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SHORT COMMUNICATION

Food supplements from a Grasshopper: A developmental stage-wise evaluation of amino acid profile, protein and vitamins in *Brachystola magna* (Girard)

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ABSTRACT

Insects can be used as an easily accessible food supplement; however, for nutritional purposes it is necessary to consider its protein content, amino acids composition and vitamin content. In this study a nutritional characterization (protein content, amino acid profile and hydrosoluble and liposoluble vitamins) was performed to the *Brachystola magna* (Girard) grasshopper in four stages of its biological cycle (egg, nymph 3, nymph 4 and adult). In the adult stage the highest values of protein content (> 59%) were observed compared with the other stages of development. At all stages of *B. magna* the presence of 9 out of the 10 essential amino acids were detected (just tryptophan amino acid was absent). Significant increases in the fat-soluble vitamins (A, D and E) were observed as the stage of development of *B. magna* increased, with the increase of vitamin E of ≈ 662% in the adult stage; however, the water-soluble vitamins of the B complex remained constant at all stages, but vitamin C, which increased significantly in the adult stage of this insect cycle. Results suggest that *Brachystola magna* (Girard) could be a potential source of consumption for people with specific health needs.

Keywords: Amino acid profile; *Brachystola magna* (Girard); Edible insects; Hydro and liposoluble vitamins; Nutritional characterization

INTRODUCTION

Among the nutritional components, proteins (considering the number and type of amino acids) represent a basic and essential aspect since they provide quality aspects and health in all living organisms, including insects (Jonas-Levi and Martinez, 2017). In addition to this, biochemical substances known with the generic name of vitamin, are a priority for cell biological cycles because they are required (at minimum concentrations) to play important roles in metabolism and in most life cycles (Khosravi-Largani et al., 2018). As a food source, insects are potentially nutritious, since they are considered abundant in proteins, fats, and provide a certain amount of minerals and vitamins (Yi et al., 2013). Within the species of insects, Orthoptera order is one of the most common groups (over 20,000 species) in the world (Löffler and Fartmann, 2017; Monter-Miranda et al., 2018; Zielińska et al., 2018) and these stands out over the other insect species because of their high protein value (Ramos-Elorduy et al., 2012; Monter-Miranda et al., 2018; Zielińska et al., 2018). The most common species of this insect and most important in Mexico are *Melanoplus* spp., *Sphenarium purpurascens*, *Sphenarium mexicanus*, *Taeniopoda*

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eques, *Chromacris versicolor*, and *Brachystola magna* (Girard), which are found in the plateau and in the northern zone of Mexico, and according with the Mexico's agriculture ministry (Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food, SAGARPA), it reach to infest an area of approximately 300,000 hectares, where corn, bean, pasture and vegetable crops predominate (SAGARPA, 2012).

Brachystola magna (Girard) is considered a polyphagous insect and a problem of priority importance for agriculture in Mexico because it generates additional costs, plus required the use of pesticides to its control (Lozano and España, 2009; Mena-Covarrubias, 2009; Monter-Miranda et al., 2016). Out of *Brachystola magna* insect several studies have been performed focus to its biological control with agents or natural enemies such as *Beauveria bassiana* (Lozano and España, 2009) and *Nosema Locustae* Canning (Mena-Covarrubias, 2009) and through its control with laboratory essays using *Metarhizium* spp. y *Beauveria* spp. (Bustillos-Rodríguez et al., 2016). On the other hand, considering that *Brachystola magna* may represent a viable and sustainable alternative for the supply of biopolymer materials and nutritive compounds, since recently this research group reported the extraction and characterization of the physicochemical, morphological and structural properties of chitin and chitosan of this insect in adult stage (Monter-Miranda et al., 2016). In a previous study (Monter-Miranda et al., 2018), our research group reported on the nutritional content of fatty acids and minerals during different stages of development in *B. magna* (egg, nymph 3, nymph 3 4 and adult). The protein content and lipids varied from 62.46 to 64.70% and from 2.88 to 8.24%, respectively, and the highest values of these macronutrients were registered in the adult state. The fatty acid profile showed significant differences for different stages of *B. magna* evaluated; however, it can be said that this insect is an appreciable source of unsaturated fatty acids such as arachidonic (0.038-0.127%), palmitoleic (0.133-0.183%) and oleic acid (0.319-5.435%). Moreover, during the different life cycles of the grasshoppers, the mineral content analysis evidenced significant differences. Compared with nymph 3, nymph 4 and adult stages, a smaller quantity of the majority of the determined minerals (Fe^{+3} Cu^{+2}, Zn^{+2}, Na^{+1}, Ca^{+2}, Mg^{+2} and K^{+1}) was observed in the egg (Monter-Miranda et al., 2018). Despite the above described, studies about the evaluation and the content of protein, amino acids and vitamins of this insect are null or scarce. So, the objective of this study was to perform an evaluation of the content and molecular weight of proteins, amino acid profile and vitamins of *Brachystola magna* (Girard) during four stages (egg, nymph 3, nymph 4 and adult) of its development.

MATERIALS AND METHODS

Materials

Specimens of *Brachystola magna* (Girard) in stages of nymph 3, nymph 4 and adults were collected from the surroundings of the "Valle de Allende", Chihuahua (Mexico) in bean crops and pasture. Once collected, the insects were washed with distilled water for the removal of external contaminants and the regurgitated of the insect. To obtain eggs (ootheca), hatcheries were established in plastic containers according to the methodology recently reported by Monter-Miranda et al., (2018). The reagents used for the proximal chemical analysis of proteins were of analytical grade, while those used in the chromatographic analyzes were of HPLC grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA). In order to determine the amino acid profile a high-purity L-amino acid kit was purchased from Sigma-Aldrich (Toluca, Estado de Mexico, Mexico).

Methods

Obtaining Brachystola magna flour

For the analyzes of proteins content, vitamins and the amino acid profile of the *Brachystola magna* samples in the different stages of development, flour samples were obtained, using the methodology recently reported by Monter-Miranda et al. (2018). Samples were washed and lyophilized at a temperature of -40 °C at a vacuum of -133 mbar with a lyophilizer (Labconco 77540-00, MO, USA). Lyophilized samples were milled (mill IKA, model M20, IKA Works, Inc., NC, USA) and sifted (Retsch of 425 μm, number 40, Haan, Alemania) to standardize the particle size. Flour samples were stored in airtight bags (Ziploc®, Johnson y Sons, Inc., Racine, WI, EUA) in a dry and light-free environment for further analysis.

Protein molecular weight quantification and determination

Protein quantification of *B. magna* was performed on egg, nymph 3, nymph 4 and adult samples in the form of flours using the official method 928.08 of the Association of Official Agricultural Chemists (AOAC, 2002). Proteins molecular weight determination (soluble proteins aggregates) was performed by polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE) according to the methodology recently reported by Mishyna et al., (2018) with slight modifications. Samples were dissolved in a 0.2 M Tris buffer and incubated for 2 h at 40 °C. Subsequently, they were centrifuged (3,000 × g/30 min), an aliquot was taken mixed with the sample buffer and incubated at 65 °C for 10 min. Samples were evaluated using a 12% gel (Tris-Glycine) with a standard marker in the range of 10-250 kDa.

Amino acid profile

Amino acid profile was determined according to the methodology reported by González Paramás et al., (2006). Amino acids were quantified using a Varian chromatographic system, which consisted of a 9012Q pump, a 9100 auto-injector and a 9075 fluorescent detector. The samples (1.0 g in dry basis, for each of the stages of insect development) were submitted to an automatic precolumn reaction using 100 µL of derivatizing reagent. The chromatographic conditions were as follows: Flow 0.1 mL/min until minute 3 and then 1.5 mL/min; solvents, A, sodium phosphate buffer (10 mM, pH 7.3):methanol: tetrahydrofurane (80:19:1) and B, sodium phosphate buffer (10 mM, pH 7.3):methanol (20:80). The gradient consists of: 100% A during 3.5. min, 0-15% of B in A for 6 min, 15% B isocratically for 5 min, 15-30% of B for 5 min, 30-40% of B for 4 min, and 40-80% of B for 12 min. Separation was performed on a C18 Waters Nova-Pack reverse phase column (particle size 4 µm, 150 × 3.9 mm internal diameter). A specific column guard Nova-Pack was placed between the auto-injector and the column. All the chromatographic information was re-processed in a Star workstation (Version 4.5) supplied by Varian.

Vitamins determination

The contents of liposoluble, water-soluble and B-complex vitamins of the different *B. magna* samples were quantified using the methodology reported by Albalá-Hurtado et al. (2000). Analyzes were performed in an HPLC system (Hewlett-Packard, Waldbronn, Germany) which consisted of an HP 1050 series de-gasification device, a HP 1100 auto-sampler (for the analysis of water-soluble vitamins), or a Waters 717 (for fat-soluble vitamins analysis) (Waters, Milford, MA, USA). Both equipped with a fixed loop injector of 20 µL, and a UV detector in series HP 1050 of variable wavelength. Water-soluble vitamins were determined from 8 g of sample (in dry basis, for each of the stages of insect development) with 10 mL of Milli-Q water to a 10 mL volumetric flask. The mobile phase used in the HPLC described above contained 5 mM octanesulfonic acid (ion pairing reagent), 0.5% trimethylamine, 2.4% glacial acetic acid, and 15% of methanol in double-destilled water. Nicotinamide, pyridoxine dihydroclorhidre, riboflavin, folic acid, and thiamin hydroclorhidre were from Sigma Chemical Co. (St. Louis, MO, USA). The precision of the water-soluble vitamins method showed an overall relative standard deviation lower than 7.5% and the recovery was higher than 78% for folic acid and thiamine and higher than 96% for the remainder of the vitamins.

Vitamin A and vitamin E were determined from 25 g of sample (in dry basis, for each of the stages of insect development) saponified at room temperature (≈ 25 °C) overnight using absolute ethanol, potassium hydroxide solution, and ascorbic acid as an antioxidant. The samples were extracted with n-hexane, and butylated hydroxytoluene (BHT) was added as an antioxidant, then evaporated and redissolved in methanol. The extracts were injected into the HPLC system described above. Water-acetonitrile-methanol (4:1:95, v/v/v) was used as the mobile phase. Working conditions involved dim light and nitrogen atmosphere in order to avoid vitamin degradation. All-trans-Retinol (vit. A) and dl-Tocopherol (vit. E) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The relative standard deviation was lower than 3.5% for both vitamins, and the recovery was higher than 85%.

The data acquisition was performed with a system Chemstation HP 3365-II (Hewlett-Packard). The separation was carried out using a C18 column in reverse phase Tracer Spherisorb ODS2 C18 (TR-011019) de 250 × 4.6 mm, with a particle diameter of 5 µm (Teknokroma, Barcelona, España), with a protective cartridge. Analyzes were performed isocratically with a flow speed of 1 mL/min.

Statistical analysis

For each determination, a minimum size of three replicates ($n \geq 3$) was used in all samples. For the analysis of results, a one-way analysis of variance (ANOVA, $P \leq 0.05$) was applied using the statistical program MiniTab, version 17 (Minitab Inc., State College, Pennsylvania, USA). The differences between treatments/samples were determined by Tukey's test (Walpole et al., 1999).

RESULTS AND DISCUSSION

Quantification and molecular weight of proteins

The results of protein content in *Brachystola magna* at different stages of insect development indicated significant increases ($P < 0.05$) from ≈ 24% (egg) to ≈ 59% (adult) without significant differences ($P > 0.05$) between the stages of nymph 3 and nymph 4 (Table 1). These values

Table 1: Content and molecular weight of *Brachystola magna* proteins at different stages of their development.*[a,b]

Sample	Proteins (%)[1,2]	Molecular weight (kDa)[3]
Egg	24.15±0.15[c]	10-25 (25); 25-50 (25); 100-125 (30); 150-200 (20)
Nymph 3	55.76±0.25[b]	10-25 (30); 25-50 (30); 100-125 (20); 150-200 (20)
Nymph 4	56.56±0.38[b]	10-25 (35); 25-50 (35); 100-125 (20); 150-200 (10)
Adult	59.46±0.56[a]	10-25 (40); 25-50 (35); 100-125 (15); 150-200 (10)

*Arithmetic mean of three determinations±standard error. Equal letters in the same column are not statistically significant (p>0.05). [a]Dry base, [2]Factor N_2=6.25, [b]The approximate percentage is in parentheses.

are slightly lower than those recently reported by Monter-Miranda et al. (2018) in similar samples of *B. magna*, with values ranging from ≈ 62% (for the stages of development of nymph 3 and nymph 4) to ≈ 65% for the adult stage of insect development; however, these investigators did not determine the content of protein in the egg stage in the insect *B. magna* nor determined the molecular weight of proteins quantified at different stages of development *B. magna*. According to these results, it was observed that in the egg stage of *B. magna* there is a relatively important amount of proteins, which can be linked to the exoskeleton that covers the ootheca (egg sac) and could even be linked to other structural components such as chitin (Barker et al., 1998; Mena-Covarrubias, 2009; Yi et al., 2013). The analysis of SDS-PAGE allowed classifying the proteins according to their molecular weight in four groups comprised between 10-25 kDa, 25-50 kDa, 100-125 kDa and 150-200 kDa (Table 1) according to the appearance of various bands (images not shown). Variations were observed between the molecular weights of the different bands as a function of insect development.

The data in relation to the molecular weight of *B. magna* proteins are null or scarce. Mishyna et al. (2018) reported the molecular weights of the soluble proteins of two edible insects belonging to two different orders, one was the orthoptera *Schistocerca gregaria* (a grasshopper, as in this study) and the other was the hymenopter known as the honey bee (*Apis mellifera*). These researchers subjected the native proteins of these insects to degreasing treatments, alkaline extractions and sonication, and observed the presence of bands, which were classified into eight groups of different molecular weight (10-25, 25-50, 50-75, 75-100, 100-125, 125-150, 150-200 and 200-250 kDa). The bands that predominated in both insects were those of 10-15 kDa, 25-50 kDa, 50-75 kDa; while the band at 200-250 kDa was only observed in *S. gregaria* in its native state. In accordance with Mishyna et al. (2018) proteins of low molecular weight in *S. gregaria* could correspond to fatty acids linked with proteins involved in the muscular flight of this orthoptera. This would explain the predominance of the low molecular weight bands in the nymph and adult stages of the development of *B. magna*; however, further studies are needed to explain the bands with higher molecular weights.

Amino acid profile

The amino acid profile at the various stages of the development of *B. magna* is shown in Fig. 1 At all stages a relatively high amount of glutamic acid (a non-essential amino acid) was observed with values ranging from 7.60% (egg) up to 11.90% (nymph 3), with intermediate values in stage nymph 2 (≈ 9.00%) and adult (≈ 10.00%). These values are slightly higher than those recently reported in a study in which the nutritional composition of five edible insects were evaluated [(three species of larvae of beetles, *Allemyrina dichotoma* (8.69%), *Protaetia brevitarsis* (5.54%), and *Tenebrio molitor* (5.78%), and two species of adult crickets, *Teleogryllus emma* (6.51%) and *Gryllus bimaculatus* (6.39%)] in South Korea (Ghosh et al., 2017).

In all stages of the development of *B. magna*, the presence of 9 out of the 10 essential amino acids (isoleucine, leucine, lysine, methionine, phenylalaline, threonine, valine, histidine, arginine and tryptophan) was detected, and only the amino acid of tryptophan was not detected. Significant increases ($P < 0.05$) were observed in all the essential amino acids as the growth stage of *B. magna* increased, which was related to the greater development of exoskeletal structures and with the highest content of structural proteins quantified in the analysis proximal chemical (Table 1). Some studies have reported that amino acid contents vary

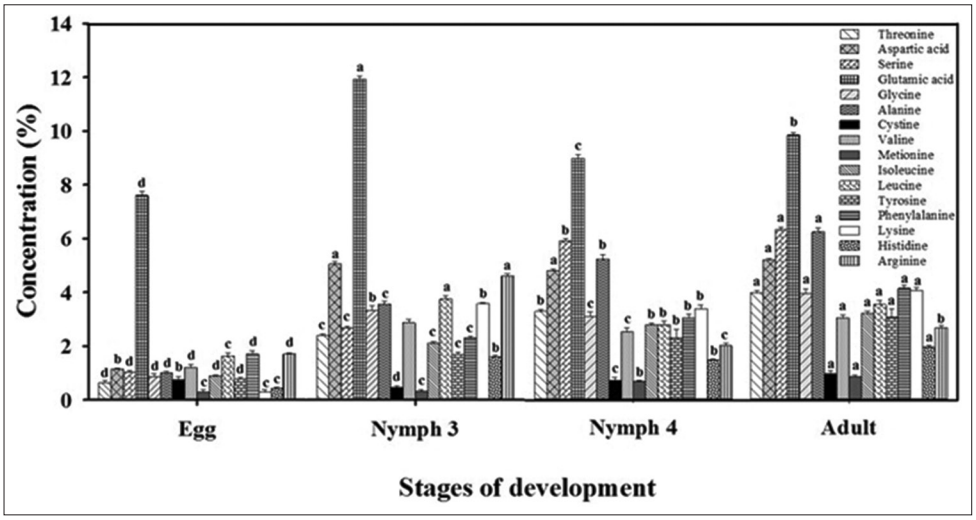

Fig 1. *Brachystola Magna* amino acids profile(%) during its development

Table 2: Vitamin content (mg/100 g) of *Brachystola magna* during its development

Analysis	Vitamin content*			
Water-soluble vitamins	**Egg**	**Nymph 3**	**Nymph 4**	**Adult**
Niacin (B_3)	0.540±0.060[a]	0.620±0.100[a]	0.730±0.130[a]	0.670±0.220[a]
Thiamin (B_1)	0.075±0.020[a]	0.080±0.070[a]	0.065±0.050[a]	0.090±0.070[a]
Pyridoxine (B_6)	0.430±0.030[a]	0.550±0.030[a]	0.630±0.040[a]	0.580±0.090[a]
Folic acid (B_9)	0.020±0.008[a]	0.025±0.008[a]	0.019±0.009[a]	0.023±0.010[a]
Riboflavin (B_2)	0.018±0.005[a]	0.021±0.010[a]	0.024±0.070[a]	0.027±0.080[a]
Vitamin C	19.860±1.000[d]	21.560±0.590[c]	27.780±0.610[b]	34.550±0.910[a]
Fat-soluble vitamins				
Vitamin A	0.150±0.030[c]	0.310±0.040[b]	0.310±0.030[b]	0.390±0.050[a]
Vitamin D	2.710±0.080[d]	2.340±0.020[c]	4.370±0.020[b]	5.250±0.060[a]
Vitamin E	21.930±0.310[d]	37.110±0.240[c]	130.710±0.630[b]	145.360±0.710[a]

*Arithmetic mean of three determinations±standard error. Equal letters in the same column are not statistically significant (p>0.05).

considerably among different insect species (Bosch et al., 2014; Zielińska et al., 2015; de Castro et al., 2018). In this sense, some authors attribute these differences (besides the type of habitat and feeding of the insect) to the genetic variation that may even exist between species of the same family (Ramos-Elorduy et al., 2012; Makkar et al., 2014; Monter-Miranda et al., 2018).

Vitamins quantification

For more than a decade it has been reported in the scientific literature that insects have a wide variety of water-soluble and liposoluble vitamins (Finke, 2002, 2005; Xiaoming et al., 2010; Oonincx and Dierenfeld, 2012; Kouřimská and Adámková, 2016). The contents of them are shown in Table 2. Among the water-soluble vitamins, the relatively greater amount of vitamin C in relation to the content of the other water-soluble vitamins is highlighted (Table 2). Vitamin C content increased with the development of *B. magna* with values ranging from ≈ 19.9 mg/100 g (egg) to ≈ 34.6 mg/100 g (adult). These contents are similar to those reported (from 23.8 to 25.5 mg/100 g) in 25 edible species of the order orthoptera (studied in the stage of larvae and adults) collected from different parts of Mexico (Ramos-Elorduy et al., 2012). Regarding to the content of the remaining water-soluble vitamins (vitamins of the B complex), relatively low amounts were observed, and these quantities remained constant during the stage of insect development (Table 2). The values reported in the Table 2 for *B. magna* during all its stages of development were lower than those recorded by Kinyuru et al. (2010) for niacin (3.01-3.22 mg/100 g), riboflavin (0.84-0.96 mg/100 mg) and folic acid (0.34- 0.35 mg/100 g) in in green and brown grasshoppers (*Ruspolia differens*). On the other hand, the content of pyridoxine during all stages of development of *B. magna* was higher than that reported for green (0.40 mg/100 g) and brown (0.14 mg/100 mg) grasshoppers (*R. differens*). Recently it has been reported that insects can not synthesize the 8 B-complex vitamins that function as co-enzymes in several required enzymatic reactions, so it has been hypothesized that most insects get their vitamin B requirements from the diet, of microbial symbiosis, or some combination of these complementary sources (Douglas, 2017). If one starts from the above hypotheses (namely, that insects can not synthesize the B-complex vitamins), then, it would have to be assumed that the quantity of B vitamins quantified in the egg stage of the insect would be a product of the structure of the sack of the ootheca (bag in which the eggs are found) and that later, when *B. magna* hatches and develops in the nymphal and adult stage, it would obtain the requirements of vitamin B from its environment (as previously was mentioned), in any way, the ability to obtain B vitamins and their use in their cellular metabolism would make the levels of this B vitamins complex remain constant. As for fat-soluble vitamins, the content of vitamin A, D and E increased significantly (P<0.05) with the stage of development of *B. magna*. This increase in liposoluble vitamins was directly proportional to the lipid content previously reported in *B. magna* (Monter-Miranda et al., 2018), where a higher lipid content was observed with the development of the insect. In this regard, Melo-Ruiz et al. (2013) reported that fat-soluble vitamins are part of the lipid content of the insect. A high amount of vitamin E increased across the developmental stages of *B. magna* (21.930-145.360 mg/100 g). This vitamin E content was higher than reported for escamoles ant eggs (*Liometupum apiculatum*) (2.22 mg) (Melo-Ruiz et al., 2013) and crickets (*Acheta domesticus*) (33.13 mg/100 g) (Ayieko et al., 2016). The content of vitamin E in egg and nymph 3 of *B. magna* was in the same order that reported by Kinyuru et al. (2010) for green (16.145 mg/100 g) and brown (17.060 mg/100 g) grasshopper (*Ruspolia differens)*, but the it was much higher in nymph 4 and adult (Table 1). Similar to the vitamin E, the vitamin D content increased across the different life cycles of the grasshoppers ranged from 2.710 to 5.250 mg/100 g. These values were greater than reported for escamoles ant eggs (*Liometupum apiculatum*) (0.00361 mg) (Melo-Ruiz et al., 2013). And finally, with respect to vitamin A, the values ranged from 0.150 to 0.390 mg/100 g, in the egg sample,

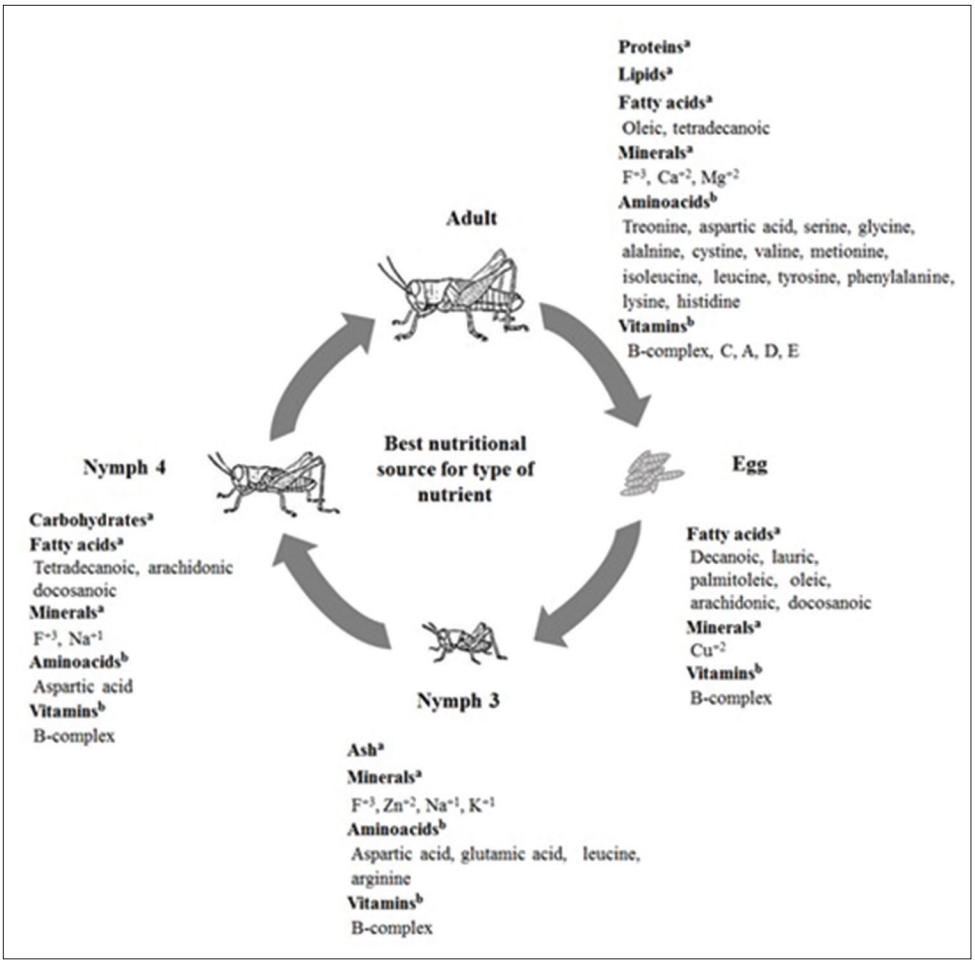

Fig 2. Life cycle diagram remarking which stage is best nutritional source. B-complex: Niacin (B_3), Thiamin (B_1), Pyridoxine (B_6), Folic acid (B_9), Riboflavin (B_2). [a]Monter-Miranda et al. (2018). [b]This study.

a significantly smaller quantity ($P < 0.05$) was observed as compared with the nymph 3, nymph 4 and adult stages. There were no significant differences between nymph 3 and nymph 4, and the highest value was observed in adult stage. These values were greater than reported in green (0.106 mg/100 g) and brown (0.221 mg/100 g) grasshopper (*Ruspolia differens*) (Kinyuru et al., 2010).

The vitamins content and other nutrients in insects is very varied and it is difficult to make comparisons (Payne et al., 2016). Further, studies carried out according to the stages of development in this type of insects are scarce, and most of them have been focused on the larval and adult stages of edible insects (Hyun et al., 2012; Oonincx and Dierenfeld, 2012; Melo-Ruiz et al., 2013; Kouřimská and Adámková, 2016). In addition, the state of development of the insects analyzed is not specified in some studies and the techniques used in their analysis may vary with the authors. On the other hand, the content of nutrients in insects also be strongly influenced by their state of development, depends on each species, seasonality and feeding, which are critical factors in wild insects.

The results of this study of vitamin content during different life cycle of *B. magna* suggest that this grasshopper could be an important source of water-soluble or liphophilic vitamins in diets with reduced consumption of meat products and fruits (Millward and Garnett, 2009).

In another hand, as previously mentioned in the introduction, the results generated in this study are complementary to those previously published by our research group (Monter-Miranda et al., 2018). In this sense, we have made a diagram of the life cycle of *B. magna* indicating which state of development is the best for each type of nutrient (Fig. 2). In general, nymph 3 presents the best source of ash, nymph 4 showed the highest concentration of carbohydrates, while the adult was the best source of proteins and lipids. The eggs and nymph 4 are the main sources of polyunsaturated fatty acids (arachidonic acid). However, *B. magna* eggs represent the best source of monounsaturated fatty acids (oleic and palmitoleic acid). On the other hand, B-complex vitamins were found in the same concentration during all the stages of development evaluated. Regarding the rest of the

vitamins, the adult specimens of *B. magna* represented the main source of vitamin C, A, D and E. Undoubtedly, in this point, more in-depth studies are required and it is a subject that can revert priority importance in future research (Luan et al., 2015; Snyder and Rio, 2015; Douglas, 2017).

CONCLUSIONS

In the adult stage of development of *Brachystola magna*, the highest values of protein content (> 59%) were observed in comparison with the other stages of development. In all stages of *B. magna* the presence of 9 out of the 10 essential amino acids was detected (only tryptophan was absent). All the fat-soluble vitamins and vitamin C increased, while the B-complex vitamins remained constant as the stage of development of *B. magna* increased. Due to the presence of proteins, essential amino acids and vitamins it is suggested that this insect could be a potential source of consumption for people with specific health needs. The results of this study and those previously reported by our working group resulted in a complete characterization of the main nutrients present in *B. magna* during different stages of development. Based on this, we can say that this grasshopper could be an excellent source of saturated and unsaturated fatty acids, high-quality protein due to its content of essential amino acids, with a significant contribution of minerals and vitamins. The adult specimens were the best source for most of the nutrients evaluated in the grasshopper.

CONFLICT OF INTEREST

There are no conflicts of interest.

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Authors contributions

P.B. Zamudio-Flores (the corresponding author) designed the research plan, execution of experimental work, interpretation of results. J.M. Tirado-Gallegos contributed to the writing of the manuscript and designed the Figures. M. Espino-Díaz, E. Ochoa-Reyes and F. Hernández-Centeno participated in the experimental design. M. Hernández-González and H. Yajaira López-De la Peña, R. Salgado-Delgado, V.G. García-Cano and O. Sánchez-Ortíz performed some analysis and contributed in the translation of the manuscript.

REFERENCES

Albalá-Hurtado, S., M. T. Veciana-Nogués, M. C. Vidal-Carou and A. Mariné-Font. 2000. Stability of vitamins A, E, and B complex in infant milks claimed to have equal final composition in liquid and powdered form. J. Food Sci. 65: 1052-1055.

AOAC. 2002. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, MD, USA.

Ayieko, M. A., H. Ogola and I. Ayieko. 2016. Introducing rearing crickets (gryllids) at household levels: Adoption, processing and nutritional values. J. Insects Food Feed. 2: 203-211.

Barker, D., M. P. Fitzpatrick and E. S. Dierenfeld. 1998. Nutrient composition of selected whole invertebrates. Zoo Biol. 17: 123-134.

Bosch, G., S. Zhang, D. G. A. Oonincx and W. H. Hendriks. 2014. Protein quality of insects as potential ingredients for dog and cat foods. J. Nutr. Sci. 3: e29.

Bustillos-Rodríguez, J. C., C. Rios-Velasco, R. Valdéz-Licano, D. I. Berlanga-Reyes, J. J. Ornelas-Paz, C. H. Acosta-Muñíz, M. F. Ruiz-Cisneros, M. A. Salas-Marina and O. J. Cambero-Campos. 2016. Laboratory assessment of *Metarhizium* spp. and *Beauveria* spp. isolates to control *Brachystola magna* in Northern México. Southwest. Entomol. 41: 643-656.

de Castro, R. J. S., A. Ohara, J. G. D. Aguilar and M. A. F. Domingues. 2018. Nutritional, functional and biological properties of insect proteins: Processes for obtaining, consumption and future challenges. Trends Food Sci. Technol. 76: 82-89.

Douglas, A. E. 2017. The B vitamin nutrition of insects: The contributions of diet, microbiome and horizontally acquired genes. Curr. Opin. Insect Sci. 23: 65-69.

Finke, M. D. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol. 21: 269-285.

Finke, M. D. 2005. Nutrient content of insects. In: J. L. Capinera (Ed.), Encyclopedia of Entomology. 1st ed. Dordrecht, Springer, The Netherlands, pp. 1563-1575.

Ghosh, S., S. M. Lee, C. Jung and V. B. Meyer-Rochow. 2017. Nutritional composition of five commercial edible insects in South Korea. J. Asia Pac. Entomol. 20: 686-694.

González Paramás, A. M. G., J. A. G. Bárez, C. C. Marcos, R. J. García-Villanova and J. S. Sánchez. 2006. HPLC-fluorimetric method for analysis of amino acids in products of the hive (honey and bee-pollen). Food Chem. 95: 148-156.

Hyun, S. H., K. H. Kwon, K. H. Park, H. C. Jeong, O. Kwon, H. Tindwa and Y. S. Han. 2012. Evaluation of nutritional status of an edible grasshopper, *Oxya chinensis formosana*. Entomol. Res. 42: 284-290.

Jonas-Levi, A. and J. J. I. Martinez. 2017. The high level of protein content reported in insects for food and feed is overestimated. J. Food Compost. Anal. 62: 184-188.

Khosravi-Largani, M., P. Pourvali-Talatappeh, A. M. Rousta, M. Karimi-Kivi, E. Noroozi, A. Mahjoob, Y. Asaadi, A. Shahmohammadi, S. Sadeghi, S. Shakeri, K. Ghiyasvand and M. Tavakoli-Yaraki. 2018. A review on potential roles of vitamins in incidence, progression, and improvement of multiple sclerosis. eNeurologicalSci. 10: 37-44.

Kinyuru, J. N., G. M. Kenji, S. M. Njoroge and M. Ayieko. 2010. Effect of processing methods on the *in vitro* protein digestibility and vitamin content of edible winged termite (*Macrotermes*

subhylanus) and grasshopper (*Ruspolia differens*). Food Bioprocess Tech. 3: 778-782.

Kouřimská, L. and A. Adámková. 2016. Nutritional and sensory quality of edible insects. NFS J. 4: 22-26.

Löffler, F. and T. Fartmann. 2017. Effects of landscape and habitat quality on Orthoptera assemblages of pre-alpine calcareous grasslands. Agric. Ecosys. Environ. 248: 71-81.

Lozano, G. and L. M. España. 2009. Enemigos naturales y control biológico de *Brachystola magna* (Girard) y *B. mexicana* (Bruner) (*Orthoptera*: *Acrididae*) con *Beauveria bassiana* en Zacatecas, Mexico. Vedalia. 13: 91-96.

Luan, J. B., W. Chen, D. K. Hasegawa, A. M. Simmons, W. M. Wintermantel, K. S. Ling, Z. Fei, S. S. Liu and A. E. Douglas. 2015. Metabolic coevolution in the bacterial symbiosis of whiteflies and related plant sap-feeding insects. Genome Biol. Evol. 7: 2635-2647.

Makkar, H. P. S., G. Tran, V. Heuzé and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197: 1-33.

Melo-Ruiz, V., T. Quirino-Barreda, C. Calvo-Carrillo, K. Sánchez-Herrera and H. Sandoval-Trujillo. 2013. Assesment of nutrients of escamoles ant eggs *Liometupum apiculatum* M. by spectroscopy methods. J. Chem. Chem. Eng. 7: 1181-1187.

Mena-Covarrubias, J. 2009. Control biológico del chapulín *Brachystola* spp. (*Orthoptera*: *Acrididae*) con el uso del protozoario *Nosema locustae* Canning (*Microsporidia*: *Nosematidae*) en Zacatecas, México. Vedalia. 13: 97-102.

Millward, D. J. and T. Garnett. 2009. Plenary lecture 3 food and the planet: Nutritional dilemmas of greenhouse gas emission reductions through reduced intakes of meat and dairy foods: Conference on over and undernutrition: Challenges and approaches. Proc. Nutr. Soc. 69: 103-118.

Mishyna, M., J. J. I. Martinez, J. Chen and O. Benjamin. 2018. Extraction, characterization and functional properties of soluble proteins from edible grasshopper (*Schistocerca gregaria*) and honey bee (*Apis mellifera*). Food Res. Int. 116: 697-706.

Monter-Miranda, J. G., J. M. Tirado-Gallegos, P. B. Zamudio-Flores, C. Rios-Velasco, J. D. J. Ornelas-Paz, R. Salgado-Delgado, V. Espinosa-Solis and F. Hernández-Centeno. 2016. Extracción y caracterización de propiedades fisicoquímicas, morfológicas y estructurales de quitina y quitosano de *Brachystola magna* (Girard). Rev. Mex. Ing. Quim. 15.

Monter-Miranda, J. G., P. B. Zamudio-Flores, J. M. Tirado-Gallegos, F. J. Molina-Corral, E. Ochoa-Reyes, C. Rios-Velasco, M. Hernández-González, F. Hernández-Centeno and H. Y. L. de la Peña. 2018. Nutritional characterization of fatty acids and minerals in *Brachystola magna* (Girard) during their development. Emir. J. Food Agric. 30: 389-395.

Oonincx, D. G. A. and E. S. Dierenfeld. 2012. An investigation into the chemical composition of alternative invertebrate prey. Zoo Biol. 31: 40-54.

Payne, C. L. R., P. Scarborough, M. Rayner and K. Nonaka. 2016. A systematic review of nutrient composition data available for twelve commercially available edible insects, and comparison with reference values. Trends Food Sci. Technol. 47: 69-77.

Ramos-Elorduy, B. J., M. J. M. Pino and C. V. H. Martínez. 2012. Could grasshoppers be a nutritive meal? Food Nutr. Sci. 3: 12.

SAGARPA. (2012). Reglas de Operación de los Programas de la Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, DOF. P. 31.

Snyder, A. K. and R. V. M. Rio. 2015. "Wigglesworthia morsitans" folate (Vitamin B9) biosynthesis contributes to tsetse host fitness. Appl. Environ. Microbiol. 81: 5375-5386.

Walpole, R. E., R. H. Myers and S. L. Myers. 1999. Probabilidad y estadística para ingenieros. Prentice-Hall Hispanoamericana, S.A., México.

Xiaoming, C., F. Ying, Z. Hong and C. Zhiyong. 2010. Review of the nutritive value of edible insects. In: P. B. Durst, D. V. Johnson, R. N. R Leslie and K. Shono (Eds.), Forest Insects as Food: Humans Bite Back. Vol. 85. FAO, Bangkok, Thailand, pp. 85-92.

Yi, L., C. M. M. Lakemond, L. M. C. Sagis, V. Eisner-Schadler, A. van Huis and M. A. J. S. van Boekel. 2013. Extraction and characterisation of protein fractions from five insect species. Food Chem. 141: 3341-3348.

Zielińska, E., B. Baraniak, M. Karaś, K. Rybczyńska and A. Jakubczyk. 2015. Selected species of edible insects as a source of nutrient composition. Food Res. Int. 77: 460-466.

Zielińska, E., M. Karaś and B. Baraniak. 2018. Comparison of functional properties of edible insects and protein preparations thereof. LWT-Food Sci. Technol. 91: 168-174.