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RESPONSES OF SOMATIC TISSUES OF DEVELOPMENTAL STAGES OF VARIEGATED GRASSHOPPER, *ZONOCERUS VARIEGATUS* (L.) (ORTHOPTERA: PYRGOMORPHIDAE) TO STARVATION

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Abstract - Nymphs and adult stage of *Zonocerus variegatus* respond to environmental stress differently. The response of somatic tissues to 96 hours starvation across the post embryonic developmental stages of *Zonocerus* was investigated in this study. The organic substances (lipids, glucose and protein) concentrations were measured by standard methods before and after starvation exercise in the somatic tissues (femoral muscles, fat body and haemolymph) and the difference calculated. The adult stage experienced highest weight loss of 0.22g from the initial weight, while the 1st instar stage had the least weight loss of 0.10g from the initial weight. Glucose was the most depleted haemolymph metabolite (0.50-3.40mg/dl) and the 1st instar stage lost the least amount of metabolites. In the fat body, the highest glucose concentration was lost by the adult stage (5.70mg/dl) while the least was loss by the 1st instar stage. Lipids were the most depleted metabolite in the femoral muscles and the adult stage similarly lost the highest concentration. Comparison of means showed this pattern of metabolites loss in the three somatic tissues: glucose>lipids>protein. Despite the highest loss in concentration of metabolites, the adult stage still fared better than other stages of development during starvation because of higher stored reserves. Early instar stages of *Z. variegatus* are thus less complicated and easier to control than the tough adult stage.

Key words: somatic tissues, starvation, instar, adult stage, *Zonocerus variegatus*, metabolites

Izvleček – ODZIV TELESNIH TKIV RAZVOJNIH STOPENJ KOBILICE *ZONOCERUS VARIEGATUS* (L.) (ORTHOPTERA: PYRGOMORPHIDAE) NA STRADANJE

Ličinke in odrasla stopnja kobilice *Zonocerus variegatus* se različno odzivajo na okoljski stres. V tej raziskavi smo preučevali odziv telesnih tkiv postembrionalnih razvojnih stopenj kobilic na 96 ur stradanja. Koncentracije organskih snovi (lipidov, glukoze in proteinov) smo merili s standardnimi metodami pred in po stradanju v telesnih tkivih (stegenskih mišicah, maščobnem telesu in hemolimfi) in izračunali razliko. Odrasla stopnja je doživela največjo izgubo teže, 0,22g, od začetne teže, prva stopnja ličinke pa z 0,10g najmanjšo izgubo teže od začetne. Glukoza je bila najbolj izčrpan hemolimfni metabolit (0,50 – 3,40 mg/dl) in ličinka prve stopnje je izgubila najmanj metabolitov. V maščobnem telesu se je koncentracija glukoze najbolj zmanjšala pri odrasli stopnji (5,70 mg/dl), najmanj pa pri ličinki prve stopnje. Lipidi so bili najbolj izčrpan metabolit v stegenskih mišicah in odrasla stopnja je podobno izgubila največ koncentracije. Skupna primerjava izgube metabolitov v treh telesnih tkivih je pokazala vzorec: glukoza>lipidi>proteini. Kljub največji izgubi koncentracije metabolitov je odrasla stopnja bolje kot druge razvojne stopnje preživela stradanje zaradi več shranjenih zalog. Zgodnje razvojne stopnje *Z. variegatus* zato nadziramo lažje in z manj zapleti kot zdržljivo odraslo stopnjo.

KLJUČNE BESEDE: telesna tkiva, stradanje, razvojne stopnje, odrasla stopnja, *Zonocerus variegatus*, metaboliti

Introduction

In Nigeria, *Zonocerus variegatus* occurs in cultivated land with the nymphs and adult stage sharing same habitat which extends from rainforest zone to Guinea Savannah in the north (Youdeowei, 1974). The life cycle consists of six nymphal stages and adult stage, which is the reproductive stage. Ademolu *et al* (2013) reported that all nymphal stages together lasted for 111.1 days while female and male adults lived for 127.9 and 101.2 days respectively. Hence, the total life cycle of *Z. variegatus* was between 210.8 and 237.5 days (Ademolu *et al*., 2013).

Though living in the same habitat, the various developmental stages behave differently: 1st -3rd instars are gregarious in nature and prefer exclusively *Chromolaena odorata* while later instars and adult stage feed predominantly on *Manihot esculenta* and live dispersed (Toye, 1982). Similarly, an initial rise in the concentrations of tissue metabolites was observed during the 1st -5th nymphal stage of *Z. variegatus* which dropped at the 6th instar and rose again at the adult stage (Ademolu *et al.,* 2007).

Muse (2003) observed that starvation had significant influence on the longevity of the adult stage. Likewise, Idowu and Idowu (2001) reported that starvation reduced the volume of the repellent gland obtained from *Z. variegatus*. In *Locusta migratoria*, lipid reserves were mobilized from the fat body during starvation, resulting in increase in total haemolymph lipids and decrease in the fat body lipids (Jutsum *et al*., 1975).

Similarly, in *L. migratoria* and fruit beetles (*Paechnoda sinuata*) glycogen stores are metabolized during the initial stage of starvation and later switch to lipid and protein metabolism happens when carbohydrates are exhausted (Hill and Goldswothy, 1970; Auerswald and Gade,2000).

Starvation process reduced the colony forming unit (cfu) in the midgut, as well as the concentration of organic and inorganic substances in the haemolymph, fat body and femoral muscles of adult *Zonocerus* (Ademolu *et al*.,2011). Since the physiology of each stage of development differs, will their tissues respond differently to starvation exercise? This study attempts to examine the response of each developmental stage of *Z. variegatus* to the process of starvation.

Materials and methods

Insect samples

Newly hatched 1st instar nymphs of *Z.variegatus* were collected from a known oviposition site on the campus of Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria. Being gregarious in behavior at this stage, hundreds were collected with the aid of sweep net and were transferred into wire cages (30x30x45 cm). On arrival at the insectary of Pure and Applied Zoology, FUNAAB, the insects were separated into seven cages each containing 60 individual insects and allowed to molt into its different developmental stages. The 2nd instar to the 6th instar stage individuals were maintained on leaves of *M. esculenta* (cassava) before molting into the next stage of development.

Each developmental stage was starved for 96 hours (4 days) at 2nd day after emergence following methods adopted by Ademolu *et al* (2011).

Data Collection

(a) Body weight

The body weight of the insects was taken both before and after the four days starvation experiment using sensitive digitalized weighing balance (Mettler Toledo, AE, 240).

(b) Tissue collection and preparation

Three somatic tissues (haemolymph, fat body and femoral muscles) were harvested from *Z. variegatus* before and after the 96 hours starvation period.

Haemolymph was collected following methods described by Ademolu *et al* (2011). A micro needle was inserted into the mid ventral axis of the thorax and the haemolymph coming out was collected into a calibrated syringe. The sample was centrifuged and the supernatant was kept in refrigerator for further analysis.

The peripheral fat body was carefully picked and 0.5g of it was homogenized in 5mls of distilled water and the homogenate was used for analysis.

Following the opening of the hind femora, the femoral muscles were removed by forceps into the petri dishes, dried to constant weight at 50^{o}C in an oven at 12 hours and from it, 0.5g was macerated in 0.05M KCl. The homogenate was centrifuged and supernatant obtained was kept in refrigerator until further usage.

(c) Analysis of samples

The protein, glucose and lipids of the samples were determined following the methods of Henry *et al* (1997), Baunmniger (1974) and Grant *et al* (1997) respectively.

Statistical analysis

All analyses were carried out in triplicates and the data were subjected to analysis of variance. Separation of significant means was done by Duncan Multiple Range Test.

Results

The influence of starvation on the body weight of developmental stages of *Z. variegatus* is shown in Table 1. The body weight of all developmental stages was significantly ($p<0.05$) affected by starvation. The weight loss was progressively increasing as the insect passed through the developmental stages with the adult stage having the highest weight loss.

Table 2 shows the influence of starvation on the haemolymph metabolites of developmental stages of *Z. variegatus*. There was a significant difference in the amount of glucose loss due to starvation by the different stages of development with the adult stage recording the highest glucose loss. Although no significant difference was recorded in the amount of protein and lipids loss to starvation by the developmental stages, adult stage had the highest numerical loss.

Table 1: Effects of 96 hours starvation on the body weight (g) of developmental stages of *Zonocerus variegatus* (L).

Stages of development	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	6th Instar	Adult
Average initial weight	0.10^{c}	0.12^{c}	0.14^{c}	0.18^{c}	0.30^{b}	0.50^{b}	0.97^{a}
Average final weight	0.09^{c}	0.11^{c}	0.12^{c}	0.16^{c}	0.25^{bc}	0.45^{b}	0.75^{a}
Average weight loss	0.01^{c}	0.01^{c}	0.02^{c}	0.02^{c}	0.05^{b}	0.05^{b}	0.22^{a}

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

Table 2: Haemolymph metabolites loss during 96 hours starvation of developmental stages of *Zonocerus variegatus* (mg/dl).

Developmental Stages	Glucose	Protein	Lipids
1st Instar	1.1^{b}	0.3	1.2
2nd Instar	1.7^{b}	0.2	1.4
3rd Instar	1.8^{b}	0.4	1.3
4th instar	1.2^{b}	0.4	1.1
5th Instar	3.4^{a}	0.3	1.5
6th Instar	0.5^{c}	0.4	0.7
Adult	3.2^{a}	0.6	1.9

Mean values in the same column having the same superscript are not significantly different (p>0.05).

The loss of metabolites in the fat body of developmental stages of *Z.variegatus* after 96 hours starvation is shown in Table 3. There was progressive increase in the amount of metabolites loss as the insect passed through 1st instar stage to the adult stage (except lipids). The results also show that more of glucose and lipids were lost to starvation than protein.

The loss of femoral muscle metabolites due to 96 hours starvation of developmental stages of *Z.variegatus* revealed differences in the amount of metabolites lost by each stage of development (Table 4). The adult stage recorded the highest glucose and lipids loss while the earlier instars had lowest loss.

Table 3: Fat body metabolites loss during 96 hours starvation of developmental stages of *Zonocerus variegatus* (mg/dl).

Developmental Stages	Glucose	Protein	Lipids
1st Instar	0.9^{c}	0.5^{b}	1.5^{b}
2nd Instar	1.5^{c}	0.4^{b}	2.3^{b}
3rd Instar	1.0^{c}	0.8^{b}	2.5^{b}
4th instar	3.2^{b}	2.7^{ab}	2.1^{b}
5th Instar	3.5^{b}	2.1^{ab}	8.1^{a}
6th Instar	0.2^{c}	2.0^{ab}	1.7^{b}
Adult	5.7^{a}	3.3^{a}	2.1^{b}

Mean values in the same column having the same superscript are not significantly different (p>0.05).

Table 4: Femoral muscles metabolites loss during 96 hours starvation of developmental stages of *Zonocerus variegatus* (mg/dl)

Developmental Stages	Glucose	Protein	Lipids
1st Instar	0.8	0.1	1.5^{c}
2nd Instar	0.6	0.5	1.1^{c}
3rd Instar	0.7	0.5	2.3^{c}
4th instar	2.3	1.5	1.7^{c}
5th Instar	0.9	2.2	1.5^{c}
6th Instar	2.7	1.8	5.0^{c}
Adult	3.0	2.1	7.5^{a}

Mean values in the same column having the same superscript are not significantly different ($p>0.05$).

Discussion

The body weight loss of *Z. variegatus* due to 96 hours starvation increased gradually from 1st instar to the adult stage. This reflects trend of energy depletion, that is, energy depletion was more in the later instars than the early instars. The food reserves as a result of food consumption by insects increased with nymphal growth owing to increased food requirement for growth and metabolic function, thus during starvation stress, such reserves are lost or depleted in high quantity (Omkar and Jones, 2003). Also, the active nature (dispersal) of the later instars and adult stage deplete their resources more compared to the early instars that are gregarious in habit and therefore conserve or less exhaust their resources (Toye,1982). Furthermore, the difference in the way the nymphal stages and adult responded to food deprivation might be related to their water balance, because adults are more susceptible to water loss than the nymphs.

Somatic tissues of *Z. variegatus* lost significant quantity of their metabolites concentration due to starvation. Similar observation was made by Perez-Mendoza *et al.* (1999) where starvation reduced the lipids content and body weight of Lesser grain borer, *Rhyzopertha dominica.* Similarly, Auerswald and Gade, (2000) reported that in *P. sinuata,* carbohydrates were first metabolized during the initial stage of starvation, then shift to lipids and proteins happened when carbohydrates were exhausted. However, the fact that protein component of these tissues was also depleted possibly suggests that lipids and glucose metabolism were not sufficient to meet the requirement of the insect, resulting in the net reduction of protein concentration.

Comparison of means revealed that less of protein and more of glucose and lipids were lost by the somatic tissues due to starvation. This is not unexpected as carbohydrates and lipids are the predominant metabolites utilized during initial stage of starvation (Hill and Goldworthy, 1970; Hainsworth, 1981). In an experiment using *Oxya* *japonica* (Acrididae: Orthoptera), a significant decrease in the total haemolymph lipids and carbohydrates was observed when deprived of food for 96 hours (Lim and Lee, 1981). During starvation trials in two *Drosophila* species, rates of lipid and protein metabolism were similar, but carbohydrate metabolism was several fold higher (Marron *et al*., 2003).

The fat body of *Z. variegatus* experienced higher metabolites loss due to starvation than other two somatic tissues examined. This might likely be due to the higher amount of metabolites present there and thus expectedly higher loss to starvation. Ademolu *et al* (2007) observed that fat body stores more protein and lipids than the haemolymph and femoral muscles during postembryonic development of *Z. variegatus*. Similarly, the fat body was reported to have stored more glycogen, fat, protein and other substances brought into it by the haemolymph (Hannerland and Shirk, 1995)**.**

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