Journal of Aquatic Biology & Fisheries, Vol. 2(1) 2014: 168-177

STUDY OF BIOCHEMICAL MOIETIES IN FRESHWATER FISH, *LABEO ROHITA* FED ON FORMULATED FEED

Nadaf, S.B. and Bhilave, M.P.*
Division of Fisheries Science, Department of Zoology, Shivaji University, Kolhapur.
**Corresponding author: drmadhavbhilave@yahoo.com*

Received on: 11.07.2013; accepted on: 10.11.2013

Abstract: On a global scale, fish and fish products are the most important source of protein in the human diet. The fish protein is relatively of high digestibility compared to other protein source. It comprises of ten essential amino acids in desirable quantity for human consumption. All these properties bring the fish flesh to be in the superior class as compared to milk, beef protein and egg albumen. The biochemical composition indicates the fish quality. Study of biochemical composition of fish helps to assess its nutritional and edible value in terms of energy units. Hence the present work was undertaken to 'study biochemical moieties in fresh water fish, *Labeo rohita* fed on formulated feed'. Twelve week experiment was conducted in glass aquaria to study biochemical moieties in fingerlings of freshwater fish, *L. rohita* fed on conventional and combinations of formulated feeds from earthworm belonging to the species *Eisenia faetida* and deoiled groundnut cake. The fishes were fed regularly at the rate of 5% of the total body weight once in a day. After specific time intervals the fishes were weighed and sacrificed for tissues like liver, intestine and muscle. The tissues were quickly excised and cleaned off extraneous material, weight and used for biochemical estimations like total protein, total glycogen and total lipid, by standard methods. Results revealed that the biochemical parameter like protein content was highest in intestine followed by muscle and liver. The glycogen was found to be highest in liver tissue followed by muscle and intestine; while intestine was rich in lipid followed by liver and muscle. The biochemical concentrations were highest in the fishes fed on combinations of formulated feed as compared to conventional group.

Key words: *Labeo rohita, Eisenia faetida,* biochemical parameter, formulated feed

INTRODUCTION

Fish and fish products are recognized as the most important source of protein in the human diet. This protein is relatively of high digestibility compared to other protein source. It comprises of all the ten essential amino acids in desirable quantity for human consumption. All these properties bring the fish flesh to be in the same class as chicken protein and are superior to milk, beef protein and egg albumen. In general, the biochemical composition of the whole body indicates the fish quality. Therefore, proximate biochemical composition of a species helps to assess its nutritional and edible value in terms of energy units compared to other species. Variation of biochemical composition of fish flesh may also occur within same species depending upon the fishing ground, fishing season, age and sex of the individual and reproductive status. The spawning cycle and food supply are the main factors responsible for this variation (Love *et al.*, 1988). Knowledge of biochemical composition is of great help in evaluating not only fish nutritive value but also helps in quality assessment and optimum utilization of these natural recourses (Rodriguez-Gonzalez *et al.,* 2006). This in turn can help in processing the fish into products and other byproducts without wastage or loss of constituents such as free amino acids, proteins and fats. Biochemical investigations on fish help to evaluate the impact of environment.

Fish are known to need a high proportion of protein in their diet because they metabolize

protein as an energy source. Production of fish protein ingredients is growing throughout the world. Increased demands for traditional raw materials for production of fish protein ingredients are leading to great pressure on fish stocks (Hultin *et al*., 2000). This has led to over-fishing of many of the more traditional species and has required governmental intervention to prevent the collapse of important species (Hultin*et al.,* 2005). Since a variety of fishes as animal protein source has a high nutritive value, consumption of them not only meets many nutritive requirements of body, but also is useful to improve human health. So many countries have tried to increase per capita consumption of this nutritive source (Dvorak, 2002). Fish protein is a healthy, sustainable and high nutritive product that sanitisedly produced from fishes (Dong, 1993). Proteins are the major organic materials in most fish tissue, and form an important component of the diet. One of the major requirements of fish culture is the efficient transformation of dietary protein into tissue protein (Weatherley and Gill, 1987). However, protein is essential for normal tissue function, for the maintenance and renewal of fish body protein and for growth. Because of the cost of the protein the feed will be more cost effective if all the protein is used for tissue repair and growth and little catobolized for energy (Jauncey, 1998). From a practical point of view, the ideal situation should tend to maximize the use of dietary protein for growth, minimizing the use of proteins for functional protein synthesis, gluconeogenesis, lipogenesis and energy (Lovell, 1998).

Glycogen is a molecule that serves as the secondary long-term energy storage in animal and fungal cells, with the primary energy stores being held in adipose tissue while lipids , along with proteinsare the major organic components of fish and in most cases of their feeds also; carbohydrates are at least quantitatively less important. In fish, the lipids and their constituent Fatty Acids (FA) along with their metabolic derivatives, such as the eicosanoids, play significant roles in various functions of the organism, including growth, health and reproduction (Sargent *et al.,* 2002). Dietary lipids provide energy and Essential Fatty Acids (EFA) to the fish and they also assist the absorption of fat-soluble vitamins (NRC, 1993). Lipids are of great importance not only to fish nutrition but also to human nutrition.

MATERIALS AND METHODS

Formulation of Feeds

Fully grown earthworms of species *Eisenia faetida* of about 20 to 30 cms were collected. They were brought to the laboratory, washed, cleaned and weighed. Then they were sacrificed by introducing in boiling water. Sacrificed earthworms were then squashed using mortar and pestle. Ingredients such as, corn flour, milk powder, agar powder, turmeric powder, garlic paste, cumin powder and pepper powder were added. The mixture was boiled till it became semisolid mass. Then it was cooled to room temperature. After cooling vitamin mixture and cod liver oil was added. The mixture in semisolid form was kept in refrigerator at temperature 15^0C for 12 hrs. After 12 hours it was removed from refrigerator, brought to room temperature and then squeezed over polythene sheet and dried for 48 hrs. The dried nodules were crushed into small pellets. Pellets were sun dried to avoid fungal infection, weighed and stored in the bottles.

Following the above procedure the feeds were formulated in five combinations, viz. 100% conventional feed (100% deoiled groundnut cake), 100% formulated feed (100% earthworm),75% formulated feed (75% earthworms + 25% deoiled ground nut cake), 50% formulated feed (50% earthworms + 50% deoiled groundnut cake) and 25% formulated feed (25% earthworms + 75% deoiled groundnut cake).

Experimental Protocol

The fingerlings of freshwater fish *Labeo rohita* were brought to the laboratory and acclimatized in rectangular glass aquaria of 36x12" with 60 liters capacity containing aerated water for seven days. During acclimatization adequate aeration was provided and temperature was maintained from

28°C to 30°C. The fishes which survived during acclimatization were distributed randomly into five aquaria (15 in each) and labeled as per the feed combination. They were fed at the rate of 2% of total body weight. The feeding was done once in a day. The body weights and lengths were recorded at each time interval i.e.30, 45, 60, 75 and 90 days throughout the experimental period.

Biochemical Studies

After time intervals of 30,45,60,75 and 90 days fingerlings of fishes (five from each group) were taken, weighed and sacrificed for tissues like liver, intestine and muscle. The tissues were quickly excised and cleaned off extraneous material, weighed and used for biochemical estimations like total protein by Garnall *et al.* (1949), total glycogen by DeZwaan and Zandee (1949) and total lipid by Barnes and Blackstock (1973).The experiments were repeated for three times. The mean value was calculated and expressed in mg/100 mg of tissue.

RESULTS AND DISCUSSION

Table 1. Protein content of Liver from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Duration in days	100% Conventional fish feed	100% Formulated fish feed	75% Formulated fish feed	50% Formulated fish feed	25% Formulated fish feed
30	19.24±1.57	31.24±1.50***	25.17±1.52***	30.02±1.45 NS	22.53±1.67***
45	23.53±1.55	33.45±1.62***	27.97±1.93***	30.65±1.17***	25.17±1.72***
60	27.87±1.75	37.30±1.41***	32.58±1.59***	37.65±1.81***	30.55±1.52***
75	26.93±1.98	40.35±1.43***	37.52±2.11***	45.38±1.43**	32.65±1.83**
90	31.83±1.62	47.41±1.69***	45.46±1.61***	50.21±1.41***	35.61±1.76**

(Value expressed is mean of n (n=5) ± SD)

*P<0.05, **P< 0.01, ***P< 0.001, NS – Non Significant

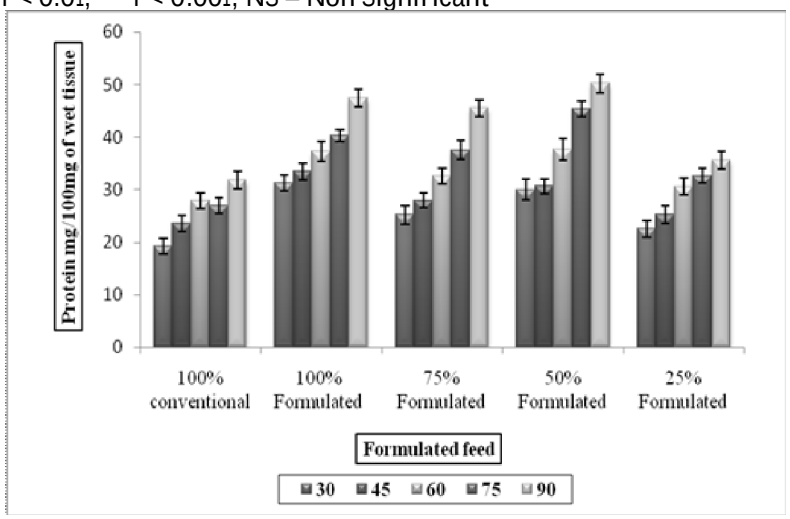

Fig. 1. Protein content of Liver from*Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Table 2. Glycogen content of Liver from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Duration in days	100% Conventional fish feed	100% Formulated fish feed	75% Formulated fish feed	50% Formulated fish feed	25% Formulated fish feed
30	27.49±1.005	30.26±1.816**	30.23±1.137***	35.27±1.076***	30.25±1.240***
45	32.45±1.009	34.47±1.010***	40.33±1.283***	37.51±1.305***	35.16±1.159***
60	35.19±1.087	37.61±1.180***	47.43±1.123**	45.47±1.249**	37.60±1.376*
75	37.46±1.414	32.47±1.337***	55.09±1.358***	52.36±1.329**	40.31±1.548**
90	40.47±1.466	42.49±1.266***	62.53±1.218***	60.25±1.651***	52.39±1.382***

(Value expressed is mean of n (n=5) ± SD)

*P<0.05, **P< 0.01, ***P< 0.001, NS – Non Significant

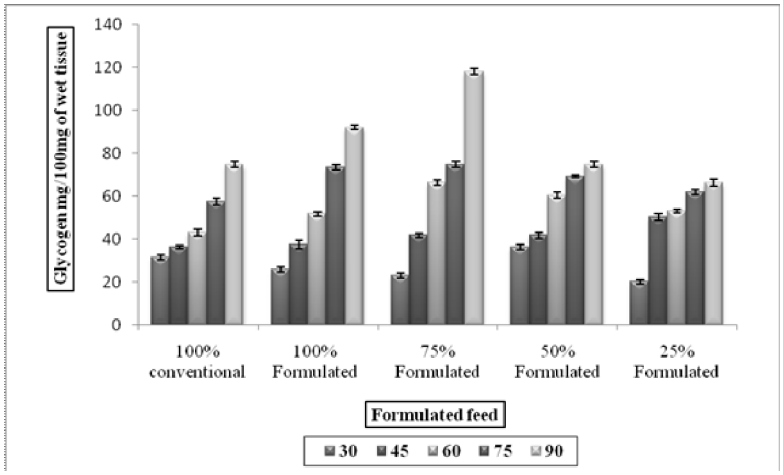

Fig. 2. Glycogen content of Liver from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Table 3. Lipid content ofLiver from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Duration in days	100% Conventional fish feed	100% Formulated fish feed	75% Formulated fish feed	50% Formulated fish feed	25% Formulated fish feed
30	31.47±1.295	25.92±0.856***	23.05±1.653**	36.24±1.573**	20.16±1.556*
45	36.33±1.201	37.44±2.061***	41.76±1.034***	41.75±1.085***	50.42±1.099**
60	43.23±1.249	51.85±1.130***	66.24±1.234***	60.46±1.285***	53.26±1.299***
75	57.49±1.218	73.44±1.435***	74.86±1.404**	69.13±0.5724***	61.93±1.322***
90	74.87±0.9866	92.15±1.586***	118.05±0.794***	74.98±1.365**	66.23±1.727 NS

(Value expressed is mean of n (n=5) ± SD)

*P<0.05, **P< 0.01, ***P< 0.001, NS – Non Significant

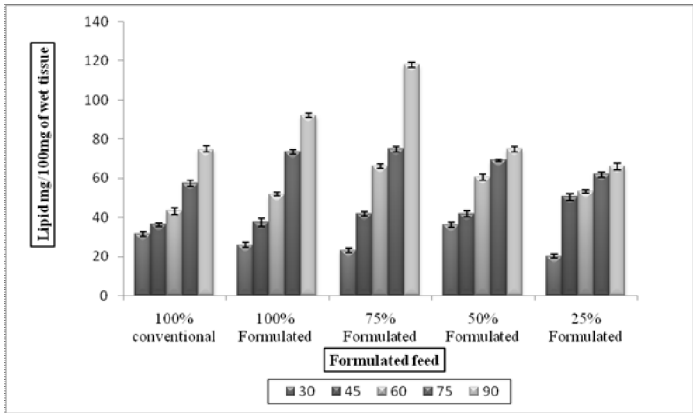

Fig. 3. Lipid content ofLiver from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Table 4. Protein content of Intestine from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100 mg of wet tissue)

Duration in days	100% Conventional fish feed	100% Formulated fish feed	75% Formulated fish feed	50% Formulated fish feed	25% Formulated fish feed
30	21.87±1.551	30.46±1.541***	32.01±1.416***	25.67±0.936***	27.49±1.714***
45	32.65±1.457	33.29±2.047***	37.51±1.483**	40.34±1.161***	34.26±1.363*
60	37.74±1.600	47.41±1.264***	42.59±1.886***	47.67±1.397***	40.52±1.495***
75	41.21±1.445	54.86±1.624***	52.63±1.429***	50.23±1.518***	42.56±1.683*
90	43.35±1.571	60.01±1.356***	60.25±1.415**	62.42±1.178***	45.15±1.398**

(Value expressed is mean of n (n=5) ± SD)

*P<0.05, **P< 0.01, ***P< 0.001, NS – Non Significant

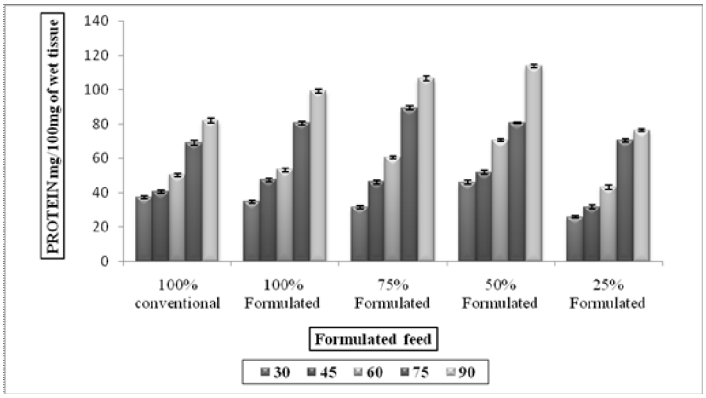

Fig. 4. Protein content of Intestine from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100 mg of wet tissue)

Table 5. Glycogen content of Intestine from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Duration in days	100% Conventional fish feed	100% Formulated fish feed	75% Formulated fish feed	50% Formulated fish feed	25% Formulated fish feed
30	12.61±1.150	17.52±1.676***	22.43±1.351***	20.23±1.700*	15.24±1.909***
45	17.39±0.9564	22.39±1.130**	30.38±1.377***	25.44±1.171***	20.12±1.560***
60	22.40±0.9218	25.38±1.106***	32.45±1.337**	32.50±1.102*	25.50±1.092*
75	20.23±1.184	29.61±2.083**	45.13±1.648***	42.40±1.803***	27.51±1.527***
90	25.36±1.519	34.48±0.8471***	45.16±2.476***	40.57±0.959***	32.63±1.187**

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

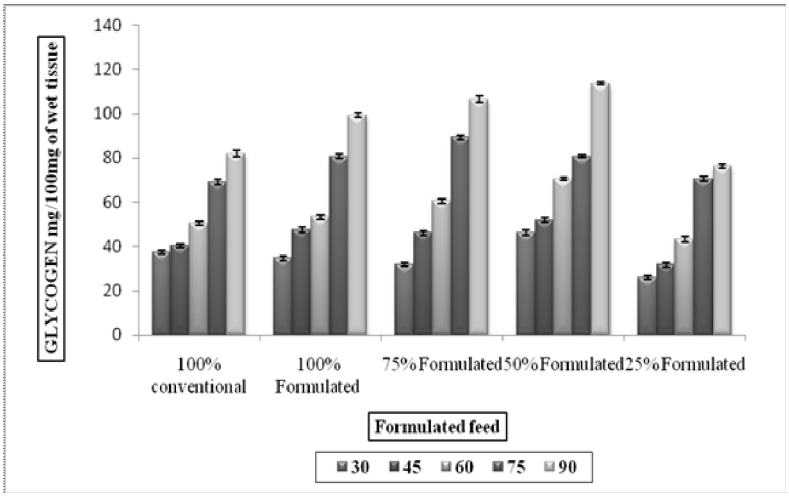

Fig. 5. Glycogen content of Intestine from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Table 6. Lipid content of Intestine from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100 mg of wet tissue)

Duration in days	100% Conventional fish feed	100% Formulated fish feed	75% Formulated fish feed	50% Formulated fish feed	25% Formulated fish feed
30	57.61±0.990	64.81±1.283***	46.08±1.942***	51.85±1.293***	50.41±1.109***
45	69.13±0.990	60.48±1.444***	56.17±0.600***	59.06±1.478**	60.48±1.114**
60	73.45±1.328	69.13±1.840***	84.97±1.771**	74.88±1.274***	69.12±0.935**
75	83.53±1.024	84.96±1.624**	106.56±1.181***	87.84±1.346**	90.72±0.987***
90	99.36±0.9765	103.68±0.834**	126.72±0.659***	148.32±0.642**	112.32±1.353*

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

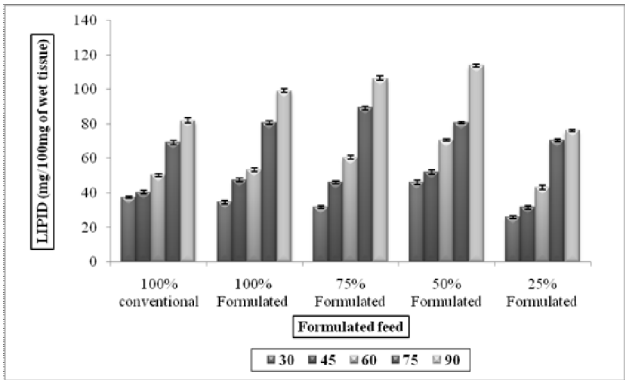

Fig. 6. Lipid content of Intestine from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100 mg of wet tissue)

Table 7. Protein content of Muscle from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Duration in days	100% Conventional fish feed	100% Formulated fish feed	75% Formulated fish feed	50% Formulated fish feed	25% Formulated fish feed
30	22.19±1.680	33.23±1.467***	27.85±1.632***	32.57±1.540***	24.65±1.566*
45	32.03±1.433	35.23±1.712***	32.77±1.664***	35.61±1.507*	32.68±1.406**
60	33.52±1.312	42.65±1.347***	44.90±1.732*	42.76±1.571***	35.58±1.579***
75	35.26±1.558	50.37±1.610***	47.63±1.423***	47.30±1.655**	37.61±1.774**
90	42.41±1.316	57.47±2.142***	52.64±1.104***	57.39±1.295***	47.45±1.266**

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

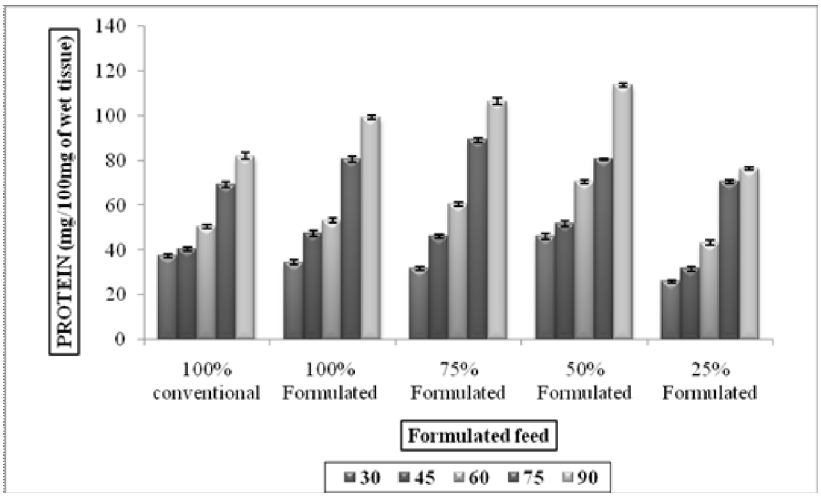

Fig. 7. Protein content of Muscle from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Table 8. Glycogen content of Muscle from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Duration in days	100% Conventional fish feed	100% Formulated fish feed	75% Formulated fish feed	50% Formulated fish feed	25% Formulated fish feed
30	22.43±1.437	25.28±0.972**	27.50±1.603***	30.30±1.881***	25.34±0.959*
45	25.31±2.116	27.39±1.311***	32.58±1.790***	30.16±1.440**	27.60±1.312***
60	27.44±1.377	30.22±1.197***	40.23±1.622***	37.61±1.583***	32.44±1.686**
75	30.26±1.492	35.16±1.363***	50.10±1.388***	47.43±1.539**	35.27±1.692***
90	35.20±1.440	37.47±1.357***	50.33±1.408***	47.28±1.453***	42.27±1.211**

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

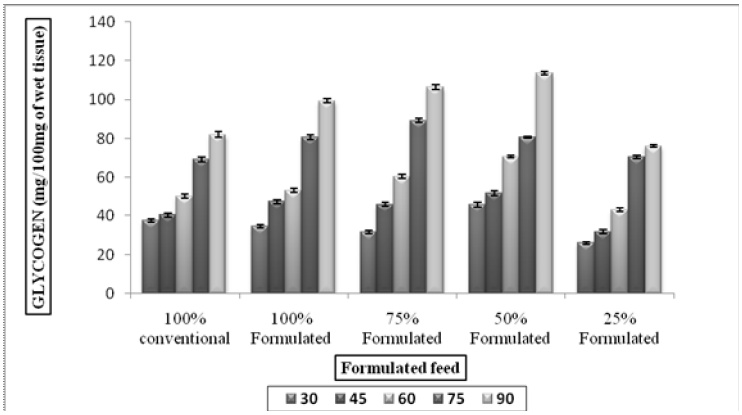

Fig. 8. Glycogen content of Muscle from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Table 9. Lipid content of Muscle from *Labeo rohita* fed on conventional and combinations of formulated feed(mg/100mg of wet tissue)

Duration in days	100% Conventional fish feed	100% Formulated fish feed	75% Formulated fish feed	50% Formulated fish feed	25% Formulated fish feed
30	37.45±0.782	34.57±0.943***	31.67±0.874***	46.07±1.340***	25.94±1.425*
45	40.33±0.921	47.50±1.191***	46.08±1.053***	51.85±1.121***	31.67±1.031**
60	50.40±0.8014	53.25±0.978*	60.48±0.959*	70.57±1.069**	43.22±1.341**
75	69.13±1.219	80.63±1.173**	89.28±0.661***	80.64±0.572*	70.55±0.761***
90	82.07±0.7314	99.36±1.074***	106.56±1.178**	113.75±0.946**	76.32±0.659**

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

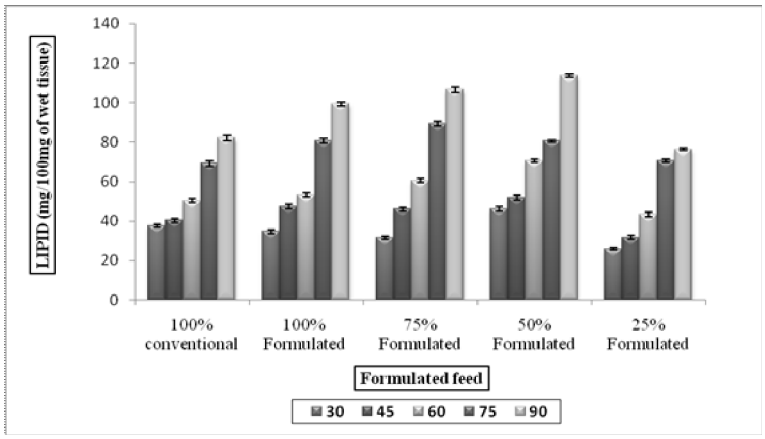

Fig. 9. Lipid content of Muscle from *Labeo rohita* fed on conventional and combinations of formulated feed (mg/100mg of wet tissue)

Fish has been recognized as an excellent food source for human beings for centuries and is preferred as a perfect diet not only due to its excellent taste and high digestibility but also because of having higher proportions of unsaturated fatty acids, essential amino acids and minerals for the formation of functional and structural proteins (Anonymous, 2003; Kumar, 1992). The total lipid contents of fish are reported to vary significantly with gradual increase in the weight and length of the fish and also due to seasonal changes aside from the available nutrients in varied habitats. Fish diets containing high levels of proteins are necessary for the economic growth of fish in intensive rearing conditions (De-Silva and Anderson, 1998). These increments of body lipid by increasing dietary lipid level have been reported in previous studies (Kaushik and Oliva-Teles, 1985; Medale *et al.,* 1991).

Glycogen levels are found to be highest in liver, as it is the chief organ of carbohydrate metabolism in animals, followed by muscle. Muscle rich in proteins, forms mechanical tissues intended for mobility and do not participate in metabolism. Liver being the centre for various metabolisms is also rich in proteins.

From biochemical observations of *Labeo rohita,* it was concluded that, the protein,glycogen and lipid content of liver was highest in fishes fed on 75% formulated feed as compared to 100% conventional feed. Protein and lipid content of intestine was highest in fishes fed on 50% formulated feed while lipid content of intestine was highest in fishes fed on 75% formulated feed. The biochemical observations from muscle shows, highest protein content in fishes fed on 100% formulated feed, glycogen content in 75% formulated feed and lipid in 50% formulated feed.As far as tissues were considered, the biochemical parameter like protein content was highest in intestine followed by muscle followed by liver. The glycogen deposition was found highest in liver followed by muscle and by intestine while intestine was rich in lipid followed by liver and muscle.

ACKNOWLEDGEMENT

Authors are thankful to the Head, Department of Zoology, Shivaji University Kolhapur, for providing laboratory and other infrastructure facilities.

REFERENCES

Anonymous (1952). The Wealth of India. Council of Scientific and Industrial Research, NewDelhi, India, pp. 35-36.

Barnes, H. and J. Black Stock (1973). Estimation of lipids in marine animals, detail investigation of sulphophosphovanillin method. *J. Exp. Mar. Biol. Ecol.,* 12:103-118.

De Silva, S.S. and T.A. Anderson (1998). Fish nutrition in aquaculture. Chapman and Hall, 208 pp.

DeZwaan, A. and D. I. Zandee (1972). Body distribution and seasonal change in glycogen content of common sea mussel, *Mitilus edulis*. *Comp. Biochem .Physio*.,43:53-55.

Dong, F. M., N. F. Hardy, F. T. Barrows, B. A. Rasco, W. T. Fairgrieve and I. P. Foster, (1993). Chemical composition and protein digestibility of poultry byproduct meals for salmonid diets. *Aquaculture*, 116: 149-158.

Dvorak, P. (2002). "Something fishy is going on in Japan in the ice cream,"*Journal of Wall Street*. Eastern edition. Pg. A.1.

Garnall, A. G., J. Charles, Bardwell and Maxima, M. David (1949). Determination of serum protein by means of the biurrete reagent. *J. Biol. Chem*., 177: 751-766.

Hilton, J. W. and J. L. Atkinson,(1982). Response of rainbow trout *Salmo gairdneri* to increased levels of available carbohydrate in practical trout diets. *British J. Nutr*., 47: 597-607.

Jauncey, K. and Ross, B. (1998): A guide to tilapia feed and feeding. University of Stirling, Scotland, UK**.**

Kaushik, S. J. and A. Oliva-Teles,(1985): Effects of digestible energy on nitrogen and energy balance in rainbow trout. *Aquaculture*, 50: 89-101.

Kumar D. (1992):Fish culture in un-drainable ponds. A manual for extension F.A.O. Fisheries Technica paper. No. 235. Rome: 239.

Lovell, T. (1989) : Nutrition and Feeding of Fish. An AVI Book, Van NostrandReinhold,New York, p 260.

Love, R.M.(1988):*The food fishes; their intrinsic variation and practical implications*. Farrand press, London.276 pp.

Medale, F., P. Aguirre and S. J. Kaushik, (1991): Utilization of dietary carbohydrates by rainbow trout at two water temperatures. In: Wenk, C., Boessinger, M. (eds), Energy Metabolism of Farm Animals. Proc. 12th Symp. Energy Metabolism of Farm Animals, KartauseIttingen, Switzerland, 1–7 September 1991. Eaap Pub., 58: 392–395.

Nutrition Research Council(1983). Nutrient requirements of cold water fishes. *Natl. Acad.Sci.,* Washington, DC.63 p.

Rodriguez-Gonzalez, Hernandez-Llamas, H. A. Villarreal,H. Sauedo, P.E..Garcia-Ulloa, M. and Rodriguez- Jaramillo (2006): Gonadal development and biochemical composition of female cryfish *Cherax quadricarinatus* (Decapoda: Parastacidae) in relation to the gonado-somatic index at first maturation. *Aquaculture,*254: 634-645.

Sargent, J. R. and A. G. J. Tacon, (1999). Development of farmed fish: a nutritionally necessary alternative to meat. *Proc. Nutr. Soc*., 58: 377-383.

Weatherley, A.H. and Gill, H.S., (1987). The Biology of Fish Growth. Academic Press, London, U.K. 443p.