Variations of Protein Content of Orthopteran Edible Insects Consumed by Tribal People in Baksa of Assam, India.

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Abstract: Insects have been being used as a part of human diet among many indigenous people in Assam, India from long before. The human population is likely to grow from six billion in 2000 to nine billion in 2050. In near future, there would be great scarcity of sources of animal proteins and then people have to search for alternative source of the protein. The insects can solve the problem of protein food requirement of humans as most suitable food since insect diet increase the food convertibility and caloric and nutritive values. The Protein, the growth factor of the body, is essential for the sustenance of life. The proportion of protein per 100g fresh body weight of various insect species compares favourably. On chemical analysis it was observed that Orthopteran insects are nutritious in terms of protein (8.2–24.6 gm/100g fresh weight), which is the main growth factor of life. The paper highlights rich content of animal protein of Orthopteran insect species. The results suggest the Orthopteran insect biomass as an alternative protein rich feed component in the feed of fish and other livestock.

Keywords: Biomass, Indigenous, Nutritive, Orthopterans, Sustenance

Introduction: Insects are specialized group of animals belonging to the largest animal phylum Arthropoda in Animal kingdom. Insect (from Latin Insectum) are the class of arthropods that have a chintinous exoskeleton, a three part body (head, thorax and abdomen, three pair of jointed legs, compound eyes and two antennas. Insects have the wide range of adaptation and they can adjust in any environmental conditions, prevailing at high temperature and altitude. Out of the more than 1,900 eaten species described in scientific literature, 13% are locusts, grasshoppers, and crickets (Orthoptera) (van Huis et al., 2013). The Baksa district is very rich in edible insect diversity and the poor tribal people of study area sell different edible insects in markets besides consumption with their normal diets. The preference of the present study was made on the basis of nutritious potentiality of the edible Orthpteran insects in a particular study area where the people extensively eat insects in their normal meal. Food and Agriculture Organization of the United Nations (2013) states that insects have a high nutritional value, their cultivation is environmentally friendlier compared to other animal protein sources. Insects play a remedial role of ecological services that are fundamental to the survival of the humankind. They play an important role as pollinators in plant reproduction, in improving soil fertility through waste bioconversion, and in natural biocontrol for harmful

pest species. Insects provide a variety of valuable products to human beings such as honey and silk and medical applications. Eri silkworm and Muga silkworm have long been considered valuable for their products in the study area. Such edible insects are reared by the ethnic people in the study area for food and livelihood. Most of the edible insects are seasonal and a few are available throughout the year.

Grasshoppers, crickets, katydids and locusts all belong to the order Orthoptera which means 'straight wings'. Most are easily recognisable by their hind legs, which are usually enlarged for jumping. They are often easily seen jumping away when disturbed or heard 'singing' at night. Human insect-eating is common to cultures in most parts of the world, including North, Central, and South America; and Africa, Asia, Australia, and New Zealand.. The people especially the ethnic tribes in Assam are traditionally consuming insects as food. Insects have played an important role in human nutrition especially as a source of protein since time immemorial. There is an increasing interest in edible insects in the Western world as palatable dishes. Insects not only represent an important role as human food but they play an vital role as food source of other animal species such as fresh water fishes and amphibians, turtle, snakes and many aquatic insects such as mayflies, stone flies etc.for their continued existence. Most of the grasshopper species in the world are edible (Ramos-Elorduy *et al.,* 2012). Being cold blooded, grasshoppers are effortlessly collected during the coldest part of the day, early in the morning (van Huis *et al.,* 2013). Even though grasshoppers produce only one generation per year, they constitute such an enormous biomass that people all over the world dry the insects and then sell and consume them. As per National Health Commission Report, the protein energy malnutrition (PEM) contributes to more than 50% of the deaths of children fewer than five years all over the developing countries.

Grasshoppers and crickets are found worldwide ranging any vegetation in size, from one to five inches in length. These are the most popular edible insect amongst the ethnic tribal in the study area. The consumption of insects, also called entomophagy, is traditionally practiced by more than two billion people worldwide, mostly in Asia, Africa, and South America. Protein is the basic constituent for cellular and physiological functions and its deficiency might lead to adverse effects on human health. Globally, edible insects, especially species from the order Orthoptera (grasshoppers, crickets, and locusts) are rich in proteins and indicate a valuable substitute protein source. The Protein contents up to 77% (on a dry matter basis) have been reported for several grasshopper species (Ramos-Elorduy *et al.,* 1997, 1998). The study by Wang *et al.* (2005) on the nutritional value of field cricket (*Gryllus testaceus*) shows that field crickets have 58.3% CP on a dry basis. Many species of insects are lower in fat and higher in protein (Dunkel V, *et. al*., 2000). Protein contribution to overall human intake varies with the stage of the insects eaten and period of availability. Some insects, for example palm weevil, have higher protein content in the mature stage compared to when it is immature (Omotoso and Adedire 2007). Most developing countries are facing difficulties of acute shortage of animal protein that adversely affects the protein intake level of the country's

population resulting in malnutrition (Das *et al.*, 2009). As a result, insects are a good alternative as they are consumed all over the world (Melo *et al.*, 2011).

The Protein, the growth factor of the body, is essential for the sustenance of life and accordingly exists in the largest quantity of all nutrients as a component of the human body (Okuzumi and Fujii, 2000). In a nutritional sense, the phrase 'a protein of high quality' implies that it contains different types of amino acids in adequate proportions and that it is highly digestible by the organisms that consume it. The developing and under developed countries are having difficulties to provide sufficient food for their people, and consequently an insufficient intake of protein is leading to malnutrition (Aylward and Morgans, 1995). Many species of insects are lower in fat and higher in protein (Dunkel V, *et. al.*, 2000) In the north-eastern region of India, particularly the tribal communities of Manipur, Assam and Nagaland, use silkworms 'late instar larvae and pupae, chiefly the *Philosomia ricini* (eri silkworm) and *Bombyx mori* (mulberry silkworm) as food. For the tribes of this region, the pupa of the eri silkworm is so highly regarded as food delicacy that the cocoon is more or less a byproduct (Hazarika, 2008).

Insect is an excellent and relatively a cheaper protein source of high biological value in the developing countries like India, the poor people are unable to provide sufficient protein food to their family. Moreover, due to ignorance about the nutritive value, some people do not prefer to take insects as substitute protein food items. Research has established that over a thousand insect species have been used as traditional foods by humans and many still form an important part of the diet and economy of many societies (Merle, 1958; Katya Kitsa, 1989; DeFoliart, 1995). In parts of the Democratic Republic of Congo, insects constitute up to 64% of the animal protein consumed by humans (Paulin, 1963; DeFoliart, 1999). Traditionally consumed unconventional food items in Assam, India, namely the pupae of non-mulberry silkworms 'Eri' (*Attacus ricinii*), 'Muga' (*Antherae assama*) and mulberry silkworm (*Bombyx mori*) were analyzed for their proximate compositions and found to be highest protein content in insect body. The protein content of insects also depends on the metamorphosis stage (Ademolu *et al.*, 2010): adults usually have higher protein content than instars. Since Old Testament times grasshoppers, uncooked or in different preparations, as snacks are eating, they are affordable to all social groups, therefore, these Orthoptera insects may represent a good choice to get better food security worldwide. More than 2 billion people all over the world consume more than 2000 edible insects species - and not only due to hunger but for their taste. From the review of literature of different authors' nutritional status of edible insects shows that insects provide essential vitamins and minerals in addition to high protein.

1.1: Study Area

The Baksa district of Assam, India is also rich in diversity of insects. The district, Baksa, (**Figure-1**) falls at the latitude- 23° 11.4′ North and longitude- 88° 54.6 ′East having a Geographical area of Baksa is 2400 sq.

km. The study area is the Baksa district, which is one of the 27 districts of Assam situated in north-eastern India. The Baksa is bounded by Bhutan in the North, Udalguri district in the East, Barpeta, Nalbari and Kamrup in the South and Chirang in the West.

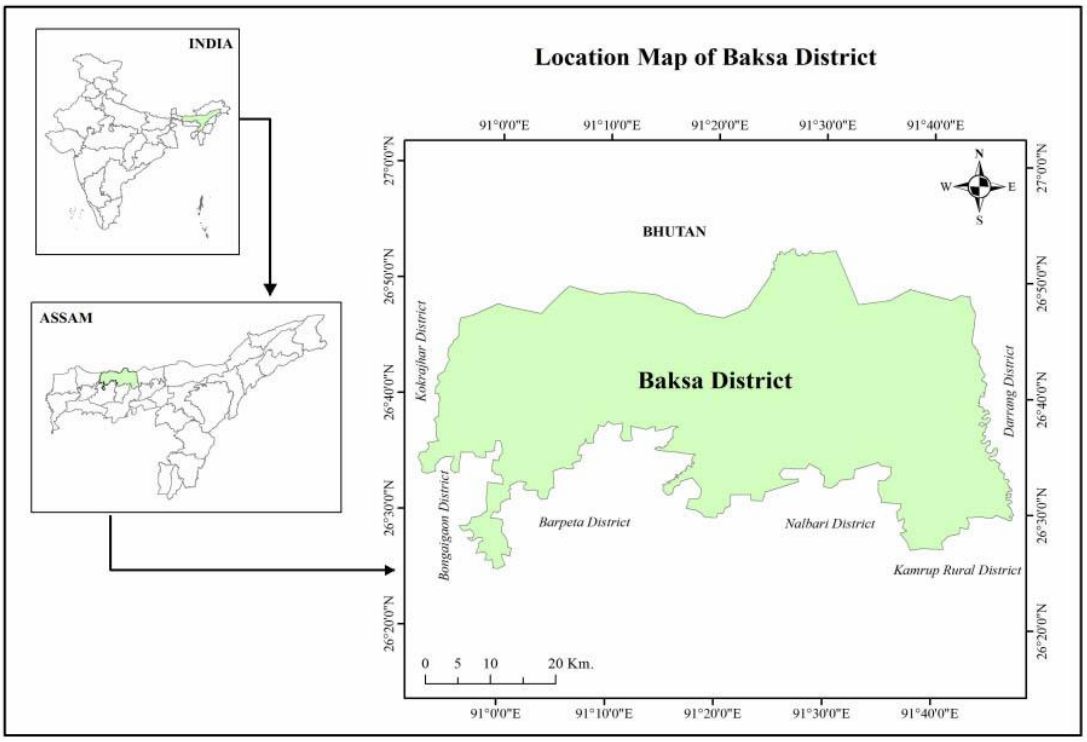

Figure: 1 Map of Baksa District, Assam

The district forms a part of the vast alluvial plains of Brahmaputra River system and Sub-basin of Rivers between the Manas and the Barnadi. Physiographically, the Baksa district is characterized by the different land forms resulting from a) denudation structural hill and b) alluvial plain.

1.2 Objectives of the study

The main objectives of the study are to evaluate the soluble protein content of Orthopteran species consumed by tribal people in Baksa, Assam. The objectives are

- To identify the collected edible insect specimen with valid key characters.
- To evaluate the protein content of Orthoptera i.e.grasshoppers and crickets consumed by tribal community in the study area.
- Comparison the amount of insect protein with Secondary data of other feedstuff.

So, this piece of my research work will inform the nutritional potentiality especially the protein value of commonly consumed Orthopteran insects by tribal people in the study area.

Materials and Methods:

Edible Orthopteran were collected from grassland and paddy fields of Baksa district (26º 32' -26º 40' N, 90º 56'- 91º 43'E), Assam by sweeping technique, using an insect net. The grasshoppers and crickets were collected early in the morning between 6.00 am and 7.00 am from grasslands and paddy fields and put in a clean plastic container. Then collected Orthopterans species were transferred into the freeze and kept at -20^{0}C and transported IASST, Boragaon for laboratory analysis. Before chemical analysing freezer samples were washed with tap water, rinsed with distilled water and kept in room temperature for a few hours. Estimation of soluble protein content of edible insects is done by Lowry's *et. al.*, (1951).The Lowry Assay protein by Folin reaction (Lowry *et. al,*.method (1951) has been most widely used method to estimate the amount the proteins in biological samples. All chemical analysis was done thrice and the mean values are reported in the result.

Procedure of identification: The edible insect specimen were identified and classified with valid taxonomic key. Few were preserved with standard methods (Ghosh and Sengupta, 1982) and identified later on comparing with the other specimens.Collected edible insects were identified on spot with the help of available literatures and books providing standard taxonomic keys, illustration, picture guide, and internet. Some insects were identified in the ZSI, Shillong, N.E, India. Some specimens were identified and confirmed by comparing with the specimens in Entomology Division of Department of Zoology, Cotton College, and a few specimens were identified by entomologist, Department of Zoology, Gauhati University, Guwahati, Assam.

Procedure for bio-chemical estimation of protein: To the tissue homogenate prepared equal volume of 10% tri-chloroacetic acid was added to precipitate the protein. Then, kept at low temperature (10^0-15^0) for about 30 minute. This was centrifuged at 6000 rpm for 10 minutes and the residue dissolved in appropriate Lowry *et. al.*, method (1951) volume (10ml) of 0.1N to dissolve the precipitated protein and used for the estimation of protein.

Suitable aliquot were pipette out in a series of test tubes [3 are unknown and 3 standard] and the volume was made up to 1 ml. with sodium hydroxide (0.1N). To each tube Sol^n C (5ml.) was added and allowed to stand at room temperature for 10 minutes. Now, 0.5ml. Folin phenol reagent was added and the contents of the tubes was mixed well and allowed to stand for 30 minutes at room temperature. Then the blue coloured test tubes were measured with the help of Spectro –photo colorimeter at 660 nm wavelength. A blank containing only NaoH and the reagents were also taken for adjusting the value of the colorimeter in Zero.

RESULTS

Table-1 shows the Orthopteran species of edible insects found in the Baksa district, Assam. The order Orthopteran shared the 12 numbers species belonging to 5 families during the field observation. The 12 species of Order Orthoptera collected in different places in Baksa, Assam are tabulated showing the scientific name, order, family along with their eaten part, seasonal availability and mode of eating (**Table-1**).

Scientific Name	**Order**	**Family**	**Consumed part**	**Seasonal availability**	**Mode of consumption**
Tarbinskiellus portentosus	Orthoptera	Gryllidae	Whole body	Whole year	Fried, burned chutney
Gryllotalpa africana (Beauvois)	Orthoptera	Gryllotalpidae	Whole body	April- - August	Fried, burned or chutney
Eupreponotus inflatus (Uvrov)	Orthoptera	Acrididae	Whole body	Sept- Nov	Fried, boiled or chutney
Choroedocus robustus (Serville)	Orthoptera	Acrididae	Adults, Whole body	May- August	Boiled, roasted and as vegetable paste
Chondracris rosea (De Geer, 1773)	Orthoptera	Acrididae	Adults, Whole body	May- Sept	Boiled, roasted and as paste
Heiroglyphus banian	Orthoptera	Acrididae	Adults Whole body	May- Sept	Fried, burned
Gryllus campestris	Orthoptera	Acrididae	Adult Whole body s	May- Sept	Fried, Boiled,chutney

Oxya hyla hyla	Orthoptera	Acrididae	Whole body	May- Sept	Fried, Boiled,chutney
Manis religiosa	Orthoptera	Mantidae	Whole body	May- Sept	Fried, Boiled,chutney
Mecopoda elongate elongate	Orthoptera	Tettigoniidae	Whole body	May- Sept	Fried, Burned ,chutney
Ruspolia baileyi	Orthoptera	Tettigoniidae	Whole body	May- Sept	Fried
Aceta domestica	Orthoptera	Gryllidae	Whole body	Whole year	Fried, Boiled,chutney

Table-1 shows the diversity of 12 Orthopteran edible insect found in the Baksa.

A total of 1920 persons were interviewed randomly through questionnaire survey for knowing the percentage of insect consumers **(Table-2**).

Table-2: Percentage of insect consumption among ethnic groups of Baksa District

Respondent groups	**Total No respondent**	**Positive response**	**% of consumption**
Bodo tribe	920	900	97.8%
Rabha	200	192	96%
Madahi	200	143	71.5%
Adivashi	250	201	80.4%
Sarania	200	157	78.5%
General (Other than tribal)	150	23	15.3%

The percentage of the insect consumers among different ethnic groups in Baksa district is graphically represented in the **figure 2**.

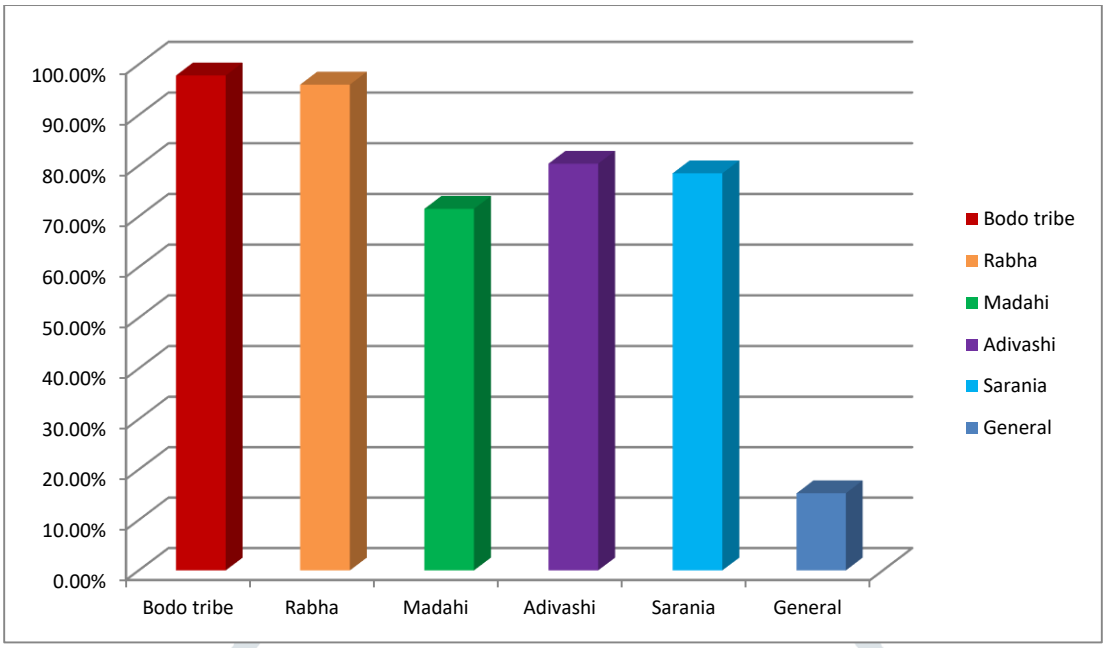

Fig-2: Graph of percentage insect consumption in Baksa District(community-wise)

All the 12 species of Orthoptera were taken for bio-chemical evaluation in the lab of IASST, Boragaon, Guwahati and biochemically estimated the soluble protein contents using Lowry *et. al*., method (1951) and the result of which is shown in **Table-3**. The table-3 shows protein content of Orthopteran species (8.2–24.6 gm/100g fresh weight), found in Baksa, Assam, India.

Table-3: Protein contents of Orthopteran species

Sl. No.	Name of the Species	**Protein Content (gm/100gm fresh weight)**
1	*Tarbinskiellus portentosus*	21.6
2	*Gryllotalpa africana* (Beauvois)	14.8
3	*Eupreponotus inflatus* (Uvrov)	16.9
4	*Choroedocus robustus* (Serville)	18.4
5	*Chondracris rosea* (De Geer, 1773)	24.6
6	*Heiroglyphus banian*	19.2
7	Gryllus campestris	13.08
8	Oxya hyla hyla	15.4
9	Manis religiosa	8.2

1o	Mecopoda elongate elongate	19.8
11	Ruspolia baileyi	22.4
12	Aceta domestica	18.3

The protein contents of these Orthopteran edible insects were expressed in mg/gm. But these values are converted into gm/100gm wet. weight and graphically represented in the **figure -3**.

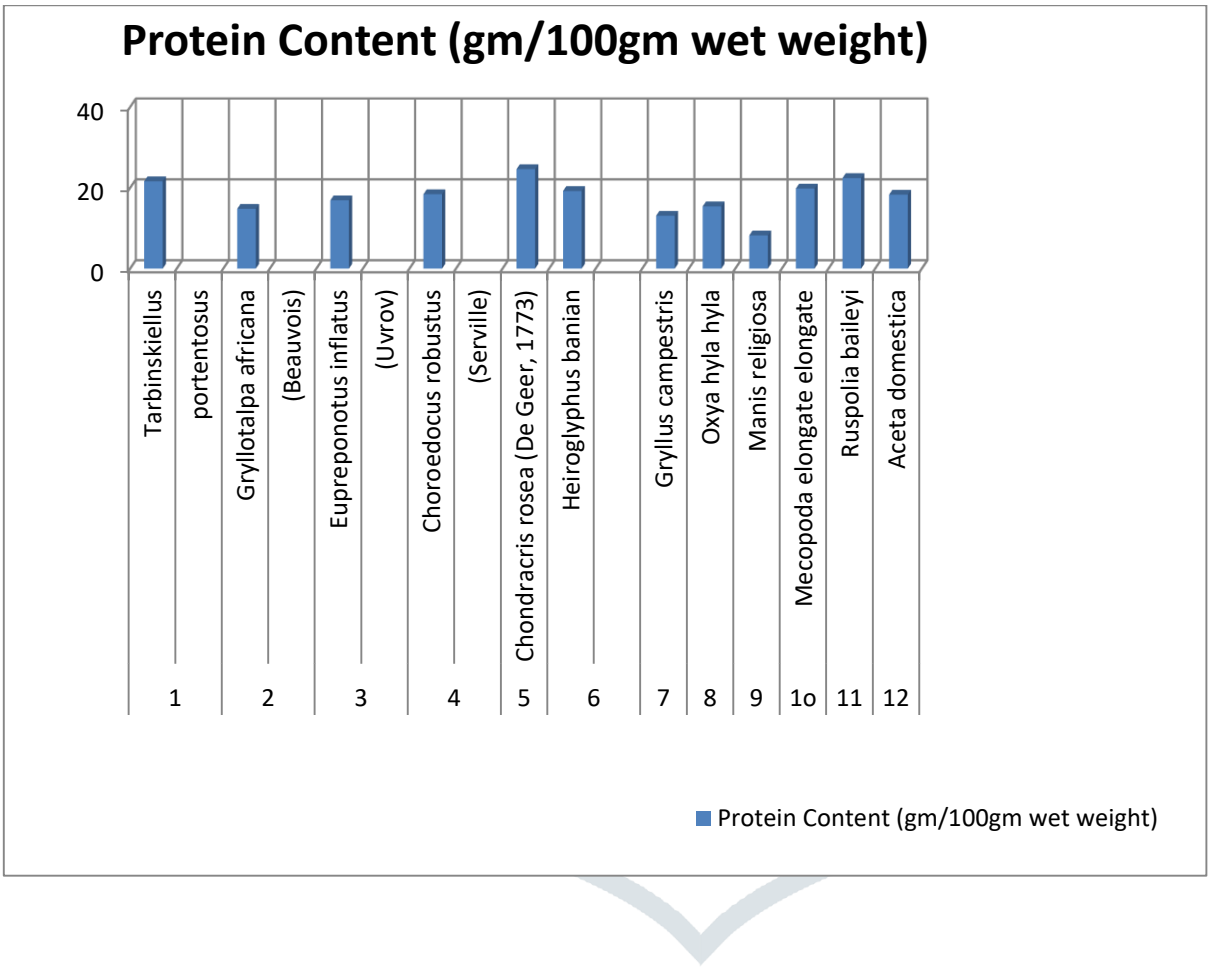

Figure-3: Variation of protein contents in Orthopteran species found in the Baksa district.

Xiaoming *et al.*(2010) assessed protein content in 100 insect species. Protein content was in the range of 13 to 77% by dry matter (**Table: 4**), reflecting the large variability of tested species.

Table 4: shows the proximate composition of Protein content in some edible insect speceis insect species (Order-wise). Source: Xiaoming *et., al.* [2010]

Orders of edible insects	Consumption Stages of Insect	Protein content (% in dry matter)
Coleoptera	Adults and larvae	23–66
Lepidoptera	Pupae and larvae	14–68
Odonata	Adults and naiads	46–65
Orthoptera	Adults and nymphs	23–65
Hemiptera	Adults and larvae	42–74
Homoptera	Adults, larvae and eggs	45–57
Hymenoptera	Adult, pupae, larvae and eggs	13–77

Discussion:

A total number of 12 species of edible Orthoptera insects belonging to 5 families are found in Baksa district Assam (**Table:1).**The tribal people especially Bodo and Rabha in the Baksa district, Assam in India also consume other edible insects such as cricket (Local name- Guchingra), grasshoppers (L.name- Guma) water giant bug (Bellostoma) (L.name-Gangjema), water scavengers (L.name- Angkhouri), termites (L.name-Chulung), eggs of red ants (*Myrmica rubra*), beetle, larvae, pupa of insects, water skater (Gerridaec) etc. Tribal people especially Bodo and Rabha people consume insects toasting, frying or making paste (chutney) with their normal diet or home-made rice beer (Jwakhai). In tribal communities of Baksa District, Assam women usually play a very important role in the society for maintaining families. The Orthoptera insects are prepared as curry, roasted, fried and raw form. Crickets (Gryllidae) and grasshoppers (Acrididae) etc. are fried in oil after having their wings removed and are then simply eaten with salt. Cricket is very much palatable dish for Bodo people in Baksa district, Assam. Moreover, the sustainable harvesting from the wild is an important hurdle that needs to be overcome. The edible Orthoptera species studied are shown in Table 1. The protein content of 12 edible Orthoptera species studied, are shown in **Table: 2**.

The ethnic tribal people of the study area consume Orthopteran insects mainly by frying, roasting, making paste or in raw stage .Similar modes of consumption were also found in the study of Alamu *et. al.*, (2013) who reported that processing of collected insects in Nigeria could be carried out by boiling, sun drying, frying and roasting methods. The mode of consumption of edible insects among ethnic tribes in Karbi Anglong district in Assam (Ronghang and Ahmed, 2010) has a congruity with the present study. The habit of insect eating is clearly seen amongst the ethnic tribal communities in the study area are Bodo, Rabha, Madahi, Sarania and Adibashi tea tribes. The Bodo people are the highest insect consumer with 97.8% amongst the other ethnic tribal people in the study area (**Table: 2**). The consumption of diverse species of Orthoptera insects reflects the diversity in wild Orthoptera insects in the study area.

The results presented here are the results on biochemical estimation of insects in fresh or wet weight condition. Results are expressed in g/100g fresh weight basis. The present biochemical analysis shows that Orthopteran insects vary widely between species in terms of protein content. According to Chen *et al.*, (2009) edible insects are rich in protein and fat but not so rich in carbohydrate. On chemical analysis, it is observed that edible insects are rich in terms of protein ranges from 8.2 g/100g to 24.6 g/100g fresh wet weight. Thus, the present study revealed that there was a significant variation in the protein content among the commonly consumed insects. Thus, the present study revealed that there was a significant variation in the protein content among the commonly consumed insects.A recent study on edible insects by Payne *et al.*, *(*2016) who revealed that the value of protein content exposed with insects containing median values of between 9.96 g and 35.2 g of protein per 100 g, compared with 16.8–20.6 g for meat. The biochemical estimation of different species of grasshopper showed a variation in terms of protein contents. Out of this Order Acrididae (*Chondracris rosea*) reveals the highest amount of protein. The present study also reveals that 100 grams fresh weight of house cricket (*Acheta domestica*) contains 18.3 grams of protein, This findings have congruity with the results of Lukiwati (2010) who revealed that 100 grams of cricket contain 121 calories, 12.9 grams of protein. Moreover, the nutritive value of 25 edible Orthoptera in Mexico were shown that protein content ranges from 43.93% to 77.13% (mix of Edible Acrididae).Values reported of protein percentage in these insects were superior to some mexican conventional foods used as source of protein (beans, lentils), and similar to those of soybean, chicken, eggs and beef(Blasquez *et.,al*. 2012).

Ramos-Elorduy *et. al.*, (2012) observed the protein content in Orthopteran insects ranging from 43.9 to 77.1% of dry matter. Similarly, Melo et ., al. 2015 found that the determined protein content of grasshoppers was very high, from 62.5% to 77.25%. The secondary data on edible insects also highlight the protein content of edible insects order-wise and the range of protein content lies from 13 to 77% by dry matter (Table: 4). All the Orthopteran species showed rich content of protein. The species *Chondracris rosea* of family Acrididae has the highest amount of protein content **(gm/100gm wet weight)** whereas *Manis*

religiosa of family Mantidae contains comparatively lowest amount of protein content**(gm/100gm wet weight).**

During the survey, it was also observed that most of the tribal people of the study area use to consume larve and pre-pupal stage of eri preferably more than larve and pupae of muga worm or other silkworm. Bodo and Rabha people in Baska district of Assam considered the curry preparation of edible insect as the top most delicious item among their other dishes. As long as protein-energy malnutrition prevails in developing countries, the search for low cost, nutritious and easy to prepare locally available complementary foods will continue. The rising cost of animal protein, food and feed insecurity, environmental pressures, population growth and growing demand for protein lead to entomophagy where insects stand as alternative solutions to conventional livestock and feed sources. Therefore insects as food and feed appear as a very significant matter of today.

The present study on the Orthopteran species shows variations in protein content among the species. Also, it can be reported that most of Orthopteran insects are rich source of protein which are found to have 8.2 gm to 24.6 gm per 100 gm fresh body weight. Thus insects represent the cheapest source of animal protein in the study region and that their consumption should be encouraged because most of the common people cannot afford fish or other meat due to high price. There is need for well-documentation of traditional rearing, cultivation and sustainable use of edible insects including Orthopterans for the greater benefit of our future generation.

CONCLUSION:

Edible insects constitute an important part of the daily diet for most of the poor tribal people of the Baksa District of Assam, India. It can be concluded that Orthoptera species have a high protein value specially those belonging to the family Acrididae i.e. the species is *Chondracris rosea* followed by the family Tettigoniidae i.e . *Ruspolia baileyi.* These values of protein content of edible insects indicate that insect could serve as an alternative source of protein supplement in human and animal diets. Though several methodical publications have reported on nutrient composition of different species of Orthoptera yet extensive research on the nutritional composition of edible insects is still required.

The further scientific study on edible insect may aware the masses to help food security for the poor & vulnerable section of the society who cannot afford to buy expensive animal food.

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