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The larvae of the insects *Oryctesgracilis* and *Rhyncophorusphoenicis*, an emerging source of nutrients.

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Abstract:
The objective of this study is to establish a comparison between the nutritional value of the larvae of Rhynchophorusphoenicis and Oryctesgracilis.
To do this, we used the standards established by the AOAC to determine the nutritional value of each larva, namely: the oven to determine the water content, the Soxhlet method to extract the fat, the Kjedahl method to determine the total protein content, the muffle furnace to know the ash rate then we deducted the carbohydrate content and finally we calculated the energy value of each species of insect studied. We thus obtained the following results: For Oryctesgracilis: Water content: 34.20%; Lipid content: 14.04%; Protein Content: 49.12%; Ash content: 1.72%; Carbohydrate content: 39.51%; Fiber content: 0.15%; Energy value: 307.02%.
For Rhyncophorusphoenicis: Water content: 34.76%; Lipid content: 49.93%; Protein Content: 11.12%; Ash content: 2.67%; Carbohydrate content: 27.79%; Fiber content: 0.05%; Energy value: 420.51%.
It appears from these results that the larvae of Oryctesgracilis are less rich in lipids than the larvae of Rhyncophorusphoenicis but richer in proteins than the latter. However, Rhyncophorusphoenicis larvae are richer in minerals than Oryctesgracilis larvae. Through these results, the larvae studied constitute a good source of nutrients, hence it is necessary to domesticate them.
Keywords: *Beetles, Nutritional value, Characterization, Loboko, Kibossi, Congo.*

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I. Introduction

The world population continues to grow and could reach close to 9.7 billion people in 2050 (United Nations, 2022). Feeding a growing global population with more demanding consumers and high malnutrition rates will necessarily lead to increased food production (Boer et *al.,* 2014). However, the contribution of conventional agriculture to environmental problems is very significant. Indeed, conventional livestock farming is one of the main causes of the most serious environmental problems, namely greenhouse gas emissions, land degradation, loss of biodiversity and water pollution (FAO, 2009).

It is therefore urgent to seek alternative sustainable and environmentally friendly solutions to meet the needs for proteins and other nutrients: Collecting and breeding insects can address this problem (FAO, 2021).

Insects have higher protein and fat levels than the plants on which they feed and also higher than those of conventionally farmed animals.

Larval forms generally tend to be richer in fat than adult forms, except for the locust which is fatter as an adult than as a nymph (De Marco *et al.,* 2015 ; Caparros*et al.,* 2016 ;Devic*et al.,* 2018; Kwiri*et al*, 2014;Paul *et al.*, 2017).

In South and Central Africa, communities collect lepidopteran insects (*Gonimbrasiabelina*, *Gynanisamai*, etc.) and beetle larvae. This activity constitutes a good source of income and food (Latham, 2016). In the Republic of Congo, the consumption of caterpillars and larvae around Brazzaville is estimated at 30 grams per day per person, however little investigation has been carried out on the nutritional potential of edible beetle species (Balinga, 2003; Lenga et *al.*, 2012).

It is with the aim of making a contribution to filling this gap that this study was carried out, with the general objective of determining the nutritional value of two beetles collected respectively in the Cuvette and in the Pool: *Oryctesgracilis* and *Rhyncophorusphoenicis*.
Oryctesgracilis is an insect of the *Scarabaeidae* family while *Rhyncophorusphoenicis* on the other hand is an insect of the *Dryophthoridae* family.

II. Material and methods

II.2. Material

II.2.1. Biological material

For the realization of this study, the biological material was constituted, larvae of *Oryctesgracilis*, collected in the villages Konda and Loboko of the district of Mossaka, located in the department of the basin, as well as the larvae of *Rhyncophorusphoenicis* coming from the village of Kibossi in the district of Goma Tsé-Tsé, located in the Pool department.

Larvae of *Oryctesgracilis* Larvae of *Rhyncophorusphoenicis*

II.2.2. Sample preparation

In the laboratory, the larvae of *Oryctesgracilis* and *Rhyncophorusphoenicis* were sorted and freed of all kinds of waste. They were then placed in an oven set at 70°C. The dry matter obtained for each sample was ground using a porcelain mortar. The ground material obtained was used to carry out the analyses.

II.2.3. Biochemical analyzes

The biochemical analyzes were carried out using reference methods recognized by the AOAC. (AOAC Standard 950.01, 1996):

II.2.3.1. Determination of water content

We used an oven to determine the water content. To do this, 50 g of larvae of each species were weighed in the petri dishes then placed in an oven set at 70°C for 72 hours until the weight stabilized. The weight of the sample being known, the water content was determined by the following formula:

$H\ (\%) = (m_1 - m_2)/(m_1 - m_0) \times 100$

With: %H: Humidity in percentage; m_0: mass of the empty petri dish; m_1: initial mass of the sample; m_2: final mass of the sample

II.2.3.2. Determination of lipid content

30g of ground larvae were weighed and then placed in a WHATMAN cartridge. The cartridge is then placed in a Soxhlet type extractor connected to a refrigerant. The Soxhlet plus refrigerant assembly is mounted on a previously weighed flask containing 200 ml of hexane then placed on a flask heater. The refrigerant is supplied continuously for approximately 3 hours with cold water in order to extract the fat from the larvae ground. At the end we obtain oil mixed with hexane. The lipid fraction is separated from the solvent using a rotary evaporator. After cooling, the flask containing the oil was weighed. The yield of extracted oil was calculated using the following formula:

$\%lipids = (M_1 - M_0)/M \times 100\%$

With: M_1: mass of the balloon containing the oil; M_0: mass of the empty balloon; M: mass of the sample used (g)

II.2.3.3. Determination of protein content

Protein determination was carried out by the Kjeldahl method. 0.5g of sample of crushed and deoiled larvae was introduced into a Matra, then a spatula tip of catalyst and 10ml of sulfuric acid were added with a few glass beads. The Matra was then placed in a mineralization ramp. The mineralization was carried out cold for 30 minutes then hot (420°C) for 2 hours. After cooling, 20ml of distilled water and 30ml of sodium hydroxide at 400g/L were added (until the solution turned brown).

The ammonia was distilled using a distiller in an excess of soda, then recovered in boric acid in the presence of a few drops of colored indicators until reaching a volume of 125 ml. The nitrogen titration was carried out using sulfuric acid at N/20, until the colored indicator changed from green to pink.
The protein content was determined by the following formula:
%N = (VH_2SO_4x0.07)/(Test sample)
With: %N: Nitrogen content; VH_2SO_4: volume of sulfuric acid; Protein rate = % N x 6.25 (which is the multiplication coefficient).

II.2.3.4. Determination of ash content

The determination of ash levels was made by mineralization of the samples. 2g of each ground sample was weighed and put into a crucible. Several 2g weighings were carried out. The crucibles were placed in a muffle furnace at a temperature of 550°C for 6 hours, until all organic matter contained in the sample was destroyed. After cooling, the crucibles containing the ashes were weighed. The mass of the empty crucible being known, the ash content of the different samples was determined by the following formula: %C= $(m_2-m_0)/(m_1-m_0)$ X100
With: m_0: mass in grams of the empty crucible; m_1: mass in grams of the crucible containing the test portion; m_2: mass in grams of the crucible containing the ashes:
%C= $(m_2-m_0)/(m_1-m_0)$ x100
With: m_0: mass in grams of the empty crucible; m_1: mass in grams of the crucible containing the test portion; m_2: mass in grams of the crucible containing the ashes.

II.2.3.5. Carbohydrate content

Carbohydrate content (G) was estimated by the difference method cited by Diallo Koffi et *al.* (2015). According to the method mentioned, it was calculated by subtracting from 100, the sum of humidity (H), fat (MG), proteins (P) and ash (C) contained in the sample: G= 100 - (H + MG + P +C)

II.2.3.6. Fiber content

The fibers were measured as follows:
In an empty flask, 1g of the delipidated ground material of the larvae was introduced, then 200 ml of sulfuric acid at 0.255N were added. The mixture was heated at reflux for 30 minutes and filtered under vacuum through muslin and adding a little distilled water. The residue was returned to the flask and treated with 200 ml of the 0.313 N sodium hydroxide solution. The mixture was heated again for 30 minutes and filtered. The residue was washed three times with distilled water and alcohol at 90°C. The wet residue was then transferred to a previously weighed dish (P_1), which was then dried in an oven for two hours at a temperature of 130°C. After cooling, the dry residue obtained is weighed (P_2) and finally calcined for 30 minutes at 600°C in a muffle furnace. After cooling the desiccator, a final weighing was carried out (P_3). The three weights being known, the percentage of fibers was determined by the formula below:
Crudefiber content = $((P_2-P_1)-(P_3-P_1)/m$ x100
With: P_1: mass of the crucible; P_2: mass of the crucible and the dried sample; P_3: mass of the crucible at the end of incineration

II.2.3.7. Energetic value

The total energy value was calculated using the method of Manzi (1999)cited by Diallo Koffi et *al.* (2015).
It is determined using the formula below:
VE (Kcal/100g) = (CHO x 4) + (CL x 9) + (CP x 4)
With CHO = % of carbohydrates, CL = % of lipids and CP = % of proteins.

III. Results and Discussion

III.1. Results

At the end of the work, we obtained the following results on the larvae studied

Table I: Results of the different analyzes

Parameters studied	Larvae	Average (%)
Lipids	*Oryctes gracilis*	14.04
	Rhyncophorus phoenicis	49.93
Proteins	*Oryctes gracilis*	49.12
	Rhyncophorus phoenicis	11.12
Fibers	*Oryctes gracilis*	0.15
	Rhyncophorus phoenicis	0.05
Energy Value (Kcal/100 g)	*Oryctes gracilis*	307.02
	Rhyncophorus phoenicis	420.51
Humidity	*Oryctes gracilis*	34.20
	Rhyncophorus phoenicis	34.76
Ashes	*Oryctes gracilis*	1.72
	Rhyncophorus phoenicis	2.67
Carbohydrates	*Oryctes gracilis*	39.51
	Rhyncophorus phoenicis	27.79

III.2. Discussion

III.2.1. Water content

The water content of the two species varies between 34.20% (*Oryctesgracilis*) and 34.76% (*Rhyncophorusphoenicis*). The water contents obtained in this study can reduce their shelf life at room temperature because these contents expose them to alterations in their organoleptic qualities (Megido et *al.,* 2014).

The water contents of *Oryctesgracilis* (34.20%) and *Rhyncophorusphoenicis* (34.76%) are very low compared to the contents obtained on edible caterpillars, specifically *Anaphe panda* (58.11%), *Imbrasiaepinethea* (80.87%) (Okangola, et *al*., 2016), *Imbrasiatruncata* (69.70%) (Fogang et *al.,* 2017) and the dipteran *Hermetiaillucens* (93.19%) (zsuzsanna et *al*., 2022), On the other hand, the moisture contents obtained in this study are higher than the results obtained in Congo on larvae of the same species of *Rhyncophorusphoenicis* collected and studied by Nzikou, et *al*. in 2010 (0%) and by Lenga et *al.* in 2012 (28.20%) and elsewhere on *Oryctesrhynoceros* larvae (16.73%) (Okaraonye et *al.,* 2009), and on *Tenebrio militor* larvae (16.2%) (Osasona in 2010). They are also higher than the water content of *Zenocerosvarigatus* larvae (11.85%) studied by certain authors (Emeka et *al*., in 2021).

III.2.2. Lipid content

The results obtained show a significant difference in terms of lipid content of the larvae of *Oryctesgracilis* and those of *Rhyncophorusphoenicis* which are respectively 14.04% and 49.93%. This difference is due to the fact that the intrinsic (the biochemical composition of the original substrate, the physiology of the larvae) and extrinsic factors are different. The chemical divergences that exist between their host substrates influence their lipid composition on a large scale. This difference is also observed in the metamorphosis and growth of the larvae of *Oryctesgracilis* which takes place slowly and over a long period of time compared to those of *Rhyncophorusphoenicis* which takes place over a short period of time because lipids constitute an energy source in insects essential for reproduction, larval growth, metamorphosis, metabolism and also carry out the detoxification process with respect to various toxicants (Sushchik et *al.,* 2013).

The lipid content of the larvae of *Oryctesgracilis* (14.04%) is lower than that of the larvae of *Oryctes rhinoceros* studied by Lenga et *al.,* in 2012 (25.85%). This difference may be due to the ages of the different larvae studied because the lipid contents vary with the different stages of development. The value of 14.04% is low compared to that of *Locustamigratorialocust*s studied by Mabossy-Mobouna et *al*., in 2020 (19.62%) and very low compared to that of *Macrotermessubhylinus* termites which is 46. 30% (Niaba-Koffi et *al.,* 2011). The difference in lipid contents between beetles can be explained by the fact that the lipid composition of the larvae depends on the physiological stage and their mobility; the less mobile the larvae are, the less they exploit the fat contained in the nutrient substrate. Knowing that the larvae used in this study were at larval stage III, their mobility was reduced due to weight, hence they are considered edible insect larvae less rich in lipids. For termites and locusts, the gap is large because they do not have the same diet and ecology and even the order of clarification is delayed (Balinga et *al.,* 2004).

However, the lipid content of *Oryctesgracilis* beetles is higher than that of *Bunaopsisaurantiaca* caterpillars (24.2%) studied by Muvundja et *al*. (2013), larger than that of *Imbrasiabelina* (10%), and the dipteran *Hermetiaillucens* which is 9.29% (Kwiri et *al.,* 2014; Emeka et *al.,* 2021, Zsuzsanna et *al.,* 2022) . This could be explained by the fact that *Oryctesgracilis* consume the tender part of the *Raphia* palm, richer in lipids and protein, whereas the caterpillars and the dipteran mentioned consume more of the leaves, which are rich in water and minerals but poor in lipids.

The lipid level of *Rhyncophorusphoenicis* larvae of 49.93% is lower than the results obtained by Agbemebia et *al.,* in 2020 (60.16%), Nzikou et *al.,* in 2010 (68.5%) and by Omotoso and Adedire in 2007 (56.88%) all working on the same species. The result of the present study is, however, very high compared to the results obtained by Idolo (2007) and who obtained a value of 25.72% and by Okounowo et *al.,* in 2017 who obtained a value of 15.36%. This difference in lipid contents with the same species of insect is due to the fact that the collection of samples of *Rhyncophorusphoenicis* was not made in the same place, nor on the same species or variety of the plant used as substrates; which could have an influence on the lipid composition of the larvae. The *Rhyncophorusphoenicis* larvae used in different works cannot have identical values on lipid content but are approximate because the physicochemical composition of these larvae depends on the varieties of oil palm trees to which they are parasitic (Agbemebia et *al.,* 2020).

III.2.3. Protein content

The protein contents obtained are 48.12% for the larvae of *Oryctesgracilis* and 11.2% for the larvae of *Rhyncophorusphoenicis*. These two types of larvae come from different palm trees. The protein level of the lavas of *Oryctesgracilis* (48.12%) is higher than the value obtained on the larvae of *Oryctes rhinoceros* (42.66%), studied by Lenga et *al*., in 2012. However the larvae of *Oryctesgracilis* used in this study lived in the trunk of the host plants and consumed plant debris certainly rich in proteins. Compared to caterpillars, this rate of 48.12% is a little low than that of the caterpillars of *Imbrasiabelina* which is 55.41% in proteins (Kwiri*et al.,* 2014) and close to that of the locust *Locustamigratoria* which is 48.20% (Next-food, 2020).

The protein level of *Rhyncophorusphoenicis* larvae of 11.12%, compared to studies carried out on the same species, allows the following conclusions to be drawn: it is lower than the levels obtained by other authors:Okounowo in 2017 (24.43%) and Nzikouet *al.*in 2010 (20%). However, the protein level obtained in this study is a little high than those obtained by Omotoso (10.5% in 2007 and 9.1% in 2017) and by Ekpo (8.38%) in 2010.

The comparison made with other families of insects shows that the protein level of the larvae of *Rhyncophorusphoenicis* is lower than those of the larvae of *Zonocerosvarigatus* (30.37%), termites *Macrotermessubhylinus* (38.2%) and *Achetadomesticus* (57.40%). (Koffi-Niaba, et *al,* 2011; Emeka et *al.,* 2021). *Oryctes* larvae could therefore constitute an essential complement of animal proteins in the human diet. According to Davidson et *al.* (1973) cited by Lenga et *al*. (2012), 100 g of these larvae can solve many problems related to the insufficient amount of daily lipids consumed in least developed countries and can substitute meat or fish.

III.2.4. Ash content

The analysis of the results obtained proves that the ash content of the larvae of *Oryctesgracilis* (1.72%) is low compared to that of the larvae of *Rhyncophorusphoenicis* (2.67%). The beetle larvae used in this study have significant ash contents.

The ash rate of *Oryctesgracilis* is very low compared to the values obtained on the larvae of *Oryctes rhinoceros* (15.25%) studied by Okaryonie et *al.* (2009) and larvae of *Oryctesmonoceros* (10.00%) studied by Emeka et *al*., in 2021. This rate of 1.72% is, however, close to that obtained by Agbemebia, in 2020 (1.24%) on the species *Oryctesmonoceros*. The comparison made with other families of insects shows that the content of 1.72% is higher than that of *Imbrasiatruncata* caterpillars (1.03%) and very low compared to that of *Anaphe panda* (26.1%) and also *Gonimbrasiabelina* (8.16%) studied by Kwiri et *al*. (2014).

For the case of *Rhyncophorusphoenicis*, the ash rate of 2.67% obtained in this study is slightly above 2.20%, the value obtained by Ekpo in 2010 on the same species. This content of 2.67% is higher than the value obtained by Okounowo (1.00%) in 2017; on the other hand, it is lower than the levels also obtained on this same species by Nzikou et *al*. in 2010 (6.3%). This rate obtained in this study is close to that of *Imbrasiapetiveri* consumed in the Democratic Republic of Congo, with a rate ash of 2.98% (Okangola et *al.,* 2016).

III.2.5. Carbohydrate contents

The carbohydrate contents of the two species of larvae studied are 39.51% for *Oryctesgracilis* and 27.79% for *Rhyncophorusphoenicis*. This divergence of the rates obtained appears normal in view of the results of the other parameters studied. The larvae of *Oryctesgracilis* from this study are richer in carbohydrates than the larvae of *Zonocerusvarigatus* (5.36%) studied by Emeka et *al*., in 2021. The larvae of

Rhyncophorusphoenicis are richer in carbohydrates than the caterpillars *Gonimbrasiabelina* (8.16%) studied by Kwiri et *al.,* in 2014 and the caterpillar *Bunaeopsisauranticia* (4.5%) studied by Muvundja et *al.,* in 2013 .

III.2.6. Fiber contents

The fiber rate results give 0.15% for the larvae of *Oryctesgracilis* and 0.05% for the larvae of *Rhyncophorusphoenicis*. The difference between these values can be explained by the fact that the larvae of *Oryctesgracilis* are equipped with chitin, exoskeleton and protective layers, thicker and more solid than those of the larvae of *Rhyncophorusphoenicis*. Consequently, in human food, the larvae are consumed with these elements which therefore constitute the fibers (Okaryonie et *al.,* 2007) The fiber rate of 0.15% of *Oryctesgracilis* is very low compared to those obtained on the larvae of *Oryctes rhinoceros* (8.70%) studied by Emeka et *al.,* in 2021 and of *Monoceros* (10.50%) studied by Idolo in 2011. This difference could be explained by the stages of development in which these larvae were found or by the stage of decomposition of the different substrates consumed by these larvae. For the case of *Rhyncophorusphoenicis*, a rate of 0.05% was determined. This rate of 0.05% is lower than that obtained by Okounowo in 2017 (2.27%). The two beetle species studied are not a good source of fiber. These differences between its two fiber contents may be due to the certainly different larval stages. Several species have fewer fibers, however the nymph and imago stages of insects are marked by a strong presence of fibers resulting from the appearance of sclerified organs (Dechambre, 2000).

III.2.7. Energetic value

We obtained the energy values of 307.02Kcal/100g for *Oryctesgracilis* and 420.51Kcal/100g for the larvae of *Rhyncophorusphoenicis*. The caloric value of *Oryctesgracilis* larvae is lower because of the low lipid content obtained in this study. On the other hand, the high rate of energy value of the larvae of *Rhyncophorusphoénicis* is the result of the abundant presence of lipids.

The energy value of *Oryctesgracilis* of 307.02Kcal/100g of fresh matter is higher than that of the edible ants *Myrmecocystusmelliger* whose rate is 116Kcal/100g and the locust *Cyrlacanthacristatarica* with a rate of 89Kcal/100g (Shantibala, 2014). The value of 307.02Kcal/100g of fresh matter is lower than those of the termites *Macrotermessubhylinus* which vary from 535 to 581Kcal and the locust *Locustamigratoria* which is 559Kcal (Kwiri et *al.,* 2014).

The *Rhyncophorusphoenicis* larvae studied have an energy value of 420.51Kcal/100g. This value is lower compared to that obtained by Abgemebia in 2020 (688.36 Kcal) on the same species. On the other hand, this value is close to that obtained by Eugene in 2005 (425Kcal) also on the same species and to that of *Bunaeopsisaurantiacia* caterpillars (433Kcal) studied by Muvundja et *al*., in 2013.

IV. Conclusion and perspectives

As part of the valorization of edible insects as sources of nutrients necessary for food security, the larvae of*Oryctesgracilis*and *Rhyncophorusphoenicis* which were the subject of this study are the beetle insects collected respectively in the basin and in the pool . Among the two species, the larvae of *Oryctesgracilis* have never been the subject of a nutritional study in Congo and are often confused with other species of the same genus. This study made it possible to achieve the set objective which was to compare the nutritional value of the larvae of *Oryctesgracilis*and *Rhyncophorusphoenicis* collected in Louboko and Kibossi in Congo Brazzaville.
In summary, the results obtained on the two species were:
- For larvae of *Oryctesgracilis*: Water content, 30.20%; Lipid content, 14.04%; Protein content, 49.12%; Ash content, 1.72%; Carbohydrate content, 39.51%; Fiber content, 0.15% and an energy value of 307.02 Kcal /100 g of fresh material;
- For *Rhyncophorusphoenicis* larvae: Water content, 34.76%; Lipid content, 49.93%; Protein content, 11.04%; Content of 2.67%; Carbohydrate content, 27.76%; Fiber content, 0.15% and an energy value of 420.51 Kcal/100 g of fresh material.
Rhyncophorusphoenicis larvae are considered an excellent source of lipids, while *Oryctesgracilis* larvae are richer in protein. This work, far from being finished, it is important to:
- measure the different vitamins and identify the minerals in the ashes of these larvae;
- separate the different proteins and establish the amino acid profiles of the different proteins;
- establish the fatty acid profiles of the extracted lipid fractions;
- address the aspects of breeding these insects.

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