



Ducetia japonica and *Phyllozelus* Sp.: Two Tettigoniid Species of Orthopteran Insects Appreciated by Tribal People of Arunachal Pradesh (North-East India) May Serve as Future Alternative Food Source

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Abstract

Ducetia japonica and *Phyllozelus* sp., two tettigoniid orthopteran insect species appreciated as food by people of Arunachal Pradesh were analyzed to assess their nutritional potentials. *Ducetia japonica* and *Phyllozelus* sp. contained 56.28% and 61.57% protein, 14.99% and 7.93% fat, 11.84% and 8.30% fiber, 4.59 % and 3.01 % ash and 11.84% and 19.19% carbohydrates respectively. In both the species 18 amino acids were detected including essential amino acids and were found satisfying the recommended dosages (scores >100). Both the species contained 22.77% and 26.84% MUFAs and 18.93% and 41.73% PUFAs, respectively. The two orthopteran insects contained superior amount of micro minerals (Cu, Fe, Zn and Mn) and moderate amounts of macro minerals (Na, K, Ca and Mg). Thus, these two-orthopteran species could serve as an alternative food source and as a tasty and promising source of essential nutrients on par with or superior to conventional food of both plant and animal origin. They should be included in plans to rear and farm nutritionally valuable species of edible orthopterans.

Keywords: Alternative Food Source; Arunachal Pradesh; *Ducetia japonica*; Nutritional Potentials; *Phyllozelus* Sp

Introduction

The population of especially India's northeast is expected to continue to increase, which means that food security for the inhabitants of the region becomes an increasingly important issue. Having noticed that members of various traditional societies made use of insects as food, [1] suggested that insects as an item in the human diet could ease the problem of global food shortages and Arunachal Pradesh is one of the regions of India where insects even today are still being valued as food [2]. Entomophagy, i.e. the consumption of insects by humans [3], has a long history and has been reported from many parts of the world [4]. It is estimated by [5] that even today worldwide there may still be 2 billion people that at least occasionally choose to eat some of the approximately 2,000 known species of edible insects [6]. Taking into consideration that many insect species are multivoltine and have a high fecundity, exhibit efficient feed conversion characteristics, possess low space

requirements and may be reared on a variety of comestibles often considered inedible or unattractive by human, certain species of insects can indeed contribute to food security and represent an interesting alternative to conventional domestic animals.

With an area of 83,743 km² Arunachal Pradesh is the largest state in North-East India and home to numerous species of insects, a plethora of which, representing at least 12 orders, have been identified as edible and as highly appreciated by members of the local tribes [2,7]. In most cases, these insects are not consumed as a nutritional supplement or to ward off starvation but are esteemed for their taste and often regarded as a -frequently not even very cheap- delicacy. Analyses of the nutrient compositions of the locally highly regarded bug *Aspongopus nepalensis* [8], the weaver ant *Oecophylla smaragdina* and the termite *Odontotermes* sp. [9] have demonstrated that such insect species are of value as a source of essential amino and fatty acids, minerals and vitamins. Some of the most popular edible insects not only in Arunachal Pradesh, but worldwide, are members of the order Orthoptera and [10] in their thorough review, covering edible members of the families

Acrididae, Pyrgomorphidae, Tettigoniidae and Romaleidae, provide a wealth of comparative data on the nutrient contents of these insects. The acridid grasshopper *Chondracris rosea* and the gryllid cricket *Brachytrupes orientalis* are two orthopterans appreciated by Arunachal Pradesh tribals belonging to the Adi, Apatani, Chakma, Deori, Galo, Nyishi, Singpho and Wancho and their nutrient contents have been published [11]. Two additional Orthopteran species, the tettigoniids *Ducetia japonica* and *Phyllozelus* sp, are equally appreciated, but have not yet had their chemical compositions analyzed, it is for this reason that the present study focuses on these two species, hoping that nutritious products based on these insects may be promoted in the future among the tribes of Arunachal Pradesh and elsewhere in India as food or feed or even a source of bioactive medicinal compounds [12].

Materials and Method

Specimen Acquisition and Sample Preparation

Both *Ducetia japonica* and *Phyllozelus* sp. were collected from a variety of places around campus, e.g. rice and sugarcane fields, forests, fruit orchards and hills, trees, shrubs, herbs and grasses. Sampling occurred throughout the year for two consecutive years, 2013 to 2015. Only adult insects were collected. The sexes were not separated, because firstly, no taboo had been reported regarding the sex of the selected insects, secondly male and female specimens differed little in size and coloration and thirdly, both were equally appreciated as food.

Sample Preparation

In the laboratory, the insects were washed thoroughly, blotted and then oven-dried (50-60°C). Ground to a powder, they were then stored in deep-freeze (-20°C) under vacuum for proximate, amino acid, mineral, and fatty acid analyses. The analyses were carried out in triplicate and completed within ten days following the collection of the insects. All of the solvents and chemicals used in this study were of analytical grade and care was taken that the glassware was meticulously clean.

Analyses

Macronutrients

Proximate analyses of the insect samples to determine moisture content, crude protein, crude fat, crude fibre, ash and Nitrogen Free Extract (NFE), were based on standard methods [13]. Moisture percentage was calculated by drying the sample in an oven at 100°C for 2 h. Crude protein was determined by the Kjeldahl method and total protein content was subsequently calculated by multiplying the nitrogen content by a factor of 6.25 [14]. Fat percentage was calculated by drying fats after extraction in a Soxhlet apparatus, using petroleum benzene. Carbohydrate content was estimated by subtracting the sum of the weights of

crude protein, lipid, ash and fibre from the total dry matter and reported as NFE by difference or as nitrogen free extracts. Crude fibre was determined through double digestions, first with sulphuric acid and then with sodium hydroxide. The ash content was determined by combusting the insect sample in silica crucibles in a muffle furnace at 620°C for 3 hours. The calorific value (kcal/100 g) was calculated by multiplying the factors for carbohydrate and protein by 4 each and that of fat by 9 and then taking the sum of the products. All of the analyses were performed in triplicate and expressed as mean \pm standard deviation.

Micronutrients

Fatty Acids

The methyl ester of the fat of the insect sample was prepared according to [15]. The fatty acid composition of the methyl ester was determined by Gas-Liquid Chromatography (GLC) according to [16] and based on three samples of 30g dry weight each.

Amino acid

Amino acid composition was determined by HPLC (Agilent 1100) according to the standard method recommended by the [13]. The ground samples were hydrolyzed in 6 N HCl for 18h at 120°C under nitrogen atmosphere and then concentrated. The concentrated samples were reconstituted with 20 mM HCl and derivatized with borate buffer. The hydrolyzed samples were analyzed for amino acid composition. All the analyses were performed in triplicate and expressed as a percentage of individual amino acid in the protein (i.e. total amino acids) fraction. Amino acid scores were calculated based on the recommendations by [17].

Minerals

Minerals were determined by Atomic Absorption Spectrophotometry (AAS) after dry-ashing the samples followed by acid dilution [13]. The ash was digested with HCl made up to 100 ml and filtered before the mineral elements were determined by AAS. All the analyses were performed in triplicate and expressed as mean \pm standard deviation.

Results and Discussions

Moisture: (Table1)

The moisture content of both species (58.48% in *Ducetia japonica* and 57.78% in *Phyllozelus* sp.) was found to be rather similar to that of beef (USDA database), but somewhat higher than that known from the orthopteran *Chondracris rosea* [11] and lower than that of the meadow grasshopper *Chorthippus parallelus* [18]. Moisture content of a food item can be an indicator of its stability and susceptibility to microbial spoilage [19] and therefore a lower value can be expected to improve the shelf life and the retention of nutrients of the food item in question.

Name of Insect	<i>Ducetia japonica</i>	<i>Phyllozelus sp.</i>
Moisture	58.476±0.421	57.777±0.362
Crude Protein	56.279±1.024	61.572±1.329
Crude Fat	14.993±0.136	7.930±1.097
Ash	4.586±0.345	3.014±0.313
Crude Fiber	11.840±0.259	8.298±0.088
Nitrogen Free Extract (NFE)	12.302±2.224	19.186±3.345
Calorific value (Kcal/100g DM)	409.261±15.372	394.402±10.252

Table 1: Proximate content of *Ducetia japonica* and *Phyllozelus sp.* (Mean ± SE).

Crude fibre: (Table 1)

Crude fibre is the nonstructural carbohydrate or the indigestible part of an insect. The present study shows that the species analysed are rich in fibre content reaching a value of 11.84% in *Ducecia japonica* and 8.30% in *Phyllozelus sp.* In terms of fibre content this places the species within the range of 3 - 12.2% for 25 orthopteran species as reported by [20] and members of the family Tettigoniidae [18]. It was also comparable to the dietary fibre content (g/100 g) of rice (4.1), wheat (12.5), whole Bengal gram (28.3), lentil (15.8), cabbage (2.8), green colocasia (6.6) and yam (4.2) [21]. Fibre enriched food is considered advantageous for our health as it reduces constipation by stimulating bowel movement, is useful in the context of weight control and fat reduction since it gives a feel of satiety even when only a small amount of such food is eaten.

Nitrogen Free Extract: (Table 1)

Besides crude fibre, considerable amount of nitrogen free extracts indicative of carbohydrates is found in *Ducecia japonica* and *Phyllozelus sp.* and amounted to 11.84% and 19.19% respectively. These values are comparable to those (0.19 to 22.64%) reported by [20,22] and for other Orthopteran species, but considerably higher than carbohydrate values given for 3 tettigoniid species listed in [10]. Differences like these may be species-specific and depend on the season, nutritional state and age of the insect, as shown for lipophilic contents of *Rhynchophorus phoenicis* larvae by [23] but are also likely due to differences in the methods used to determine carbohydrate content.

Calorific Value: (Table1)

The calorific value of *Ducetia japonica* (409.26 kcal/100g) and *Phyllozelus sp.* (394.40 kcal/100 g) places them within the range given for 78 Mexican species of edible insects (293-762 kcal/100g) studied by [24] 11 species of African Orthopterans (196-713 kcal/100 g) studied by [25] and honey bee adults and larvae (388.4 and 455.8 kcal/100g, respectively) as reported by [26]. When compared with the calorific values of chicken eggs (173 kcal), rice (345 kcal), wheat (345 kcal) and whole grain (335 kcal) given by [27], both of our insect species show that they are of substantial nutritive value.

Protein and Amino Acids: (Table 1 and 2; Figure 1,3 and 4)

Respective crude protein values of 56.28% and 61.57% of *Ducetia japonica* and *Phyllozellus sp.* were higher than those for other Orthopterans reported by [20,25] but in line with acridid grasshoppers [10,11]. When compared with the protein content of some conventional sources of meat like beef, pork and chicken, that of insects generally was often higher, indicating the potential of edible insects in mitigating protein malnutrition. The amino acid composition of proteins determines the quality of proteins. Humans and insects have the same amino acid requirements [28]. In *Ducetia japonica* and *Phyllozelus sp.* proteins are composed of 18 amino acids, including all the amino acids regarded as essential for humans and satisfying (scores > 100) the recommended dosages suggested by [17]. As with other insect species (including orthopterans: [10,29] all of the essential amino acids were present [20,30]. Of particular interest were the high levels of valine, leucine, lysine, isoleucine and threonine, which together with lysine, phenylalanine, and histidine surpass the values recommended by [17] for the “chemical score” of amino acid as an index of protein quality. Lysine and threonine are the limiting amino acids in conventional diets of rice, wheat, cassava and maize prevalent in the developing world [31]. A comparison of the amino acid composition of *Ducetia japonica* and *Phyllozelus sp.* with conventional animal foods (USDA database) indicates the supply of some of these essential amino acids from the two-insect species would either be similar or even superior to those found in conventional food items.

An appropriate balance of EAA and NEAA and other nitrogenous compounds is necessary for an effective utilization of dietary proteins. In both of our species, the NEAA were also present in substantial amounts (2.7 to 4.8 g/100 g), which were higher than or comparable to those of other edible insects [10,20,30].

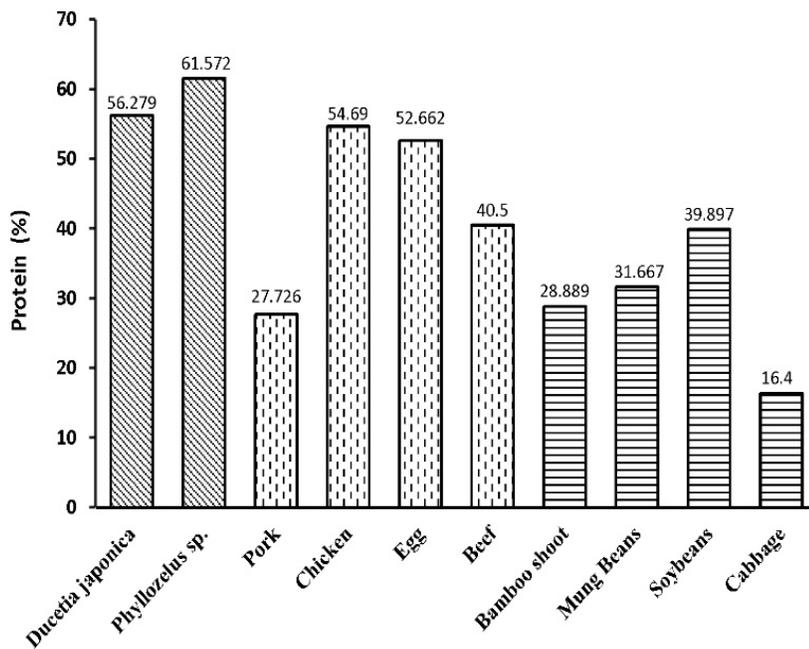


Figure 1: Protein content (% of DM) of *Ducetia japonica*, *Phyllozelus sp.* and some conventional food of plant and animal origin (source USDA database: www.ndb.nal.usda.gov).

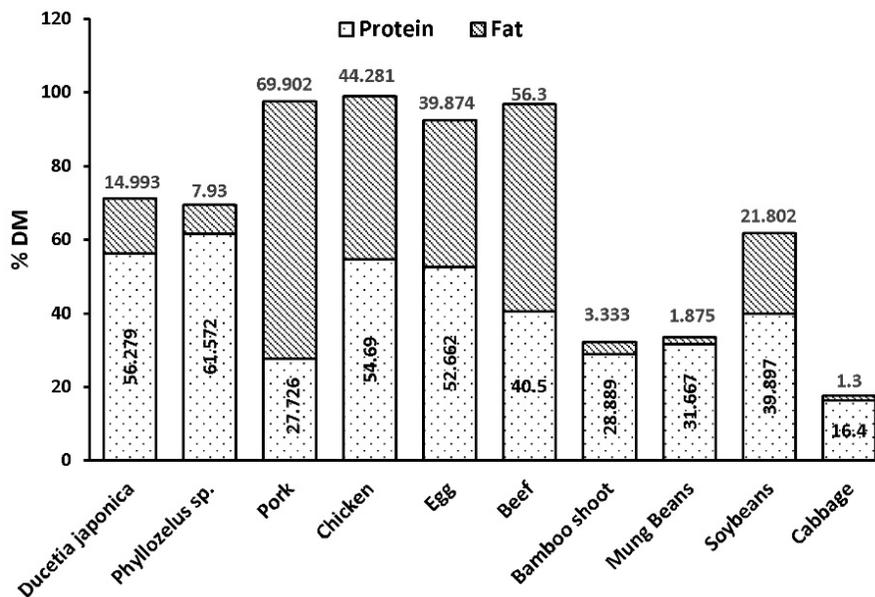
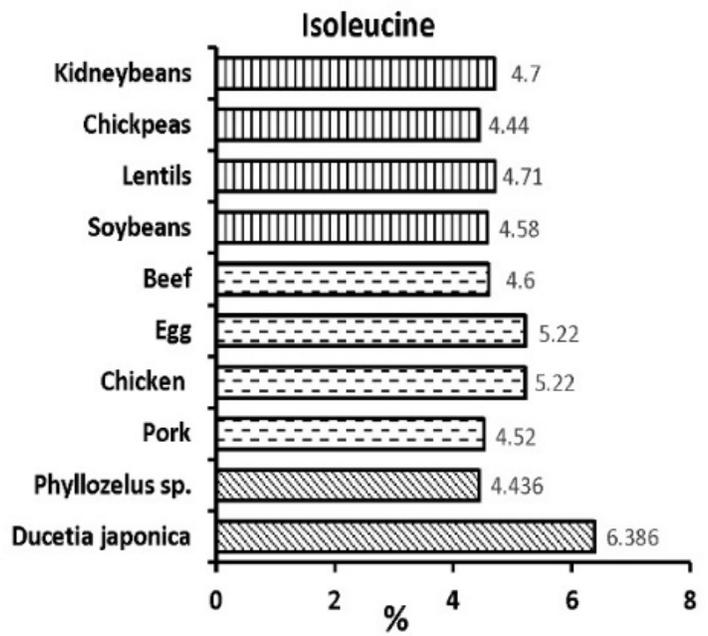
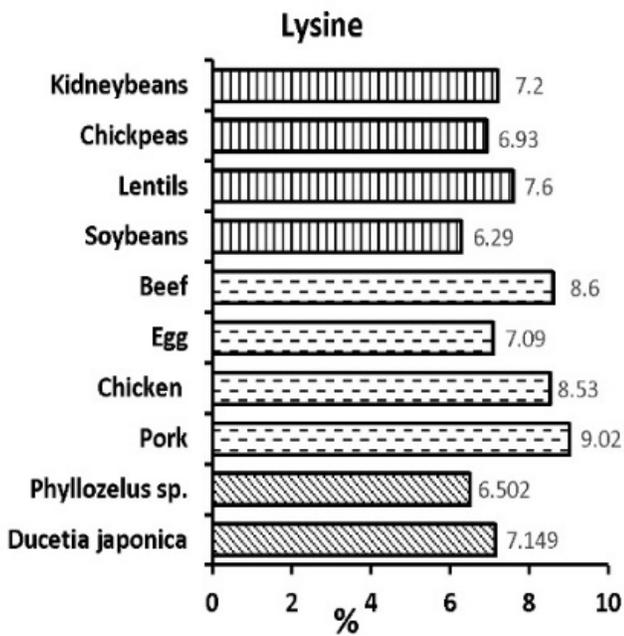
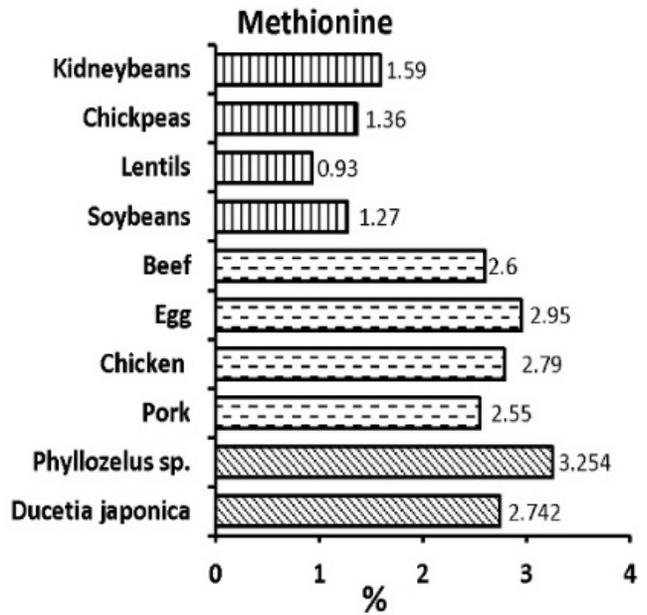
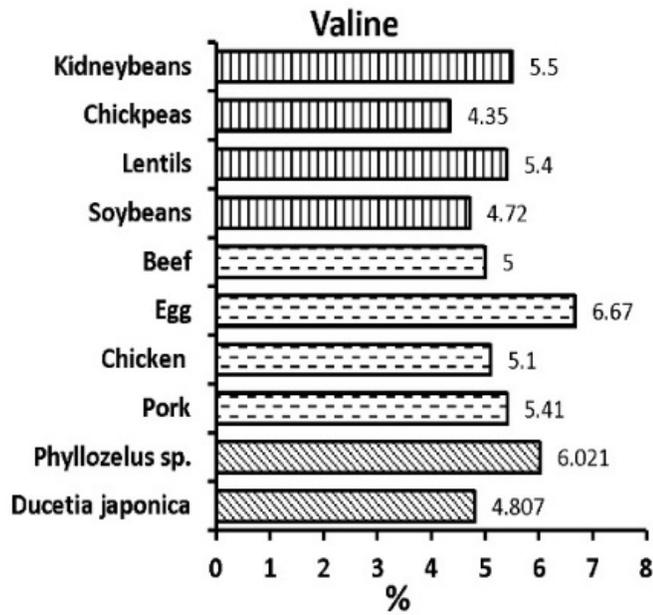
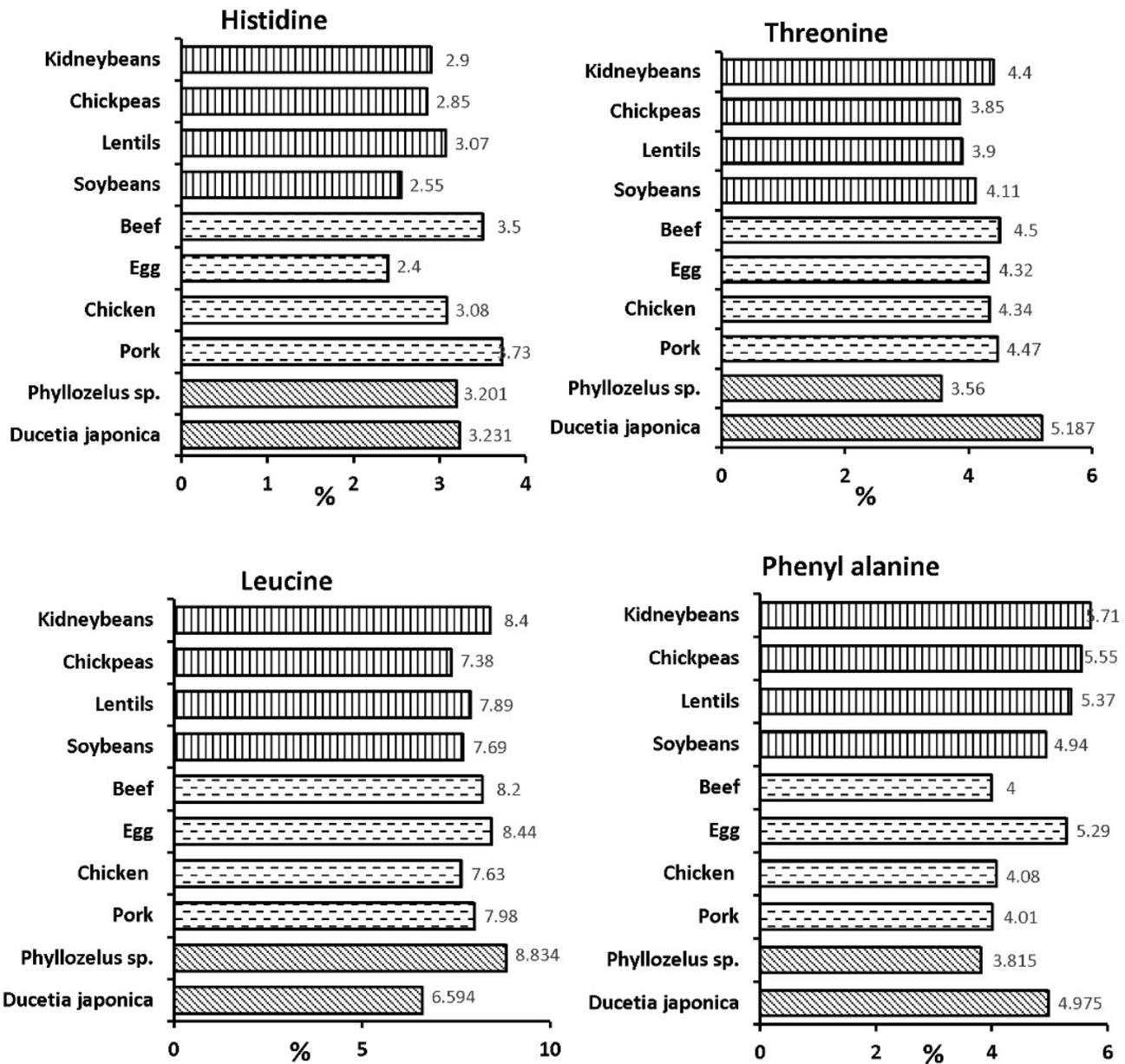


Figure 3: Comparison of protein and fat content (% dry matter, DM basis) of studied insects with conventional food sources of animal origin (data other than insects were obtained from USDA database).





Figures 4 (a and b): Comparison of amino acid composition (% of total amino acid) of *Ducetia japonica*, *Phyllozelus sp.* and some conventional foods of both plant and animal origin (source USDA database www.ndb.nal.usda.gov).

Essential amino acids	(g/100g of sample)		(% of total amino acids)		Amino acid score FAO/WHO/UNU 2007 (mg/g protein)	
	<i>Ducetia japonica</i>	<i>Phyllozelus sp.</i>	<i>Ducetia japonica</i>	<i>Phyllozelus sp.</i>	<i>Ducetia japonica</i>	<i>Phyllozelus sp.</i>
Valine	2.682±0.003	3.553±0.000	4.807	6.021	123.26	154.39
Methionine	1.530±0.000	1.92±0.000	2.742	3.254	171.4	203.4
Lysine	3.989±0.025	3.837±0.009	7.149	6.502	158.9	144.5
Isoleucine	3.563±0.004	2.618±0.008	6.386	4.436	212.87	147.87
Leucine	3.679±0.015	5.213±0.006	6.594	8.834	111.8	213.4
Phenyl alanine	2.776±0.049	2.251±0.032	4.975	3.815	346.132	261.974
Histidine	1.803±0.038	1.889±0.065	3.231	3.201	215.4	215.4
Threonine	2.894±0.003	2.101±0.025	5.187	3.560	225.5	154.8
Tryptophan	0.55±0.001	0.58±0.001	0.986	0.983	-----	-----
Total essential amino acid			42.057	40.606		
Non- essential amino acids	(g/100g of sample)		(% of total amino acids)		Amino acid score FAO/WHO/UNU 2007 (mg/g protein)	
	<i>Ducetia japonica</i>	<i>Phyllozelus sp.</i>	<i>Ducetia japonica</i>	<i>Phyllozelus sp.</i>	<i>Ducetia japonica</i>	<i>Phyllozelus sp.</i>
Serine	2.766±0.058	3.459±0.097	4.957	5.862		
Glycine	2.326±0.102	3.752±0.098	4.169	6.358		
Arginine	4.912±0.008	3.312±0.008	8.804	5.613		
Alanine	3.862±0.078	5.827±0.005	6.922	9.874		
Proline	3.117±0.003	2.341±0.010	5.587	3.967		
Tyrosine	4.563±0.025	3.623±0.003	8.178	6.14		
Aspartic acid & Asparagine	5.171±0.009	5.650±0.006	9.268	9.574		
Glutamic acid & Glutamine	5.612±0.012	7.085±0.001	10.058	12.006		
Total amino acid	55.795	59.011				

Table 2: Amino acid profile (g/100g of sample), (% of total amino acids) and amino acid score based on FAO/WHO/UNU 2007 consultation pattern of analyzed *Ducetia japonica* and *Phyllozelus sp.* (Mean ± SE).

Fat Content: (Table 3; Figure 2,3 and 5)

With respective fat contents in *Ducetia japonica* and especially *Phyllozelus* sp. of 14.99% and 7.93%, these insects were leaner than many other insect species appreciated as food or conventional food sources [8,9,20,23,32-34], something not exactly undesirable. When compared with conventional meats (USDA database) both of the species are lower in fat content.

The fatty acid composition determines the quality of the fat. The fatty acid compositions of both *Ducetia japonica* and *Phyllozelus* sp. revealed the presence of 14 and 19 Fatty acids, respectively. *Ducetia japonica* and *Phyllozelus* sp. contained 57.96% and 30.74% saturated (SFA), 22.77% and 26.84% Mono-Unsaturated (MUFA) and 18.93% and 41.73% Poly-Unsaturated Fatty Acids (PUFA), respectively. Major components of the SFA fraction commonly found in both the species were 14:0 myristic, 16:0 palmitic, and 18:0 stearic acids. The amounts of oleic acid in both *Ducetia japonica* and *Phyllozelus* sp. were 18.93% and 24.48% respectively. In *Ducetia japonica* the UFA fraction was 41.70%, which is lower than the SFAs present. In *Phyllozelus* sp. the proportion of unsaturated fatty acids (UFAs 68.57%) was higher than that of Saturated Fatty Acids (SFAs) and a similar situation was reported for many other edible insects [10,33,35-40]. In

Ducetia japonica alpha-linolenic acid was only recorded to 1.50%, but another PUFA, i.e., ω -6 linoleic acid reached 8.51% in *Ducetia japonica* and 14.60% in *Phyllozelus* sp. and was therefore more prevalent in these insects than in most other conventional foods of animal origin like lamb, veal, beef and cow milk. In *Phyllozelus* sp. alpha-linolenic acid (a PUFA) reaches 26.15%, which is significant for human nutrition as it is thought to considerably help reducing the occurrence and effects of cardiovascular, hypertensive, inflammatory, and auto immune disorders as well as depression and certain neurological functions [41,42]. Thus, *Ducetia japonica* and *Phyllozelus* sp. could be an excellent source of quality fat, due to the presence of PUFAs and stearic acid as part of the SFA content. With an SFA: UFA ratio of 1.390 and 0.448 in *Ducetia japonica* and *Phyllozelus* sp., (a little higher than the reported range of 0.262 to 0.96 for other insects: [9,30,33,37-39]. The presence of both SFA and UFA could be seen as an advantage as they may complement each other's physiological functions. However, not all the saturated fatty acids have the same effect on cholesterol synthesis in the liver. Only saturated fats with chain lengths of (12, 14, and 16: lauric, myristic and palmitic acids) have been shown to elevate blood cholesterol level and stearic acid (C18, saturated) is actually credited with lowering body cholesterol by 21% [43].

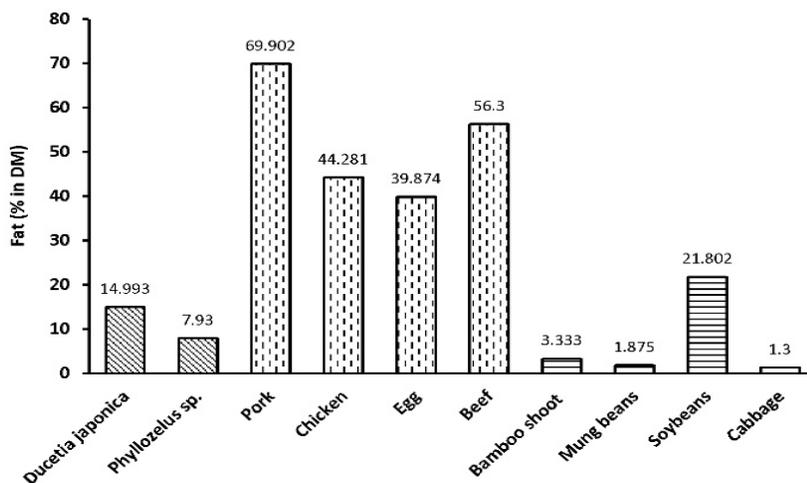


Figure 2: Fat content (% of DM) of *Ducetia japonica*, *Phyllozelus* sp. and conventional food of both plant and animal origin (source USDA database: www.ndb.nal.usda.gov).

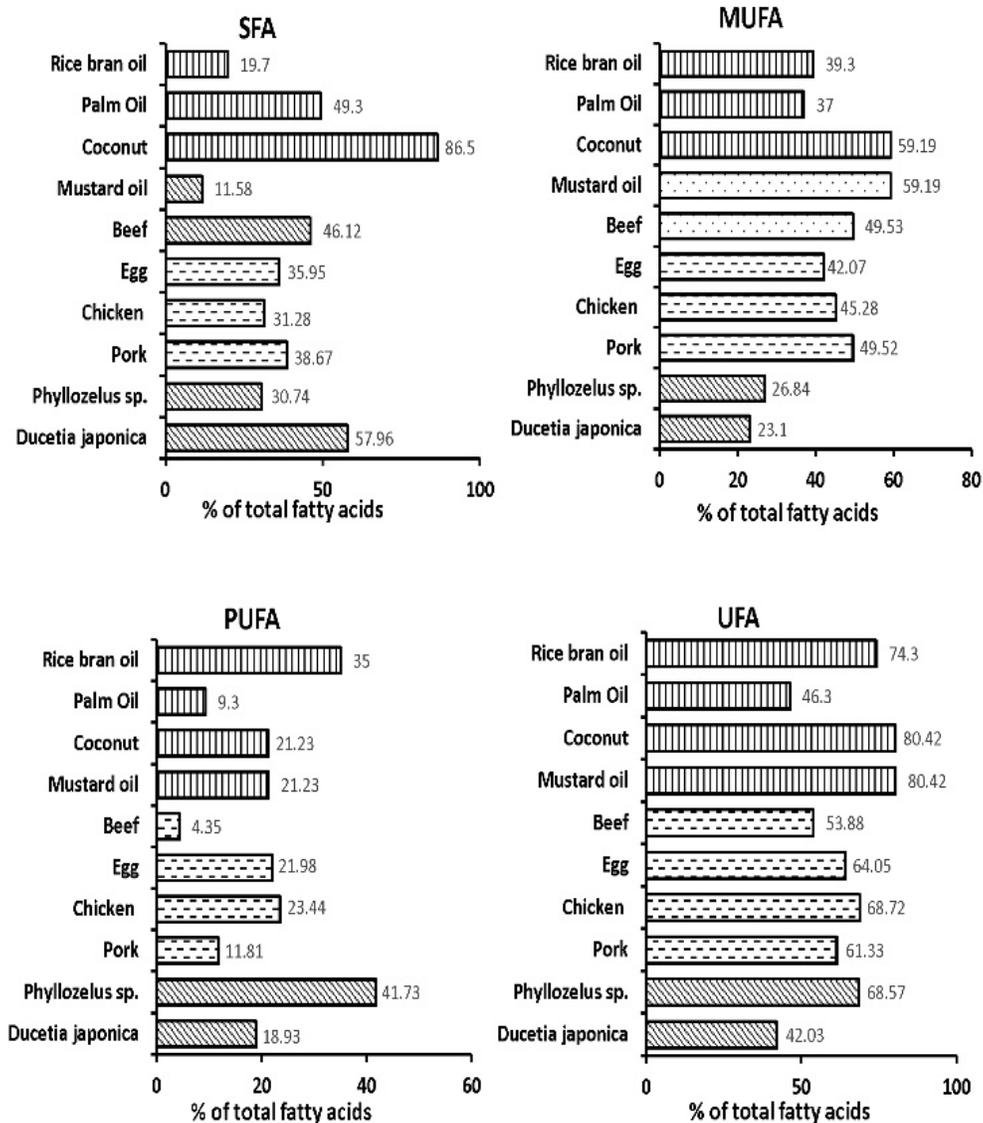


Figure 5: Comparison of different fatty acids compositions based on saturation (% of total fatty acids) of *Ducetia japonica*, *Phyllozelus sp.* and some conventional oil and foods of food of animal origin (data other than insects were obtained from USDA database 2015).

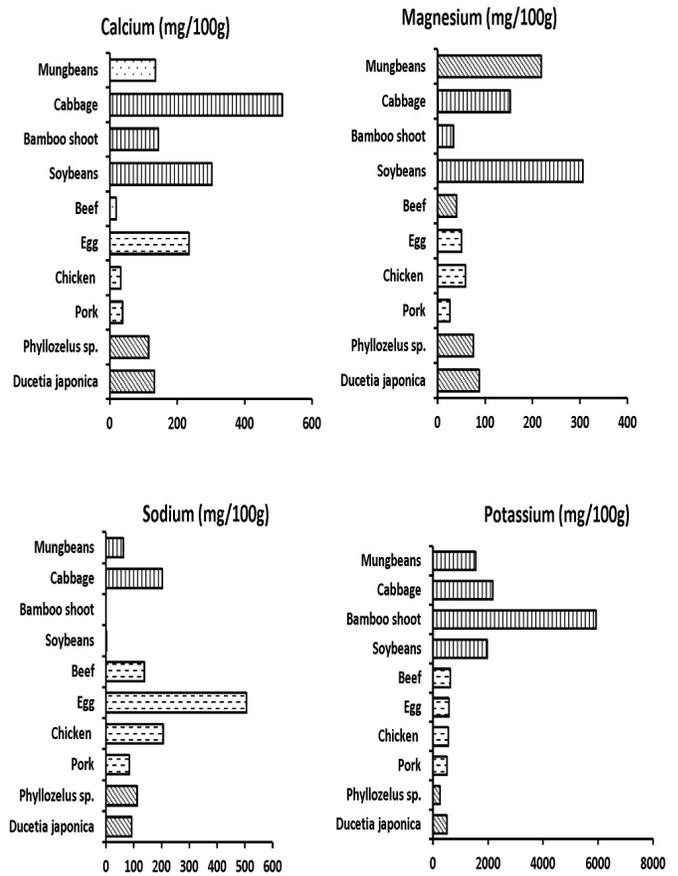
Fatty acids	<i>Ducetia japonica</i>	% of total fatty acids	<i>Phyllozelus sp.</i>	% of total fatty acids
SFA				
4:0 Butyric acid	ND		ND	
8:0 Caprylic acid	ND		0.05±0.0010	
10:0 Capric acid	ND		0.01±0.0000	
11:0 Undecanoic acid	ND		ND	
12:0 Lauric acid	0.11±0.0026	0.917	ND	ND
14:0 Myristic acid	0.55±0.0017	4.587	0.06±0.0006	0.834
15:0 Pentadecanoic acid	0.09±0.0021		0.03±0.0020	
16:0 Palmitic acid	3.80±0.0101	31.693	1.31±0.0058	18.22
17:0 Heptadecanoic acid	0.15±0.0021		0.10±0.0095	8.345
18:0 Stearic acid	2.25±0.0026	18.766	0.60±0.0021	
20:0 Arachidic acid	ND		0.02±0.0017	
21:0 Heneicosanoic acid	ND		0.01±0.0026	
22:0 Behenic acid	ND		0.02±0.0017	
23:0 Tricosanoic acid	ND		ND	
Total SFA	6.95	57.964	2.21	30.737
MUFA				
15:1 Pentadecenoic acid	0.04±0.0036		0.03±0.0029	
16:1 Palmitoleic acid	0.12±0.0026	1.0008	0.07±0.0012	0.974
17:1 Heptadecenoic acid	0.18±0.0021	1.501	0.06±0.0021	0.835
18:1 ω-9 Oleic acid	2.27±0.0020	18.932	1.76±0.0017	24.478
18:1 Elaidic acid	0.16±0.0036	1.334	0.04±0.0006	0.566
20:1 ω-9D11 Eicosenoic acid	ND			0.278
22:1 Erucic acid	ND		ND	
Total MUFA	2.77	23.102	1.98	26.843
PUFA				
18:2 ω-6 Linoleic acid	1.02±0.0031	8.507	1.05±0.0012	14.604
18:3 ω-3α- Linolenic acid	1.07±0.0021	8.924	0.07±0.0012	0.974
18:3 ω-6γ- Linolenic acid	0.18±0.0010	1.501	1.88±0.0137	26.147
20:4 Arachidonic acid	ND		ND	
Total PUFA	2.27	18.932	3.00	41.725
Total Fatty acids	11.99	41.70	7.19	UFA=68.568

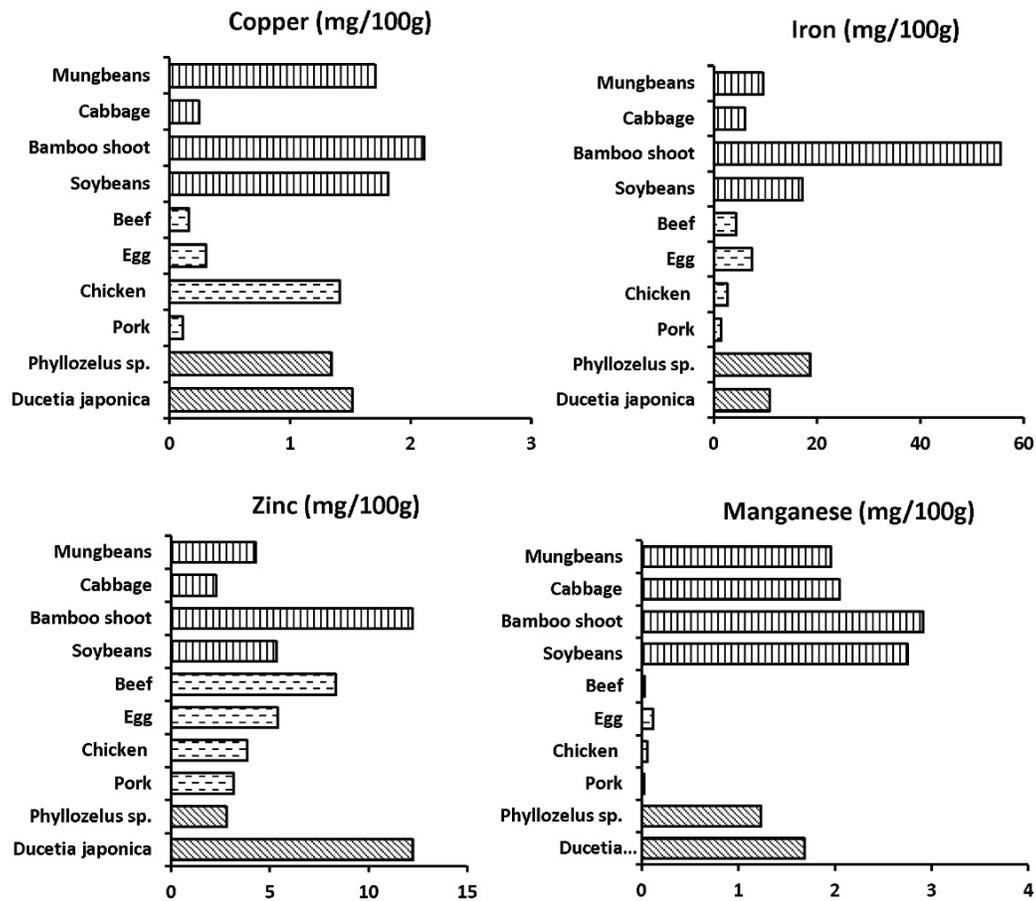
Table 3: Fatty acid profile (g/100g of sample) (% of total fatty acids) of *Ducetia japonica* And *Phyllozelus sp.* (Mean ± SE)

Ash and Mineral: (Table 1 and 4; Figure 6)

After the removal of water and organic substances by intense heating the remaining ash contents of 4.59 % in *Ducetia japonica* and 3.01 % in *Phyllozelus sp.* are comparable to those of other orthopterans [10] and insect species, generally [20,30,32,44]. Ash content of a species is an indicator of its mineral content.

The mineral composition (mg/100g) of *Ducetia japonica* and *Phyllozelus sp.* showed the presence of at-least eight important minerals. Macro-minerals (mg/100g) Ca, Mg, Na and K as well as micro-minerals (mg/100g) i.e. Cu, Fe, Zn and Mn were present in appreciable amounts. However, the rank of micro-nutrients was superior to macronutrients as per requirement proposed by [45]. Both insects contained moderate amounts of macro minerals (Na, K, Ca and Mg) and although their levels in *Ducetia japonica* and *Phyllozelus sp.* were low as per the [45] recommendation, they lay within the range known from other insect species [32,46,47]. With the exception of Zn and Fe, which were somewhat lower in our two species than what had been reported for other orthopterans [10]; the micronutrient assemblage was generally comparable to or even higher than that known from conventional foods of animal origin [48]. Availability and uptake of iron represent core public health problems, especially in developing countries, where often the food that children, elderly and nursing women get is deficient in this and some of the other micronutrient minerals [49]. About 100 g of *Phyllozelus sp.* can provide a sizeable amount of the recommended daily doses of zinc and iron and although 100 g of both *Ducetia japonica* and *Phyllozelus sp.* may not be able to supply all the minerals or their amounts required by humans, the two insects can make a considerable contribution to the provision of much needed trace elements in the nutrition.





Figures 6 (a and b): Comparison of mineral content (mg/100 g DM) of *Ducetia japonica*, *Phyllozelus sp.* and some conventional foods of both plant and animal origin (source USDA database www.ndb.nal.usda.gov).

Minerals	<i>Ducetia japonica</i> (mg/100g DM)	<i>Phyllozelus sp.</i> (mg/100g DM)	Recommended daily intakes (mg/day)
Calcium	132.14	115.32	1300 ^a
Magnesium	87.705±1.020	75.37±2.020	220-260 ^a
Sodium	92.739±0.950	112.030±2.015	1500 ^b
Potassium	507.270±1.925	254.33±5.925	4700 ^b
Copper	1.520±0.001	1.342±0.020	0.9 to 1.3 ^b
Iron	10.763±0.028	18.650±0.208	7.5 to 58.8 ^a
Zinc	12.239±0.028	2.805±0.028	3.0 to 14.0 ^a
Manganese	1.686±0.008	1.235±0.068	1.8 to 2.6 ^b

^aLinus Pauling Institute Micronutrient Centre (2012, <http://Ipi.oregonstate.edu/infocentre/>)
^bFAO (2004)

Table 4: Mineral contents (mg/100g DM) of *Ducetia japonica* and *Phyllozelus sp.* (Mean ± SE).

Conclusion

Based on our chemical analyses, *Ducetia japonica* and *Phyllozelus* sp. can be recommended as a nutritional supplement to combat malnutrition among Arunachal Pradesh's inhabitants and by inference, all those elsewhere in the world, which have access to the species under debate. Insects like the *Ducetia japonica* and *Phyllozelus* sp. studied in this paper must be seen as a tasty and promising source of essential nutrients on par with or superior to conventional meats of vertebrate origin. They should be included in plans to rear and farm nutritionally valuable species of edible orthopterans [18,50].

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