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**Research article** 

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# Nutritional compositions of two edible insects: Oryctes rhinoceros larva and Zonocerus variegatus



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# ABSTRACT

Considerably large quotas of insect species worldwide are prospective sources of food with high nutrient value, which suggests their importance in human diets. This study investigates and compares the nutrient and antinutrient contents of *Oryctes rhinoceros* larva and *Zonocerus variegatus*. The nutrient and antinutrient compositions of both *Oryctes rhinoceros* larva (palm beetles) and *Zonocerus variegatus* (grasshopper) were determined following standard procedures. The proximate results revealed that *Oryctes rhinoceros* had higher amounts of crude protein ( $34.76 \pm 0.44\%$ ) and carbohydrate ( $10.37 \pm 1.73\%$ ) compared to those in *Zonocerus variegatus* (( $30.73 \pm 1.15\%$ ) and ( $5.36 \pm 2.15\%$ ) respectively), while crude lipid ( $20.00 \pm 0.00\%$ ) was higher in *Zonocerus variegatus* variegatus. Rich mineral components were also obtained in both insects. Potassium and sodium ( $1905.01 \pm 185.01$  mg/100g and  $1656.00 \pm 46.00$  mg/100g) were moderately high in *Zonocerus variegatus* compared to *Oryctes rhinoceros*. The anti-nutrient values of both insects fall within tolerable levels, and subsequently pose no threat to life, indicating that these insects are good sources of several macro and micronutrients. *Oryctes rhinoceros*, however, may likely serve as a better source of nutrients, considering its more valuable contents of macromolecules.

# 1. Introduction

The non-availability of food resources has been an issue for most developing countries due to difficulties in providing adequate food for their population, and thus lack of protein in the body may sometimes lead to protein-energy malnutrition (Sani et al., 2014). The need therefore arises to incorporate cheap and readily alternative food sources like insects, especially due to their high abundance in nature. Different species of insects which include; grasshopper, caterpillar, winged termites have served as food for humans since ancient time (Solomon et al., 2008).

*Oryctes rhinoceros* (Coleoptera: Scarabaeidae) popularly known as palm beetles; have their breeding sites in areas of immature oil-palm, mature oil-palm, dead standing palms, and heaps of empty fruit bunch (Anugerah et al., 2020). On the other hand, *Zonocerus variegatus* (Orthoptera: pyrgomorphidae), commonly known as grasshoppers are usually predominant in the rainy season and are found on savannah land where they are harvested for food (Babayi et al., 2018). They are very

abundant in nature but are seasonal, hence may not be available all through the year (Oibiokpa et al., 2017). Ifie and Emeruwa (2011) noted that these insects may be consumed raw, boiled, smoked, or fried. Studies have shown that some of these insects which are even regarded as pests such as termites, have high nutritional qualities and are very rich in different minerals (Olowu et al., 2012; Sani et al., 2014; Paiko et al., 2016; Douglas, 2018). Chitins of arthropods are primarily involved in activation of immune cells (Paoletti et al., 2007; Roos and Van Huis, 2017). Ademolu et al. (2010), also reported that adult *Z. variegatus* serves as a means of bio-control in some areas of Ondo State, Nigeria because of its good nutritional values.

Hazards derived from insects are possible contaminants obtained via the method of production, the insect species, lifecycle or processing methods (Van Huis et al., 2013; Mézes, 2018). Inadequate nutrition is a maor challenge facing developing nations; this have adverse effect on the physical and mental development of many children, thus exposing some to infectious diseases which may cause death (Offiah et al., 2019).

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Although there have been documentations in literature on the individual nutrient composition of these insects, nothing has been documented to best of the knowledge of the authors on the comparative study of the two insects so as to ascertain the nutrient molecules predominant in each of them for possible incorporation in diets, hence this preliminary research.

# 2. Materials

# 2.1. Sample collection

Live larvae of *O. Rhinoceros* weighing 500 g were collected from dead raphia palms in kankon, Badagry town of Lagos State, Nigeria, while *Z. variegatus* weighing 400 g was sourced from Abaji, Abuja. Both samples were sourced in the month of June and were immediately washed to remove dirt while viable samples were selected and processed before been taken to the laboratory of Entomology of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, for identification by Dr. Ikechukwu Godwin Ngwu.

# 2.2. Instruments

Some of the instruments used for the analyses in this study are: Spectrophotometer (GENESYS 180 model, BioTek, Winooski, U.S.A), Micropipette (EXII MULTI model, Nichipet, Chiyoda-ku, Japan), Desiccator (Model DWA205, DWK Life Sciences Limited, Staffordshire, England), Muffle furnace (Model M-525 SII, Hamilton Instrument, Cinnaminson, U.S.A), micro-Kjeldahl digestion flask (Model K1100F, Xian Yima Optoelec Xian, China), Markham's steam distillation apparatus (L485/3 model, Jindal Medical & Scientific Instruments, Delhi India), Beaker (Model 3451305, CORNING INC, Corning, U.S.A), Porcelain crucible (Eisco, Haryana, India), Weighing balance (Model CNA403, CGOLDENWALL, U.S.A), Refrigerator (Model WHD-113FSS1, Haier thermocool, Qingdao, China), Measuring Cylinder (Eisco, Haryana, India), Hot air oven (Model FML801, MOOSOO, Kent, U.S.A), Steam bath (Model 402, LABOTEC, South Africa).

#### 3. Methodology

# 3.1. Preparation of sample

The samples collected were dried in oven regulated at 40 °C for 1h (asides from those for moisture content) and blended using an electric blender. The blend samples were stored separately in air-tight containers until further use for analyses.

# 3.2. Determination of proximate composition

Proximate composition of the insects was analysed using the method of (AOAC) 2012. Protein determination was carried out using the micro-Kjedhal's method as described by Pearson (1976).

# 3.3. Moisture content

A known quantity of the samples (2.0 g) each were weighed and dried in the oven at 40 °C to a constant weight. The dishes and sample were cooled and re-weighed. The moisture content was then calculated from equation below (1).

Moisture (%) = 
$$\frac{\text{Loss in weight}}{\text{Weight of sample (g)}} x100$$
 (1)

# 3.4. Crude fibres

The defatted samples (1.0 g) (W<sub>1</sub>) each was transferred into 300 ml conical flask. A known volume, 150 ml, of pre-heated 0.128 M H<sub>2</sub>SO<sub>4</sub> was poured into the conical flask and covered with glass lid. This was heated and boiled for 30 min and then filtered. The residue was washed three times with hot water and returned to the beaker. Another volume of 150 ml of pre-heated 0.223 M KOH was added and boiled. Some drops of anti-foaming agent was added likewise and boiled slowly for 30 min and then filtered. The residue was dried at 130 °C for 1 h and weighed (W<sub>2</sub>). This was burn to ashes at 500 °C for 3 h, cooled and weighed (W<sub>3</sub>). The percentage (%) crude fibre was calculated as shown in Eq. (2).

Crude fibre 
$$(\%) = \frac{W2 - W3}{W1} \times 100$$
 (2)

#### 3.5. Total ash

The general procedure involves weighing out 5 g of sample into porcelain dish which has previously been ignited and cooled before weighing. Then the dish and content are ignited first gently over a low flame until charred and then in a muffle furnace at 550–600  $^{\circ}$ C, cooled in a desiccator and re-weighed. The total ash content was calculated from Eq. (3) below.

Ash (%) = 
$$\frac{\text{Weight of ash}}{\text{Weight of sample}} x100$$
 (3)

# 3.6. Crude fats

A washed, dried and cooled quick-fit flask was weighed. The samples (2 g) were weighed into extraction thimble and placed into the quick-fit soxhlet apparatus with a solvent flask containing 250 ml of diethyl ether connected to a condenser. The set up was heated for 16 h for complete extraction. The extract was evaporated at 70 °C to remove any remaining solvent present. The apparatus was re-weighed and percentage crude fat calculated using Eq. (4) below.

Crude fat (%) = 
$$\frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} \times 100$$
 (4)

# 3.7. Crude protein

Two grams of each of the samples was mixed with 10 ml of concentrated  $H_2SO_4$  in a heating tube. One tablet of selenium catalyst was introduced into the tube and the mixture was heated inside a fume cupboard. The digest was transferred into distilled water. A known volume, 10 ml portion of the digest mixed with equal volume of 50% NaOH solution was poured into a kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 2% boric acid solution containing 3 drops of methyl red indicator. A total of 50 ml distillate was collected and titrated as well. At the end point, the colour turned to wine colour, indicating that all the nitrogen had been trapped as ammonium chloride. The sample was duplicated and the average value taken.

The crude protein was determined by multiplying nitrogen by a constant factor of 5.6 for *Oryctes rhinoceros* larva and 4.5 for *Zonocerus variegates* as described by Mishyna et al. (2019). See Eq. (5) below.

This is given as percentage Nitrogen = 
$$\frac{(100 \text{ x N x } 14 \text{ x VF})T}{100 \text{ x Va}}$$
(5)

where

N= Normality of the titrate (0.1N) VF = Total volume of the digest = 100 ml T = Titre Value Va = Aliquot Volume distilled

#### 3.8. Carbohydrate

**Proceedure:** This is also known as Nitrogen Free Extract (NFE). NFE was determined using Eq. (6).

% NFE = 
$$100 - (\% \text{ ash} + \% \text{ crude fat} + \% \text{ crude fibre} + \text{crude protein})$$
 (6)

# 3.9. Determination of minerals

The mineral content of the insect was determined according to methods of AOAC (2000). The dried insects were collected and ground. One gram of each sample was weighed in a crucible and placed in a muffle furnace, where they were ignited for 3h at a temperature of 500 °C. The samples were cooled, and 5 ml of 1N HNO<sub>3</sub> solution was added, after which the samples were evaporated to dryness using a steam bath. The samples from the furnace were returned and heated at 400 °C for 10–15 min until a greyish colour was obtained. The samples were cooled and 1N HCl was added. The resulting solutions were filtered into a 50 ml volumetric flask. The crucible and filter paper were washed with an additional 10 ml portion of 0.1N HCl, and the filtrates were analysed for Selenium, Iron, Calcium, Sodium, Phosphorus, Magnesium, and Potassium, using the trimetric method.

#### 3.10. Determination of vitamins

Vitamins were determined using the methods outlined by AOAC (2012).

#### 3.11. Vitamin A concentration

A quantity, 1 g of the samples was weighed accurately into 100 ml flask fitted with reflux condenser. Then 10 ml absolute alcohol and 20 ml alcoholic sulphuric acid were added. The condenser and flask were wrapped with aluminium foil. They were then refluxed for 45 min and cooled. Subsequently, 5 ml of water was poured into each flask and relocated to a separator funnel. Non-saponified matter was extracted with 30 ml of diethyl ether. The combined ether extract was then washed free from acid and dried over anhydrous sodium sulphate. The extract was evaporated at low temperature while protecting them from sunlight, final traces of solvent being removed in a stream of nitrogen and then residues dissolved immediately in 10 ml isopropanol. The extinction of the freshly prepared extract in isopropanol was read at 325 nm against a solvent blank (T1). The cuvettes were then removed, exposed to UV light until the extinction no longer fell with time and then the absorbance recorded (T2). The standard vitamin A solution was treated the same way (ST1 – ST2) using Eq. (7).

Vitamin A(mg/100g) = 
$$\frac{T1 - T2}{ST1 - ST2}$$
 x 1 x Dilution factor (7)

#### 3.12. Vitamin E concentration

One gram of the ground sample was measured into 100 ml flask and 10 ml of absolute alcohol (ethanol) was added. Twenty millilitres of 1 M alcoholic sulphuric acid and 18 ml of concentrated  $H_2SO_4$  in 1 L of ethanol were added and refluxed for 45 min and cooled in a reflux condenser. A volume of 10 ml of the clear solution was pipette into a test tube and heated in a water bath at 90 °C for 30 min and allowed to cool. A

standard and a blank were prepared and the absorbance read at 470 nm. Vitamin E was calculated using Eq. (8).

$$Vitamin E (mg/100g) = Absorbance x Dilution factor$$
(8)

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# 3.13. Vitamin C concentration

Ascorbic acid was determined by titration with diphenol indo 2, 6 – dichlorophenol (DPIP). The powdered sample (0.2 g) was mixed with 4 ml of a buffer solution made up of 1 g/l oxalic acid and 4 g/l sodium acetate anhydrous. This was titrated against a solution containing 295 mg/l DPIP and 100 mg/l sodium bicarbonate. Vitamin C content of the samples was calculated using Eq. (9).

Vitamin C (mg/100g) = 
$$\frac{MV \times 100 \times 100}{10B}$$
 (9)

M = mass of ascorbic acid tritrimetric equivalent to 0.001 M DPIP solution (mg)

100 is the dilution ratio of the sample taken, the second 100 is the scaling factor for conversion to per 100 g of raw material, 10 is the titrate volume

V = titrant volume (0.00 1 M DPIP solution) ml

B = weight of the sample extract used

# 3.14. Vitamin $B_1$ (Thiamine)

A measured volume, 2 ml of the standard and sample was put in marked test tubes. In each test tube, 5 ml NH<sub>4</sub>OH (0.1M) and 0.5 ml 4-Amino phenol solution were added and mixed well, then kept for 5 min followed by the addition of 10 ml chloroform to give two layers. The absorbance of the chloroform layer was recorded at 430 nm against blank. Vitamin B<sub>1</sub> content of the samples was calculated using Eq. (10).

Vitamin 
$$B_1 = \frac{\text{change in absorbance of sample}}{\text{change in absorbance of standard}} x \text{ Conc. of Std}$$
 (10)

# 3.15. Vitamin B<sub>2</sub> (Riboflavin) concentration

A known volume, 2 ml of the standard and sample solution was put into calibrated test tubes. In each test tube, 2 ml hydrochloric acid (1 M), 2 ml glacial acetic acid, 2 ml hydrogen peroxide, 2 ml potassium permanganate (15% w/v) and 2 ml phosphate buffer (pH 6.8) were added and mixed well and absorbance recorded at 444 nm against blank. Vitamin B<sub>2</sub> content of the samples was calculated using Eq. (11).

Vitamin 
$$B_2 = \frac{\text{change in absorbance of sample}}{\text{change in absorbance of standard}} x \text{ Conc. of Std}$$
 (11)

# 3.16. Vitamin $B_6$

A known quantity of each sample was weighed separately into a 100 ml conical flask and extracted with 10 ml 0.1M HCL with continuous agitation for 10 min. The sample was filtered through a Whatman No.1 filter paper. The filtrate was then made up to 10 ml with distilled water. Accurately measured 5 ml of the slightly acidic filtrate was treated with 1 ml 0.40% ferric chloride. The Optical Density of the resultant brown solution was measured in a spectrophotometer at 450 nm. The absorbance obtained from the sample extract was converted to pyridoxine concentration by means of calibration curve generated using different standard concentrations.

# 3.17. Vitamin $B_{12}$ (Cyanocobalamin) concentration

Weight, accurately equivalent to 1 mcg of vitamin  $B_{12}$  of sample was taken into 25 ml volumetric flask and dissolved with 10 ml of water. A known weight, 1.25 g of dibasic sodium phosphate, 1.1 g of anhydrous citric acid and 1.0 g of sodium metabisulphate were added. The volume was made up to the mark with water. The solution was autoclaved at 121 °C for 10 min. Filtered and absorbance recorded at 530 nm against sample blank as well as standard. Vitamin  $B_{12}$  content of the samples was calculated using Eq. (12).

Vitamin 
$$B_{12} = \frac{\text{change in absorbance of sample}}{\text{change in absorbance of standard}} x \text{ Conc. of Std}$$
 (12)

#### 3.18. Vitamin K

Five grams of the sample was weighed into 200 ml flask afterward, 5 g of menadione and 50 ml of (methanol) were added and mixed gently for 10 min and allowed to stand for 5 min. The mixture was diluted with 5 ml aliquot and 5 ml methanol, mixed and centrifuged with different menadione levels. The reaction was read on spectrophotometer at 575 nm wavelength using Vitamin K standards. Vitamin K content of the samples was calculated using Eq. (13).

Vitamin K = 
$$\frac{\text{change in absorbance of sample}}{\text{change in absorbance of standard}} x$$
 Conc. of Std (13)

#### 3.19. Vitamin D

One gram of the sample was weighed into a flat bottom flask. This was followed by the addition of 1g pyropanol and 25 ml ethanolic potassium hydroxide solution (60 ml ethanol, 30 ml 50% potassium hydroxide). This was extracted thrice with petroleum ether followed by washing with water. The sample was then filtered and then evaporated to dryness in the water bath. Afterwards, 1 ml 11N HCL and 1 ml trichloromethane were added to the dried extract. The volume of the mixture was made up to 7 ml with acetone and then read at 450 nm in the spectrophotometer. The absorbance obtained from the sample extract was converted to cholecalciferol concentration by means of a calibration curve generated using different concentrations of vitamin D.

#### 3.20. Determination of anti-nutrients

# 3.20.1. Oxalate

Oxalate was determined by the method reported by Munro (2000). One gram of the sample was placed in a 250 ml volumetric flask suspended in 190 ml of distilled water. A known volume, 10 ml of 6M HCl solution was added to the sample and the suspension digested at 100  $^\circ$ C for 1h. The sample was then cooled and made up to 250 ml mark of the flask. This was filtered after which a duplicate portion of 125 ml of the filtrate was measured into a beaker, and four drops of methyl red indicator were added, followed by the addition of concentrated NH4OH solution (drop wise) until the solution changed from pink to yellow colour. Each portion was then heated to 90 °C, cooled, and filtered to remove the precipitate containing ferrous ion. Each of the filtrates was again heated to 90 °C and 10 ml of 5% CaCl<sub>2</sub> solution was added to each of the samples whilst stirring consistently. After cooling, the samples were left overnight. The solutions were then centrifuged at 2500 rpm for 5 min. The supernatants were decanted and the precipitates completely dissolved in 10 ml 20%  $H_2SO_4$ . The total filtrate resulting from the digestion of 1g of the sample was made up of 200 ml. Aliquots of 125 ml of the filtrate were heated until near boiling and then titrated against 0.05M standardized KMnO<sub>4</sub> solution to a pink colour which persisted for 30 s. The oxalate content of the sample was calculated.

# 3.20.2. Phytate

Phytate was determined through phytic acid determination as described by Lucas and Markaka (1975). This entails weighing 1 g of the sample into a 250 ml conical flask. Then, 100 ml of 2% concentrated HCl was used to soak the sample in the conical flask for 3 h and then filtered through a double layer filter paper. A known volume, 50 ml of the sample filtrate was placed in a 250 ml beaker and 107 ml of distilled water added to ensure proper acidity. Moments later, 10 ml of 0.3% ammonium thiocyanate solution was added as an indicator to each sample solution and titrated with standard iron chloride solution which contained 0.00195 g iron/ml and the endpoint was signified by a brownish-yellow colouration that persisted for 5 min. The percentage of phytic acid was then calculated.

# 3.20.3. Hydrogen cyanide

The method of Onwuka (2005) was adopted in Cyanogenic glycoside determination. One gram of the sample was weighed, added to 50 ml distilled water in a conical flask, and allowed to stand overnight. To 1 ml of the sample filtrate in a corked test tube, 4 ml of alkaline picrate was added and incubated in a water bath for 5 min. The absorbance of the samples was taken at 490 nm, with that of a blank containing 1 ml distilled water and 4 ml alkaline picrate solution before the preparation of the cyanide standard curve. A colour change from yellow to reddish-brown after incubation for 5 min indicates the presence of hydrogen cyanide and was calculated from the standard curve.

# 3.20.4. Trypsin inhibitor

Trypsin inhibitor was determined by the method reported by Prokopet and Unlenbruck (2002). One gram of the sample was dispersed into 50 ml of 0.5M NaCl solution. The mixture was stirred for 30 min at room temperature and centrifuged at 1500 rpm for 5 min. The supernatant was filtered, and the filtrate was used for the assay. To the substrate of the sample, 2 ml of the standard trypsin solution was added. The absorbance of the mixture was taken at 410 nm using 10 ml of the same substrate as blank.

#### 3.20.5. Hemaglutinin

The method of AOAC (2000) was used. A known weight, 1g of the sample was added to 20 ml of 0.9% NaCl and suspension was shaken vigorously for 1 min. The supernatant was left to stand for 1h, the sample was then centrifuged at 2000 rpm for 10 min and the suspension filtered. The supernatant was collected and used as a crude agglutinating extract.

# 3.21. Statistical analysis

Statistical package for service solutions (SPSS) version 23 was used to analyse the data and presented as mean  $\pm$  standard deviation. Duncan multiple test range was used to separate and compare the means. Differences were considered significant at p < 0.05.

# 4. Results and discussion

Table 1 shows the proximate contents of *O. rhinoceros* larva and *Z. variegatus*. The moisture content of *O. rhinoceros* ( $26.17 \pm 2.06\%$ ) was significantly higher compared to that of *Zonocerus variegatus* ( $11.85 \pm 0.62\%$ ). Our findings contradicted the findings documented by Banjo et al. (2006) for *Cirina forda* (4.40%) and *Oryctes boas* (1.91%). The high moisture content of food indicates a short shelf life, as it determines the quality and susceptibility of food to microbial spoilage (Frazier and Westoff, 2003). The results of moisture content obtained in this study indicates that *O. rhinoceros* is most likely to be susceptible to spoilage than *Z. variegatus*. The ash content for *O. rhinoceros* ( $8.02 \pm 0.53\%$ ) as documented by Offiah et al. (2019), but lower than the value for *O. rhinoceros* (12.70%) as documented by Onyeike et al. (2005). The ash content obtained indicates the presence of mineral compounds (Alinnor

Table 1. Proximate Constituents of Oryctes rhinoceros larv	va and Zonocerous variegatus
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Proximate(%)	Oryctes rhinoceros	Zonocerus variegatus
Moisture	$26.17\pm2.061^a$	$11.85 \pm 0.622^{b}$
Ash	$10.00\pm0.012^{\rm a}$	$10.00\pm0.002^a$
Crude protein	$34.76\pm0.442^{\rm a}$	$30.73\pm1.151^a$
Crude lipid	$10.00\pm0.013^{\rm a}$	$20.00\pm0.002^{b}$
Crude fibre	$8.70\pm0.701^a$	$22.07\pm0.382^{b}$
Carbohydrate	$10.37 \pm 1.732^{\mathrm{a}}$	$5.36\pm2.151^{\rm b}$

Each value represents the mean  $\pm$  SD (n = 3). Values in the same row having different superscripts letters differ significantly (p < 0.05).

and Akalezi, 2010), this is a pointer that O. rhinoceros and Z. variegatus are rich in minerals. O. rhinoceros revealed a higher protein content (34.76  $\pm$ 0.44%) compared to Z. variegatus ( $30.73 \pm 1.15\%$ ) in this study, although lower than the values noted by Aiyesanmi and Adedire (2015) in the larval and adult stages of Z. variegatus (50.39  $\pm$  2.01%) ~ (53.10  $\pm$  0.56). Udoh et al. (2007) also reported higher protein values in Pachymelania bryonensis (55.00%) and Thais cattifera (56.44%). An increase in crude protein signals enzymes and antibody activation (Olowu et al., 2012). The high protein content of the insects in this study indicates that they may invariably contribute to the daily protein requirements of humans (Ifie and Emeruwa, 2011). Insects are potential sources of protein which solves global protein deficiencies (Van Huis, 2013; Van Huis et al., 2013; Montowska et al., 2019). However, the result obtained from the study reveals that O. rhinoceros would likely serve as a better protein source than Z. variegatus. The lipid content of O. rhinoceros ( $10.00 \pm 0.01\%$ ) was lower than the value obtained for Z. variegatus (20.00  $\pm$  0.00%) in this study. These results, however, differed from the value (17.30%) reported earlier in O. rhinoceros (Onveike et al., 2005), and Z. variegatus (17.65  $\pm$  $3.24\%) \sim (22.93 \pm 3.37\%)$  by Aivesanmi and Adedire (2015). Lipids are vital in the metabolism of cells and transport fat-soluble vitamins (Omotoso, 2006). Edible insects are rich in lipids and other compounds required by humans (Jantzen Da Silva et al., 2020). The crude fibre compositions of O. rhinoceros (8.70  $\pm$  0.70%) was much less than the value obtained for Z. variegatus (22.07  $\pm$  0.38%) in this study, and the value reported for Polyrhachis vicina (13.2%) (Shen et al., 2006), although higher than the fibre content in Z. variegatus (12.4  $\pm$  1.5%) (Olushola et al., 2003). The high fibre content obtained in this study for Z. variegatus may be attributed to higher chitin content, compared to that in O. rhinoceros. Fibre in diets aid in fat reduction and proper digestion of food so as prevent diverticulitis (Oduor et al., 2008). The Carbohydrate content of (10.37  $\pm$  1.73%) in O. rhinoceros was twice the value reported for Z. variegatus (5.36  $\pm$  2.15%) in this study. Omotoso (2018) reported a lower value of 5.44  $\pm$  0.01% for O. rhinoceros, while the value reported by Ademolu et al. (2010) for Z. variegatus (10.02  $\pm$  0.02%) compares favourably with the value reported for O. rhinoceros (10.37  $\pm$  1.73%) in this study. Carbohydrates are most valuable among other food components (Offiah et al., 2019).

Edible insects have high amount of micronutrients (Zielińska et al., 2015). The mineral contents of *O. rhinoceros* larva and *Z. variegatus* are

shown in Table 2. Potassium was predominant in Z. variegatus (1905.01  $\pm$  185.01 mg/100g) compared to O. rhinoceros (1070.00  $\pm$  260.00 mg/100g). These values are higher than those documented for Cirina forda and Brachytrupes membranaceus (64.02 mg/100g and 72.50  $\pm$  0.50 mg/100g) respectively (Omotoso, 2006; Paiko et al., 2013). Potassium reduces high inflow of blood in the vein and prevents cardiovascular related diseases (Paiko et al., 2013). The sodium content obtained for O. rhinoceros (931.50  $\pm$  11.50 mg/100g) was lower compared to that of Z. variegatus (1656.00  $\pm$  46.00 mg/100g) in this study, however, higher than the values (47.08  $\pm$  0.02 mg/100g, 3.06  $\pm$  0.00 mg/100g) reported for O. rhinoceros and Z. variegatus respectively (Ademolu et al., 2010; Omotoso, 2018). Sodium, whose Recommended Dietary Allowance is 1500 mg for adults, assists in the maintenance of proper acid-balance and osmotic pressure regulation (Paiko et al., 2013). O. rhinoceros had a higher concentration of calcium compared to Z. variegatus (368.00  $\pm$ 16.00 mg/100g; 328.00  $\pm$  8.00 mg/100g) respectively in this study, although higher than values reported for Z. variegatus (39.58  $\pm$  0.03 mg/100g) and *M. nigerensis* (0.21  $\pm$  0.01 mg/100g) (Paiko et al., 2016; Oibiokpa et al., 2017). Calcium enhances formation of bones and teeth in children and adults. It plays key roles in nerve conduction, blood clotting, muscle contraction, and membrane permeability (Babayi et al., 2018). Edible insects are comparably higher in iron and calcium than livestock (Sirimungkararat et al., 2010). This implies that the consummation of these insects will promote the general body functions. Magnesium was relatively higher in O. rhinoceros compared to Z. variegatus in this study, and were higher as compared to the independent reports from Oibiokpa et al. (2017) on Gryllus assimils (8.92  $\pm$  0.03 mg/100g) and Babayi et al. (2018) on sundried Z. variegatus (385.00  $\pm$  2.82 mg/100g). Magnesium modulates numerous biochemical and physiological processes in the body and is required for all biosynthetic processes (Omotoso, 2015). O. rhinoceros had lower phosphorus concentration of 38.85  $\pm$  1.50 mg/100g compared to Z. variegatus (181.77  $\pm$  0.53 mg/100g). Phosphorus modulates the activities of enzymes and aids in lipids, proteins, nucleic acids, and carbohydrate metabolism (Omotoso, 2015). The Iron concentration of O. rhinoceros was less compared to the value of Z. variegatus (1.20  $\pm$  0.16 mg/100g; 2.32  $\pm$  0.06 mg/100g respectively), and likewise lower than the value reported by Okaraonye and Ikewuchi (2009) for the same insect (4.50 mg/100g). Iron is an antioxidant which prevents cardiomyopathy and growth retardation (Buss et al., 2003) and

Table 2. Mineral contents of Oryctes rhinoceros larva and Zonocerus variegatus.			
Minerals (mg/100g)	Oryctes rhinoceros	Zonocerus variegatus	
Calcium	$368.00 \pm 16.00^a$	$328.00 \pm 8.00^{b}$	
Magnesium	$145.20\pm8.00^{a}$	$123.60 \pm 3.60^{b}$	
Phosphorus	$38.85\pm1.50^a$	$181.77 \pm 0.53^{b}$	
Potassium	$1070.00\pm 260.00^{a}$	$1905.01 \pm 185.01^{b}$	
Sodium	$931.50 \pm 11.50^{a}$	$1656.00 \pm 46.00^{b}$	
Iron	$1.20\pm0.16^a$	$2.32\pm0.06^{b}$	
Zinc	$0.65\pm0.08^a$	$0.36\pm0.03^a$	
Selenium		$1.67\pm0.04$	

Each value represents the mean  $\pm$  SD (n = 3). Values in the same row having different superscripts letters differ significantly (p < 0.05).

Table 3. Vitamin Contents of Oryctes rhinoceros larva and Zonocerous variegatus.

Vitamins	Oryctes rhinoceros	Zonocerus variegatus
Vitamin A (mg/100g)	$0.17\pm0.01^a$	$0.81\pm0.01^a$
Vitamin B <sub>1</sub> (mg/100g)	$5.90\pm0.24^a$	$\textbf{45.44} \pm \textbf{1.79}^{b}$
Vitamin B <sub>2</sub> (mg/100g)	$27.19 \pm 0.76^{a}$	$11.12\pm0.30^{b}$
Vitamin B <sub>6</sub> (mg/100g)	$0.80\pm0.05^a$	$4.36\pm0.15^{b}$
Vitamin B <sub>12</sub> (mg/100g)	$70.43\pm4.12^a$	$\textbf{276.12} \pm \textbf{21.64}^{b}$
Vitamin C (mg/100g)	$4.13\pm0.01^a$	$4.82\pm0.69^a$
Vitamin D (mg/100g)	$0.51\pm0.01^a$	$0.59\pm0.01^a$
Vitamin E (mg/100g)	$24.09\pm1.49^a$	$21.37\pm2.19^b$
Vitamin K (µg/100g)	$7.43 \pm 0.57^{a}$	$0.02\pm0.01^{b}$

Each value represents the mean  $\pm$  SD (n = 3). Values in the same row having different superscripts letters differ significantly (p < 0.05).

Table 4. Anti-nutrient Contents of C	Dryctes rhinoceros l	arva and Zonocerous	variegatus
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Anti-nutrient (mg/100g)	Oryctes rhinoceros	Zonocerus variegatus
Oxalate	$0.230 \pm 0.030^{a}$	$0.86\pm0.004^b$
Trypsin inhibitor	$0.002 \pm 0.001^a$	$3.55\pm0.060^b$
Phytate	$1.060 \pm 0.080^a$	$1.57\pm0.080^a$
Hemagglutinin	$0.002 \pm 0.001^a$	$2.18 \pm 0.260^{b}$
Hydrogen cyanide	•	$5.21\pm0.110$

Each value represents the mean  $\pm$  SD (n = 3). Values in the same row having different superscripts letters differ significantly (p < 0.05).

anaemia (Paiko et al., 2013). Zinc was relatively higher in *Z. variegatus* compared to *O. rhinoceros* ( $0.65 \pm 0.08 \text{ mg}/100\text{g}$ ;  $0.36 \pm 0.03 \text{ mg}/100\text{g}$ ) respectively. However, Ifie and Emeruwa (2011) reported a higher concentration ( $7.00 \pm 0.16 \text{ mg}/100\text{g}$ ) in the larva of *O. rhinoceros*. Zinc deficiency causes diarrhoea, delayed sexual maturation, sexual dysfunctions, and skin lesions (Ryan-Harshman and Aldoori, 2005). Generally, the wide variation in mineral concentrations could be attributed to the sampling location and the feeding habits of these insects (Babayi et al., 2018). Edible insects and food formulation with insects may boost immune system (Zielińska et al., 2015; Mishyna et al., 2019; Montowska et al., 2019).

The results of mineral analysis obtained in this study have shown that though these insects are rich sources of essential minerals, however, *Z. variegatus* contains better source of these minerals. This could be attributed to the wide range of food sources available for their ingestion, especially in their adult stage.

In vitamin compositions (Table 3), Vitamin A ( $0.17 \pm 0.01 \text{ mg}/100\text{g}$ ) in O. rhinoceros was less compared to Z. variegatus (0.81  $\pm$  0.01 mg/ 100g), but higher than the value for crickets (0.09 mg/100g), as reported by Oonincx (2010). Vitamin A has been reported in different species of insects' compound eyes and is rarely detected in some insects (Finke, 2002; Seki et al., 1998; Oyarzun et al., 1996). Vitamins B<sub>12</sub>, B<sub>1</sub>, and B<sub>6</sub> in Z. variegatus (276.12  $\pm$  21.64 mg/100g, 45.44  $\pm$  1.79 mg/100g and 4.36  $\pm$  0.15 mg/100g) respectively were all higher compared to the values for O. rhinoceros (70.43  $\pm$  4.12 mg/100g, 45.44  $\pm$  1.79 mg/100g and 4.36  $\pm$ 0.15 mg/100g respectively) in this study. These results varied differently from reports by Finke (2015): B<sub>12</sub>, B<sub>1</sub>, and B<sub>6</sub> (193.0 mg/100g, 2.0 mg/100g and 2.13 mg/100g) respectively for crickets, and 1.3 mg/100g, 1.1 mg/100g, 6.90 mg/100g respectively for mealworm. B-vitamins act as co-enzymes in several enzymes catalyses in the body (Alamu et al., 2013). Vitamin C in this study was slightly higher in Z. variegatus (4.82  $\pm$ 0.69 mg/100g) compared to O. rhinoceros (4.13  $\pm$  0.01 mg/100g), but less than the figure documented by Ademolu et al. (2010) for Z. variegatus (11.7  $\pm$  0.20 mg/100g). Vitamin C stabilizes blood vessels flow in the arteries, and plays a great role in mopping up reactive oxygen species (Alamu et al., 2013). The Vitamin D constituent of Z. variegatus (0.59  $\pm$ 0.01 mg/100g) was slightly higher compared to that of O. rhinoceros (0.51  $\pm$  0.01 mg/100g) as obtained in this study. These values were also

higher than reports for superworms; 0.01 mg/100g and crickets; 0.02 mg/100g (Finke, 2015; Oonincx, 2010). Vitamin D absorbs calcium and promotes bone growth (Ross et al., 2010). The vitamin E content in Z. variegatus (21.37  $\pm$  2.19 mg/100g) was lower when compared to that of O. rhinoceros (24.09  $\pm$  1.49 mg/100g), and the value for Cirinaforda  $(363 \pm 20 \text{ mg}/100\text{g})$  as reported by Oibiokpa et al. (2017). Vitamin K in Z. variegatus (0.02  $\pm$  0.01  $\mu$ g/100g) was lower compared to that of O. *rhinoceros* (7.43  $\pm$  0.57  $\mu$ g/100g). Finke (2015) reported higher value (78.4 mg/100g) for crickets. Vitamin E acts as antioxidants while Vitamin K maintains healthy bones and tissue formations (Bellows and Moore, 2012). The incorporation of these insects in diets would likely serve as an alternative source of vitamins needed to maintain several metabolic processes. Vitamins are supplied by food consumption, as they act as antioxidants and improve vision (Alamu et al., 2013). However, the result obtained from this study reveals that Z. variegatus may act as a better source of vitamins to livestock and likewise humans.

Anti-nutritional factors present in some insects may be toxic which discourages its direct use in some food (Agbede and Aletor, 2005; Offiah et al., 2019). The anti-nutrient compositions in this study (Table 4) revealed an oxalate content of 0.230  $\pm$  0.030 mg/100g in O. rhinoceros and higher content in Z. variegatus (0.86  $\pm$  0.004 mg/100g). Sani et al. (2014) reported a higher oxalate content in Z. variegatus (11.25  $\pm$  5.20 mg/100g), but Offiah et al. (2019) revealed a lower content in O. rhinoceros (0.01  $\pm$  0.05 mg/100g). The trypsin inhibitor content in O. rhinoceros (0.002  $\pm$  0.001 mg/100g) was lower than that of Z. variegatus (3.55  $\pm$  0.060mg/100g) as revealed in this study. Phytate obtained in this study was also lower in O. rhinoceros (1.060  $\pm$  0.080 mg/100g) compared to Z. variegatus (1.57  $\pm$  0.080 mg/100g), but was reported by Omotoso (2018) to be higher in the same insect. Phytates reduces the bioavailability of mineral elements (Oibiokpa et al., 2017). Hemagglutinin and hydrogen cyanide was present in small amounts in Z. variegatus but negligible in O. rhinoceros. Hydrogen cyanide inhibits cytochrome oxidase and interferes with the aerobic respiratory system (Oibiokpa et al., 2017). Generally, anti-nutrients reduce the assimilation of the essential nutrients by the body required for metabolic processes. However, the minute amount of the selected anti-nutrients analysed in these insects was generally low and would not cause any health concerns. These results indicate that O. rhinoceros and Z. variegatus are both low in

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# 5. Conclusion

The results obtained from this study revealed that *O. rhinoceros* and *Z. variegatus* are rich sources of micro and macro food nutrients, and therefore should be encouraged for use as food. However, *O. rhinoceros* would likely serve as a better source of food, owing to its higher macromolecule content like carbohydrate and protein, and low antinutrient contents. *Z. variegatus* on the other hand may serve as food supplements, considering its dominance in essential minerals and vitamins.

#### Declarations

# Author contribution statement

Emeka Godwin Anaduaka: Conceived and designed the experiments; Wrote the paper.

Nene Orizu Uchendu: Analyzed and interpreted the data.

Dionysius Obinna Osuji: Performed the experiments; Wrote the paper Lorreta Nwakaego Ene: Contributed reagents, materials, analysis tools or data.

Ogechukwu Peace Amoke: Performed the experiments.

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#### Data availability statement

Data included in article/supplementary material/referenced in article.

# Declaration of interests statement

The authors declare no conflict of interest.

# Additional information

No additional information is available for this paper.

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