Study Of Protein Quality Of Some Fresh And Smoke-Dried Hill Stream Fishes From Manipur, India

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Abstract: Protein quality of some hill stream fishes was studied by in-vivo experiments of feeding Albino rats for 28 days. Growth rate, apparent digestibility (AD), true digestibility (TD), protein efficiency ratio (PER), food conversion ratio (FCR), and biological value (BV) of the fish species *Neolissochilus stracheyi, Labeo pangusia, Semiplotus manipurensis, Schizothorax* sp. and *Ompok* sp. were determined. True digestibility (TD) values varied from 82.04±0.04 to 96.41±0.20%, biological value (BV) 96.10±0.25 to 97.72±0.01, PER 2.31±0.03 to 2.77±0.85 FCR 3.61±0.9 to 4.33±0.08 in the fresh fishes, while the TD value, PER, FCR, and BV were from 78.81±1.04 to 95.37±0.43%, 2.27±0.09 to 2.76±0.08, 3.62±0.29 to 4.39±0.04, 96.10±0.25 to 97.83±0.18 respectively in the smoke-dried fishes. All the fishes have high biological value compared with casein. There is no much difference between the fresh and smoke-dried samples as effect of processing. All the studied fish species were comparably good with lower value of *Ompok* sp. with reference to casein.
[Abdul Hei and Ch. Sarojnalini. **Study Of Protein Quality Of Some Fresh And Smoke-Dried Hill Stream Fishes From Manipur, India.** *N Y Sci J* 2012;5(11):1-6]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 1

Key words: In-vivo protein digestibility tests; true digestibility; protein efficiency ratio; biological value; hill stream fishes.

1. Introduction

Hill stream fishes are an important part of fish resources of Manipur as the maximum areas of the state are hill regions which have a number of streams and drainages. Along with distinctive ecological features of hill streams, the hill stream fishes stand out from the other fresh water fishes with quality. As a part of the diet of the people of the state, they are sources of many vital nutrients. Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body (Andrew, 2001). Fish protein contains all the essential amino acids and is considered to be complete protein (Devadas, 1994; Gopakumar, 1997). However protein composition varies with different species, and within the same species and with the mode of utilization. The quality of protein is determined by the amino acid composition and availability of essential amino acids. In other words, the quality is determined by the ability to supply essential amino acids and nitrogen required for manufacturing molecules and tissues. Digestibility is an important factor for determining adsorption of the essential amino acids and nitrogen by the consumers.

Smoking is an important method for preserving the hill stream fishes in the north eastern India. Smoked hill stream fishes are important among the smoked fishes of Manipur. Today smoking process is a traditional method of considerable economic importance world wide (Huda et al., 2010).The processing and preservation of fish were of utmost importance since fish is highly susceptible to quick physical deterioration, to prevent economic loses (Okenta and Ekelemu, 2005). Productions of smoked herrings are reported from Germany, the Netherlands, Scotland, England and Norway. Smoked salmon is highly valued in France (Espe et al., 2004). Mackerel, sprat, herring, salmon and trout are used as new materials for smoking fishes in Poland (Koloziejska et al., 2002, Usydus et al., 2009).Cat fish *Clarias gariepinus* is used as raw material for smoked fish in Turkey and Nigeria (Yanar,2007). *Pungasius sutchi* and catfish *Mecrones nemurus* and *Cryptoterus micronemaim* are used for smoking in Indonesia (Amin and Tjipto, 2001, Huda et al., 2010).

Evaluation of protein or amino acids through growth is one of the most rigorous of all methods, integrating most of the functions of proteins into one measurement. The nitrogen retained in growing animals is the sum of the fractions of nitrogen retained for growth and maintenance. Ammu et al. (1986) evaluated the nutritional quality of sardine soluble by feeding trials with albino rats. The authors noticed higher growth rate in whose diet dried sardines replaced half of the casein those diet had casein as the role of protein. This showed that the fish soluble had some unidentified growth factors. There are some reports on the nutritive value, in-vitro and in-vivo tests of fishes in Manipur (Sarojnalini and

Vishwanath, 1988, 1994; Singh et al.,1990; Lilabati and Vishwanath, 1996, 1998; Vishwanath et al., 1998). However there is no such information on the hill stream fishes of the state. The production of a sufficient supply of good quality protein sources to meet human requirement is a major challenge for the future as population growth continue throughout the world (Reeds et al., 2000).There is a need for accurate evaluation of quality of the protein sources as it is to be designed for the human demands, specially for women, children and adults according to their different requirements.

The present study was adopted to test in-vivo digestibility of protein of the fresh and smoke-dried hill stream fishes using 21 days old weanling rat (*Rattus norvegicus*) for the determination of growth rate, food conversion ratio (FCR), protein efficiency ratio (PER), biological value (BV), apparent digestibility (AD) and true digestibility (TD) etc.

2. Materials and Methods

2.1. Sample Collection and Preparation

The hill stream fishes *Neolishochilus stracheyi* (29-34.5cm in standard length), *Labeo pangusia* (18.5-22.5cm), *Semiplotus manipurensis* (27-28.5), *Schizothorax* sp. (21-20cm) and *Ompok* sp.(18-21.5 cm) were selected for the study. The fresh fishes were caught and collected from the different hill-streams and the smoked dried fishes from the markets of hill districts of Manipur during the month of March to June. The collected fishes consisted of six fishes of each species. The edible muscles of the fishes were taken for the various analyses**.**

2.2 In- vivo digestibility of protein

2.2.1. Preparation of diets

Diets were prepared as per the modified procedure described by Singh et al., (1990). Control diet was prepared by using 10 percent standard protein casein (Hi media) as the source of animal protein. In test diets, 10 percent of defatted and dehydrated powdered fish proteins were taken as the animal's protein source. The compositions of the diets used in the feeding experiments are shown in table1.

2.2.2. Rat feeding experiment

Rat feeding experiment for nutritional evaluation was done as per the procedure of Singh et al., (1990). Twenty one days old ± 2 days weanling male albino rats weighing 16-25g each from the same colony were used in the feeding trials The rats were divided into three groups those fed with (1) casein diet (2) Protein free diet (3) Fish protein diet. The rats were kept in separate cages for each group 6 rats were taken. The cages used in the experiment were washed and sterilized everyday with absolute alcohol and then dried. The whole body mainly the claws, tail and anal opening of the rats were also clean everyday with cotton soaked in absolute alcohol. Food and water were provided *ad labitum* and kept at room temperature (22-26^{0}C) during the feeding experiment. The weight gain and food consumed by each rat were noted down at an interval of 4 days. Faeces and urine were collected by using 5.0 percent sulphuric acid for the whole period of feeding trails. Calculation was done by using the relationship given by Pomeranz and Meloam (1971). Growth rate was calculated by direct measuring the weight of the individual rats at an interval of 4 days using electronic balance.

2.3. Statistical Analysis

Six samples were used for each determination. The data were subjected to one way-ANOVA and the significant means were compared by Duncan's multiple range tests using SPSS version 12 .0 and the data are presented as means ± standard deviations.

3. Results

Data on the in-vivo protein digestibility of the fresh and smoked hill stream fishes are shown in the table 2 and 3. Growth rate in Fig.1 & 2 and PER in Fig.4 & 5 are shown for the feeding trials of 28 days. In the fresh fish samples, the retained nitrogen was between 8.99±0.04 g and 4.58±0.44 g, and in smoked dried samples the value was between 10.05±0.72 g and 5.19±0.11g. the highest was in *Labeo pangusia* and the lowest was in *Ompok* sp. The absorbed nitrogen was between 9.20±0.04 and 4.69±0.44 g in fresh samples and between 10.15±0.72 and 5.40±0.11 g in smoked samples. The apparent digestibility was between 93.99 ±0.45 and 78.37±0.60 % in fresh samples, while in smoke dried samples it was between 92.86±0.64 and 76.21±0.48%.

True digestibility was between 96.41±0.20 and 82.04±0.39 % in the fresh samples. In smoked dried fishes, true digestibility was 95.37±0.37 and 79.29±0.81%.The lowest was in *Ompok* sp. Biological value was between 97.72±0.01 and 96.10±0.24% in all the fresh samples while it was between 97.94±0.14 and 96.10±0.25% in smoke dried fish samples. The highest biological value was in *Semiplotus manipurensis* and the lowest was in *Ompok* sp. Food conversion ratio in fresh fishes was 3.61±0.09 and 4.33±0.08% in fresh fishes and 3.62± 0.29 and 4.39± 0.4% in smoke dried fishes. Protein efficiency ratio was between 2.77±0.05 and 2.31± 0.03 % in fresh fishes and in smoked fishes it was 2.76±0.08 and 2.27±0.09 . The highest protein efficiency ratio was in *Schizothorax* sp. and the Lowest was in *Ompok* sp. The weight gained was between 53.85 g and 36.46 g in smoke-dried fishes. The highest weight gained was in *L. pangusia* in fresh fishes samples.The lowest growth rate was in *Ompok* sp.

Table 1: Composition of diets used in the feeding experiment.

Ingredients	A	B	C	D	E	F	G	H	I	J	K	L
Casein Vitamin free (g)	10.00	-	-	-	-	-	-	--	-	-	-	-
Fish powder (Lipid free) g	-	-	13.16	12.16	12.62	12.16	17.49	14.87	12.36	12.72	12.74	29.59
Refined groundnut oil (ml)	9.00	4.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Vitamin and salt mixture (g)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sucrose (g)	-	20.00	-	-	-	-	-	-	-	-	-	-
Cellulase (g)	-	5.00	-	-	-	-	-	-	-	-	-	-
Starch (g)	-	70.5	-	-	-	-	-	-	-	-	-	-
Wheat flour (g)	80.50	-	77.34	78.34	77.88	78.34	73.01	75.63	78.14	86.78	77.76	59.91

A = Casein diet, B = Protein free diet, C = fresh *N. stracheyi*, D = fresh *L. pangusia*, E = fresh *S. manipurensis,* F = fresh *Schizothorax* sp, **G =** fresh *Ompok* sp, H= smoked *N. stracheyi*, I= smoked *L. pangusia*, J= smoked *S. manipurensis*, K= smoked fresh *Schizothorax,* sp, L= smoked *Ompok* sp.

Table 2: Data on in-vivo protein digestibility tests of fresh hill stream fishes in comparison with casein.

Digestibility data	Casein	*Neolissochilus stracheyi*	*Labeo pangusia*	*Semiplotus manipurensis*	*Schizothorax* sp.	*Ompok* sp.
Total nitrogen in diet g	9.03±0.45b	8.49±0.13c	9.61±0.13c	8.73±0.15b	8. 64±0.08b	5.86±0.61a
Nitrogen in excreta (g)	0.83±0.04b	0.61±0.05a	0.62±0.09a	0.67±0.05a	0.56±0.03a	1.28±0.15c
Nitrogen retained (g)	8.20±0.24b	7.98±0.17b	8.99±0.04c	8.27±0.10b	8.47±0.07b	4.58±0.44a
Nitrogen absorbed (g)	8.41±0.04b	8.19±0.17c	9.20±0.04c	8.27±0.9b	8.68±0.05b	4.59±0.44a
Apparent digestibility (%)	90.80±0.07b	93.99±0.45cd	93.55±0.97d	92.33±0.44c	93.80±0.31d	78.15±0.37a
True digestibility (%)	92.13±0.07b	95.29±0.49d	95.40±0.49d	94.73±.039c	96.41±0.20e	80.00±0.39a
Biological value (%)	97.50±0.15c	97.44±0.06b	97.72±0.01c	97.46±0.10b	97.58±0.25b	96.1±0.24a
Food conversion ratio (%)	4.05±0.19ab	3.83±0.03a	4.00±0.32a	4.02±0.10a	3.61±0.09a	4.33±0.08a
Food intake (g)	168.56±3.7b	184.62±2.18c	215.64±2.10e	190.24±3.4d	164.45±4.66a	161.90±1.64a
Protein efficiency ratio (PER)	2.47±3.7b	2.61±0.04b	2.51±0.06b	2.49±0.10b	2.77±0.05c	2.31±0.03a

Note: Values are mean ± SD for 28 days of feeding trials.
Values with different letters in the same row are significantly different (P<0.05) in ANOVA test.

Table 3: Data on in-vivo protein digestibility tests of smoke-dried hill stream fishes in comparison with casein.

Digestibility data	Casein	*Neolissochilus stracheyi*	*Labeo pangusia*	*Semiplotus manipurensis*	*Schizothorax* sp.	*Ompok* sp.
Total nitrogen in diet (g)	9.03±0.45b	8.70±0.085b	10.35±0.92c	10.97±0.97c	8.36±0.51a	6.81±0.51a
Nitrogen in excreta (g)	0.83±0.04b	0.65±0.04	0.80±0.07b	0.95±0.13b	0.60±0.06a	1.62±0.08c
Nitrogen retained (g)	8.20±0.24b	7.27±0.20b	9.55±0.79d	10.05±0.72d	7.76 ±0.44bc	5.19±0.11a
Nitrogen absorbed (g)	8.41±0.04b	8.48±0.20d	9.70±0.79d	10.26±72d	7.82±0.44bc	5.40±0.11a
Apparent digestibility (%)	90.80±0.07b	92.86±0.64d	92.30±0.58d	91.60±0.05c	92.83±0.28d	76.21±0.48a
True digestibility (%)	93.13±0.60b	94.34±0.31d	93.53±0.17cd	92.53±0.05c	95.37±0.43cd	78.29±81a
Biological value (%)	97.50±0.15c	97.40±0.43d	97.83±0.18cd	97.94±0.14d	97.36±0.43de	96.10±0.25a
Food conversion ratio (%)	4.05±0.19abc	3.91±0.03ab	4.09±0.21abc	4.20±0.22bc	3.62±0.29a	4.39±0.4c
Food intake (g)	168.56±3.7a	185.13±4.92b	215.41±4.83c	190.01±5.00b	162.46±1.63a	162.04±2.05a
Protein efficiency ratio (PER)	2.47±3.7b	2.57±0.07b	2.50±0.03b	2.48±0.04b	2.76±0.08c	2.27±0.09a

Note: Values are mean ± SD for 28 days of feeding trials.
Values with different letters in the same row are significantly different (P<0.05) in ANOVA test.

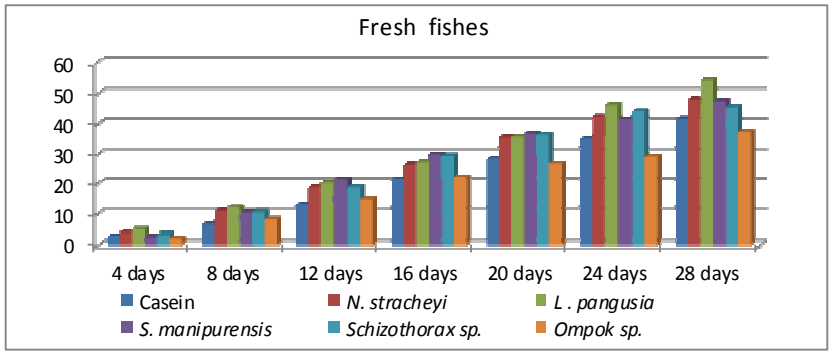

Fig.1. Weight gained by the experimental rats feeding on the fresh fish during 28 days

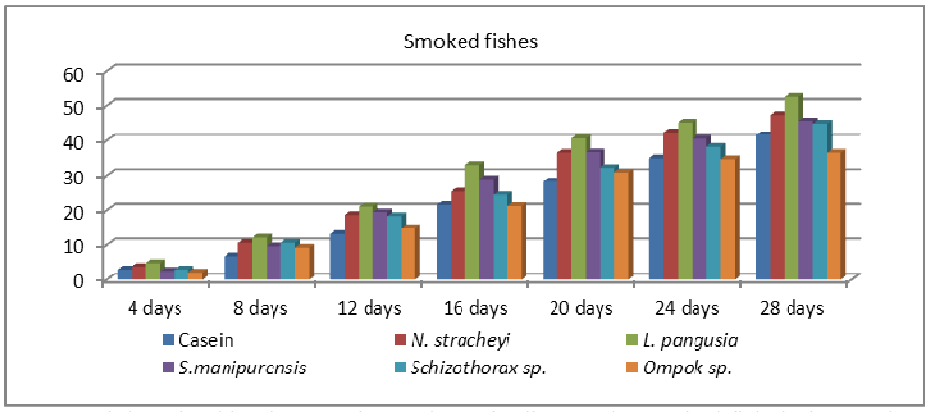

Fig.2. Weight gained by the experimental rats feeding on the smoked fish during 28 days

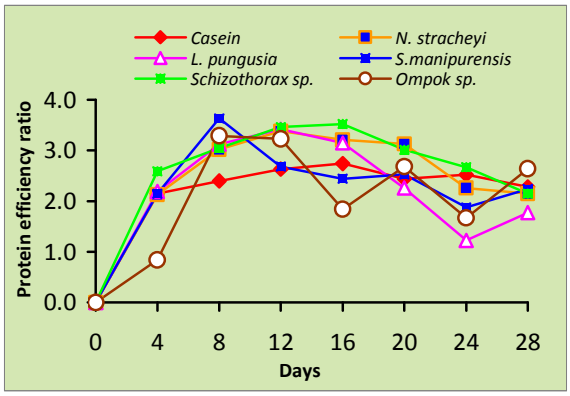

Figure 4. PER at every interval of four days growth during 28 days of feeding trials for fresh fish.

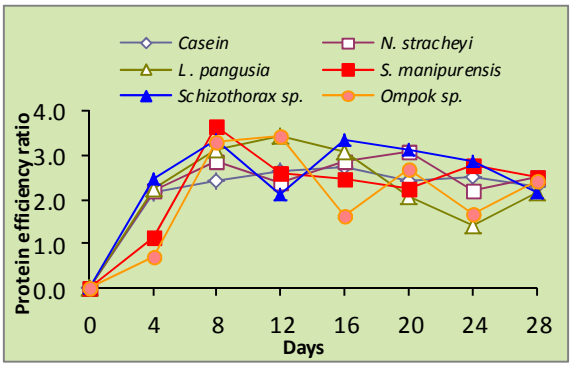

Figure 5. PER at every interval of four days growth during 28 days of feeding trials for smoked fish.

4. Discussion

The studied fishes live in an environment with unique ecological features of hill streams. With seasonal variations, they face strong current of muddy water in rainy seasons or clear water in rocky and green environment in non-rainy seasons. The fishes should have strong and healthy bodies for survival. They are noted for their taste and values. The fish based diets show different growrh rates. All the fishes *Neolissochilus stracheyi, Labeo pangusia, Semiplotus manipurensis* and *Schizothorax* sp. except *Ompok* sp. show higher growth rate than reference casein (Figure 1&2) and also higher protein efficiency ratio (PER) than reference casein (Figure 4&5).The fresh fishes shows slightly higher growth performance and higher PER than the smoked fishes. This may be due to denaturation of protein during smoking process. The rate of growth of an animal depends upon the increased consumption of protein. Amino acids viz, histidine, isoleucine, leucine, methionine, threonine, phenylalanine, lysine, valine, and tryptophan are essential in diets for growth and other physiological activities of the living system. The amino acid composition of a protein is directly correlated to its nutritive value and thus, essential amino acid is a chief limiting factor for the animal nutrition. The lower value of the PER may be due to the loss of the essential amino acids on processing. Bhuiyan et al. (1986) reported a significant decrease of PER value of Mackerel in the process of hot smoking.According to Indian standard specifications, protein rich concentrated nutrient supplementary foods should have protein efficiency ratio of 2.0 (ISI, 1982). So all the fresh and smoke- dried fishes can be considered as foods which meet this specification. However smoke processing saves the fishes from further deterioration and preserves the fishes.

According to Eves and Brown (1993), smoking preserves the fishes and promotes the digestibility.

The protein digestibility values of the present study are higher than the values reported by Singh et al. 1990, and Sarojnalini and Vishwanath,1994 on sundried and smoked fishes of Manipur. They reported that the growth rate of rats fed on the fishes based diets were higher than the casein diet. Vishwanath et al. (1997) reported that the digestibility value and PER were 91.5 % and 2.51 respectively in fresh *Monopterus albus* and 84.4 % and 2.31 in the smoked fish sample. According to FAO report (1970), protein digestibility value is 100% in the fish, South African Hake, 95% in Sardine and 90% in canned Tuna respectively.

Talabi et al. (1979) evaluated the nutritional value of big eye fish (*Brachydeuterus auritus*) for fish meal using feeding tests. They observed that the digestibility of big-eye fish meal was not affected by protein concentration in the dietary range of 10% to 20% crude protein. Even though NPU and PER decreased slightly, weight gain and protein intake increased with increasing dietary concentration and suggested that higher levels of incorporation of fish meal resulted in increased growth.

Nitrogen retention of a body determines the quality of a protein. Allison (1949) stated that the retention of nitrogen in the animal body is a function of essential amino acids. The amino acid composition of a protein is directly correlated to its nutritive value. Thus, essential amino acid is a chief limiting factor for animal nutrition.

Nitrogen retention in an animal can be evaluated by the differences between the nitrogen intake and nitrogen excreted. This difference called nitrogen balance shows whether an animal is maintaining or loosing or gaining nitrogen. The nitrogen retained in growing animals is the sum of the fractions of nitrogen retained for growth and for maintenance. In adults the retained nitrogen is utilized only for the maintenance of the nitrogenous integrity of the tissues while in the growing animals it is used for increases in body protein as well as maintenance (Barnes, 1946). Biological value, protein efficiency ratio and net protein utilization (NPU) are capable of demonstrating qualitative differences in the nutritive value of various protein sources (Young et al., 1973). The biological value (BV) of protein is the fraction of the absorbed protein nitrogen i.e. retained in the body. According to concepts developed by Mitchell, the BV of a protein is the sum of the nitrogen utilized for maintenance and growth expressed as a percentage of the absorbed nitrogen. It can be evaluated through nitrogen balance, protein intake, growth, tissue regeneration and amino acid analysis etc.

Determination of protein efficiency ratio (PER) is a measure of protein quality because the estimation of nutritional value obtained depends upon the amount of food consumed and the protein content of the diet. In determining the protein quality (PER, BV, true digestibility, NPU) of some processed fish using rats and comparing to values for casein, Udvarbe et al. (1985) added that increased fish consumption would improve the protein quality of diets. Discussing all these values in the present experiments of the hill stream fishes, the protein quality is slightly better in the fresh hill stream fishes than in the smoke dried fishes which is still well comparable with the standard casein. The studied fishes may be designed as a very good source of dietary animal protein that will promote growth, and maintain health and reproduction for all the human beings specially, children, women and adults.

5. Conclusion

The present studies show that the fishes are a very good dietary source of highly digestible protein with good protein efficiency ratio and high biological value, not only being a source of other nutrient lipid, macro and micro mineral elements, vitamins and a safe food from the hill stream ecosystems.

Acknowledgement

Authors are grateful to the Head, Department of Life Sciences, Manipur University for providing the laboratory facilities.

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9/1/2012