
Chapter 07: Biochemical (Protein)

Chapter – 07

Biochemical (Protein)

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7.1 INTRODUCTION

Air-breathing fishes play an important role in the diet as a source of protein, vitamin and minerals which are essential for healthy growth and development of human body. The demand for fish is rising continuously, as it is a source of food and ready income. The availability of water resources for fisheries and aquaculture are not only limited but are continually getting reduced with time for multiple reasons. Fish protein is one of the most important sources of animal protein and has been widely accepted as a good source of protein and other elements for the maintenance of health Arannilewa et al., (2005). Fish provides a good source of readily digested high quality animal protein, fat, mineral and vitamins especially vitamin A, D and E. Fish plays an important role in prevention and management of many human diseases such as heart disorder, neurological diseases and mood swings. Biochemical composition have been related with many parameters such as body growth (Groves, 1970), maturation (Bull, 1928), spawning migration (Idler and Bitners, 1960), water temperature (Elliot, 1976). In the present work, seasonal variation of biochemical (protein) changes of these fishes in relation to different parameters have been studied. This study is under-taken to assess the chemical composition of muscle of the Indian cat fishes and their variations within season and sex. An approximately 85% to 90% of fish protein is digestible and all the dietary essential amino acid is found in the

fish muscle. *Clarias batrachus, Heteropneustes fossilis* and *Anabas testudineus* are carnivorous air-breathing species.

7.2 MATERIALS AND METHODS

Chandrashekhar et al., (2008), Ali et al., (2003), Love (1970)

Biochemical character has been studied for three freshwater air-breathing fishes such as *Heteropneustes fossilis*, *Clarias batrachus* and *Anabas testudineus.* For the present study, fish samples are collected seasonally from freshwater ponds located at Hotar, Magrahat, South 24-Parganas, West Bengal, India during November, 2014 to October, 2016. Fishes were being brought to laboratory and washed the fishes in running water to remove the mucus from the outer surface of the body of fishes and muscle was collected. Wet muscle was dry in hot air oven and drying fish muscle was taken in pestle and made into fine powder and analysed by digestion, distillation and titration to estimate of crude protein percentage by Kjeldahl method. The amount of protein was then calculated from the nitrogen concentration of the food.

After pressing the anal zone of a fish, if it oozes out a brownish milt, it is sure to be a mature male fish. But, in case of a young but premature male fish, the milt looks whitish. On the other hand, when the anal zone of an adult and mature female fish is pressed, a good number of brownish eggs come out. In case of young but immature female fish, the eggs should be greenish in colour (Jhingran, 1975).

Protein

Central Institute of Fisheries

Weight mechine

Laboratory

Photo plate no. IX

Protein

Reagent of Sulphuric acid

Laboratory Work

Digestion of dry fish muscle

Distillation of sample (protein)

Digestion solution

Photo plate no. IX

Titration work of protein

Dry-weight basis: Determination of crude protein in dry fish muscle.

Aim: To study about the analysis of crude protein percentage in the dry fish muscle of the freshwater, air-breathing fishes of *Heteropneustes fossilis*, *Clarias batrachus* and *Anabas testudineus* on seasonal basis.

Principle: The organic nitrogen of muscle sample when digested with conc. H_2SO_4 in presence of catalyst (digestion mixture- H_2SO_4: $CuSO_4$ = 9:1) is converted to ammonium sulphate. Ammonia is liberated by making the solution of alkaline with 40% of NaOH and is distilled in a known volume of receiver solution (4% of boric acid with mixed indicator). The ammonia is then quantitatively titrated against standard H_2SO_4 solution with known strength. Crude protein can be determined by Kjeldahl method.

D. M. basis (Kjeldahl method):

Procedure -The Kjeldahl method can conveniently be divided into three steps:

Digestion, distillation and titration.

Digestion: Analysed food sample is weighed in a digestion flask and then digested by heating it in the presence of sulphuric acid (an oxidizing agent which digests the food), anhydrous sodium sulphate (to speed up the reaction by raising the boiling point and an catalyst copper to speed up the reaction). Digestion converts into nitrogen in the food (other than that which is in the form of nitrates) into ammonia and other organic matter of CO_2 and H_2O. Ammonia gas is not liberated in an acid solution as because the ammonia is in the form of the ammonium ion (NH_4^+) which bond to the sulphate ion (SO_4^-) and thus remains in solution.

N (food) $\rightarrow$ $(NH_4)_2\,SO_4$

Take 0.5 to 1 gm. dry muscle sample is digestion tube

Add 2 to 5 gm. (mean 3 gm.) digestion mixture

Add 20 ml conc. H_2SO_4 in the digestion tube

Mix the content well and place the digestion tube in the tube holder and cover with the lid which is connected with the tap water by 2 pipes. After switching on, set the digestion bench temperature at 250℃ (pre-heated temperature).

Place the digestion tube along with lid in the digestion bench with the help of tube holder.

Open the tap water and be sure that water is coming on from exit pipe.

After reaching the temperature of digestion bench to 250 ℃. Temperature, kept it for 5 minutes.

Again set the temperature of digestion bench at 350 ^{o}C.

After reaching the temperatures at 350 ^{o}C wait for 15 minutes.

After that, set the final temperature at 420 ^{o}C.

After reaching the final temperature wait for 45 minutes. The Appearance of clear blue colour digestion solution indicates completion of digestion. If the desired colour does not appear within the stipulated time period, continue the digestion for few more minutes for its completion.

Take out the digestion tube along with tube holder and lid from the digestion bench and wait for cooling the solution.

After cooling dilute the digested sample in distilled water and make the final volume of 100 ml in volumetric flask. Add little bit of water with digested solution and transfer to a volumetric flask. Keep 2 to 3 times repeated washing of digestion tube and transfer the content to volumetric flask. Make the final volume after proper cooling.

During digestion reaction:

$$\text{Organic N} + H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_2$$

Distillation: After the digestion has been completed, the digestion flask is connected to a receiving flask by a tube. Distillation is done with the help of semi-automatic nitrogen analysis.

Keep a container containing distilled water and connected to the analyser with the pipe at the tap.

Keep the container containing 40% of Na OH and connected to the analysed with the pipe on the platform at the right side of the analyser.

Open the knob of the water container.

Open the tap water (be sure that water in coming on through exit pipe).

Operation of N – Analyser

Switch on the main switch. Switch on the power button of the distillation machine. A sound will appear immediately after on the power button, sometimes sound may not appear. The indicator will appear when every arrangement in the machine will be completed. Take 10 ml of digested solution (aliquot) in a digestion tube and place it inside the distillation assembly at the left corner after opening the door of the assembly with insert the tube connected to the alkali container through distillation assembly. Take 10 ml of boric acid with mixed indicator in a conical flask and place it at the right corner of the distillation assembly and insert the ammonium flowing tube located in that position inside the conical flask (Sure that the tip of the tube has been completely submerged in the receiver solution).

Close the door of distillation unit. Press the alkali button to take desired quantity (40 ml) of 40% of Na OH solution in the digestion tube. Set the distillation time (9 minutes), within this period 60 to 80 ml of distillate may be collected using arrow key. Press the time setting button. After 9 minutes the distillation process will be automatically cut off with giving a sound. During distillation ammonia will be liberated and dissolved in pink colour receiver solution (4% of boric acid solution with indicator) and gradually the colour will be change to clear blue colour. After completion of distillation, take out the conical flask containing distillate from the distillation assembly for titration.

Reaction during distillation

a) $(NH_4)_2\ SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$

The ammonia gas that is formed in liberated from the solution and moves. The low pH (borate ion) of the solution in the receiving flask converts the ammonia gas into the ammonium ion and simultaneously converts the boric acid to the borate ion.

b) $3NH_3 + H_3BO_3 = (NH_4)_3BO_3$

(Ammonium, Borate complex) excess H_3BO_3

Titration: The nitrogen content is then estimated by titration of the ammonium borate formed with standard sulphuric acid (H_2SO_4) using a suitable indicator to determine the end point of the reaction.

Titrate the distillate of the conical flask against $\frac{N}{10}$ H_2SO_4 is taken in a burette. Appearance of pinkish colour indicates the end point of titration.

Note the volume of $\frac{N}{10}H_2SO_4$ consumed during titration.

Reaction during titration

c) $(NH_4)_2\ SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$

d) $3NH_3 + H_3BO_3 = (NH_4)_3BO_3$

(Ammonium Borate complex) Excess (H_3BO_3) borate.

Calculations

$2(NH_4)_3\ BO_3 + 3H_2SO_4 \rightarrow 3(NH_4)_2SO_4 + 2H_3BO_3$

1 ml $\frac{N}{10}$ H_2SO_4 = 0.0014 gm. of nitrogen.

Crude protein % = $\frac{0.0014 \times V \times D \times 100}{W \times A} \times 6.25$

Where,

V = Volume $\frac{N}{10}H_2SO_4$ consumed.

D = Dilution factor (the final volume of digested solution made, here it is 100).

W = Weight of sample taken for digestion.

A = Aliquot taken during distillation (10 ml).

Statistical Analysis

Statistical co-relation between the protein percentage and the body weight as well as the protein percentage and the total length have been computed using the Statistical software R.

7.3 RESULTS

As shown in (Table-48 & 49 and Fig. 42 & 43) seasonal variation in percentage value of total protein in body muscles like selected fishes like *Heteropneustes fossilis, Clarias batrachus* and *Anabas testudineus* collected from pond during November, 2014 to October, 2016.

This showed that a better amount of protein present in three different fishes is favourable for growth. This result interpreted of protein in fish depends on season, sex and reproductive cycle. Fish muscles of fishes are dehydrated by closed loop drying system using hot air convection.

Seasonal variations of total protein content (%) in body muscles of *Heteropneustes fossilis, Clarias batrachus* and *Anabas testudineus* collected from different seasons during November, 2014 to October, 2015

Muscle Analysis Report (DM basis)
(Kjeldahl Method)

Table- 48

Sl. No	Fish species / Sex	Parameter (Crude protein in % on the basis of DM)			
		Winter	Summer	Monsoon	Post Monsoon
1	*Heteropneustes fossilis* (Adult Male)	67.31 ±1.09	75.66 ±0.77	77.49 ±0.81	75.07 ±0.62
2	*Heteropneustes fossilis* (Adult Female)	73.18 ±0.38	68.75 ±0.64	75.09 ±0.12	71.88 ±0.61
3	*Heteropneustes fossilis* (Young Male)	71.3 ±0.33	73.18 ±0.32	74.8 ±0.46	73.06 ±0.04
4	*Heteropneustes fossilis* (Young Female)	68.05 ±0.40	75 ±1.18	74.06 ±0.46	71.19 ±0.03
5	*Clarias batrachus* (Adult Male)	72.64 ±0.03	69.49 ±0.02	74.43 ±0.06	73.19 ±0.03
6	*Clarias batrachus* (Adult Female)	72.76 ±0.02	68.06 ±0.06	72.92 ±0.05	71.82 ±0.03
7	*Clarias batrachus* (Young Male)	71.18 ±0.03	68.05 ±0.03	72.77 ±0.02	73.06 ±0.02
8	*Clarias batrachus* (Young Female)	72.76 ±0.01	64.18 ±0.02	74.21 ±0.01	71.04 ±0.02
9	*Anabas testudineus* (Adult Male)	68.99 ±0.13	69.36 ±0.02	68.89 ±0.03	66.5 ±0.29
10	*Anabas testudineus* (Adult Female)	67.68 ±0.01	66.58 ±0.02	66.33 ±0.01	68.36 ±0.02
11	*Anabas testudineus* (Young Male)	68.11 ±0.04	67.3 ±0.17	61.64 ±0.02	64.36 ±0.03
12	*Anabas testudineus* (Young Female)	66.1 ±0.09	66.11 ±0.03	62.97 ±0.02	65.79 ±0.02

Estimation of dry muscle protein (November, 2014 to October, 2015)

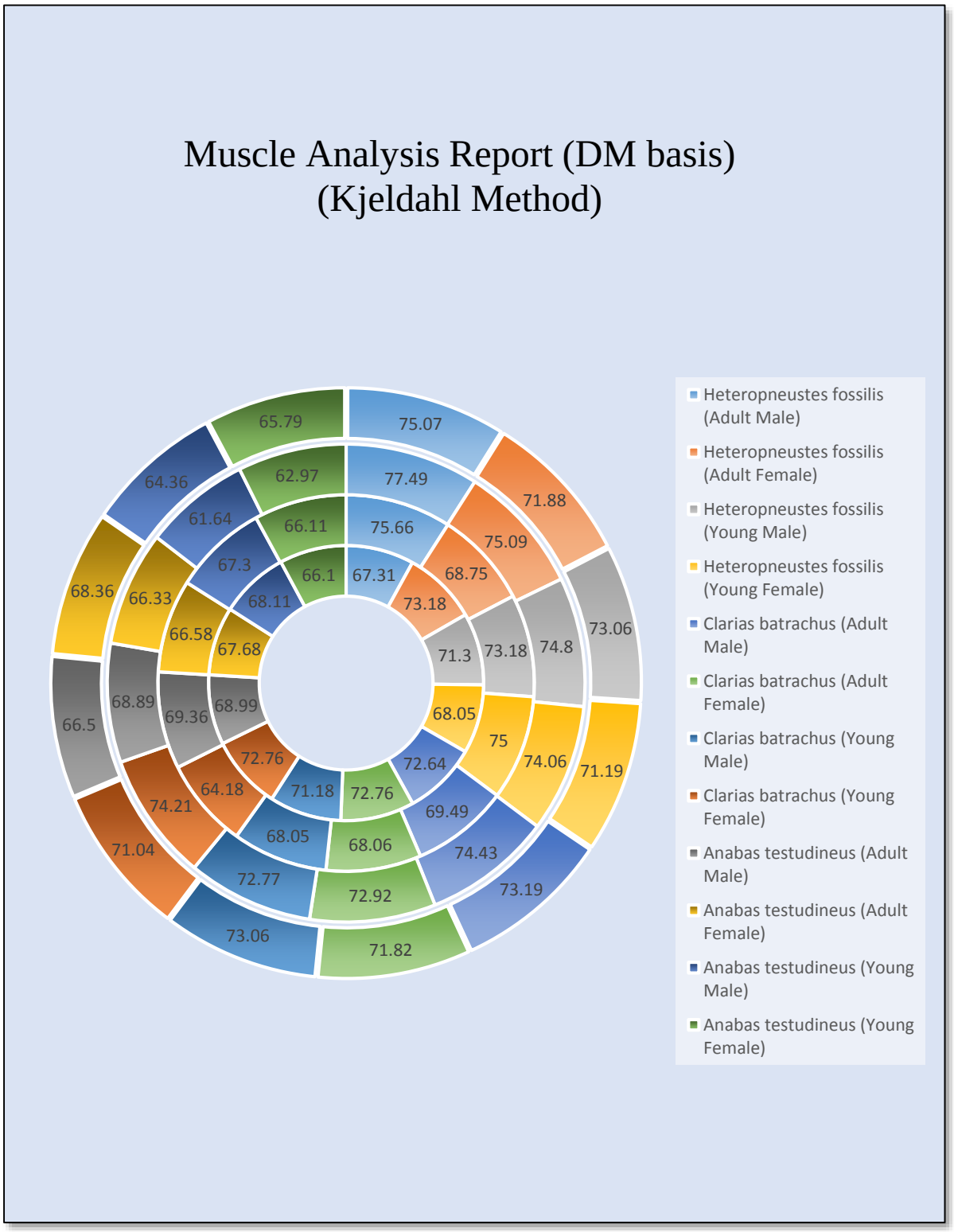

Fig. 42 Percentage contribution of muscle protein of *Heteropneustes fossilis, Clarias batrachus* and *Anabas testudineus* (adult & young, male & female) in 1st annual cycles.

Seasonal variations of total protein content (%) in body muscles of *Heteropneustes fossilis, Clarias batrachus* and *Anabas testudineus* collected from different seasons during November, 2015 to October, 2016

Muscle Analysis Report (DM basis)
(Kjeldahl Method)

Table-49

Sl. No	Fish species / Sex	Parameter (Crude protein in % on the basis of DM)			
		Winter	Summer	Monsoon	Post Monsoon
1	*Heteropneustes fossilis* (Adult Male)	66.74 ±0.52	72.37 ±0.36	76.69 ±0.56	73.34 ±0.25
2	*Heteropneustes fossilis* (Adult Female)	76.69 ±0.93	65.92 ±0.59	74.26 ±0.37	70.26 ±0.28
3	*Heteropneustes fossilis* (Young Male)	68.06 ±0.41	71.29 ±0.53	74.36 ±0.47	72.49 ±0.34
4	*Heteropneustes fossilis* (Young Female)	66.79 ±0.03	71.78 ±0.03	72.77 ±0.04	73.18 ±0.03
5	*Clarias batrachus* (Adult Male)	71.88 ±0.04	72.65 ±0.03	72.05 ±0.02	72.64 ±0.02
6	*Clarias batrachus* (Adult Female)	72.38 ±0.02	71.29 ±0.03	72.13 ±0.02	70.86 ±0.03
7	*Clarias batrachus* (Young Male)	72.2 ±0.11	67.3 ±0.84	73.06 ±0.01	71.46 ±0.03
8	*Clarias batrachus* (Young Female)	72.92 ±0.02	63.14 ±0.02	72.49 ±0.02	69.72 ±0.03
9	*Anabas testudineus* (Adult Male)	67.56 ±0.02	67.08 ±0.03	69.86 ±0.02	67.5 ±0.25
10	*Anabas testudineus* (Adult Female)	65.43 ±0.02	69.75 ±0.02	66.45 ±0.02	69.44 ±0.02
11	*Anabas testudineus* (Young Male)	69.86 ±0.03	68.05 ±0.02	60.64 ±0.02	63.98 ±0.04
12	*Anabas testudineus* (Young Female)	64.36 ±0.03	67.83 ±0.02	64.33 ±0.02	67.71 ±0.01

Estimation of dry muscle protein (November, 2015 to October, 2016)

Fig. 43 Percentage contribution of muscle protein of Heteropneustes *fossilis*, *Clarias batrachus* and *Anabas testudineus* (adult & young, male & female) in 2nd annual cycles.

Heteropneustes fossilis (Table-50 & fig. 44)

The proximate compositions of fish protein of dry muscles were presented in (Table-48 & 49). The protein contents are measured to be the highest in dry muscles in the four seasons of adult and young, male and female fishes. The mean values of protein content was highest in muscle, adult male 77.09±0.8 in monsoon and young male 74.58±0.5 in monsoon and adult female 74.94±1.8 in winter and young female 73.42±0.72 in monsoon. The protein content was lowest in adult male 67.03±0.9 in winter and young male 69.68±1.66 in winter and adult female 67.34±1.54 in summer and young female 67.42±0.69 in winter season. In the fish *Heteropneustes fossilis* protein level increased during the summer to monsoon and it decreases in post-monsoon to winter (Table-50, Fig. 44) spawning period. Jan et al., (2012) studied seasonal variation in the protein content of the muscles of *Heteropneustes fossilis* and they observed highest protein content in summer and lowest in winter season. During spawning, muscle protein started to transfer into ovaries to meet energy requirement of fish. It had been reported by Srikar et al., (1979). Protein level increased of both male and female in monsoon and decreased in winter of both male and female.

Mean values of dry muscle protein of *Heteropneustes fossilis* from November, 2014 to October, 2016

Table- 50

	Winter	Summer	Monsoon	Post-monsoon
Adult male	67.03±0.9	74.02±1.75	77.09±0.8	74.21±0.98
Young male	69.68±1.66	72.24±1.04	74.58±0.5	72.78±0.37
Adult female	74.94±1.8	67.34±1.54	74.68±0.49	71.07±0.94
Young female	67.42±0.69	73.39±1.81	73.42±0.72	72.19±0.99

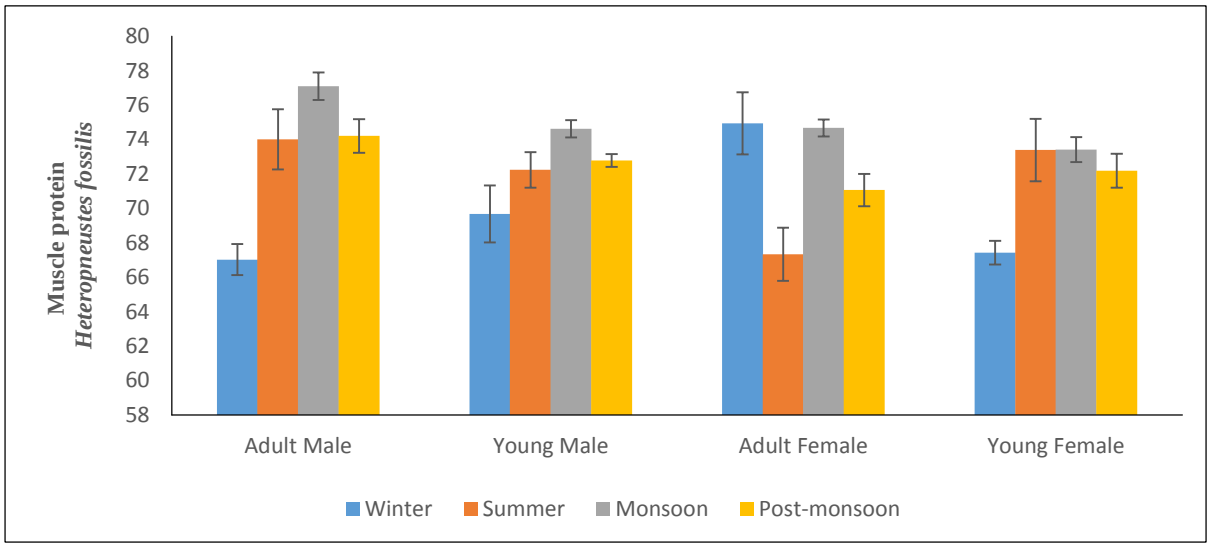

Fig. 44 Graphical representation showing mean protein in *Heteropneustes fossilis* (two annual cycles)

Clarias batrachus (Table-51 & fig. 45)

The mean of crude protein content of adult and young male and female are highest in muscle of adult male 73.24±1.19 in monsoon and young male 72.92±0.14 in monsoon and adult female 72.57±0.19 in winter and young female 73.35±0.86 in monsoon respectively. Similarly the protein content is also lowest in dry muscle of adult male 71.07±1.58 in summer and young male 67.68±0.39 in summer and adult female is 69.68±1.61 in summer and young female 63.66±1.52 in summer. Male and female, adult and young are increased in same monsoon whereas decreased in summer season. Protein level increases of both male and female in monsoon and decreases in summer. Protein level increases in November and starts decreasing again in June and July. Protein normally increases from January to June and then starts decreasing till the month of December in (Table-51 and Fig. 45).

Mean values of dry muscle protein of *Clarias batrachus* from November, 2014 to October, 2016

Table- 51

	Winter	Summer	Monsoon	Post-monsoon
Adult male	72.26±0.38	71.07±1.58	73.24±1.19	72.92±0.27
Young male	71.69±0.51	67.68±0.39	72.92±0.14	72.26±0.8
Adult female	72.57±0.19	69.68±1.61	72.53±0.39	71.34±0.48
Young female	72.84±0.08	63.66±1.52	73.35±0.86	70.38±0.66

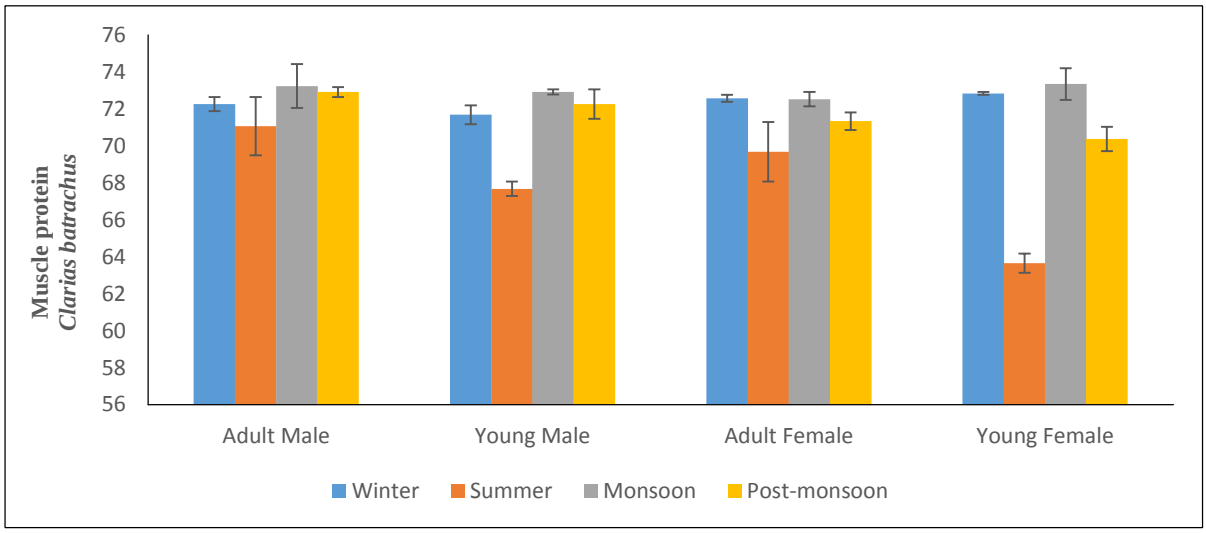

Fig. 45 **Graphical representation showing mean protein in *Clarias batrachus* (two annual cycles)**

Anabas testudineus (Table-52 & fig. 46)

The mean of crude protein content of adult and young male and female were highest in dry muscle of adult male 69.38±0.48 in monsoon and young male 68.99±0.87 in winter, whereas adult female 68.9±0.54 in post-monsoon and young female 66.97±0.63 in summer as well. Similarly, the crude protein content of adult and young male and female were lowest in dry muscle of adult male 67±0.56 in post-monsoon and young male 61.14±0.5 in monsoon and adult female 66.39±0.06 in monsoon and young female 63.65±0.68 in monsoon too. Protein level of *Anabas testudineus* increased in adult male in monsoon and young male in winter and decreased in adult male during post-monsoon whereas young male in monsoon and adult and young female increased in summer and decreased in monsoon in (Table-52 and Fig. 46).

The seasonal changes occurred in the protein contents of freshwater fishes vary with the variation of environmental changes, which depended on the fishing ground, seasons, age and sex and reproductive status of the individual. The spawning cycle and food supply are the main factors for responsible of this variation (Love, 1970).

Mean values of dry muscle protein of *Anabas testudineus* from November, 2014 to October, 2016

Table- 52

	Winter	Summer	Monsoon	Post-monsoon
Adult male	68.28±0.72	68.22±1.14	69.38±0.48	67±0.56
Young male	68.99±0.87	67.68±0.39	61.14±0.5	64.17±0.19
Adult female	66.56±1.12	68.17±1.58	66.39±0.06	68.9±0.54
Young female	65.23±0.87	66.97±0.63	63.65±0.68	66.75±0.96

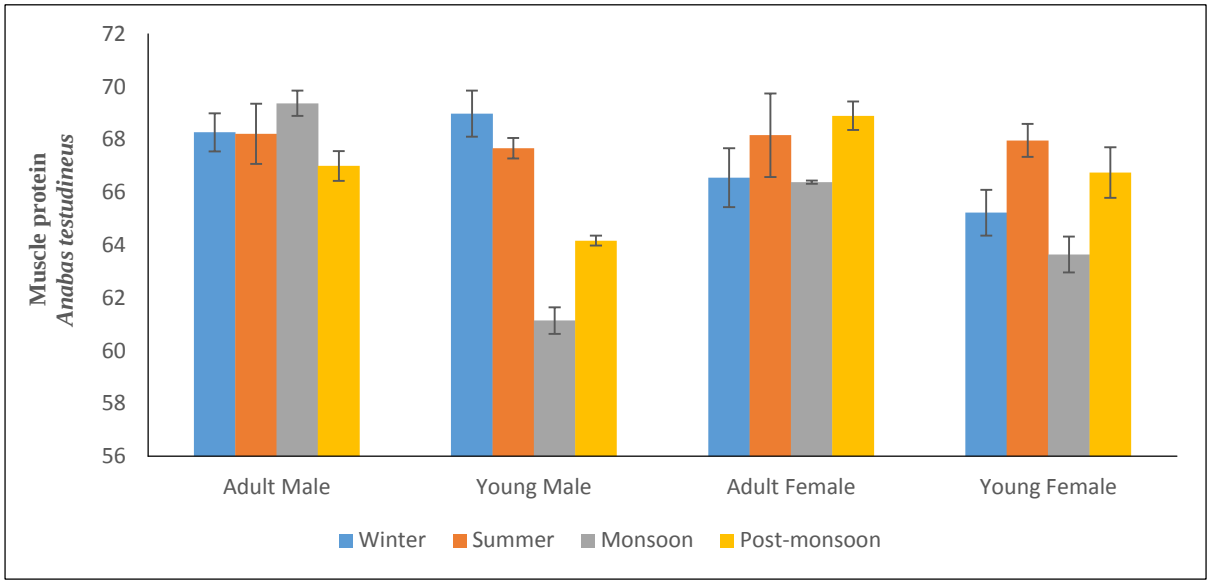

Fig. 46 Graphical representation showing mean protein in *Anabas testudineus* (two annual cycles)

Table-53

Statistical correlation between the protein % and the body weight as well as the total length of *Heteropneustes fossilis*.

Heteropneustes fossilis (Adult male)						*Heteropneustes fossilis* (Adult male)					
1st. year during November, 2014 to October, 2015						2nd. year during November, 2015 to October, 2016					
Sl. No	Descriptive	1st. year during November, 2014 to October, 2015				Sl. No	Descriptive	2nd year during November, 2015 to October, 2016			
		Winter	Summer	Monsoon	Post-Monsoon			Winter	Summer	Monsoon	Post-Monsoon
1	Body weight (gm.)	119.8	44.23	49.48	38.68	1	Body weight	110.4	49.2	58.3	48.13
2	Protein %	67.31	75.66	77.49	75.07	2	Protein %	66.74	72.37	76.69	73.34
3	Total length (cm)	29.1	21	23	2.04	3	Total length	28.2	22.1	24.5	22

Heteropneustes fossilis (Adult female)						*Heteropneustes fossilis* (Adult female)					
1st. year during November, 2014 to October, 2015						2nd. year during November, 2015 to October, 2016					
Sl. No	Descriptive	1st. year during November, 2014 to October, 2015				Sl. No	Descriptive	2nd year during November, 2015 to October, 2016			
		Winter	Summer	Monsoon	Post-Monsoon			Winter	Summer	Monsoon	Post-Monsoon
1	Body weight (gm.)	119.8	44.23	49.48	38.68	1	Body weight	110.4	49.2	58.3	48.13
2	Protein %	67.31	75.66	77.49	75.07	2	Protein %	66.74	72.37	76.69	73.34
3	Total length (cm)	29.1	21	23	2.04	3	Total length	28.2	22.1	24.5	22

Heteropneustes fossilis (Young male)						*Heteropneustes fossilis* (Young male)					
1st. year during November, 2014 to October, 2015						2nd. year during November, 2015 to October, 2016					
Sl. No	Descriptive	1st. year during November, 2014 to October, 2015				Sl. No	Descriptive	2nd year during November, 2015 to October, 2016			
		Winter	Summer	Monsoon	Post-Monsoon			Winter	Summer	Monsoon	Post-Monsoon
1	Body weight (gm.)	30.77	38.82	40.41	38.94	1	Body weight	36.7	50.7	47.73	43.65
2	Protein %	71.3	73.18	74.8	73.06	2	Protein %	68.06	71.29	74.36	72.49
3	Total length (cm)	19	20	21.03	19.07	3	Total length	21.2	22	22.2	22.5

Heteropneustes fossilis (Young female)						*Heteropneustes fossilis* (Young female)					
1st. year during November, 2014 to October, 2015						2nd. year during November, 2015 to October, 2016					
Sl. No	Descriptive	1st. year during November, 2014 to October, 2015				Sl. No	Descriptive	2nd year during November, 2015 to October, 2016			
		Winter	Summer	Monsoon	Post-Monsoon			Winter	Summer	Monsoon	Post-Monsoon
1	Body weight (gm.)	34.64	50.07	48.15	35.79	1	Body weight	40.63	58.6	53.18	40.81
2	Protein %	68.05	75	74.06	71.19	2	Protein %	66.79	71.78	72.77	73.18
3	Total length (cm)	19.5	22	21.07	19	3	Total length	22.1	23.3	23.1	21.8

Correlation Study

Heteropneustes fossilis

	Correlation between protein % and body weight	p. value	Correlation Between protein % and total length	p. value
Adult male	-0.878249883	0.004109832	-0.454152402	0.258308
Adult female	0.141470809	0.738260227	0.435625163	0.280655
Young male	0.2977969	0.473764434	0.031274558	0.941398
Young female	0.534302184	0.172519112	0.274260373	0.510967

Statistical correlation between the protein % and the body weight as well as the total length of *Clarias batrachus.*

Table-54

Clarias batrachus (Adult male)						*Clarias batrachus* (Adult male)					
1st. year during November, 2014 to October, 2015						2nd. year during November, 2015 to October, 2016					
Sl. No	Descriptive	1st. year during November, 2014 to October, 2015				Sl. No	Descriptive	2nd year during November, 2015 to October, 2016			
		Winter	Summer	Monsoon	Post-Monsoon			Winter	Summer	Monsoon	Post-Monsoon
1	Body weight (gm.)	117.79	120.58	141.31	61.89	1	Body weight	115.31	130.1	138.4	70.4
2	Protein %	72.64	69.49	74.43	73.19	2	Protein %	71.88	72.65	72.05	72.64
3	Total length (cm)	24.7	26.2	29.9	23	3	Total length	24.4	26.6	28.7	23.3

Clarias batrachus (Adult female)						*Clarias batrachus* (Adult female)					
1st. year during November, 2014 to October, 2015						2nd. year during November, 2015 to October, 2016					
Sl. No	Descriptive	1st. year during November, 2014 to October, 2015				Sl. No	Descriptive	2nd year during November, 2015 to October, 2016			
		Winter	Summer	Monsoon	Post-Monsoon			Winter	Summer	Monsoon	Post-Monsoon
1	Body weight (gm.)	100.58	190.41	130.6	67.65	1	Body weight	111.38	187.3	140.32	72.8
2	Protein %	72.76	68.06	72.92	71.82	2	Protein %	72.38	71.29	72.13	70.86
3	Total length (cm)	23	29.7	28.5	23.2	3	Total length	22.24	28.8	28.9	23.7

Clarias batrachus (Young male)						*Clarias batrachus* (Young male)					
1st. year during November, 2014 to October, 2015						2nd. year during November, 2015 to October, 2016					
Sl. No	Descriptive	1st. year during November, 2014 to October, 2015				Sl. No	Descriptive	2nd year during November, 2015 to October, 2016			
		Winter	Summer	Monsoon	Post-Monsoon			Winter	Summer	Monsoon	Post-Monsoon
1	Body weight (gm.)	77.6	112.29	82.9	59.02	1	Body weight	80.2	113.15	90.7	60.2
2	Protein %	71.18	68.05	72.77	73.06	2	Protein %	72.2	67.3	73.06	71.46
3	Total length (cm)	22.6	24.9	24.01	20.01	3	Total length	23.4	24.8	24.4	20.2

Clarias batrachus (Young female)						*Clarias batrachus* (Young female)					
1st. year during November, 2014 to October, 2015						2nd. year during November, 2015 to October, 2016					
Sl. No	Descriptive	1st. year during November, 2014 to October, 2015				Sl. No	Descriptive	2nd year during November, 2015 to October, 2016			
		Winter	Summer	Monsoon	Post-Monsoon			Winter	Summer	Monsoon	Post-Monsoon
1	Body weight (gm.)	46.85	106.84	91.41	60.48	1	Body weight	48.5	109.9	87.5	67.32
2	Protein %	72.76	64.18	74.21	71.04	2	Protein %	72.92	63.14	72.49	69.72
3	Total length (cm)	18.9	24.6	24.5	21.05	3	Total length	19.3	24.7	24.8	22.2

Correlation Study

Clarias batrachus

	Correlation between protein % and body weight	p. value	Correlation between protein % and total length	p. value
Adult male	-0.06850844	0.871948	0.164154036	0.697696
Adult female	-0.485690245	0.222411	-0.409890894	0.313198
Young male	-0.767490563	0.026199	-0.474420559	0.234924
Young female	-0.675075548	0.066219	-0.462440243	0.248611

Statistical correlation between the protein % and the body weight as well as the total length of *Anabas testudineus*.

Table-55

***Anabas testudineus* (Adult male)**						***Anabas testudineus* (Adult male)**					
1st. year during November, 2014 to October, 2015						**2nd. year during November, 2015 to October, 2016**					
Sl. No	**Descriptive**	**1st. year during November, 2014 to October, 2015**				**Sl. No**	**Descriptive**	**2nd year during November, 2015 to October, 2016**			
		Winter	**Summer**	**Monsoon**	**Post-Monsoon**			**Winter**	**Summer**	**Monsoon**	**Post-Monsoon**
1	**Body weight (gm.)**	**57.68**	**80.55**	**78.35**	**48.51**	**1**	**Body weight**	**60.58**	**76.3**	**80.21**	**57.2**
2	**Protein %**	**68.99**	**69.36**	**68.89**	**66.5**	**2**	**Protein %**	**67.56**	**67.08**	**69.86**	**67.5**
3	**Total length (cm)**	**14.7**	**17**	**17**	**15.03**	**3**	**Total length**	**14.8**	**17.3**	**16.9**	**15.4**

***Anabas testudineus* (Adult female)**						***Anabas testudineus* (Adult female)**					
1st. year during November, 2014 to October, 2015						**2nd. year during November, 2015 to October, 2016**					
Sl. No	**Descriptive**	**1st. year during November, 2014 to October, 2015**				**Sl. No**	**Descriptive**	**2nd year during November, 2015 to October, 2016**			
		Winter	**Summer**	**Monsoon**	**Post-Monsoon**			**Winter**	**Summer**	**Monsoon**	**Post-Monsoon**
1	**Body weight (gm.)**	**61.07**	**76.47**	**60.54**	**51.71**	**1**	**Body weight**	**65.8**	**81.47**	**72.13**	**60.7**
2	**Protein %**	**67.68**	**66.58**	**66.33**	**68.36**	**2**	**Protein %**	**65.43**	**69.75**	**66.45**	**69.44**
3	**Total length (cm)**	**16.2**	**17**	**15.1**	**15.05**	**3**	**Total length**	**17.3**	**17.4**	**15.8**	**15.7**

***Anabas testudineus* (Young male)**						***Anabas testudineus* (Young male)**					
1st. year during November, 2014 to October, 2015						**2nd. year during November, 2015 to October, 2016**					
Sl. No	**Descriptive**	**1st. year during November, 2014 to October, 2015**				**Sl. No**	**Descriptive**	**2nd year during November, 2015 to October, 2016**			
		Winter	**Summer**	**Monsoon**	**Post-Monsoon**			**Winter**	**Summer**	**Monsoon**	**Post-Monsoon**
1	**Body weight (gm.)**	**28.35**	**34.18**	**40.8**	**41.06**	**1**	**Body weight**	**35.25**	**40.1**	**51.6**	**45.3**
2	**Protein %**	**68.11**	**67.3**	**61.64**	**64.36**	**2**	**Protein %**	**69.86**	**68.05**	**60.64**	**63.98**
3	**Total length (cm)**	**12.3**	**14.2**	**14.05**	**14.05**	**3**	**Total length**	**12.2**	**15.3**	**15.6**	**15.1**

***Anabas testudineus* (Young female)**						***Anabas testudineus* (Young female)**					
1st. year during November, 2014 to October, 2015						**2nd. year during November, 2015 to October, 2016**					
Sl. No	**Descriptive**	**1st. year during November, 2014 to October, 2015**				**Sl. No**	**Descriptive**	**2nd year during November, 2015 to October, 2016**			
		Winter	**Summer**	**Monsoon**	**Post-Monsoon**			**Winter**	**Summer**	**Monsoon**	**Post-Monsoon**
1	**Body weight (gm.)**	**29.38**	**37.32**	**31.55**	**42.05**	**1**	**Body weight**	**31.14**	**49.35**	**42.55**	**50.5**
2	**Protein %**	**66.1**	**66.11**	**62.97**	**65.79**	**2**	**Protein %**	**64.36**	**67.83**	**64.33**	**67.71**
3	**Total length (cm)**	**13**	**14.5**	**13.01**	**15.03**	**3**	**Total length**	**10.3**	**16.4**	**15.4**	**15.7**

Correlation Study

Anabas testudineus

	Correlation between protein % and body weight	**p. value**	**Correlation between protein % and total length**	**p. value**
Adult male	0.648748682	0.081805036	0.386447834	0.34431929
Adult female	0.019313759	0.963795707	-0.054557996	0.89790657
Young male	-0.783138842	0.021529696	-0.610833392	0.10768881
Young female	0.688146548	0.059193583	0.608237658	0.10961113

Findings

The computed values of correlation coefficients along with the p-values have been displayed in the table-53 to 55.

For the *Heteropneustes fossilis*, the correlation coefficient between protein % and the body weight for the adult male is significant at 5% level. For other three categories (adult and young female and young male), no significant correlation coefficient is observed amongst the protein percentage and the body weight as well as the former and the total length.

For *Clarias batrachus*, we observe that only for the young male category, the correlation between the protein percentage and the body weight is significant at 5% level but for the rest categories, values of correlation coefficient are insignificant.

For *Anabas testudineus*, for the young male and young female categories we observe the significant correlation coefficient values between protein % and body weight whereas for other categories the values of protein % and total length as well as protein % and total length are insignificant.

Significant correlation coefficient values indicate the existence of linear association amongst the variables under study. The insignificant correlation coefficient values are very interesting findings here as it suggests that there might be nonlinear association amongst those traits.

7.4 DISCUSSION

As per the results are concerned about the protein content of *Heteropneustes fossilis, Clarias batrachus* and *Anabas testudineus* during November, 2014 to October, 2015 and November, 2015 to October, 2016 are discussed in a scientific ways as follows:

The muscles of body contain more amount of protein. The *Heteropneustes fossilis* exhibits maximum amount of protein in muscle in comparison to other two species of *Clarias batrachus* and *Anabas testudineus.* In *Heteropneustes fossilis*, protein content increases in summer and

winter and decreases in winter and summer in both male and female specimen. In *Clarias batrachus* protein content increases in monsoon and winter and decreases in summer of both male and female, young and adult. In *Anabas testudineus* protein content increases in monsoon and winter and decreases in post-monsoon in case of male adult only and in young male in monsoon whereas in adult female and young female, protein content increases in summer and decreases in monsoon as happened on the studies of Elagba et al., (2013) in case of female only. *Latas niloticus* (protein 74.2%), *Heterotis niloticus* (protein 78.8%), *Clarias lazera* (protein 59.9%), *Protopterus annectans* (protein 68.00%) whereas the percent value in case of *Heteropneustes fossilis*, *Clarias batrachus* and *Anabas testudineus* are as follows:

Heteropneustes fossilis (protein 77.49 %), *Clarias batrachus* (protein 74.21%) and *Anabas testudineus* (protein 69.86%). These findings of major changes in the body composition of fishes have been brought by the changes in the environmental conditions in the ponds water in different seasons, nutritional status and breeding period of fish Ali et al., (2003).

The protein content of dry muscle increased gradually to reach the highest in spring in both the sexes of fish whereas the lowest values are found in the rainy season. It has been found that the spawning periods of fishes are the rainy season. Siddiqui et al., (2010) examined the protein level of *Mystus tangara*, highest in pre-monsoon and lowest in monsoon in cat fish.

The fishes studied show increase in body protein in pre-monsoon period whereas in post-monsoon these values decline because of use of protein in preparation of eggs and growth of the body. Further decrease in body protein in summer also throw light towards the higher temperature and scarcity of food (Love 1970).