Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 3, Issue, 12, pp.069-075, December, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOENERGETICS, AMINO ACID ABSORPTION AND *IN VIVO* PROTEIN BIOSYNTHESIS IN A SILUROID FISH, *CLARIAS BATRACHUS* (LINN.)

Bidhan Chandra Patra

Aquaculture Research Unit, Department of Zoology, Vidyasagar University, Midnapore 721 102, West Bengal, India

ARTICLE INFO

Article History:
Received 17th September, 2011
Received in revised form
14th October, 2011
Accepted 18th November, 2011
Published online 31st December, 2011

Key words:
Bioenergetics,
Satiation time,
Feeding time,
Protein requirement,
Essential amino acid requirement,
Intestinal absorption,
Protein biosynthesis.

ABSTRACT

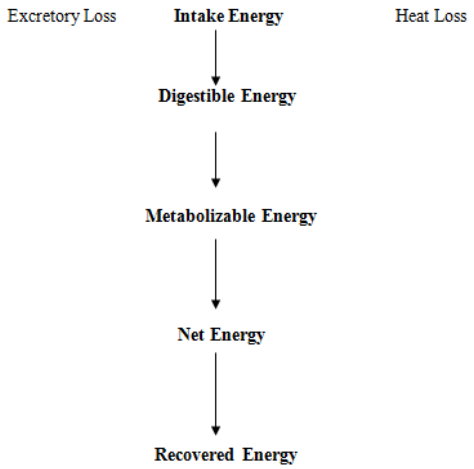
A three months experimental trial was conducted with variable percentage levels of protein (25, 30, 35, 40, 45 & 50) diet fed to the common Indian catfish, *Clarias batrachus* to study the satiation time, maximum feed intake in different hours of the day, protein/dietary energy requirement for optimum growth in different size groups, amino acid absorption and quantitative requirement, *in vivo* protein biosynthesis etc. and finally to calculate the requirement of digestible energy for the production of one gram fish to thousand gram in weight. It was observed that the maximum feed intake recorded with smaller size groups (5.0 g) as compared to 20.0 g and 50.0 g, satiated within one hour and highest appetite was recorded at 18.00 hours of the day. Maximum growth were observed with 45.0% protein (5.0 ± 0.3g) followed by 40% (20.0 ± 2.1 g) and 35% (50.0 ± 3.6 g) in *Clarias batrachus,* although growth continued till 50% of dietary protein but not significant at the 0.5% level. Maximum weight increase recorded with 1: 14.85 digestible energy / digestible protein ratio. Maximum amino acid absorption takes place in the posterior serosal layer of the intestine. It was also recorded that the same 10 amino acids are essential and their requirement was almost at par with the other fish species, and as in higher vertebrates. However, the importance of cystine and tyrosine for the growth of *Clarias batrachus* should not be ignored and should be considered as two additional essential amino acids. The protein biosynthesis *in vivo* is highly correlated with the dietary protein/energy, feed intake and growth performance of fish.

INTRODUCTION

Dietary protein is always considered to be of primary important in fish feeds as the protein requirements of fish are higher than those of terrestrial animals as well as the protein is the basic building nutrient of any growing animal and muscle constitutes, anatomically, the major component of the fish body (68-85% dry weight). The degradation products of protein are absorbed from the intestinal content as amino acids or peptides (Matthews, 1975; Patra, 2011). Individual amino acids are readily absorbed against concentration gradients and their absorption appears to be completed to transport of inorganic ions (Forman-farmaian *et al*., 1972). Protein and peptides in the intestinal content are probably also taken up to some extent, without previous degradation by pinocytosis or related processes (Patra, 2011).

A satisfactory level and balance of amino acids in a diet do not guarantee that ingestion of the diet will satisfy the amino acid requirements of the fish. This could be made only by proper digestion and most important is the absorption from the intestinal content i.e. low molecular weight protein, peptides and amino acids.. And, as there is no significant work is available in the absorption and incorporation of protein by the common Indian freshwater catfish fish, *Clarias batrachus* (gastric i.e. with stomach), the present study has been designed to find out / detect the site of absorption of protein macromolecules/ peptides/ amino acids in the Gastro Intestinal tract, rate of absorption of protein molecules and amino acids from the intestinal content and finally assimilation efficiencies of amino acids in the liver, body muscle. Fate of ingested energy as feed has been estimated by Cho and Kaushik (1990) in rainbow trout (16.0 to 145.0g in size) as fasting metabolic heat production, in kcal $fish^{-1}$ day^{-1}, to be $8.85W^{0.82}$, where W is body weight. However, Smith (1989) reported this for the same fish as $4.41W^{0.63}$ in lower size group (4.0 to 50.0 g). National Research Council (1993) stated that there are several places where energy is lost between ingestion and weight gain. A dietary excess or deficiency of useful energy can reduce growth rate. In practice, fish nutritionists have given priority to meeting the requirements for protein (but not the quantity of plant and animal protein and amino acids thereof), major minerals, and the vitamins, and generally have allowed energy to take care of itself but not fat (ratio of plant and animal fat) and carbohydrate (digestible and indigestible ratio). Also, practical feeds (30% protein) for most species made with commonly available ingredients, contains approximately 2.9 kcal g^{-1} which provides a digestible energy (kcal) to digestible protein (gram) ratio of 10:1, but for maximum weight gain for several fish species are ranging from 8.5 to 12.3 kcal g^{-1}. Protein and amino acid allowances as a function of dietary

***Corresponding author:** bcpatra@yahoo.com*



Growth in terms of protein deposition in muscle

energy plane, that is, as the energy concentration of the diet increases, the protein percentage increases proportionately. The rationale here is that in *ad libitum* feeding, energy intake regulates feed consumption and thus the amount of nutrients the animal ingests daily. Fish, however, are not fed *ad libitum*, and often not to satisfy, and therefore nutrient consumption would be controlled by feed allowance and not energy concentration of the diet. Fishes evolved in an aqueous environment where carbohydrates are scarce, their digestive and metabolic systems to be better adapted to utilization of protein and lipids for energy than carbohydrates. Some fishes, however, such as warm-water herbivore or omnivores, like Indian major carps can digest and metabolize carbohydrates relatively well. Since fish do not regulate body temperature they spend less energy in maintaining position in water than do terrestrial animals.

Energy requirements may be calculated empirically, based on energy losses and energy recovery. Cho and Kaushik (1990) constructed a model for calculating the digestible energy required to grow 1.0 kg of rainbow trout, from 1.0 g to 100 g size at 15^0C, based on derived heat and excretory losses and estimated recovery of energy in the fish, and indicated that, 3.56 Mcal of digestible energy was required to produce 1.91 Mcal of recovered energy in 1kg of fish biomass with a recovered energy / digestible energy efficiency ratio of 0.54. Most monogastric animals, including Siluroids, cat fish, *Clarias batrachus* and other fishes, required the same 10 essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Amino acid composition of practical feedstuffs is usually presented on a total content basis. Thus, in formulating fish feeds to meet amino acid requirements, the total amino acid contents of the feed ingredients must be corrected for availability. The digestibility of protein may be used in estimating the availability of amino acids in the feedstuff when digestibility of individual amino acids is not known, but digestibility of the protein is known. Fish were fed animal (fish meal) or plant (soyabean meal) protein-based diets and killed 3 or 6 hr after feeding. Intestinal tube of carp has been divided into five equal parts and their contents have been analysed. In carp digesta 3 hr after feeding the amount of released essential amino acids from dietary protein in the first 20% of intestine constituted 58.0–93.7% of the total with the exception of threonine—36.3%. Apparent absorption of several essential amino acids was the highest in the first 20% of carp intestine; lysine 51.0, arginine 50.2, tyrosine 52.5, phenylalanine 47.7% already 3 hr after feeding fishmeal-based diet. Fish fed soyabean-meal-based diet have shown the lower level of free amino acids in digesta and lower apparent absorption than that in fish fed animal-protein-based diet. Amino acid absorption from soyabean meal was much lower in carp than that found in other fish, rainbow trout, catfish and grass carp, as well as in the pig **(** Dabrowski, 2003a). The ratio of free amino acid concentration in intestinal content to blood of carp most commonly was between 10 and 20 and decreased as digestion proceeded. Free amino acid concentration in gut content of fish appeared several times higher than that in reptiles or mammal. Release rate of amino acids from dietary protein was most intense in the second segment of carp intestine where most of amino acids were also absorbed. Requirements of essential amino acids based on their absorption rate were determined **(** Dabrowski, 2003b).

Reports in the technical literature have indicated that the optimum level of protein in feeds for growth of fish has ranged from 25% to 50%. In all of these studies the researchers were probably justified in making their conclusions that a specific percentage of protein was optimum under their experimental conditions because a number of factors influence the growth response of fish to feeds containing different levels of protein, some of these are size of the fish, ambient water temperature, feed allowance, feeding frequencies, amount of non-protein energy, quality of the protein, and natural food availability. Fish has higher protein requirements during early growth than during later phases of growth.

MATERIALS AND METHODS

Halver's synthetic diets with variable and increasing levels of protein was used as **e**xperimental diets for evaluating the protein requirements prepared from highly purified ingredients (Casein, Gelatin as protein; Dextrin, Alpha-cellulose & Carboxymethyl cellulose as carbohydrate source, Fish oil & Vegetable oil as lipid source; Amino acid mixture, vitamin premix, Mineral premix, etc.) to allow maximum control over the nutrient being tested. All diets were alike in all respect, such as palatability, particle size & texture, water stability, and nutrient like digestible protein, lipid & carbohydrate and dietary energy content. Fishes were acclimatized in the continuous flow glass aquaria having temperature, photoperiod, air and water flow control facility for 2 weeks with test diet prior to beginning the experiment. The water quality was monitored as per the standard methods of APHA, 1989. The fish was fed *ad libitum* twice daily @ 6.0% body weight of fish for enhancing sensitivity to died differences and maximum growth. Feed allowance was adjusted each week on the basis of weight increment and fed twice daily at 18 and 06 hours of the day. Fish from continuous flow aquarium chemically analyzed at the beginning and after 90 days i.e. at the end of the feeding trial for moisture, protein and fat (AOAC, 1984), and rates of *in vivo* protein biosynthesis. Scanning Electron Microscopy of different portion of gastrointestinal tract was done to study the protein absorption site by using protein macromolecule Ferritin, HRP and IgG. After administration through orally or anus with Pasture pipette and the tissues from gut sections at different interval

was fixed in primary fixative gluteraldehide for 15 minutes and then change with the same fixative and kept for 24 hours. Then washed by cacodylate buffer with three changes by 15 minutes interval. Then the tissue was fixed in 1% Osmium tetroxide for 2 hours. Then the tissue was dehydrated throw graded ethanol. The tissue was kept in acetone to make ready for CPD. After CPD gold plating was done and studied under Scanning Electron Microscope to get the photograph.

At the beginning of the experimental trial for absorption of amino acids or protein macro molecules like Ferritin, the two weeks indoor laboratory conditioned fishes were starved for 24 hours to allow emptying of stomach & intestinal (gastric fish) food residues. Thread ligatures was placed at esophageal (upper and lower), stomach, duodenal, anterior, middle and posterior intestinal junctions in anaesthetized fish. Thorough and clean washing is essential for correct estimation of absorption. Two small incisions were given at the opposite ends of each ligatured section and the part of the gut was washed with Krebs-Ringer bicarbonate solution or by fish saline at 30°C. Legations at 5-6 cm apart was given in washed lower esophagus, gastric, duodenum and ileum. One ml. Krebs-Ringer bicarbonate solution / fish saline at 100 mg % protein or amino acid concentration at 30°C was injected into each of the above segment. The vicera and the ligatured segments were kept moist with Krebs-Ringer bicarbonate/ fish saline solution at 30°C in incubator. Absorption was allowed for 30, 60 and 90 minutes, following which the segments were separated from the rest of the gut and removed. The post absorption fluid was collected in vials and the wet weight of the separated segments was recorded. An aliquot of the fluid was taken for spectrophotometric determination of protein/ amino acids and was expressed as µg protein / amino acid absorbed $hour^{-1}sqcm^{-1}$ of intestinal serosal layer. This experiment was repeated for six times.

Quantitative requirements of amino acids were determined in fish by feeding a purified diet containing isolated crystalline amino acids as a control diet and feeding test diets similar to the control except that one amino acid at a time has been removed. Test diet that produce no growth or markedly less than the control represented amino acids that were essential to fish. Quantitative requirements for essential amino acids was determined by feeding graded levels of one amino acid at a time in a test diet containing crystalline amino acids. The amino acid profile of the test diet is usually designed to be similar to that in the fish muscle. In fact, the essential amino acid profile of fish muscle has been found to agree closely with the dietary amino acid profile for optimum growth of the fish. The rates of protein bio-synthesis in the liver and muscle of the experimental fish were measured as incorporation of amino acid (^{14}C-L–leucine) in the liver and muscle. The fish from each of the experimental sets was injected intramuscularly with the isotope ^{14}C-L–leucine (Specific activity 282 M Ci / MMOL ; solution in 0.01 N HCl; obtained from Bhaba Atomic Research Centre, Trombay, Bombay – 400 085) both at the initial stage and at the termination of 90 day experimental period at the rate of 0.05 µ Ci $100g^{-1}$ body weight of the fish. During the commencement of the experiment, the injected fish was sacrificed to measure the steady state of the level of incorporation. The liver and muscle tissues were weighed and processed as follows for counting the radioactivity:

Homogenized with 2.0 ml of the ice cold 0.6N NaCl solution and 20.0 µl of homogenate was kept for estimation of protein by the method of Lowry *et al*., 1951.

⇩

1.0 ml of 20% trichloroacetic acid (TCA) was added to the remaining homogenate. The volume of all the tubes was equalized by adding 20% TCA and mixed thoroughly.

⇩

Keep in deep freeze for 15 minutes. Centrifuged in refrigerated centrifuge (Indian Equipment Corporation, Bombay) at the temperature of 0 – 5°C at 7,000 rpm for 15 minutes.

⇩

Supernatant was discarded and 2.0 ml of 10% TCA was added to the precipitate and mixed well. Cooled for 15 minutes in deep freeze.

⇩

Centrifuged in refrigerated centrifuge for 15 minutes at 7,000 rpm and Supernatant was discarded.

⇩

2.0 ml of 5% TCA will be added to the precipitate and mixed well. Cooled for 15 minutes in deep freeze. Centrifugation in refrigerated centrifuge for 15 minutes at 7,000 rpm.

⇩

Supernatant was discarded and the precipitate was kept in a deep freeze and finally the precipitate dissolved in 0.5 ml 1% NaOH solution, boiled and mixed well.

⇩

7.5 ml of Scintillation fluid [(i) POPOP – [1, 4 – { 2 – (5 – Phenyloxazolyl)} benzene], Scintillation grade; (ii) POP – [2,5 – Diphenyloxazole] Scintillation grade. For 500 ml, 0.2g of (i) and 2.0g of (ii) was added with 200 ml toluene, 100 ml methanol and 200 ml methyl cellosolve] was added in the sample in Scintillation tubes.

⇩

The radioactivity was counted in an Automatic Liquid Scintillation system (Perkin Elmer, Wallac OY 1409 – 01 Routine counting system) and Radioactivity was expressed as cpm / mg of tissue protein.

RESULTS AND DISCUSSION

Proximate composition of the diets prepared with highly purified ingredients on a dry matter basis, is presented in Table 1. Protein share of the diet varied from 25% to 50% , content of lipid fixed at 6% level and carbohydrate percentage gradually reduced as their digestive and metabolic systems to be better adapted to utilization of protein and lipids for energy than carbohydrates. Although digestible energy content was kept constant within the limit of 3.03 to 3.05 kcal g^{-1}. The ratio of digestible energy / digestible protein gradually increased

Table1. Percentage composition of different ingredients and protein on dry matter basis, and digestible energy content (Kcal g^{-1}) in the prepared diets

Ingredients	Percentage of ingredients					
Casein	20	24	28	32	36	40
Gelatin	05	06	07	08	09	10
Dextrin	40	33	26	19	12	05
α-Cellulose	21	21	21	21	21	21
Carboxy - methyl cellulose	5	7	9	11	13	15
Amino acid mixture	0	0	0	0	0	0
Vitamin premix	1	1	1	1	1	1
Mineral premix	2	2	2	2	2	2
Animal fat	3	3	3	3	3	3
Plant fat	3	3	3	3	3	3
Protein %	25	30	35	40	45	50
Digestible Energy (DE) (KCalg^{-1})	3.05	3.04	3.04	3.04	3.03	3.03
Protein /DE	8.20	9.87	11.51	13.16	14.85	16.50

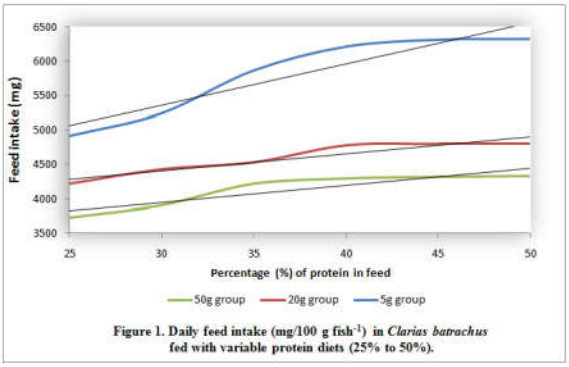


Figure 1. Daily feed intake (mg/100 g $fish^{-1}$) in *Clarias batrachus* fed with variable protein diets (25% to 50%).

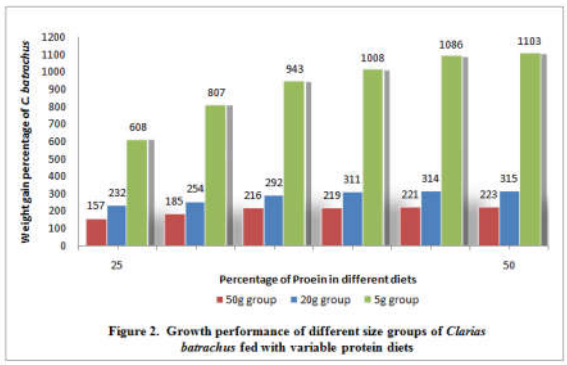


Figure 2. Growth performance of different size groups of *Clarias batrachus* fed with variable protein diets

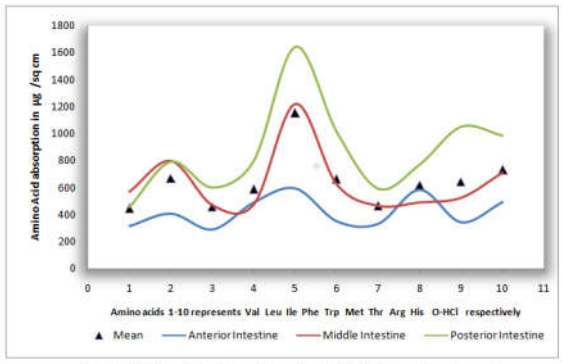


Figure 3. Absorption of essential amino acids (EAA) in µg $hour^{-1}$ sq cm^{-1} through intestinal epithelium of *Clarias batrachus*.

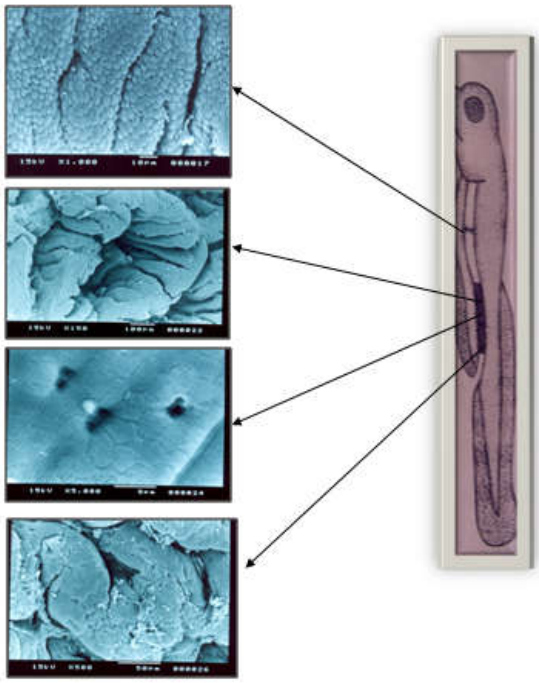


Plate 1. Absorption sites of protein/peptide/amino acids in the gastro-intestinal tract of *Clarias batrachus* justified with Scanning Electron Micrographs [Top –Stomach, Bottom - 2 cm, 4 cm & 8 cm apart from the end portion of stomach].

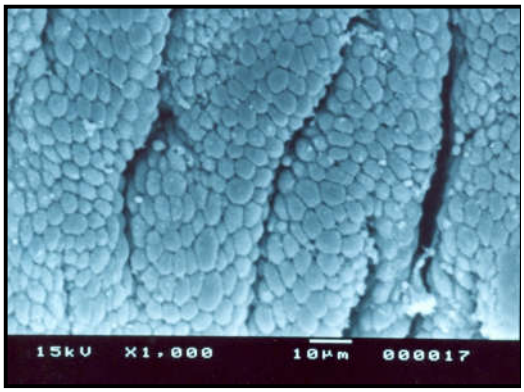


Plate 2. SEM showing the inner wall of stomach of the *C. batrachus, a gastric catfish*

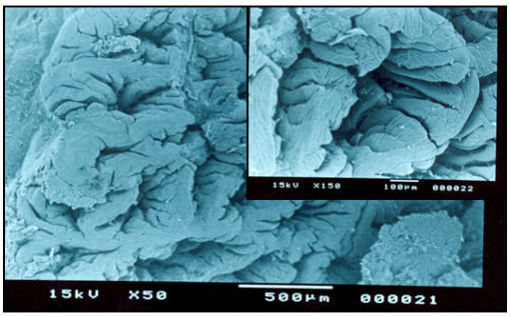


Plate 3. SEM showing the anterior intestinal serosal layer *C. batrachus* with absorption site (2 cm apart from the end of stomach) [Inset is enlarged view of the plate 2]

from 8.20 to 16.50 and it highly correlated with the findings of NRC (1993). In the present investigation, the maximum percentage growth was observed in smaller size group (5± 0.64 g) followed by larger size groups 20 ± 1.85 g and 50 ± 3.20 g of *Clarias batrachus*. It was also recorded that the satiation time in case of *Clarias batrachus* was about 60-90 minutes and maximum feed intake recorded at 18 hours , followed by 00.00 hours, 06.00 hours and 12.00 hours of the day, presented in Figure 1 (Patra, 1993). Highest feed intake recorded in smaller size groups of *Clarias batrachus* (5 ± 0.64 g) was 6318 mg $100g^{-1}$ body weight of fish followed by

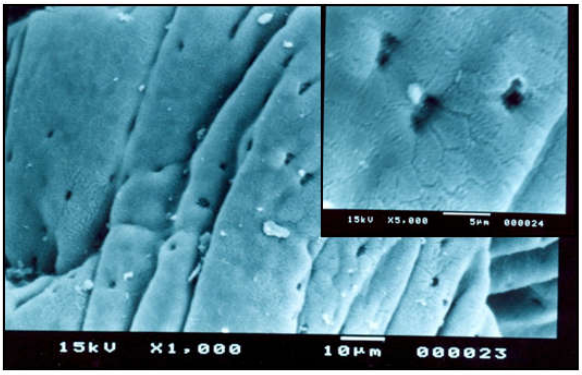


Plate 4. SEM showing the middle intestinal serosal layer with absorption sites of *C. batrachus* (4 cm apart from the last part of stomach) [Inset is the enlarged view of plate – 2]

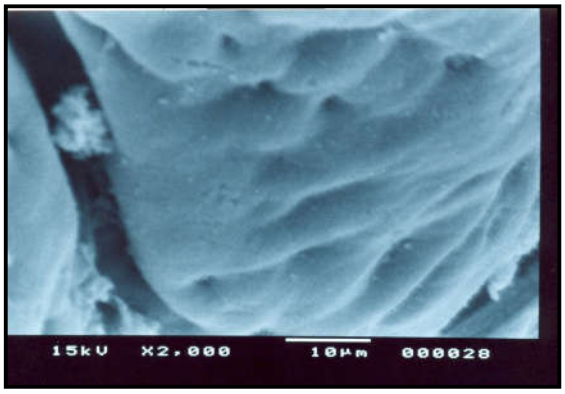


Plate 5. SEM view showing the presence of absorption sites in the last posterior part of the intestine of *C. batrachus* (8 cm apart from the last part of stomach)

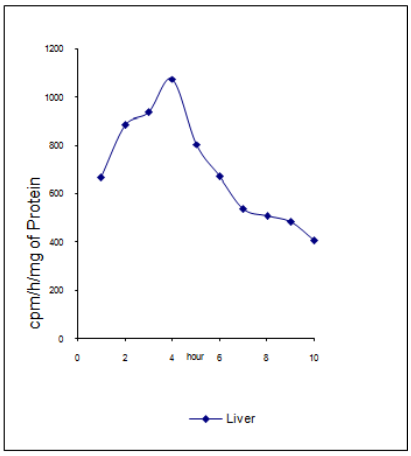


Fig. 4. Rate of *in vivo* protein biosynthesis in liver of *Clarias batrachus* after feeding

larger size groups 20 ± 1.85 g (4782 mg $100g^{-1}$) and 50 ± 3.20 g (4217 mg $100g^{-1}$) as presented in linear curve of Figure 1. The protein requirement in terms of growth performance and weight gain was very high in smaller size groups of *Clarias batrachus* (5± 0.64 g) followed by larger size groups 20 ± 1.85 g and 50 ± 3.20 g respectively as presented in Figure 2. Weight gain is not a measure of true growth. True growth is an increase in muscle both smooth & striated, skeletal and organ tissue, whereas weight gain can be represented as an increase in adipose tissue with relatively less change in the other

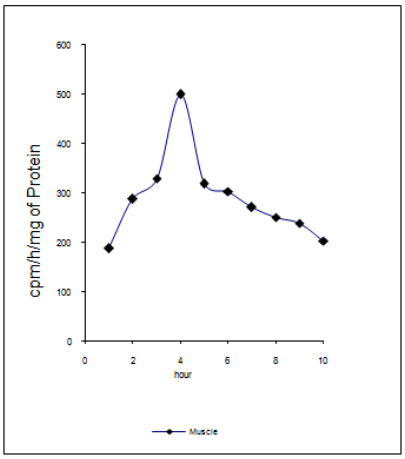


Fig. 5. Rate of *in vivo* protein biosynthesis in muscle of *Clarias batrachus* after feeding

tissues. Because, muscle is the main marketable product in fish grow out for food and good animal protein source. Jobling (1994) recorded that, deposition of 1.0g of fat represents weight increase of 1.0g, whereas, deposition of 1.0g of protein leads to the gain of about 4.0g of tissue. Feed conversion is more efficient in smaller size group (5g: 1.32) and faster growing fish than in higher size group (20g: 1.81 and 50g: 2.10). In most situations, protein gain is an acceptable measure of growth in fish. In small fish, weight gain correlates well with protein gain but as fish increase in size there is no significant correlation (Lovell and Li, 1992). It was estimated that, for calculating the digestible energy required to grow 1.0 kg of *Clarias batrachus* in controlled condition from 1.0 g to 100 g size at 25-30^{0}C water temperature based on estimated recovery of energy in the fish which indicated that 3.71Mcal of digestible energy is required to produce 1.0 kg of fish biomass equivalent to 1.91Mcal recovered energy. Protein retention rarely exceeds 50% in fish fed either purified or practical diet.

Apparently *Clarias batrachus* respond to supplementation of isolated (crystalline) amino acids when the diet is deficient in the amino acid, but little is known about the relative bioavailability of isolated amino acids compared to protein-bound amino acids. Theoretically it should be possible to grow Indian major carps at an acceptable rate on a diet containing 15% essential amino acids, and an additional 10% of non-essential amino acids. Highest rate of EAA absorption recorded through the posterior intestinal segment as compared to anterior and middle segment and presented in Figure 3 (Kim Jeong *et al*., 2001; Berge *et al.,* 2004;Dabrowski *et al.,* 2004; Manuel *et al.,* 2994; Morais i2005; Xuemei i 2005; Patra, 2004, 2008a,c; Lovell, 2009). Amino acid requirements are found that, among the EAA, maximum quantity required in the diet is Lysine (5.6) followed by Phenylalanine (4.6), Arginine (4.2), Threonine (3.8), Leucine (3.4), Valine (2.7), Isoleucine (2.5), Histidine (2.2), Methionine (2.0), Tyrosine (1.9) and Cystine (1.2). Protein macromolecules and peptide were introduced in the digestive tract of *Clarias batrachus* and was absorbed through anterior, middle & posterior intestinal segments and transferred to the general circulation but not through the stomach region (Plate 1, 2, 3, 4 & 5) which supported by Le Bail et al.1989. Results obtained by radioimmunoassay showed that the protein macromolecules of

higher molecular weight transferred through the intestinal serosal layer without changing the structure, may be entered through pinocytosis. Similar observations were confirmed by the work of Moriyama *et al.,* 1990, Hertz *et al.,* 1991, L.-Z Sun and Farman-farmaian, 1992 and Stroband *et al*., 2005.

Maximum incorporation of U-^{14}C L-leucine takes place at 2 hours in liver (4000 cpm mg^{-1} of protein) and 4 hours in muscle (605 cpm mg^{-1} of protein) in case of *Clarias batrachus* (Figure 4 &5). There were no significant differences in assimilation rate either in liver or in muscle with formulated diets and synthetic diets in *Clarias batrachus*. It was recorded that maximum assimilation takes place in smaller size groups (4100 cpm mg^{-1} of protein in liver & 604 cpm mg^{-1} of protein in muscle at 18 hours of the day and 637 cpm mg^{-1} of protein in liver & 241 cpm mg^{-1} of protein at 12.00 hours of the day) as compared to larger one which directly correlated with the feed intake, protease enzyme activity, growth performance, fish flesh quality in terms of nutrients and palatability (Patra, 1994, 1995 and 2008b).

ACKNOWLEDGEMENT

The Author is grateful to the Department of Science & Technology, for providing SERC Visiting Fellowship and necessary grant & Indian Council of Agricultural Research, for award of Research Projects, Government of India to complete the work.

REFERENCES

AOAC, 1984. Official methods of analysis. *Association of Official Agricultural Chemist.* 13th edition, edited by S. Williams, Arlington, VA. 1141 pp.

APHA. 1989. Standard methods for the examination of water and waste water. American Public Health Association, American Water Works, Water Pollution Control Federation. Washington D.C., USA.

Berge, G. E., Goodman, M., Espe, M. and Lied E., 2004. Intestinal absorption of amino acid in fish : kinetics and interaction of the in vitro uptake of L-methionine in Atlantic salmon (Salmo salar L.)., *Science Direct Aquaculture,* 229 (1-4) : 265-273.

Cho, C. Y. and Kaushik, S. J., 1990. Nutritional energetics in fish: Energy and protein utilization in rainbow trout (*Salmo gairdneri*). World Rev. *Nutr. Diet*. 61:132-172.

Dabrowski, K. 2003a. Digestion of protein and amino acid absorption in stomachless fish, common carp (Cyprinus *carpio* L.) *Comparative Biochemistry and Physiology Part A: Physiology,* 74 (2): 409-415.

Dabrowski, K. 2003b. Comparative aspects of protein digestion and amino acid absorption in fish and other animals. *Comparative Biochemistry and Physiology Part A:Physiology*, 74(2): 417-425

Dabrowski, K., Zhang, Y., Arslan, M. and Terjesen, B. F. 2007. Indispensable amino acid deprivation does not cause rapid amino acid depletion in fish body and this principle has a potential to be used as the new strategy in nutrition. *Rev. Col. Cienc. Pec.,* 20:4.

Formanfarmaian, A., Ross, A. and Mazal, D. 1972. *In vivo* intestinal absorption of sugar in the toat fish (Marine teleost, *Opnanus tua*). *Biol. Bull.,* 142: 427-445.

Hertz, Y., Tchelet, A., Madra, Z. and Gartler, A. 1991. Absorption of bioactive human growth hormone after oral administration in the common carp (*Cyprinus carpio*) and its enhancement by deoxycholate. *J. Comp. Physiol.,* 161B:159-163.

Jobling, M. 1994. Fish bioenergetics. New York: Chapman & Hall.

Jeong–Dae, Kim. & Santosh, L. P. 2001. Recent Advances in Protein and Amino Acid Nutrition of Marine Fish. Commonwealth A, 1030-1230.

Le Bail, P. Y., Sire, M. F. and Vernier, J. M. 1989. Intestinal transfer of growth hormone into the circulatory system of rainbow trout, *Salmo gairdneri.* Interference by granule cells. *J. Exp. Zool.,* 251: 101-107.

Lovell, R. T. and Li. M. 1992. Comparison of feed conversion, dressing yield, and muscle composition for second- and third- year channel catfish. *Prog. Fish Culturist*. 54: 171-173.

Lovell, T. 2009. Nutrition and feeding of fish. (Editor Tom Lovell) Springer International Edition, Springer Science, 2671pp.

Matthews, D. M. 1975. Protein absorption from intestinal content. *Physiol. Rev.,* 55, 537 – 608.

Manuel, A. Segovia-quintero and Robert, C. R. 2004. Coating crystalline methionine with tripalmitin- polyvinyl alcohol slows its absorption in the intestine of Nile Tilapia, *Oreochromis niloticus*., Science Direct Aquaculture, 238(1-4): 355-367.

Morais, S., Kkoven, W., Ronnestad, I., Dinis, M. T., and Conceicao L. E. C. 2005. Dietary protein/lipid ratio affects growth and amino acid and fatty acid absorption and metabolism in Senegalese sole (Solea senegalensis kaup 1858) larvae. *Science Direct Aquaculture,* 241(1-4): 347-357.

Moriyama, S., Takahashi, A., Hirano, T. and Kawauchi, H. 1990. Salmon Growth hormone is transported into the circulation of rainbow trout, *Onchorhynchus mykiss*, after intestinal administration. *J. Comp. Physiol.,* 160b: 251-257.

National Research Council. 1993. Nutrient requirement of fish. Washington, D.C.: *National Academy of Sciences.*

Patra, B.C. 1993. Satiation time, appetite and daily pattern of feed intake and faeces release by an air-breathing fish, *Anabas testudineus* (Bloch). *J. Aqua. Trop.,* 8, 41-46.

Patra, B.C. 1994. Growth performance and metabolism of the air-breathing fish, *Anabas testudineus* (Bloch) at varying dietary protein levels. *Philippine J. Sci*., 123 (1), 41-50.

Patra, B.C. 1995. Influence of different protein level and source on the GOT and GTP level and protein synthesis in an air-breathing fish, *Anabas testudineus* (Bloch). *J. Inland. Fish. Soc. Ind*., 24(2), 50-55.

Patra, B.C. 2004. Absorption of amino acids at different sites of the gut in an Indian major carp *Labeo rohita* (Hamilton). *7th Asian Fisheries Forum*, Penang, Malayasia. NFM, 11.

Patra, B.C. 2008a. Evaluating the nutritional condition of an Indian climbing perch, *Anabas Testudineus* (Bloch) fingerlings by the RNA/DNA, ca/p ratio in muscle and protein bio-synthesis rate in liver and muscle. *Aquaculture America'08*, Florida, USA FFN 10, 2.

Patra, B.C. 2008b. Intestinal serosal absorption of essential amino acids in *Labeo rohita* (Hamilton). *Aquaculture America'08*, Florida, USA FFN 9, 1.

Patra, B. C. 2008c. Absorption of protein in the gastrointestinal tract of teleost fish. *Indian Journal of Biological Sciences,* 14:1-12.

Patra, B. C. 2011. Nutritional Energetics of an Indian Major Carp *Labeo Rohita* (Ham.), Family Cyprinidae. *International Journal of Current Research,* 3(11): 259-263.

Smith, R. R. 1989. Nutritional energetics. In *Fish nutrition*, ed. J. E. Halver, San Diego, Calif: Academic Press.

Sun, L.-Z. and Farmanfarmaian, A. 1992. Biphasic action of growth hormone on intestinal amino acid absorption in striped bass hybrids. *Comparative Biochemistry and Physiology Part A: Physiology, 103(2*): 381-390.

Stroband, H. W. J. and Veen F. H. Van der. 2005. Localizatioin of protein absorption during transport of food in intestine of the grass carp, *Ctenopharyngodon idella. Journal of Experimental Zoology,* 218 (2): 149-156.

Xuemei, K., Yuan, T. Y., Chun, F. C., Yong, L. W. and Jiang, R. 2005. Study on the absorption and utilization of L-leucine and L-tyrosine by the intestine of grass carp (*Ctenopharyngodon idella*). *Acta Hydrobiologica Sinica,* Vol. 29, No. 4, pages 424-429.

****