PROTEIN QUALITY OF COMMONLY CONSUMED EDIBLE INSECTS IN ZIMBABWE

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ABSTRACT

Consumption of edible insects as alternative animal protein-source is a potential long-term solution to curb protein deficiency in resource-limited communities where diets lacking in protein are predominant. Entomophagy has been expressed in both developed and developing countries, and previous studies have proven that edible insects are high in protein. However, there is paucity of information on protein quality of edible insects to adequately guide populations on their utilization as good alternative protein sources. The aim of this study was to evaluate protein quality of three edible insects commonly consumed in most regions of Zimbabwe, namely *Imbrasia belina* (mopane worms), *Locusta migratoria* (locust) and *Encosternum delegorguei* (stinkbug). Kjeldahl method was used to evaluate crude protein of edible insects and a 20-day mice-feeding trial was conducted to evaluate protein efficiency ratio and protein digestibility in comparison to a control protein (casein). Crude protein was higher in *Locusta migratoria* (71.2%) compared to *Imbrasia belina* (57.7%) and *Encosternum delegorguei* (31.3%). Protein efficiency ratio was lower in insect samples *L. migratoria* (2.3), *I Belina* (1.96), *E. delegorguei* (2.0) compared to control casein (2.5). There was a significant difference ($p<0.05$) in protein efficiency ratio between the three edible insects and casein. Protein digestibility of the three insects (*I. belina*-92%, *L. migratoria*-90%, *E. delegorguei*-92%) was high and comparable to that of casein (96%). There was no significant difference ($p>0.05$) in protein digestibility between the three insect protein sources and casein. The results showed high protein quality of three edible insects commonly consumed in Zimbabwe comparable to casein, a high quality animal protein. High protein digestibility of edible insects indicated ease in absorption and improved utilisation in the body. The lower PER values for *I. Belina* and *E. delegorguei* could possibly indicate that these edible insects may be limiting in the amino acids that support body tissue building and growth. Edible insects are a good source of quality protein that could meet protein requirements in resource-limited populations to curb protein deficiency. There is a strong need to further promote edible insects as a good alternative animal protein source.

Key words: Protein Efficiency Ratio, Digestibility, Protein quality, Edible Insects

INTRODUCTION

The human practice of consuming insects as food, (entomophagy) is common in many parts of the world, especially in resource-limited countries (RLCs) [1]. It has been estimated that approximately two billion people worldwide eat insects as part of their normal diet [2]. Generally, insects that are mostly consumed are those that can be gathered in large numbers and meet organoleptic preferences of consumers [3]. In Zimbabwe, entomophagy has been reported as a common practice in both rural and urban areas in the country's 10provinces [4]. A significant number of insect types are consumed by the Zimbabwean population, and in other areas some insects are sold for money or bartered for other necessities [3].

The natives of Southern Africa used a number of insects as food, including caterpillars, locusts, ants, termites and beetles [5, 6]. Insects commonly consumed in Zimbabwe include *Imbrasia belina* (mopane worm)*, Cerina forda* (locusts), *Encosternum delegorguei* (stinkbug), *Eulepinda* sp (Christmas beetle), *Ruspolia differens* (locally known as dzambarafuta) and *macrotermes* (locally known as ishwa) [3, 4]. Entomophagy especially for *Imbrasia belina* and *E. delegorguei* is a long-standing practice in Zimbabwe and its continuity has not been affected by changes in lifestyle with passage of time. This is evidenced by young and old individuals who still eat these insects regularly. Therefore, focusing on evaluating protein quality of these edible insects is important.

Imbrasia belina is consumed at the larval stage and is found mainly on mopane trees, which are a source of their food [7, 8]. These caterpillars bury themselves in order to re-appear as emperor moth [9]. *Encosternum delegorguei* also commonly known as the edible stink bug is also consumed at the larval stage and is mainly harvested during the winter season from its host plants (*Dodonaea visosa* and *Diospyros mespilifomis)* [10, 11]. *Locusta migratoria* (edible locusts) are a group of locust species that become gregarious and migratory when their populations are dense enough, with a single swam containing up to 10 billon insects [12]. The swarming behaviour makes locusts relatively easy to harvest for food [13]. Most consumers prefer degutted, fried, and then dried insects. The dried insects are perceived to present improved organoleptic qualities [3].

Food security is dependent upon food availability, accessibility, and utilization by the body for energy and healthy purposes [14]. Protein is generally the most expensive dietary macronutrient and visits to Zimbabwean markets showed most of the commonly consumed sources of animal proteins to be expensive [3]. Chavunduka [6] argued that edible insects are the cheapest source of animal protein and emphasised that their use as food should be encouraged. The author also indicated that insects averted many potential cases of protein deficiency in remote rural areas. Insects are considered a ready source of food that can be easily accessed, highly nutritious, and cheap and require little or no processing. Other authors argue that because insects are readily available in large numbers when in-season and consist of high protein content in a small meal, insect farming could be considered as a potential strategy to address food insecurity and malnutrition [15]. The United Nation' Sustainable Development Goals (SDGs) to eradicate hunger, end poverty, and ensure food security and nutrition are hindered by

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increased protein-energy malnutrition (PEM) in the sub-Saharan African region. Thus, improvement of dietary diversification through consumption of edible insects is a possible solution to achieve these targets [2].

Despite high interest in the possible use of insects as food, information on proximate and biological evaluation of protein quality in edible insects is lacking [15]. Protein quality describes the characteristics of a protein in relation to its ability to achieve defined metabolic functions [16]. As the understanding of protein actions expands beyond its role in maintaining body protein mass, the concept of protein quality must expand to incorporate these newly emerging actions of protein into the protein quality concept [17]. Therefore, with the broad existing evidence regarding consumption of edible insects in Zimbabwe and other resource limited countries (RLCs), several authors have recommended mass production of insects as a way of achieving food security and reducing protein deficiencies [6,2]. However, with lack of adequate information on protein quality of these edible insects, recommendations that encourage people to consume insects as a way of achieving protein security could be misleading. Hence, the need to conduct this study and evaluate protein quality of three commonly consumed edible insects in most parts of the country.

MATERIALS AND METHODS

A laboratory experimental study was conducted to determine nutritional composition of *Imbrasia belina, Locusta migratoria and Encosternum delegorguei*. A randomized animal feeding trial was conducted to evaluate protein quality of the edible insects**.**

Collection of samples and sampling technique
A non-random convenience sampling method was employed to select edible insects from local insect traders' in Harare (capital city of Zimbabwe) and Bikita district (located in Masvingo province of Zimbabwe). *I. belina* and *L migratoria* samples were collected from the largest food market centre in the country, Mbare traditional foods marketplace in Harare. *E. delegorguei* samples were collected from a local market at Nyika Growth Point in Bikita district. This study was carried out at the University of Zimbabwe- Faculty of Agriculture over a period of 6 months.

Sample Preparation
Insect samples were prepared utilising traditional methods used to prepare edible insects for human consumption in Zimbabwe.

Preparation *of Imbrasia belina*
Degutted dried *I. belina* samples were washed and boiled for one hour to rehydrate them prior to cooking. Salt was added and the samples were fried until crispy and ready for human consumption. They were sun dried for twenty four hours to enhance the dryness. Samples were then ground to powder by a laboratory mill (Thomas –Wiley Laboratory mill Model 4 using an 8 mm grinding plate).

Preparation of *Locusta migratoria*

Samples were degutted and boiled for twenty minutes prior to being fried and sun dried for 24 hours. Samples were then ground to powder using a laboratory mill (Thomas – Wiley Laboratory mill Model using an 8 mm grinding plate).

Preparation of *Encosternum delegorguei*

Samples were soaked in a bucket of water and stirred with a wooden spoon for 10 minutes. The bugs were then rinsed with warm water and the process repeated three times. When the bad odour was removed, the insects were blanched briefly (15 minutes) in hot water prior to being roasted and sun dried for 48 hours. The samples were then ground to a powder using a laboratory mill (Thomas – Wiley Laboratory mill Model 4 using an 8 mm grinding plate).

Measuring of protein content

Samples divided in triplicate of the ground insect powder were weighed (0.4 g – 0.5 g) using an analytical scale and added to a digestion flask containing 98% concentrated sulphuric acid, anhydrous sodium sulphate and copper catalyst. The mixture was transferred to a Macro-*Kjeldahl* digestion apparatus which was set at digestion temperature of 420ºC for 40 minutes. The distillation process followed in which 4% boric acid was added into an Erlenmeyer flask with each of the samples being run and placed in the *Kjeldahl* unit. The digestion flask was connected to a receiving flask which contained the boric acid using a receiving tube. Water and sodium hydroxide (450 g NaOH flakes/ litre N_2 free) were slowly added and attached to the condenser. Distillation was carried out for thirty minutes. The liquid (ammonium borate) was titrated in an Erlenmeyer flask using 0.1N standard hydrochloric acid (HCL) with 0.1% methyl red and 0.2% bromocresol green in alcohol as an indicator. The quantity of hydrochloric acid used was read from a burette and recorded. A reagent blank and at least one sample in house standard was run as control checks to ensure accuracy of the procedure. Protein concentration was determined as concentration of hydrogen ions (moles) required to reach end point as being equal to concentration of nitrogen in original sample. Then % protein was found using nitrogen conversion factor of 6.25.

Determining Protein Efficiency Ratio

Preparation of feed

Components of the diet included casein from Blair Research Laboratories (BRL), Harare, as reference protein, mouse missal powder which is a feed for mice, (National Foods Limited Harare). The mouse missal powder used for this study had all other nutrients except protein and was prepared at National Foods Harare and inset samples (Table 2). Insect samples were ground into powder using a laboratory mill (Thomas –Wiley Laboratory mill Model 4 using an 8mm grinding plate). The reference protein (casein) and each of the three test sample diets *(I belina, L. migratoria, and E. delegorguei)* were separately mixed with the mouse missal powder. Rations of the diet formulation was done using Pearson's square. Composition of the rations is outlined in Table 1. The mixtures were individually pelleted for the convenient feeding of mice. The total mouse diet prepared for each protein source was as follows:

Diet requirement= Xg/ day × number of days × number of mice per treatment
= 15g/day ×20 days × 24 mice
= 7200g (7.2kg)

Following diet preparation for each type of insect powder and reference protein (casein), crude protein analysis was conducted to ensure that the diet formulation was done correctly. Each test protein was standardised to deliver eight percent protein concentration [18].

Experimental Animals
A total of 24 mice of the species Balb C (bred at the University of Zimbabwe Animal House Unit) were used in this feeding trial. The mice used were 21 days old. Equal numbers of males and females were selected and divided into four treatment groups of six mice per group receiving different diets *I. belina*, *L. migratoria*, *E. delegorguei* and one control group that was fed on casein. Each mouse was caged separately within the group so as to efficiently monitor metabolic study factors. Mice that showed any symptoms of ill health were excluded from the experiment. The mice were kept at the Animal House Unit at the University of Zimbabwe for 23 days.

Feeding Regime
Before the beginning of the feeding regime, the mice were weighed to obtain initial weights. They were introduced to their different diets and allowed a three-day adjustment period followed by a 20-days feeding trial. Weighed feed was placed in small porcelain mortars and water was provided in separate containers. Spilled feed and faecal matter were collected daily and dried. The dried spilt feed was weighed together with the dry unconsumed feed to total amount of food actually consumed by each mouse. The proximate daily food consumption was determined by weight difference between the served feed and unconsumed feed and spillages. Feed intake and weight was measured and recorded after every 24 hours for individual mice. Each group of test mice consumed a specific feed of *I belina, L. migratoria, E. delegorguei* and casein for the control group.

Protein Efficiency Ratio (PER)
PER was calculated as weight gain (in grams) divided by protein intake in grams.

In Vivo Apparent Protein Digestibility (AD)
Collection and Treatment of Faecal matter
Faecal output for each test mouse was collected daily for ten days (day 7 to day 16) of the 20-day feeding period and recorded. The faecal samples were dried to a constant weight and stored in a stoppered glass bottles for analysis.

Faecal Matter Analysis
Dried faecal matter was sampled and analysed for nitrogen content using the Kjeldahl method. The values obtained were converted to protein content in faecal matter.

Apparent Digestibility = (Nitrogen in diet- Nitrogen in faeces) / Nitrogen in diet) × 100% [19].

Sacrifice of test animals

Following the 20 days of the feeding trial, the mice were weighed and their physical conditions such as fur, appearance and agility were observed and recorded prior to sacrificing them. The test mice were put to sleep by placing them in a sealed container containing diethyl ether.

Ethical Considerations

Ethics approval was granted from the Joint University of Zimbabwe College of Health Sciences and Parirenyatwa Group of Hospitals Ethics Committee (JREC).

Statistical Analysis

Protein concentration was determined and expressed as mean ±standard deviation. The significance of difference in protein efficiency ratio and digestibility between the control group and test animal groups was measured using one way ANOVA with post-hoc Dunnett test (Dunnett Multiple Comparison test). Data was analysed using Graph pad Prism 4 at 95% significance level.

RESULTS AND DISCUSSION

Data analysis (calculated on dry matter basis) indicated that crude protein was high in the three edible insects, 71.2% in *L. migratoria*, 57% in *I. belina and* 31.3 in *E. delegorguei* (Table 3). The protein content of insects studied was observed to vary widely but results were similar to published data for various edible insects in Zimbabwe and elsewhere especially for *I. belina* [3, 4, 19]. In other studies, authors have reported varying protein content from our results for *Imbrasia belina* [20]. These variations could be attributed to metamorphic stage of the insects, their habitat as well as varying diets prior to analysis [21]. Like most foods, the difference in preparation and processing methods (including drying, frying and boiling) applied before consumption might also have effect on nutritional composition of the edible insects[22].

Imbrasia belina is one of the most consumed and economically valuable edible insects in Southern Africa and could contribute substantially to improving intake of protein animal food in the region. In previous studies it has been shown that in addition to protein, *Imbrasia belina* also has high iron content ranging between 31 mg and 77mg/ 100g [23, 24]. In some parts of Southern Africa, *I belina* is used in health care where it is crushed and mixed with porridge to help relieve symptoms of kwashiorkor in children [20], showing its potential in curbing protein malnutrition in developing countries.

Encosternum delegorguei had a lower protein content value compared to *I. belina* and *Locusta migratoria*. Other studies elsewhere have reported consistent results of lower protein content of *E. delegorguei*[25]. *E. delegorguei* has also been reported to contain high levels of essential amino acids including phenylalanine, tyrosine, threonine and tryptophan reported to mimic levels of those amino acids found in beef and chicken. This suggests that despite its lower protein content compared to other insects and protein sources, consumption of *E. delegorguei* provides substantial amounts of most essential amino acids. In addition to protein, *E. delegorguei* was also found to have a relatively high concentration of essential minerals such as iron, potassium, phosphorus and

selenium [25]. Thus *E. delegorguei* is not only a traditional delicacy to many different tribes in developing countries, but a significant dietary contribution in terms of quality protein and minerals.

Locusta migratoria which had the highest crude protein content (71.2%) in comparison to the other insect samples had lower protein content as compared to reference protein casein. The value we reported was within the range presented in literature which is above 50% and can also range as high as 82% although other authors have reported lower protein levels (<30%) [26].

In the mouse bioassay, all mice survived until the end of the feeding trial and gained body weight (Figure 1). Mice fed with *E. delegorguei* had a rapid weight gain and the highest mean body weight (22.6 g) after 15 days as well as after the 20-day complete feeding trial compared to other groups. The lowest mean body weight gain after 15 days (19.2 g) and 20 days (21.2 g) was recorded for the mice group fed on *I. belina.* Casein-fed mice had a mean weight of 21.2 g at day 15 which was lower than *L. migratoria* (21.4 g) and *E. delegorguei* (23.7g) groups. There was no significant difference (p>0.05) in mean body weight after 15 days between casein-fed mice and groups fed on insect formulated-diets. There was also no significant difference (p=0.41) in mean body weight gain between the three groups fed on different insect-formulated diets. Mice fed on casein presented lower average weight gain compared to groups fed on *L. migratoria* and *E. delegorguei. Encosternum delegorguei* has been reported to consist high fat content (51% on dry weight basis) [25]. This high fat content could explain the rapid weight gain in the mice group fed on *E. delegorguei* in this study. Rate of weight gain started to diminish around day 15 for test mice fed on *L. migratoria, E. delegorguei* and casein. Rate of weight gain for test mice fed on *I. belina* reduced around day nine. Mice fed on E. *delegorguei* initiated weight loss from day 15 and regained after day 18. Test mice on diet containing case in initiated weight loss from day 18 and test mice fed on *L. migratoria* and *I. belina* did not lose weight during the feeding period. Weight loss may have been triggered by decreased food intake (Figure 1).
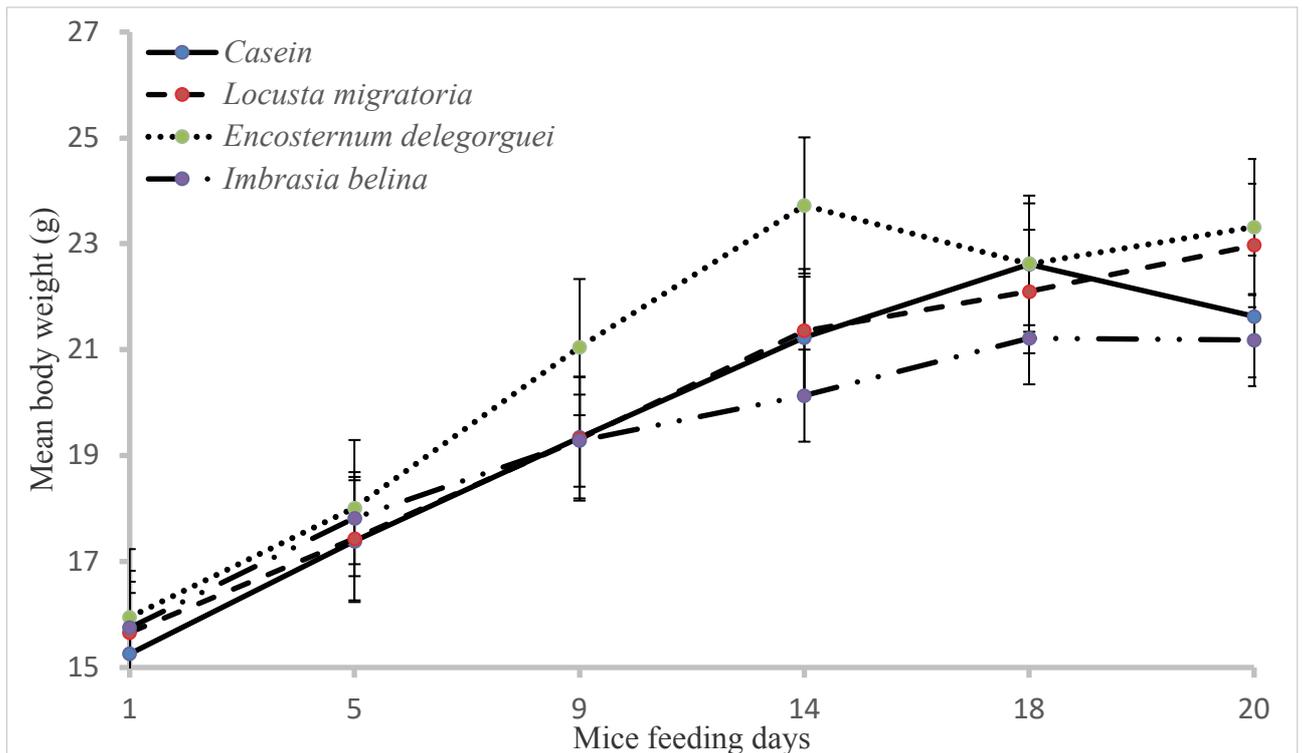
Figure1: Mean body weight gain of mice fed with diets of *L. migratoria*, *E. delegorguei*, *I. belina* and control protein casein

The group of mice fed on *E. delegorguei* had the highest feed intake (121.8 g) after 15 days as well as after 20 days of feeding. Casein-fed mice had the lowest feed intake rate (95.4 g) at day 15 compared to the insect-fed groups (Table 4). Mice fed on insect formulated diets recorded high feed intake compared to casein during the 20-day feeding period. This was consistent with studies elsewhere that reported lower feed intake among test animals fed on casein as a protein source, which can be attributed to low DL-methionine in the mouse diet [27].

Control protein casein had the highest PER compared to the three insect- substituted diets (Table 4). Among the test insect- substituted diets, *L. migratoria* had the highest PER value and *I. belina* had the lowest PER recorded. There was significant difference (p<0.05) between PER of casein and insect-substituted diets and between the three insect proteins as well. Lower PER for insect protein sources compared to casein have been reported in other parts of Africa as well [19]. If weight gain is used as a measure of growth, then lower feed intake is supposed to correlate with apparent growth failure [19]. However, in this study, test mice fed on *I. belina* had a high feed intake than *L. migratoria* and casein but the lowest average weight gain. In addition, *I. belina* had a relatively low PER. This could be an indication that *I. belina* consists of high protein content inefficient for growth utilisation. Presence of anti-nutritional factors could reduce absorption of essential amino acids required for optimum growth [28]. *Locusta migratoria* had the highest PER among the insect samples and was comparable to PER for casein. This high PER is comparable to PER levels for locusts assessed in other regions [29].

Test mice fed on *E. delegorguei* had the highest feed intake (121.8g) which could have supported the rapid growth. However, *E. delegorguei* had the lowest PER compared to other treatment groups. This follows that *E. delegorguei* is a poorer protein source compared to the other edible insects commonly consumed by humans. Rapid weight gain for mice group fed on *E. delegorguei* could be attributed to the high fat content of the edible insect. Although *E. delegorguei* has relatively low protein content, most of the protein was available for utilisation by the test animals. PER for *L. migratoria* was high compared to *I. belina* and *E. delegorguei* despite lower intake of the insect feed, suggesting it could be a more superior quality protein.

Digestibility for casein (96%) was comparable to protein digestibility of insect protein sources *E. delegorguei* (92%), *L. migratoria* (92%) and I. belina (90%) (Table 5). Protein digestibility was not significantly different between the three insect protein sources. Protein digestibility (PD) is a reflection of the extent to which amino acids in a particular protein source are digested, absorbed and ultimately available for biological processes which is an important factor in considering the nutritional quality of a protein [8]. Results from this study showed that apparent digestibility of casein was higher compared to edible insect protein s though comparable. These digestibility values were slightly higher compared to those reported elsewhere [19], showing good quality protein insects in the Zimbabwean region.

Lower digestibility of *Imbrasia belina* despite high protein content further supports the possibility of presence of anti-nutritional factors being available in the insect. For instance, chitin which is believed to be present in insect cuticle resist digestion and may also protect some protein materials in the insect from breakdown [30]. Thus, as we promote intake of edible insects as protein-rich sources comparable to other animal proteins, presence of anti-nutritional factors should be considered as they limit absorption of protein and various minerals. As promotion of consumption of edible insects is expanded, there is need for further investigate on strategies to produce these insects in large amounts to support demand of alternative protein sources. Strategies that include insect farming could be potential solutions to protein-lacking diets in resource-limited and rural communities with conditions supporting growth of these edible insects. Production of large amounts of edible insects could also be linked with nutrition programmes for prevention of protein-energy malnutrition as a prevention strategy to address PEM in developing countries. Besides use of edible insects in resource-poor communities, there is also need to invest in food development to come up with new food products from edible insects that are easily acceptable by modern populations.

CONCLUSION

Imbrasia belina, *Encosternum delegorguei* and *Locusta migratoria* are high in crude protein, and comparable to the casein reference protein. These edible insects are good protein sources, readily absorbed and well retained for use in the body. Protein Efficiency Ratio of *I belina*, *E. delegorguei* and *L. migratoria* is significantly low compared to casein suggesting these insects are limiting in the amino acids that primarily support building and growth of body tissues. Edible insects are a good source of protein in people's diets, thus are recommended to be promoted in resource-limited communities

as a strategy to alleviate protein deficiency. Further research is warranted to explore on nutritional composition of edible insects including full amino acid profiling and levels of antinutritional factors in order to confidently promote edible insects as good alternative animal-source proteins.

ACKNOWLEDGEMENTS

Table 1: Mouse diet composition with 8% test protein (g/ 100g feed)

Protein source	Protein concentration (g/100g feed)	Mouse missal powder (g/100g feed)
Casein	8.91	91.09
I. belina	16.1	83.8
L. migratoria	12.6	87.3
E. delegorguei	34.4	65.5

Table 2: Composition of mouse missal powder used in the mice-feeding trial

Diet composition	g/kg feed
Corn starch	10.3
Cellulose	1.2
Moisture	0.1
Vitamin mixture	1.4

Table 3: Crude Protein Content for edible insects

Sample	Crude protein (%)±SD
Locusta migratoria	71±2.1
Encosternum delegorguei	31.3±1.01
Imbrasia belina	57±2.46

Data presented in the mean values, mean values bearing different superscripts differ significantly ($p < 0.05$

Table 4: Protein Efficiency Ratio (PER) Values for edible insects in comparison to casein calculated after 15 days of feeding

Protein Source	Feed Intake (g)	Protein Intake (g)	Total weight gain (g)	PER	PER (% of Casein)
Casein	95.36	8.2	21.22	$2.5^a \pm 0.3$	100
I. belina	113.06	9.4	19.28	$1.96^b \pm 1.5$	78
L. migratoria	106.41	9.04	21.36	$2.3^c \pm 1.2$	92
E. delegorguei	121.8	9.86	23.73	$2^d \pm 0.8$	80

Mean values within the column bearing different superscripts differ significantly ($p < 0.05$)

Table 5: Percent in-vivo Apparent Digestibility of casein, *L. migratoria*, *I. belina* and *E. delegorguei*

Protein source	Feed Intake in 10days (g)	Nitrogen consumed in10days (g)	Faeces dry weight (g)	Nitrogen dried faeces (%)	Nitrogen dried faeces (g)	Apparent digestibility (%)
Casein	59.9	5.2	89.43	4.04	0.2	95.96 ± 0.32^a
L. migratoria	72.3	6.2	83.53	8.039	1.3	91.96 ± 0.41^b
I. belina	78.6	6.5	89.41	10.337	1.6	89.66 ± 0.84^b
E. delegorguei	98.7	7.9	89.48	7.984	4.6	92.01 ± 0.7^b

Mean values within the same column bearing different superscripts differ significantly ($p < 0.05$)

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