Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(1): 193-197

Received: 25-11-2015
Accepted: 28-12-2015

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Evaluation of nutrient quality of a short horned grasshopper, *Oxya hyla hyla* Serville (Orthoptera: Acrididae) in search of new protein source

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Abstract
The demand of protein rich feed stuffs has been escalated with the needs of feeds for aquaculture and live-stock management. Being nutrient rich, grasshoppers may be an alternative to resolve the problem. The present study evaluated nutrients quality in a grasshopper species, *Oxya hyla hyla*. Results revealed that this species contains about 64% protein, considerable amount of essential amino acids, unsaturated fatty acids, minerals and vitamins. On the other hand, anti-nutritional factors are detected to be negligible. These results suggests for considering *O. hyla hyla* biomass as an alternative protein rich feed ingredient in the feed of fish and livestock.

Keywords: *Oxya hyla hyla*, Biomass utilization, Protein source, Nutritional aspect.

Introduction

The demand of feed for aquaculture and livestock has been escalated with the rapid growth in these sectors to meet up the needs of protein rich food for increasing human population. Fish meal is the traditional protein rich feed ingredient in the feeds of fish and livestock. The resource limitation for fish meal production led scientists to find out alternative protein rich feed stuffs from various plant and animal sources (Mukhopadhyay and Ray 1997 [25], 1999 [26]; Yang *et al*. 2004 [37]; Turker 2005 [33]). Various researches suggest that insects are rich in nutrient contents and can be an alternative protein rich natural food (Ueckert *et al*. 1972 [34]; De Foliart 1999 [12]; Rumpold and Schlüter 2013 [32]; Finke 2013 [14]). In this context, grasshoppers have immense potential because these insects are rich in protein and other nutrients (Haldar *et al*. 1999 [20]; Wang *et al*. 2007 [35]; Ganguly *et al*. 2013 [15]). Grasshoppers are found suitable as feed ingredient for poultry (Wang *et al*. 2007 [35]; Anand *et al*. 2008 [2]). Ganguly *et al*. (2014) [16, 24] have reported that upto 50% of fish meal can be replaced by the biomass of *Oxya fuscovittata* in the feed of black molly fish, *Poecilia sphenops* without compromising their growth and reproductive performance. *Oxya hyla hyla* is widely distributed multivoltine short-horned grasshopper and can be reared in "hopper farm" for biomass production (Haldar *et al*. 1999 [20]; Das *et al*. 2012 [10]; Ghosh *et al*. 2014 [18]). The aim of present study is to evaluate the nutritional quality in terms of proximate composition, amino acids, fatty acids, minerals, vitamins and anti-nutritional factors contents in the biomass of a grasshopper species *Oxya hyla hyla* Serville to explore its suitability as food material.

Materials and Methods

Maintenance, procurement of specimen and sample preparation

Oxya hyla hyla, a multivoltine, polyphagous, short horned grasshopper under the family Acrididae, order Orthoptera were collected from grassland and paddy fields of Santiniketan (23° 39'N/ 87° 42'E), West Bengal by sweeping technique, using an insect net and were maintained in the insectariums of the Department of Zoology, Visva-Bharati University, following a standard method (Haldar *et al*. 1999 [20]). Insect were fed *ad libitum* with fresh leaves of *Sorghum helepense*. Five thousand adult individuals were procured from the insectariums and kept without food for 24 hours before being sacrificed so that their guts become empty. The insects were freeze-killed, wet weight taken and then dried in hot air oven at 40ºC until body weight became constant. The moisture content was calculated using the formula: Moisture (%) = 100 - [(dry weight × 100)/wet weight]. The dried insects were ground to powder and subjected to further analyses. The insects were maintained and used according to the guideline of the Institutional Animal Ethics Committee, Visva-Bharati University, West Bengal, India.

Estimation of proximate composition

The standard methods (AOAC 1990) were used for analysis of the proximate composition of *O. hyla hyla* biomass on dry matter basis. All analysis was done thrice and the mean proximate values were reported in percentage. Percentage nitrogen content was estimated by micro Kjeldahl digestion and distillation method. Nitrogen content was converted into crude protein (%) using the factor N × 6.25. The crude lipid was estimated by ether extraction method. The samples were subjected to 40-60 ºC petroleum ether for 8 hours in a Soxhlet extraction apparatus (Instrumentation India, Kolkata, India). Total fiber content of the samples was determined by digestion method with 1.25% H_2SO_4 and 1.25% NaOH followed by washing in distilled water. Ash content was calculated by keeping the dried sample in Muffle furnace (Instrumentation India, Kolkata, India) at 550 ºC for about 6hours. Nitrogen free extract and carbohydrate (%) were calculated by difference method. Carbohydrate was calculated by the difference of the sum of percentage of crude protein, crude fat and ash from 100. Nitrogen free extract (NFE) was calculated by subtracting the sum of percentage of crude protein, crude lipid, ash and crude fiber and from 100.

Estimation of amino acids, fatty acids, minerals and vitamins

Amino acids, fatty acids, minerals and vitamins were estimated through Vimta Lab Limited, Hyderabad, India (an organization duly accredited by National Accreditation Board for Testing and Calibration Laboratories, DST, GOI as per ISO/IEC 17025; ISO 15189:2007). Amino acids were analyzed through HPLC with UV detector (Model-LC-2010A and C, Shimadzu) following HPLC method (Kwanyuen and Burton 2010) and tryptophan estimation was done by HPLC method (Zhang *et al.* 2009) through HPLC with fluorescence detector (Model-LC-10vp Series, Shimadzu). After extraction of fatty acids and their methyl esters were prepared and purified methyl esters of fatty acids were subjected to Gas Chromatographic (Model-3900, Varian) analysis (Bettleheim and Landesberg 1997 [5]). Percent compositions of the samples were computed from GC peak areas. Mineral contents such as Mg, K, Na, Fe, Zn, Cu, and Mn were estimated by ICP-MS methods. Vitamins like retinol, ascorbic acid, niacin and riboflavin were estimated by HPLC with UV detector (Model-LC-2010A and C, Shimadzu) and ascorbic acid estimated through HPLC with fluorescence detector (BIS 1970).

Estimation of anti-nutritional factors

Tannin was estimated with vanillin-HCl reagent and catechin solution and oxalate was estimated by titration using methyl red as indicator following the procedures (Gupta *et al.* 1988 [19]). Phytin phosphorus was estimated by titration using ferric chloride ($FeCl_3$) as indicator and phytin content was calculated by multiplying of the value of phytin phosphorus with 3.55 following the method (Agbede and Aletor 2004 [1]).

All data were presented as mean of three samples ± standard deviation (SD).

Results

O. hyla hyla retains about 54% moisture in their body and proximate compositions such as crude protein, fat, carbohydrate, nitrogen free extract, crude fiber and ash content as percentage of dry matter are shown in Table-1. The insect contains quite high amount of protein (about 64%), good amount of carbohydrate (28%) and nitrogen free extract (19%) while fat content is found only 2.58%.

Twenty amino acids have been assayed and their amounts are shown in Table 2. Among the estimated amino acids, glutamic acid and glutamine were found in quite high content about 30% of the dry matter of the insect followed by serine about 6% of dry matter while phenylalanine was found in non-detectable amount. The amino acid profile of the insect represents all essential amino acids such as lysine (4.29%), threonine (3.75%), cysteine (3.5%), isoleucine (2.85%), and leucine (2.53%) in good amount while arginine, tyrosine, histidine, methionine, tryptophan and valine are detected in low amount.

Twenty two fatty acids were detected in this species as shown in Table 3 and among them five fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were the major and constitute about 89.6% of total fatty acids. The content of linoleic acid was found to be the highest followed by palmitic acid, stearic acid, oleic acid, and linolenic acid.

Six minerals have been estimated (Table 4) among which magnesium content was found to be the highest (84.84±0.68mg/100gm) followed by zinc (17.34±0.40 mg/100gm), iron (16.19±0.51mg/ 100gm) and copper (4.36±0.17mg/100g). Manganese and phosphorus were detected in low quantity.

Four vitamin contents retinol, ascorbic acid, niacin and riboflavin have been assayed (Table 4) of which niacin (vitamin-B3) was found to be highest (29.59±0.58mg/100gm) followed by ascorbic acid (vitamin C) (26.73±0.31mg/100gm). But retinol (vitamin A) and riboflavin (vitamin B2) were detected in fewer amounts.

Four anti-nutritional factors such as tannin, oxalate, phytin bound phosphorus (phytin P) and phytin have been estimated (Table 5) and all were found in very less amount.

Table 1: Proximate composition of *O. hyla hyla* (% of dry matter).

Proximate composition	Mean	SD
Crude Protein	64.67	1.61
Crude Carbohydrate	28.17	1.44
Crude Fibre	9.23	0.70
Crude Fat	2.58	0.09
Nitrogen Free Extract	18.94	1.76
Ash	4.59	0.18

Table 2: Amino acids content in *O. hyla hyla* (% of dry matter).

Amino acids	Mean	SD
Alanine	1.76	0.12
Arginine	1.91	0.11
Aspartate & Asparagine	1.90	0.18
Cystine	3.57	0.16
Glutamic acid & Glutamine	30.01	0.71
Glysine	0.56	0.09
Histidine	1.12	0.11
Isoleucine	2.85	0.15
Leucine	2.53	0.15
Lysine	4.29	0.15
Methionine	0.97	0.18
Proline	0.39	0.06
Serine	6.23	0.46
Threonine	3.75	0.17
Tryptophan	0.52	0.14
Tyrosine	1.51	0.17
Valine	0.19	0.06
Phenylalanine	nd	

nd=not detectable

Table 3: Fatty acids content in *O. hyla hyla* (% of dry matter).

Fatty Acids	Mean	SD
Caprylic acid	0.003	0.001
Lauric acid	0.006	0.002
Myristic acid	0.023	0.001
Pentadecanoic acid	0.006	0.002
Palmitic acid	0.503	0.004
Palmitoleic acid	0.015	0.001
Heptadecanoic acid	0.044	0.004
Cis-10-Heptadecanoic acid	0.003	0.001
Stearic acid	0.487	0.006
Elaidic acid	0.005	0.001
Oleic acid	0.470	0.003
Linoleic acid	0.555	0.015
Arachidic acid	0.061	0.002
Gama-Linoleic acid	0.007	0.001
Cis-11-Eicosenoic acid	0.010	0.001
Linolenic acid	0.294	0.004
Heneicosanoic acid	0.005	0.001
Behenic acid	0.035	0.004
Cis-8,11,14-eicosatrienoic acid	0.001	0.001
Arachidonic acid	0.014	0.004
Lignoceric acid	0.007	0.001
Cis-4,7,10,13,16,19-docosahexaenoic acid	0.025	0.001

Table 4: Minerals and vitamins content in *O. hyla hyla* (mg/ 100g of dry matter).

Minerals	Mean	SD
Iron	16.19	0.51
Zinc	17.34	0.40
Magnesium	84.84	0.68
Copper	4.36	0.17
Manganese	2.30	0.07
Phosphorous	0.75	0.05
Vitamins	**Mean**	**SD**
Niacin (Vit-B3)	29.59	0.58
Ascorbic acid (Vit-C)	26.73	0.31
Riboflavin (Vit-B2)	2.55	0.20
Retinol (Vit-A)	0.12	0.03

Table 5: Anti-nutritional factors in *O.hyla hyla* (% of dry matter).

Anti-nutritional	Mean	SD
Tanin	2.316	0.024
Oxalate	0.474	0.029
Phytin P	0.031	0.002
Phytin	0.109	0.009

Discussion

The nutritional qualities of edible insects vary from species to species and even within the members of same species as a function of metamorphic stages, habitats and diets. Rumpold and Schlüter (2013) [32] have observed nutrient compositions for 236 edible insects and have found that insects provide with satisfactory amounts of energy and protein and meet amino acid requirements for human. Ramos-Elorduy *et al*. (2012) [30] observed the protein content in Orthopteran insects ranging from 43.9 to 77.1% of dry matter. Other works support the presence of rich protein contents in these insects (Haldar *et al*. 1999 [20]; Wang *et al*. 2007 [35]; Ganguly *et al*. 2013) [15]. Grasshoppers are also found to be suitable as feed ingredient for poultry feed (Wang *et al*. 2007 [35]; Anand *et al*. 2008 [2]), fish feed (Ganguly *et al*. 2014 [16, 24]) and for human consumption (Ramos-Elordy 2009). *Oxya hyla hyla* is a short horned grasshopper of high biotic potential and biomass yielding capacity (Ghosh *et al*. 2015 [17]) and suitable feed stuff for Japanese quail (Das and Mandal 2014 [9]). The present study on the proximate composition reveals that protein content of *O. hyla hyla* is about 64% of dry matter which is quite good amount when compared to that of other grasshopper species (Wang *et al*. 2007 [35]; Ganguly *et al*. 2013 [15]; Ramos-Elordy *et al*. 2012) and protein rich food materials such as fish meal (De Silva and Anderson 1998 [13]). Thus, from this point of discussion it can be concluded that *O. hyla hyla* is a potential source of protein rich food ingredients.

Quality of the protein source depends on its essential amino acids (EAA) content. Chen *et al.,* (2009) [7] analyzed more than hundred edible insects and have reported the presence of EAA. A substantial amount of EAAs has been reported in a grasshopper, *Oedaleus arbutus* (Ganguly *et al*. 2013 [15]). This study has assayed for 20 amino acids in *O. hyla hyla* and found all EAAs required for fish diets (Yang *et al*. 2010 [36]). Among the estimated amino acids, glutamic acid and glutamine are found to be quite high contributing more than 48% of the amino acids while phenylalanine is found in trace amount. High glutamine and glutamic acid content has been reported in different protein rich food materials such as in fish (Mohanty *et al*. 2014 [24]), meat and in grasshoppers (Wang *et al*. 2007 [35]). Glutamic acid is a nonessential amino acid but plays an important role in nutrition and synthesis of key molecules, such as glutathione which are required for removal of highly toxic peroxides and the polyglutamate folate cofactors. This study reveals the EAAs profile like lysine, threonine, isoleucine and leucine in good amount (>2.5% of dry matter) whereas arginine, tyrosine, histidine, methionine, tryptophan and valine are detected to be low as compared to fish (Mohanty *et al*. 2014 [24]). However, except valine and phenylalanine EAAs contents are found higher than the need of amino acid percentages in fish diets (Nose 1979; De Silver and Anderson 1998).

Edible insects are a good source of fat and polyunsaturated fatty acids (De Foliart 1991 [11]). Among grasshoppers the fat content of adult *Oxya chinensis* has been found to be only 2.2% (Chen *et al*. 2009 [7]) and 5.75% in *S. purpurascens* (Melo *et al*. 2011 [23]). The present study reveals only 2.58% crude fat contents in *O. hyla hyla*. Though amount of fat is less than the optimal need of 10-20% in fish diets (Cowey and Sargent 1979 [8]) the fat content of this insect biomass can play an important role in fish nutrition due to presence of polyunsaturated fatty acids. Chen *et al*. (2009) [7] report that like other animal fat edible insects have a higher content of essential fatty acids and eight fatty acids have been detected in a grasshopper, *Acrida cinerea* (Wang *et al*. 2007 [35]). The present investigation reveals twenty two fatty acids in *O. hyla hyla* and among them five poly unsaturated fatty acids namely linoleic acid, linolenic acid, palmitic acid, stearic acid and oleic acid contributes more than 89.6% of the fatty acids.

According to Chen *et al*. (2009) [7] edible insects are rich in protein and fat but not so rich in carbohydrate. However, carbohydrate contents have been reported to be 20-30% in some grasshoppers (Ramos-Elorduy *et al*. 2012 [30]; Ganguly *et al*. 2013 [15]). The present study also reported about 28% carbohydrate in *O. hyla hyla*.

Micronutrients (minerals and vitamins) play an important role in the nutritional value of food. The present study reveals the highest amount of magnesium followed by zinc, iron, copper and manganese in a good amount with respect to the need in carp diet (NRC 1993). Ramos-Elorduy *et al*. (1988) [31] observed that edible insects are rich in vitamin B group such as thiamine, riboflavin and niacin. The present investigation found that *O. hyla hyla* biomass contains considerable amount of vitamin B group and ascorbic acid but deficient in vitamin A. However, all vitamin contents are much lower than the plant sources.

Anti-nutritional factors are usually present in plants but many phytophagous insects may retain these chemicals (Berenbaum 1993). Among them tannin, oxalate and mineral bound phytate (i.e. phytin P and phytin) were detected in the grasshopper in the present study. The tannin content in *Oxya* is about 2.31% which is much lower than the plant food materials (Hassan *et al.* 2011) while oxalate, phytin P and phytin are detected to be very low.

Conclusion

From this study it may be concluded that the short-horned grasshopper species *O. hyla hyla* being rich in protein, amino acids, fat, fatty acids and carbohydrate and low in anti-nutritional factors is suitable for use as a protein rich feed supplement in fish and animal diets.

Acknowledgement

We thank the Head of the Department of Zoology, Visva-Bharati University for providing the laboratory and infrastructural facilities and the University Grants Commission (UGC), Government of India for financial support in the form of a Major research project F.No. 40-358/2011(SR). We thank to Vimta Labs Limited, Hyderabad, Andhra Pradesh, India for valuable assistance in analyzing amino acids, fatty acids, minerals and vitamins.

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