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NUTRIENT PROFILE OF INDIAN CLIMBING PERCH, *Anabas testudineus*

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ABSTRACT

The proximate composition, fatty acid, amino acid and fat soluble vitamins of Indian climbing perch, *Anabas testudineus*, locally called "*Koi"* were assayed in relation to its body weight. The fish samples were collected from different geographical locations and were grouped as small (10-50g) and big (52-150g) sizes. The proximate composition, essential amino acid (EAA) and non-essential amino acid (NEAA) contents in Koi did not differ significantly between the groups. The monounsaturated fatty acid (MUFA) content was significantly ($P<0.05$) higher in bigger sized Koi. The polyunsaturated fatty acid (PUFA) content was 23.67±0.85 and 13.62±1.02 (%) respectively in the small and big sizes of Koi, while the docosahexaenoic acid (DHA) was significantly higher in small Koi. The vitamin A content was 85.77±0.35 and 93.90 ±1.34 (I.U./100g) respectively in small and big Koi. Vitamin D content was significantly higher in small Koi compared to big one. Vitamin E and K were significantly ($P<0.05$) higher in big Koi. The results indicated that *Anabas testudineus* is a good source of protein, fat, vitamins, amino acids and fatty acids.

Keywords: *Anabas testudineus*, Proximate composition, vitamins, amino acid profile and fatty acid profile

INTRODUCTION

Fish protein occupies an important position in human nutrition (Nargis 2006). Fish is consumed by human being for centuries and is preferred as a perfect diet not only due to its taste and high digestibility but also because of having higher proportions of unsaturated fatty acids, essential amino acids and vitamins and minerals (Kumar, 1992). The high nutritional value of fish is mainly related to their readily digested proteins which are good source of essential amino acid (Mohanty et

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al., 2014). Fish is also a good source of polyunsaturated fatty acids (PUFAs), viz., the ω-3 and ω-6 PUFAs, which are well known for beneficial effect to human health (Das et al., 2009; Paul et al., 2015; Mohanty et al., 2016 and Giri et al., 2010). Fat-soluble vitamins viz., Vitamin A, D, E and K act as essential nutrients in important biological processes of the human body (Sau and Paul, 2004).

Anabas testudineus, commonly called as climbing perch or Koi, is a small fish widely distributed throughout south and south-east Asia. This is a highly priced fish owing to its consumer preference compared to carps. There are few reports available on nutrition and aquaculture of *A. testudineus* (Mukhopadhyay and Paul, 1996; Kumar et al., 2013 and Bungas et al., 2013). Also, not much information is available on the nutrient profile of Indian climbing perch (Wimalasena and Jayasuriya 1996 and Bogard et al., 2015). Keeping in view the importance as food fish; the nutrient profile of Indian climbing perch, *A. testudineus* was determined to document the information of amino acid and fatty acid profiles, along with some selected vitamins.

MATERIALS AND METHODS

Collection of samples

The samples of *Anabas testudineus* (n=40) were collected from West Bengal, Odisha and Andhra Pradesh. The collected fish samples were categorized into two groups as per their bodyweight i.e., small (10-50g) and big (52-150g). The fish samples were eviscerated and the head was removed. The representative portion of the edible part was taken and homogenized in a mixer for further analysis as per the methodology reported earlier (Sankar et al., 2010). The proximate composition of fish tissue samples was done as per AOAC (2005).

Fatty acid analysis

Pooled samples were extracted for fatty acid analysis following the method of Folch et al. (1957) using chloroform: methanol (2:1, v/v) solvent system that contained 0.01% butylated hydroxyl anisole as an antioxidant. Fatty acid methyl esters (FAMEs) were prepared by the transmethylation with boron trifluoride (BF_3) in methanol from lipids fraction as per Metcalfe et al. (1966). The fatty acid methyl esters were quantified by injecting 1µL (50:1 split ratio) into a gas chromatograph (GC) (Perkin Elmer; CLARUS 480). The oven temperature was programmed from an initial temperature at 30^{o}C rising to 140^{o}C (hold time 4 min.) and up to 200^{o}C. Nitrogen gas was used as a carrier gas. The injection port and the flame ionization detector were maintained at 260 and 300^{o}C. Identification of individual FA was identified by comparison of retention times to those of standards (SUPELCO, Cat. No. 47885-U) and quantified by comparing with respective areas, following "Total Chrome" software of Perkin Elmer.

Amino acid analysis

The amino acid analysis was done as per the method of Ishida et al., (1981) and Paul et al. (2016a). For amino-acid analysis phenylisothiocyanate (PITC) was used

for pre-column derivatization, while reversed-phase gradient elution high-performance liquid chromatography (HPLC) separates the phenylthiocarbamyl (PTC) derivatives which are detected by their UV absorbance of Pico Tag method of Waters Associates.

Vitamin analysis

The fat soluble vitamins Retinol (Vitamin A), Cholecalciferol (Vitamin D), α-Tocopherol (Vitamin E) and Vitamin K were analyzed in high performance liquid chromatography system. Fish tissue (30g) was grinded with anhydrous sodium sulfate. Then extracted the oil using 2:1 chloroform: methanol after adding BHA as antioxidants (Folch et al., 1957). The sample preparation and vitamin analysis was done as per Sankar et al. (2010). To about 2.0 g oil in a round bottom flask, added 25 ml alcohol, and 1.5 ml of 150% KOH. Reflux in water bath for 30 min. Transfer the contents in to a 250 ml separating funnel after cooling; wash the flask with 50 ml petroleum ether and add to the separating funnel; shake the contents thoroughly and allowed to separate. Extract the aqueous layer twice more and pool the solvent layer. Wash the solvent layer with 20 % of water (v/v) to make it alkali free. Concentrate non-saponifiable matter in the ether extract fraction using a flash evaporator at 30-40^{o} C to a definite volume. The non safonifiable matter is filtered through 0.45 µ syringe filter and stored under refrigerator. Then the fat soluble vitamins were analyzed by injecting 20 µL of sample in HPLC equipped with C_{18} Bondapack column. The mobile phase of HPLC consists of water (HPLC grade) solvent A and acetonitrile as solvent B with 1% TFA. A gradient system was used (solvent A/solvent B), starting from 50/50, 80/20 to 100/0 at the rate of 1 mL/min for 20 min. The fat soluble vitamins were identified and quantified by comparing with the retention times and peak area of respective vitamins standards.

The data were analyzed using t-test as per Snedecor and Cochran (1968) and have been presented as Mean±S.E.

RESULTS AND DISCUSSION

The proximate composition of Koi is presented in table 1. The moisture, crude protein, crude fat and ash content did not differ significantly between the size groups. The protein and fat content were 16.47±0.11 and 16.91±0.59; 6.68±1.35 and 6.98±1.49 (%), respectively in small and big Koi. The moisture content of Koi in the present study was lower than the freshwater Eel (*Mastacembelus armatus*) and *Anguila bengalensis bengalensis* as reported by Pal and Ghosh (2013). Fat content of Koi was higher in the present study in comparison to that of many freshwater fish species (Pal and Ghosh, 2013; Swapna et al., 2010, Ackman, 2002 and Paul et al., 2016b). Fat content of Koi of the present study is in agreement with earlier report by Nargis (2006). The protein content of Koi was lower than other freshwater fish (Pal and Ghosh, 2013). Generally an inverse relationship between tissue moisture and lipid content is observed with the increase of age and body weight of fish (Wheeler

and Morrissey, 2003; Jankowska et al., 2007), which was not observed in the present case. The amino acid contents of Koi of two weight ranges are presented in table 2. The essential amino acid contents (EAA) are 44.67±1.79 and 44.65±1.54g/100g whole body protein, respectively in small and big size groups. The Leucine content was maximum among EAA. The non essential amino acid (NEAA) content was 54.48±0.26 and 55.80±0.91g/100g protein, respectively in small and big size of Koi; where Glycine was maximum among NEAA in small size range group. However both the groups contain a good proportion of the essential as well as non-essential amino acids with non significant mean values.

Table 1. Proximate composition (% as such basis) of Koi (*Anabas testudineus*) of different body size.

Particulars	Small	Big
Moisture	68.65±0.68	68.00±1.77
Crude Protein	16.47±0.11	16.91±0.59
Crude Fat	6.68±1.35	6.98±1.49
Ash	5.03±0.04	5.50±0.49

Data are presented as Mean ± S.E. (n=50)

The present study for the amino acid content of Koi shows that Isoleucine, Leucine, Valine and Phenylalanine among the essential amino acids (EAA) and Aspartate, Glutamine, Glycine and Alanine among the non essential amino acids (NEAA) are higher in quantity. However, Iwasake and Harada (1985) reported that the main amino acids of fish are Aspartate, Glutamate and Lysine. Over the last 20 years, increasing evidence suggests the importance of Glutamine for the proper functioning of many organ systems (Christina et al., 1999). Our study denotes that Koi of both sizes contain glutamine about 13.14-14.41 (%) which is very effective for human health. It is observed that the EAA/NEAA ratio is 0.82 and 0.80 in the small and big Koi. Wessilinova (2000) reported the variation in amino acid content of fish with season and location. However, in this study there was no significant variation between the two size groups of Koi.

The fat soluble vitamin content in Koi of different body weights are presented in table 3. The vitamin A content was85.77±0.35 and 93.90± 1.34(I.U/100g) in two weight groups, which did not differ significantly. The vitamin D content in Koi is significantly ($P<0.05$) higher in smaller Koi. The Koi of bigger size (50-152 g) contains significantly higher vitamin E (1.27 I.U/100g) and vitamin K (1.15 µg/100g) than the Koi of smaller size.

Table 2. Amino acid profile (g/100g protein) of Koi (*Anabas testudineus*) of different body weights

Particulars	Small	Big
Essential Amino Acids (EAA)		
Arg	2.10±0.32	2.01±0.29
His	3.99±0.44	4.12±0.39
Ile	5.36±0.10	5.58±0.46
Leu	8.13±0.25	8.42±0.66
Lys	3.02±0.40	2.53±0.24
Met	1.60±0.06	1.44±0.05
Phe	6.26±0.29	5.95±0.31
Thr	5.48±0.46	5.88±0.23
Try	1.39±0.50	1.09±0.08
Val	7.32±0.06	7.61±0.28
ΣEAA	44.67±1.79	44.65±1.54
Non-Essential Amino Acids (NEAA)		
Asp	10.84±0.69	10.95±0.38
Ser	5.07±0.43	5.30±0.13
Glu	13.14±0.07	14.41±0.30
Pro	1.63±0.32	1.42±0.03
Gly	15.29±0.56	14.90±0.18
Ala	7.26±0.44	7.77±0.96
Cys	0.22±0.07	0.14±0.00
Tyr	1.01±0.07	0.89±0.38
ΣNEAA	54.48±0.26	55.80±0.91
EAA/NEAA	0.82	0.80

Data are presented as Mean ± S.E. (n=8)

Fish is a good source of fat soluble vitamins. Vitamin A content from fish is more readily available to the body than from plant sources (Liu, 2003). Vitamin A is responsible for normal vision and bone growth is well known and its derivative retinoic acid regulates gene expression in the development of epithelial tissue (Roos et al., 2003). Vitamin D functions to activate the innate and dampen the adaptive immune systems (Hewison, 2011). As Koi contains a good amount of vitamin D, it plays a major role for immune system. Vitamin E is an indispensable nutrient

required to maintain flesh quality, immunity, normal resistance of red blood corpuscles to haemolysis, permeability of capillaries and heart muscles (Halver and Hardy, 2002). Vitamin E content in Koi ranges from 0.70-1.27 (I.U/100g). The Koi contains 0.53-1.15 (µg/100g) of vitamin K. Vitamin E also functions as lipid soluble antioxidants and protects biological membranes, lipoproteins and lipids against oxidation (Hamre, 1998 and Sau et al., 2004). The human body needs vitamin K for post translational modifications of certain proteins required for blood coagulation and in metabolic pathways in bone and other tissue (Halver and Hardy, 2002).

Table 3. Vitamin content of Koi (*Anabas testudineus*) of different body weights

Particulars	Small	Big
A (I.U/100g)	85.77±0.35	93.90±1.34
D (I.U/100g)	85.60^{b} ±1.29	43.12^{a} ±1.03
E (I.U/100g)	0.70^{a} ±0.04	1.27^{b} ±0.03
K (µg/100g)	0.53^{a} ±0.03	1.15^{b} ±0.02

Superscript ^{a,b} in a row differs significantly (P<0.05). Data are presented as Mean ± S.E. (n=8)

The fatty acid profile of Koi is presented in table 4. The saturated fatty acids (SFA) were 66.19±3.33 and 60.73±1.25 (%) respectively in small and big Koi. Among SFA the palmitic and stearic acid are found to be higher in both the groups. The palmitic acid is significantly (P<0.05) higher in big Koi compared to small one; conversely stearic acid was higher in small Koi than big one. Other SFAs viz., pentadecanoic, heptadecanoic and arachidonic acid were significantly (P<0.05) higher in small size of Koi.

The monounsaturated fatty acid (MUFA) contents were 10.39±0.51 and 25.70±0.89 respectively in small and big groups. Palmitoleic acid was significantly (P<0.05) higher in big size of Koi. The MUFA content is significantly (P<0.05) higher in big Koi. The polyunsaturated fatty acids (PUFA) are 23.67±0.85 and 13.62±1.02 respectively in the small and big koi. The PUFA content is significantly higher in small group compared to the bigger group. Among PUFA, α-linolenic acid is significantly (P<0.05) higher in small Koi. The docosahexaenoic acid (DHA) is also significantly (P<0.05) higher in smaller group. Σω3 PUFA are significantly (P<0.05) higher in smaller Koi but Σω6 PUFA are significantly (P<0.05) higher in the bigger size of Koi. It is also observed that SFA and MUFA are higher in bigger sized Koi whereas the PUFA content is higher in Koi of smaller size.

Fatty acid composition of aquatic animals is influenced by intrinsic variables, such as species, sex, age and size; as well as extrinsic factors, such as diet, salinity, temperature, geographical regions, and the general rearing conditions (Abd Rahman

Table 4. Fatty acid profile (% of total fatty acid) of Koi (*Anabas testudineus*) of different body weights

Particulars	Small	Big
C12:0 (Lauric Acid)	0.43±0.03	0.68±0.05
C14:0 (Myristic Acid)	1.33±0.18	1.35±0.13
C15:0 (Pentadeconoic Acid)	1.27^{b}±0.08	0.48^{a}±0.09
C16:0 (Palmitic Acid)	40.56^{a}±1.60	52.56^{b} ±2.12
C17:0 (Heptadeconoic Acid)	2.70^{b}±0.28	0.11^{a}±0.02
C18:0 (Stearic Acid)	15.32^{b} ±2.04	5.19^{a} ±0.64
C20:0 (Arachidic Acid)	0.71^{b} ±0.06	0.31^{a}±0.03
C21:0 (Heneicosanoic Acid)	3.21±0.21	ND
Other	0.66^{b} ± 0.03	0.05^{a}±0.01
Σ SFA	66.19±3.33	60.73±1.25
C15:1 (Pentadecenoic Acid)	0.51±0.03	0.91±0.06
C16:1 (Palmitoleic Acid)	8.56^{b}±0.46	3.27^{a}±0.18
C17:1 (Heptadeconoic Acid)	0.66±0.05	0.46±0.11
C18:1n9c (Oleic Acid)	ND	2.49±0.11
C18:1n9t (Elaidic Acid)	ND	18.13±0.88
C20:1n9 (Eicisanoic Acid)	0.66±0.03	0.44±0.05
Σ MUFA	10.39^{a}±0.51	25.70^{b}±0.89
C18:2n6c (Linoleic Acid)	ND	8.17±0.96
C18:3n3(α Linolenic Acid)	17.83^{b}±0.85	1.91^{a}±0.06
C18:3n6 (γ Linolenic Acid)	0.37±0.04	0.11±0.01
C20:4n6 (Arachidonic Acid)	1.24±0.11	ND
C20:5n3 (Eicosapenta enoic Acid)	0.38±0.04	ND
C22:6n3 (Docosahexaenoic Acid)	2.67^{b}±0.16	1.26^{a}±0.07
Other	1.18±0.09	2.18±0.11
Σ PUFA	23.67^{b}±0.85^{b}	13.62^{a}±1.02
Σω-3 PUFA	20.88^{b}±0.66	4.11^{a}±0.03
Σω-6 PUFA	1.61^{a}±0.12	8.30^{b}±0.20
ω-3: ω-6 PUFA	1.16^{b}± 0.12	0.49^{a}±0.02

Superscript in row ^{a,b} differs significantly ($P<0.05$). Data are represented as Mean ± S.E. (n=8)

et al., 1995; Sener et al., 2005). Fatty acids in fishes are derived from two main sources, namely, biosynthesis and diet (Hearn et al., 1987, Morris et al., 1995, Kamler et al., 2001). Palmitic acid content among the SFA is maximum in big size Koi which is in agreement with earlier report (Kaya et al., 2008). The SFA content is higher in freshwater fish (Indian Major Carp) as reported by Paul et al., (2015) which is in agreement with the present result. The palmitic acid is considered to be a key to many metabolic processes in fish and other aquatic animals (Ackman and Eaton, 1966). Fish oils are known to be rich source of essential PUFA of the omega-3 family (Kenari et al., 2009). In Koi PUFA content varies from 13.62-23.67% and DHA ranges from 1.26-2.67%. This is in agreement with earlier report (Kenari et al., 2009). The n-3 PUFAs, especially the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in high concentrations in the phosphoglycerides of cellular membranes, and DHA is particularly abundant in the retina and brain, where it has a crucial role in maintaining the structure and function of the excitable membranes of these tissues (Lauritzen et al., 2001). These fatty acids have beneficial effect in the prevention of cardiovascular and inflammatory diseases (Gebauer et al., 2006) and neurodegenerative syndromes, such as Alzheimer's disease (Moyad, 2005). The consumption of fish and fish oils containing omega-3 fatty acids are beneficial for a number of biological factors like cardiovascular diseases, rheumatoid arthritis, psoriasis (Paul et al., 2016a).

CONCLUSION

The results indicated that Koi is a good source of essential amino acids, protein, fat and ash. Among the fatty acids, palmitic and stearic acid were dominant in SFA and palmitoleic acid was predominant in MUFA. Among the PUFA, linolenic and docosahexaenoic acids were found in higher contents. Vitamin A and D were also present in good quantity in Koi. Irrespective of the size groups, the nutrient profile reflected that the fish was enriched with fat, protein, fatty acids, essential amino acids and vitamins.

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