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Research Article

# Biochemical and Morphometric Parameters Support Nutritional Benefits in Laboratory Rats Fed With an Orthopteran-Based (*Sphenarium purpurascens*) Diet

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## Abstract

Edible insects are considered a sustainable food source for humans, mainly due to their high protein content and low environmental footprint. Generally, knowledge about the nutritional composition of insects stems from bromatological studies; however, *in vivo* assays are lacking. This study evaluated the nutritional benefits of consuming *Sphenarium purpurascens* (Orthoptera) as a protein source on an animal model. Over a 5-week experiment, five Wistar rat groups (7-8 week old males) were fed *ad libitum* with different diets: three based on varying concentrations of *S. purpurascens* (i.e., hypoproteic or LPD, standard or SPD, and hyperproteic or HPD), a Control Diet (CT), and a low protein Hypercaloric diet (HC). Grasshopper diets had 18.1%, 23.1%, and 28.7% of total protein, respectively, and 7.19%, 13.44%, and 20.71% of grasshopper protein. Weekly, we assessed morphometric (e.g., weight, length, abdominal circumference, BMI, and Lee's index) and biochemical parameters. In addition, we daily calculated the food consumed per individual. Our results did not show significant differences in morphometric parameters since CT exhibited the highest net weight gain, but SPD showed the best weight gain/food intake ratio. According to biochemical test results, LPD, HPD, and SPD showed significant statistical differences with the control group in hemoglobin, albumin, triglycerides, cholesterol, and glucose content; however, all biochemical parameters remained within the normal range expected for Wistar rats. Our results support the hypothesis that the addition of grasshopper protein to the diet of rats has acceptable nutritional effects (similar to conventional protein) without causing adverse effects on their health. This type of experiment may become essential to develop trials in human populations that evaluate the nutritional benefits of insect protein to support its inclusion in our usual diet.

**Keywords:** Grasshopper; Protein; Rat Diet; Nutrition; Edible Insects; Orthopteran

## Introduction

The consumption of edible insects has been reported for several centuries in diverse cultures around the world. For example, in Mexico, the first reports about edible insects date back to 1557, when Sahagún described about 96 edible species in the Florentine Codex [1]. Thus, the potential of edible insects as a nutritious food source has been constantly discussed. The first bromatological analyses to determine their carbohydrate, lipid, and protein content were carried out in the 1930s [2]. For instance, under this approach, Yamafuji and Yonezawa [3] analyzed the pupa of *Bombyx mori* (Lepidoptera), bringing about the differences between protein and non-protein nitrogen content. For almost 100 years, numerous investigations have replicated these methods. However, the greatest problem in determining the nutritional potential of insects (especially their protein content) is assessing digestible protein versus total nitrogen contents, specifically, the N-acetyl glucosamine content within the cytoskeletal chitin, which is non-assimilable [4]. Moreover, the quantity of non-protein nitrogen can change among phylogenetically close species, life cycle stages, and diets, mainly due to the cytoskeletal protein composition [5]. It was not until the 80's when the first research on digestibility and assimilation of insect proteins was carried out *in vivo* [6]. Back then, Dreyer and Wehmeyer [7] reported high digestibility and assimilation values on rats fed with lepidopteran larvae (*Gonimbrasia belina*), and recently, Oibiokpa, et al. [8] conducted a study to determine the nutritional effect of four insect species on Wistar rats finding that *Melanoplus foedus* (Orthoptera) possessed a protein content of 75.08%. However, *in vivo* studies to determine the nutritional effect of edible insects are still scarce.

Within Insecta, order Orthoptera stands out for its large number of edible species. These insects inhabit a wide variety of geographical regions within natural environments and agroecosystems [9]. Early analyses on orthopterans already mentioned a high protein content revealed by conventional biochemical methods. For example, dry weight values are 61.1%, 63.5%, and 75% for *Nomadacris septemfasciata* [10], *Schistocerca gregaria* [11], and *Locusta migratoria* [2], respectively. In addition, Banjo, et al. [12] determined a protein content of 63.1% in *Trimerotropis* spp., while values up to 58%, 49.7%, and 26.8% are reported for *Nomadacris fasciata, L. migratoria,* and *Zonocereus varigatus,* respectively [13]. All these species are considered pests in agriculture and controlling them has been challenging. For instance, *L. migratoria* causes millionaire losses per year, risking alimentary security in Africa, which worsens during crises like the COVID-19 pandemic [14]. Also, *Sphenarium purpurascens* is considered one of the most devastating species in central Mexico [15]. Some studies on pest control report slight effectiveness through conventional methods but with negative environmental and health consequences due to the constant use of pesticides [16-18]. Therefore, the development of sustainable methods of pest control management is needed.

In Mexico, grasshoppers are both a pest and an edible insect of great importance for local gastronomy [19-25]. Thus, exploiting insects for consumption is feasible and could contribute as a pest control mechanism [23-25]. Furthermore, the harvest and commercialization of only a fraction of grasshopper populations from agroecosystems could result in significant economic incomes [20]. In addition, these species are rich in nutrients with protein contents ranging from 52.6% to 71.5% (dry weight), lipids from 5.75% to 19.56%, fiber from 1.58% to 11.05%, and carbohydrates from 8.11% to 21% [21, 26]. Grasshoppers are also a good source of vitamin D (164.91 U.I./100 g) and vitamin B: thiamine (0.27 mg/100 g), riboflavin (0.59 mg/100 g), and niacin (1.56 mg/100 g) [22]. This species could potentially produce approximately 350,000 tons of grasshoppers annually and sustainably. With this yield, it is possible to feed around 9 million people per year, considering the protein recommended daily intake of 25 g per day [25] or 0.8-1 g/kg for adults and 0.9 g/kg for children and adolescents [27]. Exploiting this nutritional potential could be of great importance in regions where malnutrition prevalence is very high, such as Africa, Asia, and Latin America. For instance, the United Nations Children's Fund (UNICEF) indicates that Mexican children have severe malnutrition and obesity problems [28]. Mexico ranked first in childhood obesity in 2019 because one in three children between the age of 5 and 11 were overweight, while 36% of adolescents between the age of 12 and 19 had this condition [28]. To address these issues, insect consumption is a promising alimentary alternative due to their high nutrient content and because several species grow into high population densities. Parameters to assess the nutritional condition of humans can be classified into two broad categories: morphometric measures like weight, size, and abdominal circumference, which are needed to obtain the body mass index or BMI [29,30], and biochemical measures such as creatinine/height, nitrogen balance, and 3-methyl-histidine, prealbumin, retinol-bound protein, transferrin, somatomedin, and cholesterol contents [29]. In laboratory rats, the same parameters are used; mostly, albumin and prealbumin contents and morphometric measures necessary to calculate BMI and Lee index, which is an assessment of the BMI commonly used in rats [8, 31, 32]. The parameters quantified in basic blood profiles are also significant for detecting diseases (e.g., hemoglobin, glucose, and hematocrit, among others) [33, 34].

Due to ethical and biosecurity concerns, *in vivo* assays to determine the nutrient quality of any novel food for regular human consumption must be tested on animals. These studies allow evaluating adverse or potentially negative effects on vulnerable subpopulations through detecting alterations on: 1) absorption

and secretion mechanisms; 2) biochemical, physiological, and metabolic responses; and 3) organs functioning [35]. In particular, assays are frequently conducted on Wistar rats because they are easily handled and the analyzed parameters can be extrapolated to humans, despite their phylogenetic distance [36]. For example, Cossio-Bolaños et al. [37] accurately reported weight and size changes in rats fed with a normal diet. In this work, we evaluated the effect of three diets added with different concentrations of *S. purpurascens* protein, assessing growth and development parameters on Wistar rats. Then, we compared the results with a control diet and a nutritionally deficient diet (i.e., high in carbohydrates and low in proteins, similar to recent human diets consisting of highly processed and refined products). This type of protocol is needed to support orthopterans as a healthy protein source for human populations.

## Materials and Methods

### Collection and nutritional assessment of *Sphenarium purpurascens*

Grasshoppers were collected with a conical net at Village Santa María Zacatepec, Puebla, and adjacent areas. Most insects were in their adult stage. Afterward, grasshoppers were manually separated from the rest of the material and cleansed thrice in 50 L of water at room temperature. Then, they were dehydrated in three phases: 1) mechanical drying in a centrifuge with heat for two 5 min cycles; 2) roasting in a metallic sheet (comal) at 100°C for approximately 30 min until they acquired a reddish color; and 3) solar exposition for 5-6 h, moving them constantly. Finally, the nutrient composition of a sample was analyzed (Table S1) in the Laboratorio de Química y Análisis de Alimentos of the Departamento de Alimentos y Biotecnología, Facultad de Química, UNAM, in November 2018. Grasshoppers were stored at room temperature.

### Dietary formulation

The experimental set-up was based on five treatments with different protein quantities (Table 1, S2), as follows: 1) Control diet (CT): commercial feed *Nutricubo*; 2) Standard protein diet (SPD): formulated with *S. purpurascens* protein in a percentage similar to commercial feed *Nutricubo*; 3) Hyperproteic diet (HPD): *S. purpurascens*-based with protein content 50% higher than commercial feed; 4) Hypoproteic diet (LPD): *S. purpurascens*-based with protein content 50% lower than commercial feed; and 5) Hypercaloric diet (HC): feed high in carbohydrates without grasshopper protein and added with highly processed foods (Maruchan® Ramen instant noodles, chicken flavor). Rats fed with CT, SPD, HPD, and LPD were provided with water, while HC rats had access to a caloric soft drink (i.e., Coca-Cola® soda type diluted to 50% with water).

Orthoptheran-based diets were elaborated with dehydrated grasshopper flour, corn flour, dried amaranth, roasted peanut without salt, and barley flour. These ingredients were selected because they are all locally produced and have a Mesoamerican origin (except barley) so they are easily accessible. The quantities of these ingredients were determined according to NOM-062-ZOO-1999 recommendations [38] and based on the nutrients reported for the commercial feed Rodent Laboratory Chow 5001. Nutritional values were obtained from the food composition tables of INCAP/OPS [39], and PROFECO [40] for instant noodles, and from bromatological analysis carried out by the Facultad de Química of the UNAM for the commercial feed *Nutricubo* (Table S3). Ingredients specific to each diet were mixed manually and shaped into pellets. These were baked at 200°C for 10 min on both sides and then at 180°C for one hour, turning them every 20 min for uniform cooking and to avoid contamination. Finally, they were left to cool on paper and stored in labeled Styrofoam containers.

### Experimental design

Fifty young male Wistar rats (approximately 7-8 weeks old) were donated by the animal facility center of the Facultad de Ciencias, UNAM. The animals, with an initial weight of 210 ± 28g and identified through ear punches, were randomly divided into 5 experimental groups of 10 individuals. Each group had ad libitum access to feed and liquids for five weeks and were housed in groups of five rats in labeled polypropylene cages at a temperature of 22 ± 1°C and lighting cycles of 12h light/ 12h dark. At the end of the experiment, animals were euthanized by an overdose of isoflurane following NOM-062-ZOO-1999 [38] guidelines, to avoid pain and stress after the experimental period. This study was approved by the Comité Interno de Cuidado y Uso de Animales de Laboratorio de la Facultad de Medicina, UNAM, and animal handling and all experiments were performed under the guidelines of international and national regulatory instruments for animal welfare, such as NOM-062-ZOO-1999 [38]. During the entire experimental period, the pain and discomfort of the animals were minimized as much as possible.

### Blood sampling and biochemical parameters

Blood collection was performed weekly from the beginning of the study. Rats were individually anesthetized with isoflurane and 500 µL of blood were drawn through a tail cut following NOM-062-ZOO-1999 [38] guidelines. The latter indicates that it is safe to extract 7.5% of blood every 7 days [41]. This procedure was performed from 8 to 10 in the morning each time. Blood samples were placed in heparinized Eppendorf tubes and stored in cold conditions to quantify hemoglobin, albumin, glucose, cholesterol, and triglycerides by spectrophotometry using Spinreact® kits.

### Morphometric parameters

Morphometric parameters were measured at the beginning of the experiment and monitored weekly. Rats were weighed using an electronic scale (ADIR®) and body weight gain was estimated as follows: $Body\ weight\ gain = Weekly\ body\ weight - Initial\ body\ weight$. Body size measurements, i.e., body length (or nasal-anal length, from the tip of the nose to the base of the tail), total length (from the tip of the nose to the tip of the tail), and abdominal circumference (just below the ribs), were taken with a metric tape measure [31]. Body length changes were assessed following the same logic of the weight gain formula. Additionally, body weight and body length measures were used to calculate the BMI and Lee's index, as follows [31]:

$$Body\ mass\ index\ (BMI) = \frac{weekly\ body\ weight\ (g)}{weekly\ size^2\ (cm^2)}$$

$$Lee\ index = \frac{\sqrt[3]{weekly\ body\ weight\ (g)}}{weekly\ size\ (cm)}$$

### Feed intake

Every 24 hours, the initial and final feed amount per box was weighed, and the intake was calculated through a subtraction, similar to that described by Oibiokpa et al. [8]. Then, the result was divided by the number of individuals per box to obtain the average feed intake per individual.

### Statistical analyses

Morphometric parameters and blood biochemistry data were analyzed with a factorial ANOVA and an ANOVA, both of repeated measures. To test for normality, Shapiro-Wilk and post hoc Tukey tests were performed. These analyses were carried out with STATA® software (version 12.0). Finally, the correlation between gained weight and feed intake was examined through the Pearson linear correlation coefficient. Extreme data in the blood chemistry parameters were eliminated and replaced with the mean obtained from the rest of the individuals in the same experimental group.

## Results

### Diet design

We designed four of the five diets assessed in this study. Three of them had different grasshopper protein concentrations (i.e., low: LPD; standard: SPD; and high: HPD), while the fourth (HC) sought to resemble a Western urban diet for humans, without protein content from *S. purpurascens* (Table 1). We compared these treatments with a control group fed with a diet (CT) based on the commercial feed for Wistar rats regularly used in the animal facility center (Facultad de Ciencias, UNAM). The average amount of protein from commercial feed (CT) was 23%, while lipids and carbohydrates were 8. 22% and 57%, respectively. In insect-based diets, total protein contents were 23.1% (SPD), 18.1% (LPD), and 28.7% (HPD), lipid values were 11.4% (SPD), 12.2% (LPD), and 10.9% (HPD), and grasshopper protein contents were 13.44% (SPD), 7.19% (LPD), and 20.71% (HPD). Finally, in the diet based on highly processed products and sugary drinks (HC), protein, lipids and carbohydrates values were 12%, 12.9%, and 65.8%, respectively (Table 1). The bromatological analysis revealed that *S. purpurascens* has 59.75 g of protein per 100g of insect in dry weight (Table S1). Thus, with this information we were able to determine the amount of grasshopper protein needed for each experimental treatment (Table S2).

| | | Parameter (%) | | | | |
|---|---|---|---|---|---|---|
| **Diet** | **Description** | **Protein** | **Lipids** | **Fiber** | **Carbohydrates** | **Ash** |
| **CT** | Commercial food (*Nutricubo*) and water | 23.0 | 8.22 | 6.0 | 57.0 | 7.0 |
| **SPD** | Feed with grasshopper protein and water | 23.1 | 11.4 | 11.7 | 51.4 | 2.4 |
| **LPD** | Low protein feed with grasshoppers and water | 18.1 | 12.2 | 10.9 | 56.6 | 2.2 |
| **HPD** | High protein feed with grasshopper and water | 28.7 | 10.9 | 12.8 | 45.1 | 2.5 |
| **HC** | Hypercaloric feed added with instant noodles and soft drink | 12.0 | 12.9 | 7.6 | 65.8 | 1.6 |

**Table 1:** Nutritional composition (% dry basis) of the diets administered.

For each treatment to be comparable to the control in terms of nutrient contents (protein, fiber, lipids, carbohydrates, and essential vitamins and minerals), we added the following secondary ingredients: nixtamalized corn flour, amaranth seeds, roasted peanuts, and seeds of barley. Table S2 shows the nutritional contribution of each ingredient per 100 g in each diet.

### Morphometric parameters and feed intake

The five-week experiment revealed significant differences ($P<0.05$) among treatments in weight, total size, body length, abdominal circumference, BMI, and Lee index (Figures 1 and 2). Rats fed with CT gained more weight and abdominal circumference from the first week than the other treatments (Figures 1A, D); however, regarding total and body lengths, the SPD and LPD groups showed an increasing behavior similar to CT (Figures 1 B, C). Throughout the study, there were no significant differences among grasshopper-fed groups and CT. Still, individuals fed with LPD obtained low weight gain and slight abdominal circumference increase than the other three groups, while SPD and HPD obtained the same weekly weight gain as CT. Grasshopper-based diets showed oscillating BMI and Lee index values like CT (Figure 2), ranging within intervals considered normal. At the end of the study, CT and SPD displayed similar behaviors in both indexes.

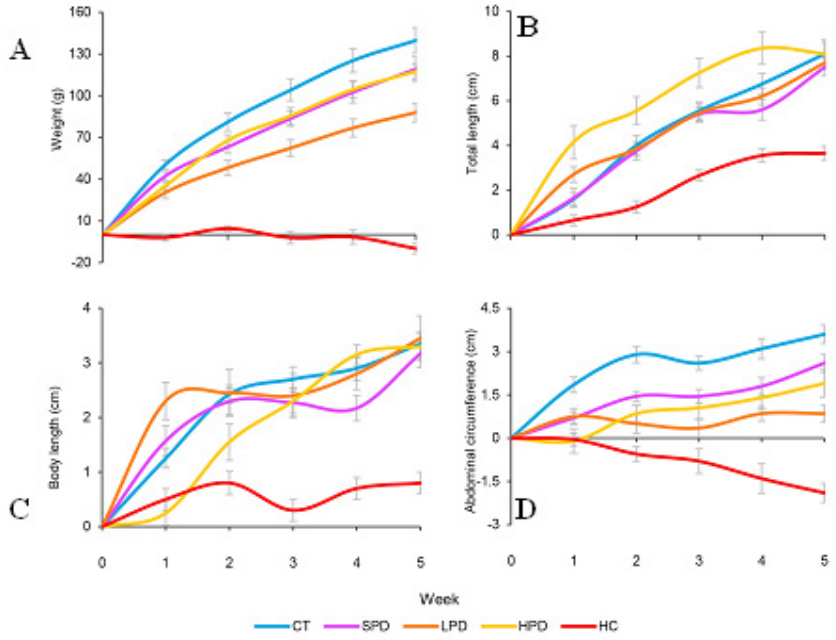


**Figure 1:** Weekly increase in morphometric parameters compared to the initial measurement: weight gain (**A**), $F= 78.05$, $P<0.05$; total length (**B**), $F= 47.0$, $P<0.05$; body length (**C**), $F= 25.03$, $P<0.05$; and abdominal circumference (**D**), $F= 19.32$, $P<0.05$. HC rats had little changes in morphometric parameters and were the only group that showed weight loss.

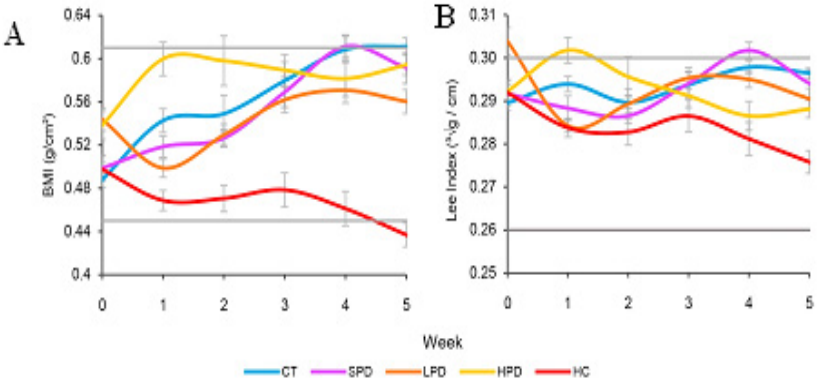


**Figure 2:** Corporal composition indices: Body mass index (**A**), $F= 16.04$, $P<0.05$; and Lee index (**B**), $F= 5.59$, $P<0.05$. The grey lines represent the intervals reported by Novelli, et al. [31] under normal conditions for 60-90 days old rodents. For both indices, the CT and SPD groups had a similar behavior, whereas the HC group had the lowest values.

In contrast, HC rats did not gain any weight ($P>0.05$), and from the fourth week, their abdominal circumference decreased ($P<0.05$) (Figure 1). Regarding size parameters, BMI, and Lee index (Figures 1B, C and 2), group HC did not show significant differences throughout the study, except in the last week, when we observed a significant increase in size parameters and a decrease in the BMI and Lee index.

Groups CT, SPD, LPD, and HPD showed a direct correlation between accumulated weight and food intake (Table 2), while HC did not (slope value of -0.018 and $R$= -0.23). Figure 3 shows similar slope values between the control group and HPD (0.154 and 0.150, respectively); however, the highest value corresponded to group SPD (slope value of 0.179) and the lowest to LPD (slope value of 0.139). The average daily feed intake is shown in Table 2. Daily feed intake was similar between CT and HPD groups, and between SPD and LPD, while HC obtained the lowest values. Thus, HC was the group that had the poorest feeding.

| | Group | | | | |
|---|---|---|---|---|---|
| | **CT** | **SPD** | **HPD** | **LPD** | **HC** |
| Pearson's correlation coefficient (R) | 0.90 | 0.92 | 0.94 | 0.84 | -0.23 |
| Daily feed intake per rat (g/rat/day) | 25.3 ± 0.02 | 18.06 ± 0.05 | 23.0 ± 0.04 | 17.1 ± 0.05 | 11.4 ± 0.03 |

**Table 2:** Pearson's correlation coefficient and daily feed intake per individual for 5 weeks.

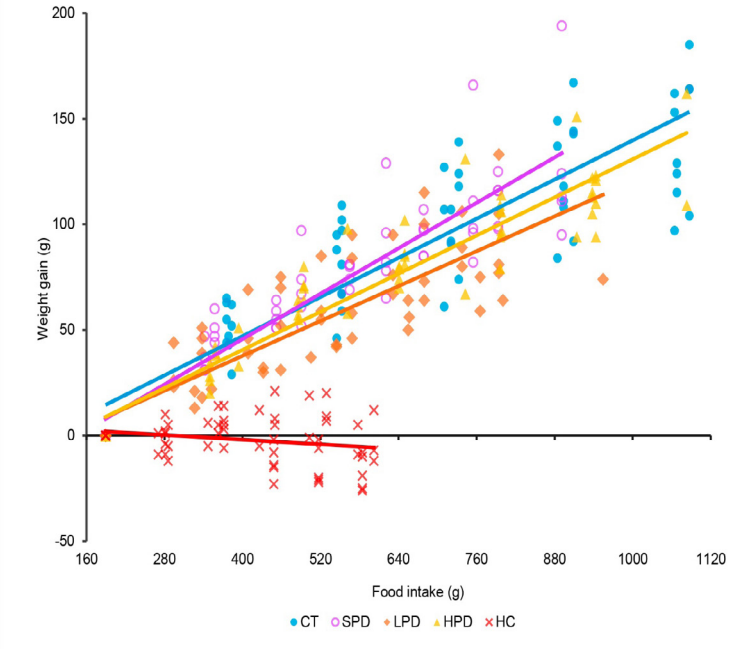


**Figure 3:** Correlation between food intake and weight gain (per individual). The HPD group had the highest value (R = 0.94) while CT (R = 0.90) and SPD (R = 0.92) showed similar responses. HC rats showed a weak negative association (R = -0.23).

**Hematological patterns and blood biochemistry**

To evaluate the effect of each diet on the health of laboratory rats, we conducted five biochemical tests: hemoglobin and albumin tests (Figure 4A, B) to determine the state of health based on protein absorption, and cholesterol, triglycerides and glucose tests (Figure 4C, D, E) to determine adverse effects. Blood biochemistry results constantly oscillated throughout the five-week experiment (Figure 4). However, parameter curves of rats fed with CT and all grasshopper-based diets (SPD, LPD, and HPD) moved within the normal intervals [38-40]. However, in the fifth week, HPD and LPD showed significant differences ($P<0.05$) with CT in glucose concentration (Figure 4E). At the end of the study, HPD rats had the highest glucose values, while LPD had the lowest of the grasshopper diets. Similarly, HPD albumin shifted below the interval in the fifth week. LPD also showed the highest triglyceride values in the second week. CT did not show significant differences in hemoglobin (Figure 4A) during the experimental period, but it did in albumin, cholesterol, triglycerides,

and glucose (Figure 4B-E). Of the grasshopper-based diets, SPD is the most similar to CT in all the parameters, except in the first week albumin concentration.

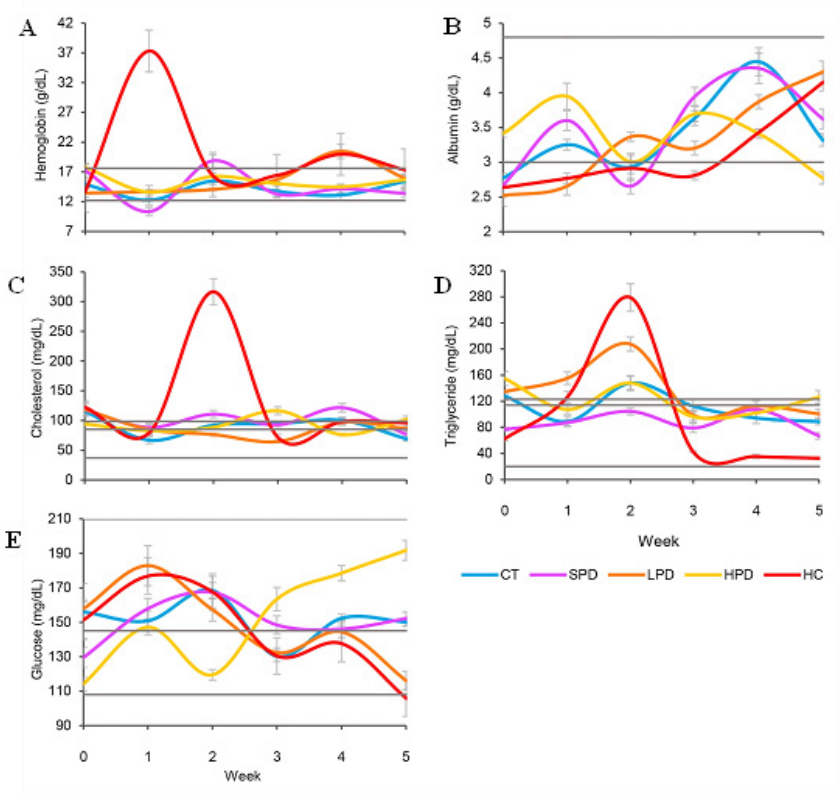


**Figure 4**: Biochemical parameters: hemoglobin (**A**), albumin (**B**), cholesterol (**C**), triglyceride (**D**), and glucose (**E**). All parameters were oscillatory throughout the experimental period. However, CT and all grasshopper diets (SPD, LPD, HPD) remained within the normal intervals (grey lines: 42, 43, 44)

On the other hand, we found that there was an interaction between diet type and experimental time in the following parameters: hemoglobin ($F$= 2.83, $P$<0.05), albumin ($F$= 5.10, $P$<0.05), cholesterol ($F$= 34.38, $P$<0.05), triglycerides ($F$= 9.45, $P$<0.05), and glucose ($F$= 9.54, $P$<0.05). Hemoglobin values showed a greater effect caused by diet type ($F$= 8.11, $P$>0.05) than experimental time ($F$= 0.94, $P$<0.05). This case was opposite to glucose values, in which there was a greater effect caused by time ($F$= 8.11, $P$>0.05) than diet type ($F$= 0.90, $P$<0.05). In albumin, cholesterol, and triglyceride parameters, both diet and time greatly affected the results.

Finally, HC blood biochemistry values had a different behavior (Figure 4), especially during the second and third weeks. This group showed the highest hemoglobin values (37.32 ± 2.86 g/dL) in the first week after the experiment onset (Figure 4A). All parameters, i.e., albumin ($F$= 29.68, $P$<0.05), cholesterol ($F$= 95.04, $P$<0.05), glucose ($F$= 5.42, $P$<0.05), and triglycerides ($F$= 52.74, $P$<0.05) showed significant differences during the experimental period. Especially in the second week when the highest triglycerides (278.98 ± 66.43 mg/dL) and cholesterol (316.58 ± 69.51 mg/dL) values were reached and cholesterol results shifted above the interval considered normal. Significant decreases were also observed in albumin at the beginning of the study and in triglycerides at the end. Albumin registered its lowest values in the first and third weeks: 2.64 ± 0.44, 2.77 ± 0.24, g/dL, respectively, and triglycerides in the last two weeks: 35.29 ± 10.84 and 32.65 ± 6.31 mg/dL. HC glucose was the only parameter that maintained its values within the normal interval at every moment.

## Discussion

Several experimental physiological studies use animal models due to their similarity with humans and because many variables are easy to measure under controlled conditions. The importance of these studies lies in their feasibility to extrapolate results to humans, in contrast to *in vitro* models that usually offer more controlled conditions but are greatly limited because they cannot mimic a complex organism [42]. Rats are commonly used animal models for investigating the causes, diagnosis, and

treatment of diseases that affect both animals and humans [43]. Specifically, Wistar rats are broadly used in diverse areas: teaching, toxicology, pharmacology, metabolism, auditory problems, oncology, ophthalmology, immunology, and malnutrition studies [8,31-34,37,44]. Unfortunately, very few studies have tested the nutritional value of insects using animal models. Of the 2,000 registered insect species, less than a dozen studies have evaluated their nutritional value with laboratory rats [8,45-47]. For example, Phelps [45] studied the nutritional effect of the termite species *Macrotermes falciger* in laboratory rats and Finke et al. [46] evaluated the nutritional effect of three insect species. However, unlike our findings, previous works recorded an unpromising effect of insect proteins compared to control diets. Although we did not evaluate it in this study, one of the main reasons for previous results is related to the amount of non-assimilable nitrogen in insects, particularly in chitin [46,47]. In a study carried out by Meza-Cureño et al. (unpublished data) in the same experimental system, they evaluated the amount of assimilated versus unassimilated nitrogen, both in urine and feces, determining the existence of a large amount of non-assimilable nitrogen in these excretions. Also, in this same study the efficiencies of food and protein assimilation were evaluated in the five different types of diets and although no significant differences were found between the control group and the diets made based on insect proteins, it was intended to find a higher rate of assimilation of the food in the HPD group, because it contained more protein of *S. purpurascens.*

Our results show that the diet type influenced all morphometric parameters from the first week of the experiment. However, we only observed significant differences in weight and abdominal circumference among the groups at the end of the experimental period (Figure 1A, D). The CT group obtained the highest increase in those parameters. Weight is considered one of the best indicators to evaluate rats' nutritional state because it provides a clear insight into the effects of different treatments in a short experimental time [37]. The weight gained by the control group (Figure 1A) had similar behavior to the results reported by Cossio-Bolaños, et al. [37] in 7-12 weeks old male Wistar rats, which is consistent with the ages of our individuals at the beginning and the end of our study.

Although protein quantities in CT and SPD were similar, the latter showed a slightly lower weight gain and abdominal circumference increase than the control. The smaller feed intake by SPD compared to CT can explain this result (Table 2). Despite these differences, the Lee index and BMI (Figure 2) indicate adequate body composition as they did not show significant differences in the fifth week and always remained within the established limits [31,48]. This observation rules out the possibility of any malnutrition status in CT and SPD, specifically obesity or overweight. The hyperproteic (HPD) and hypoproteic (LPD) diets did not lead to overweight or obesity either, as both groups were below the maximum limits established for those indexes. It is worth mentioning that BMI is the most widely used index to predict body composition in humans, especially for its relation to fat weight or adiposity, which indicates degrees of obesity [49]. However, this index has certain limitations as it does not distinguish between fat-free weight and fat weight, leading to incorrect conclusions. In this study, dissection of the animals was not carried out to obtain these data, as it requires the use of a higher number of animals to obtain meaningful, consistent and comparable results. On the other hand, the Lee index, proposed in 1928, is also considered an obesity indicator for rats subjected to diets aiming to increase fat weight [48]. Taking into account the above and as mentioned above, we can observe that the hyperproteic (HPD) and hypoproteic (LPD) diets administered did not lead to overweight or obesity conditions either, as both groups are below the maximum limits established for those indexes.

The low protein diet (LPD) has consequences on weight and abdominal circumference (Figure 1A, D). LPD rats showed the lowest values in both parameters compared to CT, SPD, and HPD, while for body length and total length the values were similar to CT and SPD. On the other hand, the high protein diet (HPD) did not cause a higher increase in body size, abdominal circumference, or weight gain during the experiment, even though the animals had a similar feed intake to CT (Table 2). HPD rats showed a higher increase in total length during the first four weeks (Figure 1B), but the body length did not follow this pattern (Figure 1C), meaning that the increase in total size was mainly due to tail growth.

Feed intake strongly influenced weight gain in all treatments. As already mentioned, the results showed that CT was the group with the highest weight gain, while SPD and HPD obtained similar values (Figure 1). However, feed intake was not the same in all groups, thus affecting weight gain. For CT and all grasshopper-based diets, the Pearson's linear correlation coefficient between 0.94 and 0.84 indicates that feed intake is directly correlated to weight gain (Figure 3). Rats can increase or decrease their feed intake depending on the nutrient content of their diet [50]. Thus, to obtain the necessary amounts of certain nutrients, rats generally tend to eat more when their diet has low protein levels and do the opposite when their diet is hyperproteic [50]. However, Du et al. [50] and Pezeshki, et al. [51] reported that, occasionally, individuals fed with low-protein diets show a different pattern where their feed intake decreases, especially when the diet provided is very deficient in protein content. The correlation between weight gain and feed intake for SPD agrees with Du et al. [50]. Therefore, although SPD and CT had the same protein content, SPD rats obtained the necessary nutrients with less feed, suggesting that they could have better nutrient assimilation than CT. Contrastingly, the feed intake reported for HPD and LPD disagrees with Du et al. [50]. Consumption tendencies were contrary to what was proposed by these authors, especially in LPD, whose protein

content is not considered low enough for the individuals to present the behavior indicated by Du et al. [50] and Pezeshki et al. [51]. For this reason, we suggest that the lower LPD intake could be attributed to palatability issues.

Hematological and biochemical parameters are good indicators of the physiological, nutritional, and pathological states of the animals [52]. Most of the groups fed with grasshopper-based diets remained oscillating within ranges considered normal for the parameters analyzed (Figure 4), indicating adequate nutrition. The non-significant differences ($P<0.05$) between CT and SPD in hemoglobin, albumin, cholesterol, triglycerides, and glucose indicate that the SPD diet is safe for consumption, as it did not cause adverse effects on the parameters evaluated, concurring with the data reported by Oibiokpa et al. [8]. They formulated six experimental isonitrogenous and isocaloric diets with four insects: termites (*Macrotermes nigeriensis*), crickets (*Gryllus assimilis*), grasshoppers (*Melanoplus foedus*), and moth caterpillars (*Cirina forda*), and fed them to male Wistar rats for four weeks. During the experiment, they measured the protein quality and examined the effects of consuming similar quantities of the four insects. The authors concluded that the protein quality provided by *Gryllus assimilis* was superior compared to the others and that their insect-based diets did not cause adverse effects on the rats based on biochemical parameters (i.e., serum concentrations of aspartate transaminase, alanine transaminase, alkaline phosphatase, total protein, HDL cholesterol, total cholesterol, albumin, triglycerides, and hematological indices).

In our study, hemoglobin, cholesterol, and glucose values in the LPD group remained similar to the control group throughout the experiment (except for glucose in the last week), while albumin and triglycerides showed significant differences (Figure 4). Differences in triglycerides appeared in the second week; however, these did not lead to adverse health effects because the final values were within the established ranges for male Wistar rats under normal conditions [53-55]. The albumin values of the LPD rats were the lowest from the begging of the experiment (Figure 4B). In the first three weeks, values fell slightly below the lower limit of the interval. This decrease in albumin was not considered very relevant because at week four its values increased again, and at week five this parameter no longer showed differences with CT and SPD. HPD rats showed constant hematological and biochemical patterns. Hemoglobin, albumin, cholesterol, and triglycerides (Figure 4A-D) remained within the normal ranges, but albumin decreased in the last week. Glucose increased steadily, causing its values to be the highest in the fifth week. Both diet type and feed intake could explain this result: the HPD formulation was characterized by the highest protein content, however, the amount of lipids, fiber, and carbohydrates remained similar to CT and LPD. On the other hand, HPD rats did not reduce their feed intake as expected by Du et al. [50], causing a higher carbohydrate intake over time, explaining the increase in glucose (Figure 1E). In the present study, the effects of the hyperproteic diet were slightly counterproductive in terms of glucose concentration in the last experimental week; however, other studies in which hyperproteic diets are formulated with 45% protein richness, reported serious adverse health effects, e.g. kidney damage [56].

In this work, we also evaluated a diet based on highly processed and refined products (HC). HC contained high amounts of sugars and saturated oils, which have been associated to a large number of non-infectious diseases [57-60]. Diets of this type have spread to a large part of the Western population, causing serious health problems, especially in children [28,61,62]. In this study, HC works as a proxy for the poor diets adopted by a large part of the Western population. In such diets, monosaccharide fructose is a predominate compound in instant foods and sugary drinks. The consumption of large amounts of fructose leads to malnutrition diseases such as obesity [63,64]. High-calorie diets tend to increase body weight in humans and rodents; however, in our study, individuals fed with HC showed significant differences compared to CT in all morphometric parameters (Figure 1). Unlike CT, HC rats showed a decrease in accumulated weight and abdominal circumference. Regarding body length, the values in this group remained constant and without any significant change throughout the experiment. In contrast, the final values of total length increased significantly compared to the initial values but remained well below the other treatment results.

Even though HC contained large amounts of carbohydrates (65.8%) and the protein content was considerably low (12.0%), it was not enough to induce obesity. These effects, especially weight loss, are similar to the results reported by Du et al. [50]. They recorded weight, body composition, and feed intake in groups fed with different concentrations of protein (casein): 2%, 5%, 8%, 10%, 15%, and 20%, but with the same concentration of carbohydrate and lipids. In their study, the authors observed that the group fed with a lower amount of protein decreased its feed intake, leading to weight loss. Our parameters show that HC rats had inadequate growth compared to expectations for their age (a definition used in the concept of malnutrition) [28]. BMI and Lee index values confirmed this condition, as their mean values were significantly lower than those of the CT rats (Figure 2). In particular, BMI values tended to decrease, which may be related to malnutrition, and were similarly reflected in weight loss, body size, and abdominal circumference. As mentioned before, BMI is not considered a good indicator to understand body composition, for identifying especially fat weight. This issue is important because if BMI is considered an index directly related to fat weight, we could infer that fat weight loss mainly causes the malnutrition observed in the HC group. However, more precise methods that involving the death of the individual describe that the decrease in body weight of rats fed with hypoproteic diets is mainly due to the

decrease in body protein and body water. That is, animals fed a low amount of dietary protein accumulate more body fat than those fed with normal protein levels [50,65].

A fundamental factor that determines the malnutrition impact caused by HC is age. Cossio-Bolaños et al. [37] indicated that depending on body weight, rodents will have grown 21% during the first 21 days of age, 50% at 56 days, and will have completed a 100% growth at 112 days. In other words, Wistar rats show the highest growth between 56 and 112 days of age [37]. Given that the malnutrition observed in HC rats occurred during the growth period, we consider the effects to be irreversible; that is, even if in the following weeks they had obtained a diet with higher protein content, they probably would not have reached the same size as the other experimental groups. This insight is important because we could expect similar results from children fed with poor diets during their main developmental stages. Furthermore, we observed that HC rats had higher drink consumption than the other groups. The HC group was provided with a solution containing 50% soft drink and 50% water. Their high drink intake is related to a decrease in food intake because they consumed 10.3g of sugars (mainly fructose) per 100 ml of liquid [63], which caused the rats to lose their appetite for feed and develop an addictive behavior toward soft drinks [66]. High amounts of fructose cause a cascade of metabolic reactions [63,64,66-70]. Fructose metabolism in the liver [67,68,71,72] induces the synthesis of ligand 2-arachidonoylglycerol (2-AG) through the expression of diacylglycerol lipase-β [68,73]. Then, the 2-AG ligands bind to cannabinoid-1 receptors in the hypothalamus producing endocannabinoid, which induces appetite and addictive behaviors like the one observed in HC rats [68,73].

Contrary to expectations, HC rats had the lowest feed intake of all experimental groups. As explained above, this behavior was caused by excessive fructose consumption from soft drinks [64,66-70]. In normal conditions, there is a correlation between adipose tissue and leptin concentrations, regulated by insulin and glucocorticoids [68,71,72]. Leptin is secreted by adipose tissue and its primary function is to regulate body weight by signaling the hypothalamus to induce or stop the sensation of hunger [68,71,72]. High amounts of fructose in the body stimulate 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) activity and glucocorticoid production in the adipocytes, allowing increased leptin secretion [64,66-70]. However, high amounts of glucocorticoids also cause central insulin resistance, preventing the hypothalamus from being signaled by leptin to regulate appetite [64,66-70]. These metabolic pathways could explain the observed feed intake in HC rats.

The addition of *S. purpurascens* to diets made with local ingredients did not result in malnutrition for any of the individuals. All groups exhibited a constant and adequate growth similar to the control group, reflected in weight gain, Lee index, and BMI. Daily consumption of SPD and LPD diets with 13.44% and 7.19% grasshopper protein did not have negative nutritional effects under the measured parameters; however, we recommend more studies to evaluate the effects of consuming a diet with larger grasshopper protein contents (20.71%). The amount of food ingested is positively correlated to weight gain in rats fed with grasshopper-based diets and CT; however, higher protein intake does not ensure greater growth. Therefore, the diet added with 13.44% grasshopper protein yielded the best results because it did not cause adverse nutritional effects, and a lower feed intake allows the individuals to carry out their essential physiological functions appropriately. Finally, all grasshopper-based diets show better nutritional effects than the hypercaloric diet. Together with sugary drinks, the latter produced adverse effects that compromised the proper development of rodents, especially in early growth stages. These results support *S. purpurascens* as a good protein source that could be sustainably exploited in agroecosystems where it is considered a pest [25,74]. Moreover, the addition of this insect into the regular diet would allow access to a healthy and protein-rich food in places where cow protein is scarce [25,56], solving public health problems such as malnutrition. This study can be used as a basis for the development of future research where *S. purpurascens* protein is tested in a more complex and realistic context.

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## Supplementary Material

| Sample (100 g) | | |
|---|---|---|
| | **Boiled grasshoppers** | **Toasted grasshopper** |
| Moisture (g) | 4.63 | 7.76 |
| Ash (g) | 14.08 | 3.35 |
| Lipids (g) (ether extract) | 10.40 | 10.40 |
| Protein (g) | 52.40 | 59.75 |
| Dietary fiber (g) | 18.49 | 18.74 |
| Digestible carbohydrates** (g) | UD | UD |
| Energy value (kcal) | 303.20 | 332.60 |
| UD: Undetected | | |

**Table S1:** Proximal analysis of grasshopper samples from two different cooking methods. The data shown in the study belonged to toasted grasshoppers. The results are an average of three repetitions with a variation coefficient fewer than 5%. Analyses were realized conducted in the Facultad de Química, UNAM.

| **Ingredients [%]** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Group | | **Grasshopper powder** | **Nixtamalized corn flour, dry mass*** | **Amaranth, burst*** | **Peanut, toasted w/o salt*** | **Pearl barley*** | **Instant noodles**** |
| SPD | **Protein** | 13.44 | 4.32 | 1.53 | 3.13 | 0.66 | N/A |
| | Lipids | 2.34 | 1.75 | 0.69 | 6.57 | 0.08 | |
| | Fiber | 4.21 | 4.44 | 0.98 | 1.06 | 1.03 | |
| | Carbohydrates | 1.11 | 35.31 | 7.00 | 2.85 | 5.14 | |
| | Ash | 0.75 | 0.73 | 0.32 | 0.48 | 0.07 | |
| LPD | **Protein** | 7.19 | 4.82 | 1.16 | 3.99 | 0.98 | N/A |
| | Lipids | 1.25 | 1.95 | 0.52 | 8.38 | 0.11 | |
| | Fiber | 2.26 | 4.96 | 0.74 | 1.35 | 1.55 | |
| | Carbohydrates | 0.59 | 39.39 | 5.29 | 3.63 | 7.70 | |
| | Ash | 0.40 | 0.81 | 0.24 | 0.61 | 0.11 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| HPD | **Protein** | 20.71 | 4.07 | 1.11 | 2.44 | 0.36 | N/A |
| | Lipids | 3.60 | 1.65 | 0.50 | 5.13 | 0.04 | |
| | Fiber | 6.49 | 4.19 | 0.71 | 0.83 | 0.57 | |
| | Carbohydrates | 1.71 | 33.26 | 5.07 | 2.22 | 2.85 | |
| | Ash | 1.16 | 0.68 | 0.23 | 0.37 | 0.04 | |
| HC | **Protein** | N/A | 4.39 | 1.55 | 3.18 | 0.66 | 2.22 |
| | Lipids | | 1.77 | 0.70 | 6.66 | 0.08 | 3.71 |
| | Fiber | | 4.51 | 1.00 | 1.07 | 1.05 | N/D |
| | Carbohydrates | | 35.81 | 7.10 | 2.89 | 5.21 | 14.83 |
| | Ash | | 0.74 | 0.33 | 0.48 | 0.07 | N/D |

*Data obtained from INCAP (39); **Data obtained from PROFECO (40); N/A: Not applicable; N/D: No data

**Table S2:** Total nutritional content per ingredient in each treatment. Values in percentages for every 100gr of processed food.

| | Diets (100 g) | |
|---|---|---|
| Parameter | *Nutricubo* (CT diet) | Standard diet (SPD) |
| Protein (g) | 28.43 | 28.94 |
| Lipids (g) (ether extract) | 8.22 | 13.06 |
| Dietary fibre (g) | 26.53 | 38.29 |
| Total digestible carbohydrates (g) | 28.74 | 9.45 |
| Energy content (kcal) | ----- | 271.10 |
| Moisture content (g) | 8.24 | 8.21 |
| Ash (g) | 8.09 | 2.05 |

**Table S3:** Bromatological analyses by the Laboratorio de Química y Análisis de Alimentos del Departamento de Alimentos y Biotecnología, Facultad de Química, UNAM.