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VARIATIONS IN TISSUES METABOLITES AND GUT MICROBIAL FLORA OF ADULT MALE *ZONOCERUS VARIEGATUS* (L) (ORTHOPTERA: PYRGOMORPHIDAE) DURING STARVATION

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ABSTRACT

A study to determine the influence of starvation on the gut microbial flora and somatic tissues (femoral muscles, fat body and haemolymph) metabolites of adult male Zonocerus variegatus was conducted. Two hundred and fifty (250) Z. variegatus individuals were collected from the field and randomly divided into five groups A-E based on the starvation duration (0, 24, 48, 72, 96 hours). The colony forming units (cfu) of the gut microbial flora decreased as the starvation period progressed with the midgut recording the highest values. Na^+ and Cl^- were the only inorganic substances significantly ($P<0.05$) affected by starvation as their concentrations in the tissues dropped significantly during the 96 hours starvation. Similarly, a strong positive relationship existed between glucose concentration in the fat body and starvation period (+0.61). The tissues' glucose concentrations dropped during the 96 hours starvation (in fat body glucose concentration dropped from 28.0mg/dl at 0 hour to 20.0mg/dl at 96 hour) while tissues' lipid and protein concentrations were not significantly affected. Glucose can then be concluded to be the most utilized metabolite during starvation in Z. variegatus

Keywords: Starvation, tissues, metabolites, microbial flora Zonocerus variegatus

INTRODUCTION

Zonocerus variegatus (L) is a tropical African grasshopper occuring in areas with warm climate (Yondeowel, 1974). It feeds on a variety of plants and is an important pest of crops in West Africa. Chiffaud and Mestre (1990) reported that *Z. variegatus* consumes more than 250 plant species among 71 families. However, it has been shown that not all the food plants eaten by it is adequate for survival and development (Bernays *et al.*, 1975 and Tamu 1990).

The condition of the host plant significantly affects the feeding, development and fecundity of the insect that feeds on it. The excision of a leaf results in water loss, chemical degradation and mechanical deterioration. Continuous enzymatic activity in cut and wilted leaves results in a considerable loss of soluble carbohydrate and conversion of proteinous nitrogen to soluble nitrogen as well as changes in the chemicals associated with water stress (Brandy, 1960). Similarly, turgid, growing cassava leaves produced distasteful hydrocyanide when eaten by *Z. variegatus* (Berneys *et al.*, 1975).

Locust (*Locusta migratoria*) and Fruit bettles (*Pachnoda Sinuata*), metabolize glycogen stores during initial stages of starvation, then switch to lipid and protein metabolism when carbohydrates are gone (Hill and Goldsworthy, 1970).Rankin and Riddiford (1977) observed that the removal of food from *Oncopeltus fasciatus* caused a decline in the activity of the Juvenile Hormone (JH) secreted by corpora allata (CA) over a 4-6 hours period.

Idowu and Akinsete (2000) reported that starvation had a positive impact on the attraction of *Z. variegatus* nymphs to the different food lures. Likewise, starvation affects the volume of repellant gland secretion obtained from *Z. variegatus* (Idowu and Idowu, 2001). Recently too, Ademolu and Idowu (2004) observed a significant influence of short term starvation on the proximate composition and fat body scores of *Z. variegatus*. However, the impact of starvation on the somatic tissues is yet to be documented in literature. The focus of this present study is to investigate the changes in the somatic tissues metabolites during starvation, thereby revealing the utilization of food reserves during the process in this insect.

MATERIALS AND METHODS

Collection and Maintenance of Insects

Five hundred (500) newly hatch 6th instar nymphs of *Z. variegatus* were collected from uncultivated farm land on the Campus of University of Agriculture, Abeokuta (UNAAB). Collections were made with sweep net very early in the morning at about 6:30am - 7:30am from September-December, 2007. The collected insects were kept and maintained in wire mesh cages (40 x 30 x 30cm) in the insectary of the Department of Biological Sciences, University of Agriculture at the temperature of 29± 2°C and relative humidity of 79-85 % until they reached the adult stage.

Starvation Experiment

Only the adult males were selected for this study because of the reproductive complications (copulation and oviposition) in the female adults. A total of [illegible] insects were used for the starvation trials.

they were randomly divided into five groups (A-E) of fifty (50) each (each treatment has 5 replicates with 10 insects each), based on the starvation durations that is 0, 24, 48, 72 and 96 hours respectively.

Data Collection

a) Weight

The weights of the insects were taken both before and after the starvation process using the electric balance (Mettler Toledo AE, 240).

b) Gut microbial examination

Twenty five (25) adults of *Z.variegatus* were used for this experiment (5 insects for each starvation period and the control).Before dissection, each insect was surface sterilized by swabbing with iodine followed by 70% ethanol.

The dissection of the insects for the gut examination was carried out following the method described by Youdeowei (1974). The body cavity was opened by ventral longitudinal cut and this exposed the alimentary canal system. The gut was separated from adjoining tissues like fat body and malphigian tubules

The gut was partitioned into 3 parts namely: foregut, midgut and hindgut by a flamed forceps. The gut contents of the various parts were emptied into labeled petri dishes while the wall was washed thoroughly with distilled water to free it from any adhering material. Using a sterile pestle and mortar each gut section was homogenized in 1 ml of sterile distilled water. They were decanted with labeled bottles containing 9 ml sterilized water.1 ml of the samples was homogenized in 9 ml sterile diluted water and 6 fold serial dilutions were made. 1 ml aliquot of 4 – 6 dilution were plated in duplicates by pour plate technique using the following media : Potato Dextrose Agar (PDA) was used for Fungi enumeration, Nutrient Agar (NA) and demann Rogoss Sharpe medium (MRS) (Oxford, England) were used for bacteria and lactobacillus enumeration respectively.

Potato Dextrose Agar plates were incubated at 30° C for 5 days, while Nutrient Agar and MRS were incubated at 37°C for 48 hours. After 48 hours the colony forming units (cfu) were determined by visual counting. Purified colonies were grouped according to their colony morphology and cell characteristics. Yeast and mould were identified after staining with cotton blue lactophenol. Further identification was done according to Kreger-venrij (1984) by pseudomycelium formation and pattern of sugar fermentation (glucose, galactose, maltose and lactose). The bacterial isolates were identified using Bergey's Mannual of Systematic Bacteriology (Sneath *et al*, 1986) and the methods of Harrigan and MacCance (1976).

c) Glucose, protein and minerals determination

The protein content of the tissues (fat body, haemolymph and femoral muscles) was determined before and after starvation process by the Biuret method (Henry *et al*, 1974). The glucose content was determined by colorimetric method (Baumniger, 1974). The lipids assay was done following the method of Grant (1987). The tissues' sodium (Na^+), Potassium (K^+), Calcium (Ca^{2+}) Phosphate (Po^-_4) and Chloride (Cl^-) composition were determined by the standard methods of AOAC (1990)

d) Statistical Analysis

At data collected from the above experiments were analyzed by One Way Analysis of Variance (ANOVA) and where significant difference existed, means were separated by Student Newmen-Keuls (SNK) test.

RESULTS

A) Weight

Results from the study shows that there was a decline in the weight of *Z.variegatus* as the starvation period progressed from 0-96 hours. The weight loss by insects starved for 96 hours was statistically ($P<0.05$) more than weight loss by the insects starved for 24 hours (Table 1).

B) Gut Microflora

The gut microbial flora load (bacteria) of male adult *Z.variegatus* starved for different hours is shown in table 2. The colony forming units of the bacteria decreased as the starvation period increased (except in the fore gut at 24 hours).

The comparison of means showed that the midgut had the highest colony forming unit out of the three gut regions. Same observation as mentioned above was observed for the gut microbial floral (fungi) (table 2).

C). Inorganic Substances of The Tissues

i) Fat Body

The concentrations of inorganic substances in the fat body of *Z.variegatus* during starvation period are shown in table 3. Of all the substances analyzed Na^+ and Cl^- were significantly affected ($P<0.05$) by the starvation process. The concentrations of Na^+ and Cl^- in the fat body of the control (0hr) insects were significantly higher than starved Insects.

ii) Femoral Muscle

Table 4 shows the concentration of inorganic substances in the femoral muscles of adult male *Z.variegatus* during starvation. Starvation did not significantly ($p>0.05$) affect the concentrations of the inorganic substances in the femoral muscles (except Na^+ and Cl^-). The concentration of Cl^- in the femoral muscles dropped from 80.1 mmol/l in the control (0hrs) to 10.3 mmol/l after 48 hours of starvation.

iii) Haemolymph

The concentrations of inorganic substances in the haemolymph of adult male *Z.variegatus* during starvation is shown in table 5. Na^+ and Cl^- were significantly affected by starvation process. The concentrations of Na^+ and Cl^- in the haemolymph dropped from 90.0mmol/l and 55.2 mmol /l at 0hr to 40.0 mmol/l and 11.1mmol/l respectively after 96 hours of starvation.

It is noteworthy that Na^+ recorded the highest concentration in the three somatic tissues examined during starvation.

D. **Organic Substances**

i) **Fat Body**

Table 6 shows the concentrations of organic substance in the fat body of the adult male *Z. variegatus* during starvation. Glutose and lipid concentrations were significantly ($P<0.05$) affected by starvation as glucose concentration drooped from 28.1mg/dl at 0hr to 20.4mg/dl at 96 hrs. On the other hand, the concentration of lipid in the fat body increased during starvation (24.2 mg/dl at 0hrs and 38.2mg/dl after 96hrs of starvation).

ii) **Haemolymph**

The concentration of haemolymph glucose ranged from 17.2mg/dl at 0hr to 13.8mg/dl at 96 hours of starvation. No significant difference ($p>0.05$) existed in the concentrations of haemolymph lipids and protein during starvation (Table 7).

iii) **Femoral Muscles**

The concentrations of organic substances in the femoral muscles of adult male of *Z. variegatus* during starvation are presented in table 8. Statistical analysis reveal that the concentration of glucose dropped significantly ($P<0.05$) from 20.4mg/dl at 0hr to 8.10mg/dl at 96hrs of starvation. However, no significant difference was observed in the concentration of lipids and protein in the femoral muscle during starvation.

The relationship between the starvation period and concentrations of the organic substances in the fat body is shown in figure 1. A strong positive relationship existed between the concentration of glucose and starvation period ($R^2 = 0.6099$) followed by lipids (0.537).

Table 1: The body weight of *Zonocerus variegatus* during starvation process (n=20)

Starvation period (hrs)	0	24	48	72	96
Final average weight (g)	0.97 ± 0.02^a	0.91 ± 0.5^b	0.86±0.1c	0.77 ± 0.22^d	0.74 ± 0.11^d
Initial average weight (g)	0.97±0.01	1.02±0.1	1.02±0.5	0.95±0.15	0.99±0.01
Average weight loss (g)	0.00 ± 0.01^d	0.11 ± 0.04^c	0.16 ± 0.14^b	0.18 ± 0.23^b	0.25 ± 0.20^a

*mean values in the same row having the same superscript are not significantly different ($p>0.05$)

Table 2: The gut regions microbial load (cfu x 10^5) of adult male *Z. variegatus* during starvation

Starvation period (hrs)	Bacteria			Fungi		
	Fore gut	Mid gut	Hind gut	Fore gut	Mid gut	Hind gut
0	20 ± 0.3^b	32 ± 0.25^a	27 ± 0.14^a	27 ± 0.35^a	29 ± 0.74^a	27 ± 0.14^a
24	29 ± 0.2^a	30 ± 0.6^a	17 ± 0.26^b	23 ± 0.8^b	25 ± 0.1^{ab}	21 ± 0.1^b
48	15 ± 0.1^c	26 ± 0.1^b	15 ± 0.7^b	16 ± 0.42^c	17 ± 0.01^b	15 ± 0.3^c
72	12 ± 0.3^d	17 ± 0.65^c	11 ± 0.4^c	13 ± 0.15^c	15 ± 0.1^b	11 ± 0.5^d
96	13 ± 0.12^d	13 ± 0.12^c	8 ± 0.23^c	8 ± 0.36^d	14 ± 0.05^b	10 ± 0.8^e

*Mean values in the same column having the different superscript are significantly different ($p<0.05$)

Table 3: Concentrations of inorganic substances* in the fat body of adult male *Z. variegatus* during starvation (mg/dl)

Starvation period (hrs)	0	24	48	72	96
Ca^{2+}	2.9±0.02	3.0±0.5	2.7±0.01	2.7±0.11	3.0±0.01
Na^+	137.1 ± 0.12^a	$97..0\pm0.3^c$	100.5 ± 0.25^c	117.0 ± 0.1^b	114.0 ± 0.01^b
K^+	2.0±0.2	1.9±0.12	2.1±0.36	3.0±0.48	2.7±0.20
PO_2^{4-}	1.9±0.1	0.2±0.6	0.4±0.51	0.4±0.20	0.6±0.15
Cl^-	70.0 ± 0.14^b	50.2 ± 0.05^c	82.0 ± 0.42^a	82.1 ± 0.62^a	68.0 ± 0.24^b

*Mean values in the same row having the different superscript are significantly different ($p<0.05$)

Table 4: Concentrations of inorganic substances in the femoral muscles of adult male *Z. variegatus* during starvation (mg/dl)

Starvation period (hrs)	0	24	48	72	96
Ca^{2+}	2.6±0.1	2.9±0.22	2.6±0.1	2.9±0.89	2.7±0.01
Na^+	112.0 ± 0.11^a	116.0 ± 0.52^a	42.0 ± 0.1^b	92.1 ± 0.25^a	105.0 ± 0.56^a
K^+	2.7±0.23	3.0±0.95	0.9±0.2	3.1±0.13	1.8±0.14
PO_2^{4-}	1.6±0.45	1.4±0.47	1.6±0.51	1.3±0.15	2.5±0.48
Cl^-	80.1 ± 0.01^a	75.3 ± 0.6^a	10.3 ± 0.62^c	50.5 ± 0.1^b	71.2 ± 0.5^a

*Mean values in the same row having the different superscript are significantly different ($p<0.05$)

Table 5: Concentrations of inorganic substances in the haemolymph of adult male *Z. variegatus* during starvation (mg/dl)

Starvation period (hrs)	0	24	48	72	96
Ca^{2+}	3.1±0.23	2.6±0.71	2.4±0.09	2.6±0.19	3.1±0.01
Na^{+}	90.0±0.57^{a}	100.3±0.19^{a}	90.0±0.58^{a}	119.0±0.23^{a}	40.0±0.12^{b}
K^{+}	2.5±0.52	3.5±0.1	2.9±0.87	2.9±0.14	2.0±0.14
PO_2^{4-}	0.1±0.19	0.2±0.58	0.2±0.17	.0.2±0.02	0.7±0.33
Cl^{-}	55.2±0.25^{b}	80.1±0.84^{a}	45.2±0.01^{b}	72.0±0.56^{a}	11.1±0.22^{c}

*Mean values in the same row having the different superscript are significantly different ($p<0.05$)

Table 6: Concentrations of organic substances in the femoral muscles of adult male *Z. variegatus* during starvation (mg/dl)

Starvation period (hrs)	0	24	48	72	96
Protein	11.2±0.01	13.5±0.45	13.0±0.14	10.5±0.35	8.0±0.02
Lipid	30.1±0.09	30.1±0.28	30.1±0.01	30.2±0.48	30.0±0.3
Glucose	20.4±0.12^{a}	10.3±0.16^{b}	10.2±0.16^{b}	10.2±0.19^{b}	8.1±0.21^{b}

*Mean values in the same row having the different superscript are significantly different ($p<0.05$)

Table 7: Concentrations of organic substances in the haemolymph of adult male *Z. variegatus* during starvation (mg/dl)

Starvation period (hrs)	0	24	48	72	96
Protein	7.5±0.22	8.0±0.28	9.2±0.01	10.4±0.02	10.1±0.95
Lipid	23.3±0.45	23.1±0.7	15.7±0.1	23.5±0.25	23.5±0.52
Glucose	17.2±0.3^{a}	20.1±0.12^{a}	17.3±0.01^{a}	8.6±0.18^{c}	13.8±0.08^{b}

*Mean values in the same row having the different superscript are significantly different ($p<0.05$)

Table 8: Concentrations of organic substances in the fat body of adult male *Z. variegatus* during starvation (mg/dl)

Starvation period (hrs)	0	24	48	72	96
Protein	15.0±0.01	9.1±0.1	10.2±0.1	12.2±0.12	14.0±0.3
Lipid	24.2±0.1^{c}	24.3±0.5^{c}	40.5±0.13^{a}	32.1±0.01^{b}	28.2±0.2^{a}
Glucose	28.1±0.2^{a}	22.1±0.6^{b}	22.6±0.5^{b}	15.4±0.27^{c}	20.4±0.1^{b}

*Mean values in the same row having the different superscript are significantly different ($p<0.05$)

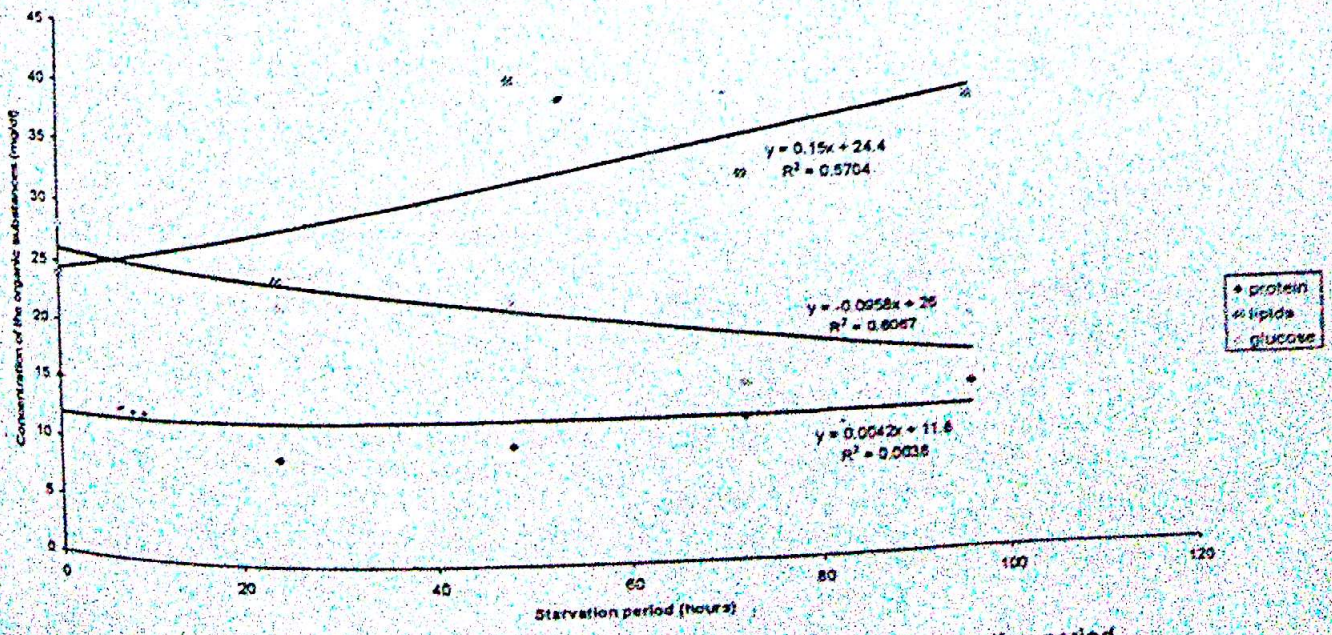

Figure 1: Relationship between the fat body organic substances and starvation period

DISCUSSION

Starvation had a significant effect on the body weight of *Z. variegatus* as it experienced weight loss during the starvation period. Also the weight loss increased as the starvation period increased. This observation parallels that of Muse (2003) and Samuel *et al* (1993). Since food intake significantly influenced body weight of insects (Chapman, 1990), food deprivation will actually produced weight loss.

The colony forming unit (cfu) of the gut microflora decreased during the starvation periods. Idowu and Edema (2004) earlier observed that gut microbial of *Z. variegatus* are horizontally acquired from their food plants, thus, as no food was consumed during starvation, the colony forming unit of the gut was not unexpectedly reduced.

The comparison of the cfu means showed that the midgut of *Z. variegatus* had the highest cfu (both Fungi and bacteria) out of the three gut regions. Midgut of *Zonocerus* had been described to have the highest enzymatic activities and the major site for digestive enzyme secretion in Orthoptera (Terra, 1990). The presence of these microbes in the gut assists in degrading cellulosaic food materials consumed by *Z.variegatus* (Idowu *et al.*, 2009)

The concentrations of Na^+ and Cl^- were significantly influenced by starvation as their concentration dropped during starvation. This had strong implication on the insect because Na^+ and Cl^- are vital ions in the osmotic pressure regulation and conduction of nerve impulses in insects (Ademolu *et al.*, 2007). This means that the somatic tissues' activities of *Z. variegatus* might be reduced during starvation.

The concentration of glucose in the somatic tissues dropped significantly during starvation period while protein and lipids concentrations did not (except lipids in femoral muscles).Carbohydrates had been reported to be the predominant metabolite utilized during initial stage of starvation in *Oxya japonica* and *Locusta migratoria* (Hill and Goldsworthy 1970) and Lee 1980). Similarly, Fruit beetle, *Pachnoda sinuata* metabolizes glycogen stored during initial stages of starvation then switched to lipid and protein metabolism. The non-utilization of protein and lipids during the starvation process in the adult male *Z. variegatus* suggests that there are enough reserves of carbohydrates to meet up with the deprivation of food. Adult stage of *Z. variegatus* is dispersal in habit (Toye, 1982 and Youdeowei, 1974), thus need to accumulate food reserves in the tissues to be utilized as fuel for jumping/hopping, unlike the gregarious earlier instars.

The concentration of lipids in the fat body rose up while glucose concentration dropped during the 96 hours starvation period. This observation compliments well with the report of Justum *et al* (1975) that the concentration of haemolymph lipids of adult male and female *Locusta migratoria* are elevated during food deprivation. This suggests mobilization of lipid reserves of the haemolymph during starvation. The comparison of means of the tissues metabolites showed that lipid had highest concentration in all the tissues and fat body had the highest concentration of protein and carbohydrates. The fat body has been reported to store glycogen, fat, protein and other substances brought into it by the haemolymph (Chapman, 1990). It can be concluded that glucose is the most utilized metabolite during starvation in variegated grasshopper, *Z.variegatus*

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