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Protein Content of *Ctenopharyngodon Idella* Fed with Soyabean Formulated Feed

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Abstract
Proteins are certainly supreme importance in the living world for biological specificity among different types of cells. Hence the protein content of the cell considered as most important tool to evaluate physiological standards. In any type of nutritional study highest priority is given protein content which shows proper growth and development of fish. The protein in the liver, muscle and intestine of *Ctenopharyngodon idella* varies as per the conventional and combinations of formulated feeds. The highest liver protein contents was found in 75% formulated feed and lowest in fishes fed with100% conventional feed, while muscle protein contents was found in 50% formulated feed and lowest in 100% conventional feed. Highest intestine protein contents was found in formulated feed as compared to fishes fed with 100% conventional feed. In the present study protein efficiency ratio was highest in formulated feed which showed the best utilization of protein content from formulated feeds.

Keywords: Protein, Nutritional study, formulated feed, *Ctenopharyngodon idella*.

1. Introduction
Aquaculture is considered as a dominant livelihood for a large section of economically under-privileged population in India. Fish culture is induced primary by the necessarily for increased protein supply. Fish generally have a higher protein requirement than land animals [1]. The gross dietary protein requirement is directly influenced by the amino acid composition of the diet. All fish species studied to date have been shown to require ten indispensable amino acids in their feed for maximum growth [2]. An indispensable amino acid deficiency may cause reduced growth and poor feed conversion [3]; therefore, satisfying the indispensable amino acid requirements of a species is of utmost importance in preparing well-balanced feeds.
Feed is considered as the most intricate input for augmenting fish production. Protein content of feed appears to be the significant factor to enhance production in fish breeding. Fish is a chief sources of proteins, vitamins and minerals, suitable for incorporation in fish diet. Several research have been carried out on the development of formulated feed for the species under controlled culture system [4, 5, 6].

2. Materials and methods
2.1 Formulation of feeds
Feed was formulated by soyabean meal as plant protein sources in present feed formulation. The other ingredients such as milk powder, corn flour, eggs, cod liver oil, vitamin mixture containing vitamin B Complex and E, agar powder, garlic paste, pepper powder, and cumin powder were also used.
Soyabean meal was taken in powder form as main ingredient. Ingredients mentioned above were added. All the ingredients were heated for some time to avoid infection and kept under refrigeration for 12 hrs. Then the mixture was squeezed over plastic tray and dried at room temperature for 48 hrs. The strands were cut into short pieces sun dried for three days to avoid fungal infection. Following the above procedure all the feeds were formulated, in the percentage composition of 25% (soyabean meal 25% +groundnut oil cake 75%), 50% (soyabean meal 50%+groundnut oil cake50%), 75% (soyabean meal 75% +groundnut oil cake 25%), 100% formulated (only of soyabean meal)and 100%conventional (only of groundnut oil cake).

2.2 Experimental protocol

Studies were conducted on *Ctenopharyngodon idella*, Fish weighing 1.34±0.089 gm with body length 5±0.7 cm and kept in aquarium for seven days of acclimatization. Then they were randomly selected and experiment was conducted in five glass aquaria has dimension (35×36.5×35 cm^3) with water volume 60 liter under continuous aeration and experimental set up were under natural light and dark cycle. For each feed i.e. conventional and different combination of feed three replicates were used and in each replicate 25 fishes were stocked. They were fed at the rate of 2% of live net weight once in a day. A water change was implemented by replacing one half of the water in each aquarium with dechlorinated water every day. After each stipulated time intervals i.e. 30, 45, 60, 75 and 90 days, the a live fishes were sacrificed and the tissues (liver, muscle and intestine) from experimental animal was removed and quickly excised and cleaned off extraneous material, weighted and then taken for biochemical estimations. These pooled samples were used for the estimation of total protein by [7].

3. Results

Table 1: Liver Protein content of *Ctenopharyngodon idella* fed with conventional and combinations of formulated fish feed: (Mg/100 mg of wet tissue).

Duration (in days)	100% Conventional fish feed	Combinations of formulated feed			
		25% Formulated fish feed	50% Formulated fish feed	75% Formulated fish feed	100% Formulated fish feed
30	3.083±0.629	4.083±0.878^{NS}	6.666±0.520***	7.833±0.381***	6.416±0.629***
45	3.666±0.629	5.583±0.629 ^{NS}	8.666±1.465***	11.916±1.283***	7.75±0.661**
60	5.083±1.041	7.416±0.763 ^{NS}	10.75±1.250***	13.916±0.629***	9±1.250**
75	5.416±0.763	7.916±0.878*	11.25±1.00***	13.75±1.00***	10.666±0.878***
90	4.666±0.520	7.25±0.500*	9.916±0.629***	11.58±0.629***	8±1.250**

(n=5); ±: SD)*P<0.05, **P< 0.01, ***P< 0.001, NS – Non significant

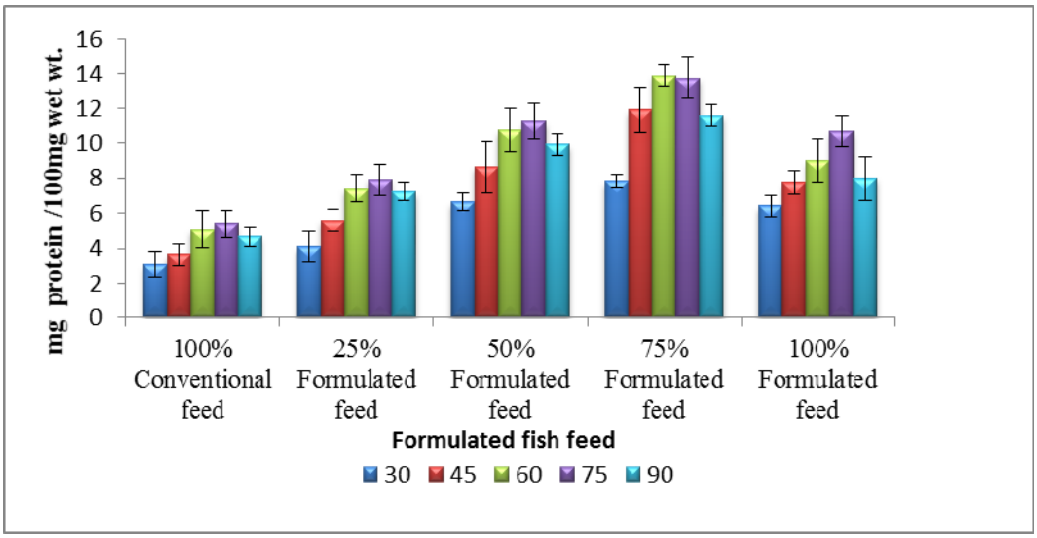

Fig 1: Liver Protein content of *Ctenopharyngodon idella* fed with conventional and different combinations of formulated fish feed

Marginal variations in liver protein content of *Ctenopharyngodon idella* on different combinations of formulated and conventional fish fed has been shown in (table 15). In all the days 75% formulated feed shows highest liver protein content > 50% formulated feed > 100% formulated feed > 25% formulated feed > 100% conventional feed. From above data it is clearly indicated that liver protein content shows tremendous increase in the formulated fed than conventional fed for the stipulated time period of 30 days, the highest liver protein content was found on 75% formulated fed (7.833±0.381 mg/100 mg of wet tissue), which followed by 50% formulated fed (6.666±0.5204 mg/100 mg of wet tissue),which followed by 100% formulated fed(6.416±0.629 mg/100 mg of wet tissue),which followed by 25% formulated fed (4.083±0.878 mg/100 mg of wet tissue) and the least liver protein content was observed in the fishes fed on 100% conventional fed(3.083±0.629 mg/100 mg of wet tissue).

For the stipulated time period of 45 days, the highest liver protein content was found on 75% formulated fed (11.916±1.283 mg/100 mg of wet tissue),which followed by 50% formulated fed (8.666±1.465 mg/100 mg of wet tissue), which followed by 100% formulated fed (7.75±0.661 mg/100 mg of wet tissue), which followed by 25% formulated fed (5.583±0.629 mg/100 mg of wet tissue) and the least liver protein content was observed in the fishes fed on 100% conventional fed (3.666±0.629 mg/100 mg of wet tissue).

For the stipulated time period of 60 days, the highest liver protein content was found on 75% formulated fed (13.916±0.629 mg/100 mg of wet tissue),which followed by 50% formulated fed (10.76±1.250 mg/100 mg of wet tissue), which followed by 100% formulated fed (9.0±1.250 mg/100 mg of wet tissue), which followed by 25% formulated fed (7.416±0.763 mg/100 mg of wet tissue) and the least liver protein content was observed in the fishes fed on 100% conventional fed (5.083±1.041 mg/100 mg of wet tissue). For the stipulated time period of 75 days , the highest liver protein content was found on 75% formulated fed (13.75±1.000 mg/100 mg of wet tissue), the least liver protein content was

observed in the fishes fed on 100% conventional fed (5.416±0.763 mg/100 mg of wet tissue).

For the stipulated time period of 90 days, the highest liver protein content was found on 75% formulated fed (11.583±0.629 mg/100 mg of wet tissue),which followed by 50% formulated fed (9.916±0.629 mg/100 mg of wet tissue),which followed by 100% formulated fed (8.0±1.250 mg/100 mg of wet tissue),which followed by 25% formulated fed (7.25±0.500 mg/100 mg of wet tissue) and the least liver protein content was observed in the fishes fed on 100% conventional fed (4.666±0.520 mg/100 mg of wet tissue).

Table 2: Muscle Protein content of *Ctenopharyngodon idella* fed with conventional and combinations of formulated fish feed (mg/100 mg of wet tissue)**.**

Duration (in days)	100% Conventional fish feed	Combinations of formulated feed			
		25% Formulated fish feed	50% Formulated fish feed	75% Formulated fish feed	100% Formulated fish feed
30	4.5±1.146	9.25±1.146***	7±0.661*	10.16±0.520***	6.25±0.091 NS
45	6.083±0.803	10.75±1.146**	14.66±2.005***	11.75±1.00**	8.25±1.090 NS
60	8.083±0.878	11.416±1.127*	18.53±1.127***	11.91±1.041**	9.083±0.763 NS
75	8.416±0.763	16.583±1.422***	21.25±1.00***	10.66±1.422 NS	8.25±1.090 NS
90	6.50±1.146	16.416±1.127***	18.41±0.946***	8.333±0.803 NS	8.833±0.629 NS

(Value expressed is mean of n (n=5); ±: SD)*P<0.05, **P< 0.01, ***P< 0.001, NS – Non Significant

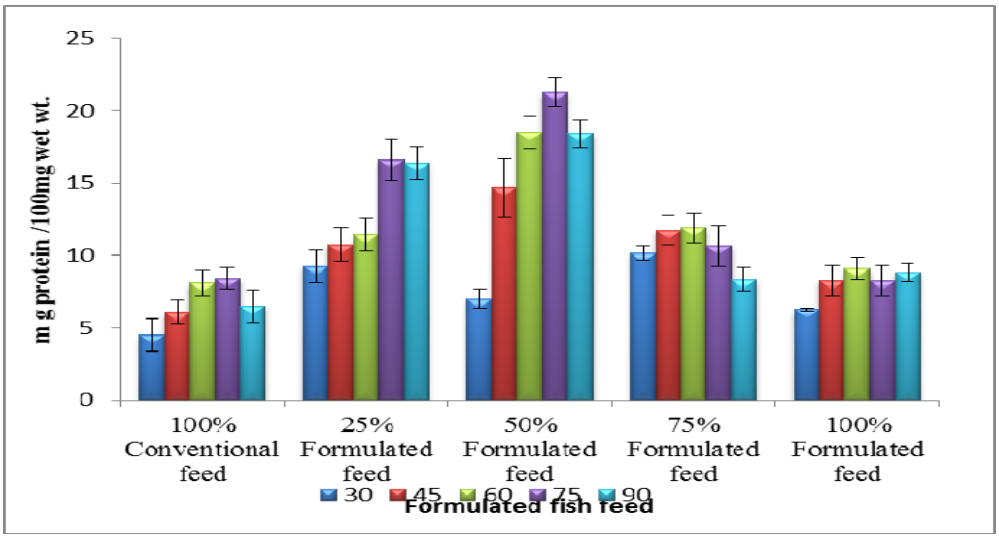

Fig 2: Muscle Protein content of *Ctenopharyngodon idella* fed with conventional and combinations of formulated fish feed

The impact of different combinations of formulated and conventional fish fed on muscle protein content of *Ctenopharyngodon idella* has been shown in the table. The muscle protein content shows an increasing order up to 50% formulated feed and goes on decreasing after that all the formulated fed shows drastic influx in muscle protein content of *Ctenopharyngodon idella*. Formulated fed shows upward trend in muscle protein content up to 50% formulated fed then it goes on decreasing movement. At the stipulated time period of 30 days, the highest muscle protein can be observed on 75% formulated fed (10.166±0.520 mg/100 mg of wet tissue), which followed by 25% formulated fed (9.25±1.146 mg/100 mg of wet tissue),which followed by 50% formulated fed (7.0±0.661 mg/100 mg of wet tissue), which followed by 100% formulated fed (6.25±0.091 mg/100 mg of wet tissue) and the least muscle protein content was observed on 100% conventional fed (4.5±1.146 mg/100 mg of wet tissue).

At the stipulated time period of 45 days, the highest muscle protein can be observed on 50% formulated fed (14.66±2.005 mg/100 mg of wet tissue), which followed by 75% formulated fed (11.75±1.000 mg/100 mg of wet tissue), which followed by 25% formulated fed (10.75±1.146 mg/100 mg of wet tissue), which followed by 100% formulated fed (8.25±1.090 mg/100 mg of wet tissue) and the least muscle protein content was observed on 100% conventional fed (6.083±0.803 mg/100 mg of wet tissue).

At the stipulated time period of 60 days, the highest muscle protein can be observed on 50% formulated fed (18.53±1.127 mg/100 mg of wet tissue), which followed by 75% formulated fed (11.916±1.041 mg/100 mg of wet tissue), which followed by 25% formulated fed (11.416±1.127 mg/100 mg of wet tissue),which followed by 100% formulated fed (9.083±0.763 mg/100 mg of wet tissue) and the least muscle protein content was observed on 100% conventional fed (8.083±0.878 mg/100 mg of wet tissue).

At the stipulated time period of 75 days, the highest muscle protein can be observed on 50% formulated fed (21.25±1.000 mg/100 mg of wet tissue), which followed by 25% formulated fed(16.583±1.422 mg/100 mg of wet tissue),which followed by 75% formulated fed (10.66±1.422 mg/100 mg of wet tissue),which followed by 100% conventional fed (8.416±0.763 mg/100 mg of wet tissue) and the least muscle protein content was observed on 100% formulated fed (8.25±1.090 mg/100 mg of wet tissue). At the stipulated time period of 90 days, the highest muscle protein can be observed on 50% formulated fed (18.416±0.946 mg/100 mg of wet tissue), and the least muscle protein content was observed on 100% conventional fed (6.5±1.146 mg/100 mg of wet tissue).

Table 3: Intestine Protein content of *Ctenopharyngodon idella* fed with conventional and combinations of formulated fish feed (Mg/100 mg of wet tissue).

Duration (in days)	100% Conventional fish feed	Combinations of formulated feed			
		25% Formulated fish feed	50% Formulated fish feed	75% Formulated fish feed	100% Formulated fish feed
30	2.5±0.661	2.166±0.381 NS	2.916±0.878 NS	2.583±0.721 NS	5.083±0.283*
45	1.5±0.5	2.75±0.250**	2.416±0.381 NS	2.25±0.250 NS	2.25±0.250 NS
60	2.333±0.144	3±0.661 NS	4.75±0.500***	4.333±0.577**	5.833±0.381***
75	1.916±0.144	2±0.250 NS	2.666±0.629 NS	2.5±0.250 NS	3.5±0.250**
90	2.25±0.250	2.833±0.381 NS	3.166±0.288 NS	5.166±0.520***	3.916±0.763*

(Value expressed is mean of n (n=5); ±: SD) *P<0.05, **P< 0.01, ***P< 0.001, NS – Non Significant

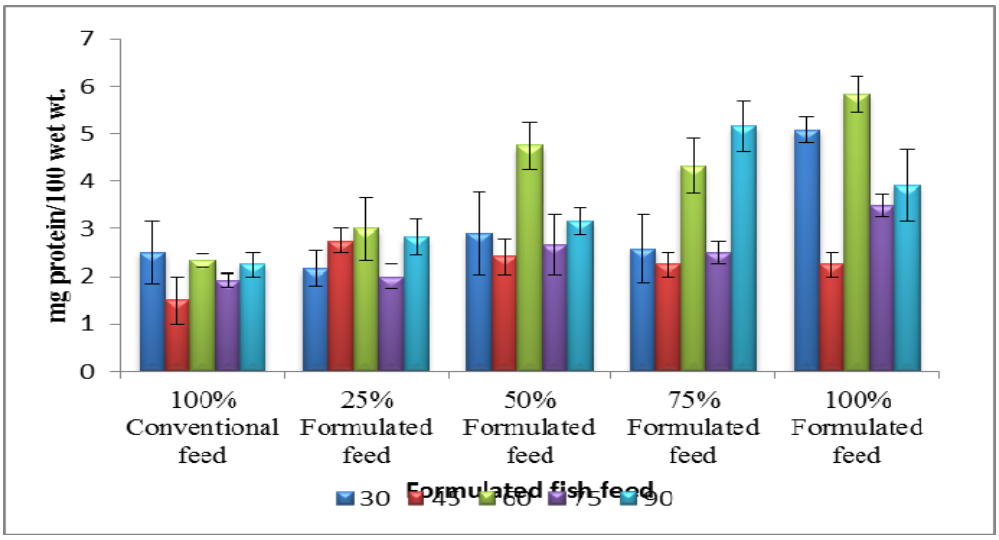

Fig 3: Intestine Protein content of *Ctenopharyngodon idella* fed with conventional and combinations of formulated fish feed:

Changes in the total protein in intestine of *Ctenopharyngodon idella*. fed on conventional and different combinations of formulated fish feed are shown in (Table 21). As compared to conventional the formulated fed shows high protein content in all stipulated time interval due to soyabean, it is rich in protein content.

For 30 days stipulated time interval there was significant increase ($p < 0.05$) in 100% formulated fed (5.0833±1.283 mg/100 mg of wet tissue), which followed by 50% formulated fed (2.9166±0.8780 mg/100 mg of wet tissue), which followed by 75% formulated fed (2.5833±0.7217 mg/100 mg of wet tissue), which followed by 100% conventional fed (2.5±0.6614 mg/100 mg of wet tissue) and least protein value has been observed on 25% formulated fed (2.1666±0.3819 mg/100 mg of wet tissue).

For 45 days stipulated time interval there was significant increase ($p < 0.05$) in 25% formulated fed (2.75±0.2500 mg/100 mg of wet tissue), which followed by 50% formulated fed (2.4166±0.3819 mg/100 mg of wet tissue), which followed by 75% formulated fed and 100% formulated fed which shows equal value i.e. (2.25±0.2500 mg/100 mg of wet tissue) and least protein value has been observed on 100% conventional fed (1.5±0.5000 mg/100 mg of wet tissue).

For 60 days stipulated time interval there was significant increase ($p < 0.05$) in 100% formulated fed (5.8333±0.3819 mg/100 mg of wet tissue), which followed by 50% formulated fed (4.75±0.5000 mg/100 mg of wet tissue), which followed by 75% formulated fed (4.333±0.5774 mg/100 mg of wet tissue), which followed by 25% formulated fed (3.0±0.6614 mg/100 mg of wet tissue) and least protein value observed on 100% conventional fed (2.3333±0.1443 mg/100 mg of wet tissue).

For 75 days stipulated time interval there was significant increase ($p < 0.05$) in 100% formulated fed (3.5±0.2500 mg/100 mg of wet tissue), which followed by 50% formulated fed (2.6666±0.6292 mg/100 mg of wet tissue),which followed by 75% formulated fed (2.0±0.2500) mg/100 mg of wet tissue, which followed by 25% formulated fed (2.0±0.2500 mg/100 mg of wet tissue) and least protein value observed on 100% conventional fed (1.9166±0.1443 mg/100 mg of wet tissue).

For 90 days stipulated time interval there was significant increase ($p < 0.05$) in 50% formulated fed (5.1666±0.5204 mg/100mg of wet tissue), which followed by 100% formulated fed (3.9166±0.7638 mg/100 mg of wet tissue), which followed by 50% formulated fed (3.1666±0.2887 mg/100 mg of wet tissue), which followed by 25% formulated fed (2.8333±0.3819 mg/100 mg of wet tissue) and least protein value observed on 100% conventional fed (2.25±0.2500 mg/100 mg of wet tissue).

4. Discussion

The intestine and liver are the most important organs in digestion and absorption of nutrients from food and therefore monitoring of these organs is considered necessary [8, 9]. In common carp (*Cyprinus carpio*) reaction to the replacement of fishmeal for soyabean meal differs as compared to the family *Salmonidae*. According to Uran *et al* [10] there is a short-term reaction and inflammation to soybean but after a period of one month, after adaptation, the intestine returns to a normal histological structure. The carp has a better tolerance to the presence of antinutritive ingredients in food, as [11] claim that tolerance is specific for the species.

Proteins are the macromolecules in living organisms which serve vital functions in all biological processes. It acts as

catalysts, which transports and stores other molecules like oxygen; it gives mechanical support and immune protection. It controls growth and differentiation in cells. To determine the physiological phases of an organism protein contents can be considered as a diagnostic tool because proteins are involved in main physiological actions. In the present study liver protein shows agreement with the liver protein fed with aquatic weeds to *Catla catla* [12]. Muscle protein shows the similar values to both the species *Catla catla* and *Cirrhinus mrigala* studied by Kalita *et al.*, but in 50% formulated feed shows highest values of protein than that found by Kalita *et al.*, (table 2). Intestine protein values are less than that of liver and muscles.

Protein is the most expensive ingredient in diet formulation. In any nutritional study protein requirement is given highest priority, it require in the largest amount for growth and development of animal [1, 13]. It is observed that, the protein percentage varied from (3.08-21.25 mg/100 mg tissue) (Table 1, 2, 3) in different combinations of formulated feed. Steady increase in percentage of protein from conventional to 75% formulated feed was observed, however decline in 100% formulated feed was noted.

Present study shows that, the combination of formulated feed shows highest protein content. Variation in feeding causes alteration in biochemical and physiological conditions of fish through changes in digestive efficiency. Olvera-Novoa [14] found in reduction in carcass crude protein content in tilapia, when higher inclusion of plant ingredient was done in the feed formulation. Patra and Ray [15] observed the similar relation between dietary protein level and muscle content in Anabas.

Significant difference in the body protein content was not found in turbot [16] and in Nile tilapia [17] when replacement of fishmeal was replaced with mixture of plant protein. Same result were found by and many other workers. Mohanta *et al* [18] reported significant protein, lipid and energy values in groundnut and soybean cake diets compared to other diets. Similar findings were observed in the present study.

5. Conclusion

A main problem in the use of plant protein is its comparatively low protein content. The protein content of plant feed may be increased with the combination of two or more plant proteins. Due to stumpy protein content plant proteins gives lower growth performance. It is observed that the increasing level of plant proteins in feed increases the protein content in liver muscle and intestine. The increase in plant protein is not consistent in all the formulated feed it also varies in stipulated time intervals. There is also reduction in protein content when 100% soybean meal is used in feed formulation while it also shows affects the acceptability of feed which directly correlated with fish body protein.

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