnightly water quality parameters (temperature, dissolved oxygen, pH, conductivity and turbidity) of the rearing tanks were measured with Vernier analogue instrument (Model LABQUEST2). Water alkalinity and hardness were measured with titramatic method following APHA [15]. Similarly, fortnightly fry weights were measured by sampling 10% of the population for growth analysis throughout the experimental period.

The proximate composition of the experimental diets was determined following standard methods of AOAC [16] in Food Research Division, Khumaltar, Nepal (Table 2). Moisture was determined by drying at 105 °C to a constant weight. Ash was determined by incinerating the samples in a muffle furnace at 600 °C for 6 h. Nitrogen was estimated by the Kjeldahl method (2200 Kjeltec Auto distillation, Foss Tecator, Sweden) and crude protein was estimated by multiplying the percent nitrogen by 6.25. Ether extract was measured by the solvent extraction method (1045 Soxtec extraction unit, Tecator, Sweden) using diethyl ether (boiling point 40-60 °C) as a solvent.

Table 1: Per cent composition of ingredients in the formulation of the test diets for *Schizothorax richardsonii* (Gray)

Ingredients	% Feedstuff (as fed)				
	25% CP	35% CP	40% CP	45% CP	50% CP
1. Basic ingredients					
Corn gluten	-	-	66	34	3
Spirulina	0	-	5	3	1
Shrimp	20	30	4	5	5
Soya de-oiled cake	20	35	3	36	69
Soya Oil	0	-	6	6	6
Black gram flour	25	-	-	-	-
Wheat flour	20	22	14	14	14
Rice bran	-	11	-	-	-
Rice flour	15	-	-	-	-
Vitamin premix	-	1	1	1	1
Mineral mix	-	1	1	1	1
2. Additional					
Vitamin E	-	-	0.25	0.25	0.25
Liver tonic	-	-	2	2	2
Phytase-plus broiler 500	-	-	0.1	0.1	0.1
Yeast	-	1	3	3	3
DL- methionine	-	0.6	0.6	0.6	0.6
L-Lysine	-	0.2	0.2	0.2	0.2
Salt (NaCl)	-	1	1	1	1
Butylated hydroxyl toluene	-	-	0.02	0.02	0.02

Table 2: Proximate analysis of formulated feed for *Schizothorax richardsonii* (Gray)

	Diet group				
Analysis (%)	25% CP	35% CP	40% CP	45% CP	50% CP
Dry matter	94.46	91.55	94.36	91.71	92.93
Crude Protein	25.0	35.0	40.0	45.0	50.0
Crude fat	4.41	3.35	7.32	6.37	7.27
Crude fibre	2.37	3.03	1.89	1.86	1.48
Ash	14.94	11.34	5.36	7.45	15.16
Carbohydrate	53.97	38.68	38.29	33.92	23.29
Energy (kcal kg^{-1})	3405.7	3379.3	3933.3	3695.3	3481.6

Growth performance of the fish was measured in terms of body weight gain day^{-1}, percent weight gain day^{-1} and specific growth rate (SGR, % day^{-1}) using the following equations:
Body weight gain day^{-1}= (W_t– W_0)/days
% weight gain day^{-1}= [(W_t– W_0)/ (W_0 x days)] 100
SGR= [(lnW_t–lnW_0)/days] l00

Where W_0 is the weight of fish at time 0 and W_t is the weight of fish at time t.
Food conversion ratio (FCR) was calculated according to the equation:
FCR = food intake (dry weight (g))/body weight gain (g)
The protein productive value (PPV) and protein efficiency ratio (PER) were calculated according to the following equations:
% PPV = [protein gain in fish (g) /protein intake in food (g)] 100
% PER= [gain in weight (g) /protein in food (g)]100

The differences between the group means of body weight gain, percent weight gain, SGR, FCR, %PPV and %PER, were tested by analysis of variance (ANOVA). Duncan's Multiple Range Tests (DMRT) was applied to determine the significance of differences between any two means. Differences in water quality in fish rearing tanks between beginning and termination of the experiment was tested by the standard t-test. All statistical tests were performed using statistical package SPSS (Version 20.0). Comparisons were made at 5% probability.

Results

Water quality was within acceptable ranges throughout the experiment (Table 3). Mean values of water quality parameters in different diet group treatments ranged between: temperature 17.2-17.9 °C, dissolved oxygen 6.1-7.9 mg L^{-1}, pH 6.8-7.5, alkalinity 44.0-68.0 mg L^{-1} as $CaCO_3$, hardness 96.0-137.3 mg L^{-1} as $CaCO_3$, conductivity 28.2-44.9 µs sec^{-1} and turbidity 23.9-56.8 NTU. Water conductivity and turbidity were increased insignificantly in tanks of all diet groups at later stage of experiment but the difference of turbidity was significant only in the 35% diet group.

Table 3: Mean and standard deviation (SD) of water quality parameters of *Schizothorax richardsonii* (Gray) rearing tanks at the start and end of the experiment

Dietary protein level	Start (S) and end (E) of the experiment	Temp. (ºC)	DO(mg L^{-1})	pH (range)	Alkalinity (mg L^{-1} as $CaCO_3$)	Hardness (mg L^{-1} as $CaCO_3$)	Conductivity (µs sec^{-1})	Turbidity (NTU)
25%	S	17.3±0.1	7.9±1.6	6.8-7.0	48.0±12.0	129.6±28.2	23.9±3.8	26.0±1.4
	E	17.7±0.4	6.4±0.6	6.9-7.1	68.0±24.0	90.6±15.2	44.3±10.9	29.7±8.3
35%	S	17.3±0.1	7.8±1.4	6.8-7.0	52.0±9.1	96.0±62.1	28.2±2.3	24.1±2.1
	E	17.6±0.3	6.0±0.1	7.0-7.2	48.0±6.0	161.0±6.1	39.9±2.6	55.0±13.3*
40%	S	17.2±0.1	7.9±1.6	6.8-7.0	46.0±17.3	109.3±14.0	31.3±3.9	25.6±3.1
	E	17.9±0.7	6.2±0.4	7.0-7.2	58.0±6.9	132.0±31.7	42.6±3.6	56.8±25.2
45%	S	17.3±0.2	7.9±1.3	6.8-6.9	44.0±3.4	97.3±7.5	28.2±4.7	27.8±3.1
	E	17.6±0.3	6.1±0.2	6.8-7.0	44.0±6.9	126.6±37.8	44.9±6.9	55.8±16.6
50%	S	17.3±0.1	7.9±1.5	6.9-7.0	52.0±9.1	96.0±26.2	30.8±4.7	23.9±1.9
	E	17.6±0.2	6.2±0.4	7.3-7.5	48.0±6.0	137.3±30.1*	41.7±3.2	50.4±15.4

* denotes significant different (α 0.05) between start and end of experiment within diet group.

Plotting of growth data indicated that the fry of *S. richardsonii* fed on 45% protein diet grew continuously with highest body weight gain in all intermediate growth samples taken at 15 days interval throughout the experimental period (Fig. 1a).The intermediate body weight gain was lowest for fish fed on 25% protein diet and such weight gain of fish fed on 35%, 40% and 50% protein diet remained intermediate of the weight gain of fish fed at 25% and 45% dietary protein. SGR of fry of *S. richardsonii* fed on different protein level diets except 45% protein diet decreased continuously as the growing period progressed (Fig. 1b). SGR of fish fed on 45% protein diet decreased sharply up to 30 days of rearing and later remained somewhat constant at high level compared to that of the SGR of fish fed on other dietary protein levels (Fig. 1b). Although, SGR of fish fed at different dietary protein levels decreased as fish size increased over the rearing period, the fish fed on 45% dietary protein maintained relatively high SGR with polynomial growth function against fish size (Fig. 2).

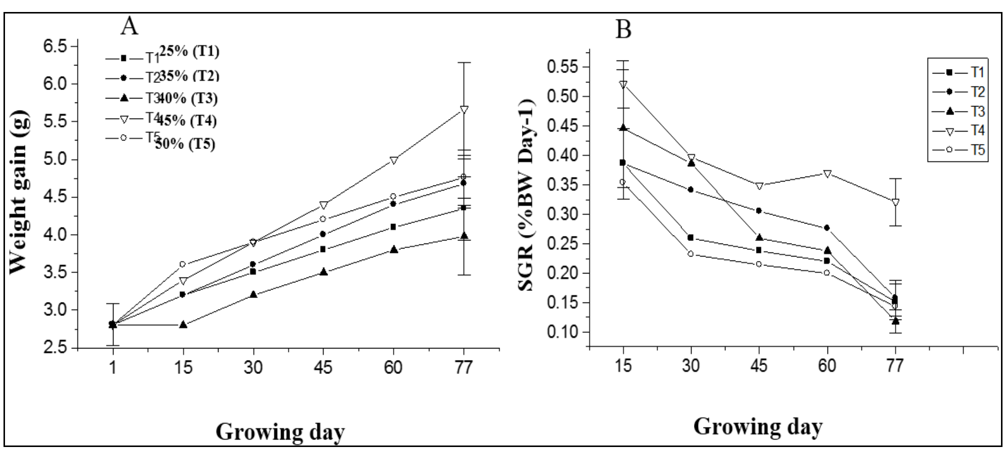

Fig 1: a) Absolute growth and b) specific growth rate (SGR) of *Schizothorax richardsonii* (Gray) fed at different dietary protein levels diets, in relation to time

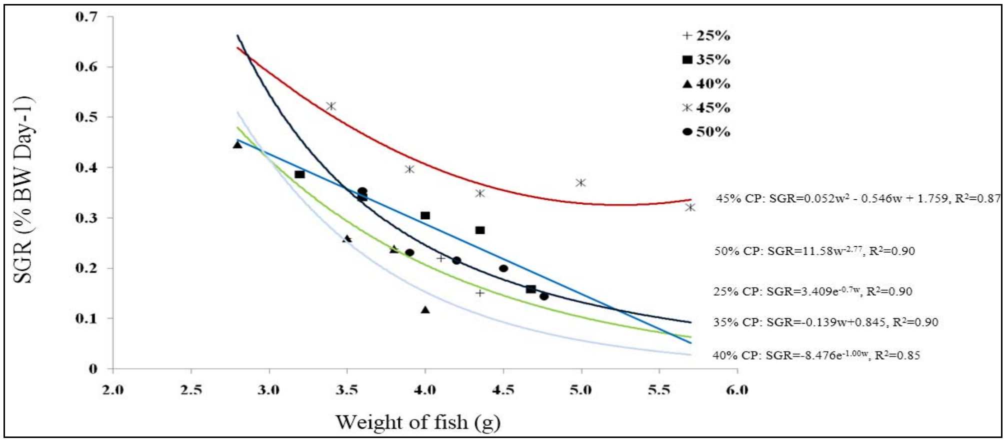

Fig 2: Specific growth rate (SGR) of *Schizothorax richardsonii* (Gray), fed at different dietary protein levels diets, in relation to body weight gain

Data on growth rate and specific growth rate (SGR) are shown in Table 4. The survival of fish among diet group was ranged between 81.3% to 94.3 and differences were not significant ($P>0.05$). The absolute weight gain was higher in fish fed a diet with 45% CP than other diet groups and the differences were significant ($P<0.05$). The relative growth performance of *S. richardsonii* at fry stage, in response to diets with varying levels of protein shows that fish fed on 45% protein diet attained best growth, while 25% protein diet exhibited least growth. Although not significant, percent weight gain and SGR of fish increased with the increase in dietary protein level up to 45% CP. Beyond this level at 50% CP the percent weight gain and SGR of fish decreased. Fish fed on diet containing 45% CP exhibited significantly ($P<0.05$) higher percent weight gain and SGR. On the basis of relative growth (% weight gain) and SGR the following trend emerged 45%>50%>40%>35%>25%.

Table 4: Growth and survival rate of *Schizothorax richardsonii* (Gray) fry during the experimental period at different dietary protein levels (mean±SD)

Dietary protein level	Growth rates			% survival rate
	Wet weight gain day^{-1} (mg)	% weight gain day^{-1}	Specific growth rate (SGR)	
25%	11.4±2.8	0.27±0.08	0.13±0.04	86.3±4.5
35%	18.0±4.2	0.38±0.08	0.20±0.04	94.3±4.7
40%	15.3±3.4	0.39±0.07	0.21±0.05	87.3±3.0
45%	36.7±9.4*	0.65±0.15*	0.40±0.13*	92.7±4.2
50%	20.4±1.2	0.42±0.14	0.23±0.01	81.3±5.0

* denotes significant different (α 0.05) within same column.

Food conversion and protein utilization by fish fed the different protein level diets are shown in Table 5. Food conversion was better in fish fed a high-protein diet (40-50%) than in those fed a low-protein diet (25-35%). Fish fed on 25% protein diet showed significantly high feed conversion ratio. Similar results were obtained with protein utilization. Although not significant, the protein efficiency ratio was high for fish fed on high protein diets. Protein retention was significantly high in fish fed on 45% protein diet followed by 50% protein diet.

Table 5: Protein productive value (PPV), protein efficiency ratio (PER) and food conversion ratio of *Schizothorax richardsonii* (Gray) fry during the experimental period at different dietary protein levels

Dietary protein level	Feed efficiency		
	Protein productive value (PPV)	Protein efficiency ratio (PER)	Food conversion ratio (FCR)
25%	14.0±3.9 [a]	57.4±14.2	8.6±2.4[a]
35%	15.9±4.1 [a]	56.7±13.9	5.1±1.2 [b]
40%	19.5±7.4 [a]	74.1±21.4	3.1±0.8 [b]
45%	29.1±3.9 [b]	86.7±19.3	2.4±0.5 [b]
50%	21.8±4.5[ab]	73.1±3.7	3.3±0.2 [b]

Different superscripted letters within same column are significantly different at α 0.05.

The percent of protein in the body of the *S. richardsonii* at the beginning and the termination of feeding experiment is given in Table 6. At the end of experiment, the protein content was significantly higher in the body of fish fed on 45% protein diet compared to mean protein content of fish fed on other diet groups. No significant differences were found in the body protein composition of *S. richardsonii* fed on low and very high protein diets. Moisture and protein (dry basis) content of fish fed on all graded protein diets increased significantly at the end of experiment compared to that the content of these variables at the start of experiment.

Table6: Body protein composition of *Schizothorax richardsonii* (Gray) at the beginning and end of the experiment (mean±SD)

Diet group	Initial			Final		
	% moisture	% protein (dry basis)	% protein (wet basis)	% moisture	% protein (dry basis)	% protein (wet basis)
25%	72.1±1.2	32.8±1.7[a]	9.1±0.2[a]	79.7±0.3[*]	65.4±0.5[a*]	13.3±1.9[*]
35%	72.2±1.5	44.4±1.7[b]	12.3±0.2[c]	79.1±2.0[*]	65.8±0.5 [a*]	13.8±1.4
40%	71.2±1.2	42.7±1.5[b]	12.3±0.1[c]	79.6±0.7[*]	65.7±0.4 [a*]	13.4±0.5
45%	72.4±1.9	41.1±3.1[ab]	11.4±0.7[bc]	79.8±0.9[*]	70.1±1.7 [b*]	14.2±0.3[*]
50%	70.8±1.5	37.8±1.6[a]	11.2±1.3[b]	79.8±0.6[*]	65.9±0.4 [a*]	13.3±0.4

Different superscripted letters within column are significantly different (α 0.05)
[*] denotes significant different (α 0.05) between initial and final values within diet group

Discussion

The dietary protein requirement for fish fry is high and ranges from 35% to 56% [17]. The best growth rates and protein gain in *S. richardsonii* were found in fish fed a high-protein diet (45% protein) rather than a low-protein diet (25%, 35%, 40%) and a very high-protein diet (50%). Similar to present observation protein requirement of 45.6% and 40% has been recorded in case of grass carp fry and Nile tilapia, *Oreochromis niloticus* fry [18, 19]. The growth rate measured in this study is in agreement with the findings of a feeding experiment conducted at Bhimtal, Uttarakhand, India for *S. richardsonii* and *Tor putitora* [13]. The difference in these two studies is that the present study used corn gluten and de-oiled soybean cake as a protein source in 45% protein diet formulation and the later study used casein supplemented with essential amino acids as a protein source in the diets [13]. In the present study, plant protein source (soybean, corn gluten) was used in high protein diets (40%, 45%, 50%) whereas both plant protein and animal protein source (shrimp) were used in low protein diets (25%, 35%). It seems that *S. richardsonii* prefer high plant protein diet for better growth and protein gain.

In this study *S. richardsonii* grew better on a diet composed of combination of corn gluten and soybean cake than on one containing a high level of shrimp. Corn gluten has been reported as excellent protein source, containing a minimum of 60% protein which is 97% digestible to trout and 95% to *Cyprinus carpio* [20-22]. It can substitute for 25–40 % of fish meal without negative effects on growth or feed conversion ratios in trout and 20% substitution in European seabass *Dicentrarchus labrax* [20, 23, 24]. In our investigation substitution of shrimp meal with a combination of corn gluten and soybean cake caused a significant ($P<0.05$) difference in growth performance including % weight gain, SGR and PPV.

Spirulina (blue-green algae) holds potential for inclusion in diets of various fish species due to its attractive nutrient profile and digestibility [25-27]. In aquaculture the feeding experiment conducted with algae are related with their use as general protein sources - due to the complete replacement of fish meal in fish feed or as additive substances in the feed for cultivated fish [28]. In the present study, incorporation of 3% *Spirulina* in 45% protein diet exhibited significantly ($p<0.05$) higher percent weight gain and SGR of *S. richardsonii* than the fish fed with 5% *Spirulina* in 40% protein diet and 1% in 50% protein diet. Most of the studies have shown that fortification of *Spirulina* at 5-10% in fish diets improves growth, coloration and immunity in several fish species *S. richardsonii, Oncorhynchus mykiss, Oplegnathus fasciatus* and *Oreochromis niloticus* [14, 28-30]. In contrary to present findings, addition of *Spirulina* at 2.5 to 10% in fish diet did not influence ($P>0.05$) growth related parameters of *Oncorhynchus mykiss* [31]. This suggests that concerted research efforts are needed to determine the inclusion level of *Spirulina* to substitute animal protein in diets of *S. richardsonii* without impairing its growth attributes.

Increase in dietary protein levels is often associated with higher growth rate in many species until it reaches the level beyond which further growth is not supported and may even decrease [32]. During the present study, the value of relative growth and SGR increased with the increasing dietary protein level up to 45% and decreased beyond this level at 50% protein diet. These results agree with the facts that the decrease in specific growth rate at protein level above the optimum may be because of a reduction in the dietary energy available for growth to deaminate and excrete excess absorbed amino acids [33].

The growth rate of *S. richardsonii* fed a diet containing 20-30% shrimp was much slower (0.27 to 0.38% per day). These results revealed that *S. richardsonii* can utilize a diet with a low percentage of animal protein, although the rate of growth is reduced. Preference of low percentage of animal protein by *Schizothorax* fish species has also been supported by the fact that natural diet of this species comprises 75% plant matter and 25% of animal matter [34].

Protein efficiency ratio (PER) and protein productive value (PPV) are used as indicators of protein quantity and quality in the fish diet and amino acids balance. So, these parameters are used to assess protein utilization and turnover, where they are related to dietary protein intake and its conversion into fish gain and protein gain. In this study, PER and PPV were affected by protein level and reflects that protein utilization in *S. richardsonii* increased by increasing dietary protein levels up to 45% and then decreased at 50% protein level. The body protein in most of the diet groups did not vary at the end of experimental period (Table 6). For maximum growth, maximum protein synthesis is needed. These results indicate the *S. richardsonii* does not accumulate a large amount of body fat, as do eels and African catfish, needs a high level of protein in its diet [35, 36]. Furthermore, these results may be because of the fact that the major part of weight gain is related to the deposition of protein, and the protein accretion is a balance between protein anabolism and catabolism. Moreover, gastric emptying rate or solubility of the protein has been shown to affect the utilization of dietary protein [37, 38].

For maximal growth, protein synthesis and sufficient energy intake is needed [17, 39]. In this study it was found that %PPV and %PER increased with an increase of protein in the diet. It seems that energy retention was higher for the high-protein diets. For the low energy (low-protein) diets, protein retention was lower presumably because a large amount of the protein was used for energy and a smaller amount was used for growth.

The present investigation revealed that dietary protein level of 45% had a positive impact on the growth performance of fry of *S. richardsonii*. Corn gluten can be incorporated to the diets of *S. richardsonii* to enhance their growth performance. Further studies will be needed to assess the suitability of other protein sources in grow out diets of this indigenous coldwater fish.

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