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Article

Changes in energy content of *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) in response to different diets

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Abstract

Nymphal instars and adults of the spined soldier bug, *Podisus maculiventris*, preferably feed on lepidopteran and colopteran larvae. Different prey diets can change fitness including energy reservoirs of the predator. In the present study, effects of different artificial and natural diets as well as starvation was studied on energy contents of the third and fourth nymphal instars of *P. maculiventris*. Total available energy calculated as the sum of the energy contents of lipids, carbohydrates, glycogen, and proteins did not significantly differ in the third and fourth nymphal instars fed on natural and artificial diets. Among the energy reservoirs, only total lipid storage of the starved nymphal instars was significantly different from that in the bugs fed on the natural diet. In conclusion, total energy reserves cannot be changed in response to artificial diet. A better understanding of the impact of artificial diets on the energy reserves of natural enemies can be considered as a biomarker for more appropriate mass rearing approaches of natural enemies.

Keywords diet; *Podisus maculiventris*; lipids; proteins; carbohydrates; glycogen.

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1 Introduction

Many species of arthropods are commercially available for augmentation programs targeting many pests such as mites and insects. One of these arthropods is *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) because of its broad host range, high reproductive capacity and receptiveness to low-cost artificial diets (Hough- Goldstein and McPhersons, 1996). *Podisus maculiventris* is a polyphagous predator known to feed on several coleopteran and lepidopteran pests (e.g., De Clercq and Degheele, 1994; De Clercq et al., 1998a; De Clercq, 2000). The predator has been used for biological control of lepidopteran pests in Europe greenhouses since 1997, but it is indigenous throughout North America (De Clercq, 2000; De Clercq et al., 2002). It is being commercialized for biological control of the Colorado potato beetle and noctuid caterpillars (De Clercq, 2000). Polyphagous predators are reared on unnatural foods easily and can be made them suitable for commercialization (De Clercq, 2002). Little is known about the impact of different nutritions upon the energy reserves of predaceous Heteroptera, especially predaceous pentatomid. Application of artificial diets for *P. maculiventris* showed physiological effects on survival, reproduction and development (De Clercq and Degheele, 1992b). Both ovarian maturation rate and fecundity levels in adults of the spined soldier bug reared on an artificial diet were significantly lower than females fed larval prey during both nymphal and adult stages (Wittmeyer, 2001). Mahdian et al. (2006) determined the effects of four natural diets and one artificial diet on development, survival and reproduction of *Picromerus bidens* L. and *P. maculiventris*, in laboratory. Developmental duration, survival and reproduction were affected by the diets in both species. Quantity of the different energy reserves of the red and green pea aphids were measured and revealed that the total energy reserves of two morphs did not differ (Ahsaei et al., 2013). The content of water-soluble carbohydrates and lipids in the red clones was higher whereas protein content was higher in green clones (Ahsaei et al., 2013). Lipid and soluble carbohydrate contents in the pale pea aphids was less than that in the red aphids (Tabadkani et al., 2013).

In the present study, we have measured the energy reserves (including water-soluble carbohydrates, glycogen, proteins and lipids) of the spined soldier bug in response to feeding from different diets. A better understanding of the impact of artificial diets on the energy reserves of a natural enemy is important for the design of biological control strategies and may aid in improving diets for augmentative rearing systems.

2 Materials and Methods

2.1 Predator and prey cultures

P. maculiventris were fed on larvae of the greater wax moth, *Galleria mellonella*. The stock colony of the bug was maintained at 23 ± 1°C, 60-70% relative humidity and a photoperiod of 16L : 8D. The greater wax moth was reared on an artificial diet containing wheat flour, honey, glycerol, yeast and old black bee wax. For preparation about one kilogram of the artificial diet, 400g flour, 200g honey, 164g glycerol, 100g dried yeast powder, 40g old black bee wax were used. The stock colony of the greater wax moth was reared at 28 ± 1°C, 50% relative humidity and photoperiod of 12L :12D. At the time of the study, the bugs had been reared under controlled conditions for at least 50 generations.The artificial diet for the bug used in this study was included from 200 g beef liver, 200 g fatty ground beef, 24 ml sucrose solution (5%) and 20 g fresh hen’s egg yolk according to Mahdian et al. (2006).

2.2 Sample preparation

The third and fourth nymphal instars of *P. maculiventris* selected and were separately homogenized in 180 μL of aqueous lysis buffer solution (100 mM KH_2PO_4, 1 mM dithiothreitol and 1 mM ethylenediaminetetraacetic acid, pH 7.4) using a plastic micropestle. There were three replicates of each of the determinations in the following assays.

2.3 Quantification of the energy reserves

The measurements of energy reserves in the whole body of the bugs were determined according to a modified method (Ahsaei et al., 2013) of Foray et al. (2012). Protein contents of nymphal instars of *P. maculiventris* homogenates were measured according to the method of Lowry (1951). Bovine serum albumin was used as the standard.

Total carbohydrates by addition of 20 μL of sodium sulfate solution (20%) to 180 homogenate were dissolved (van Handel, 1965; van Handel and Day, 1988). Water-soluble carbohydrates and total lipid by mixing the solution with 1500 μL of a chloroform-methanol solution (1 : 2 v/v) were solubilized (van Handel, 1965; van Handel and Day, 1988). Then, Each sample was centrifuged at 16,000 rpm at 4°C for 15 min. The supernatant was transferred to a new microtube for water-soluble carbohydrate determination.

For determination of the glycogen content was used from the pellet. Glycogen and water-soluble carbohydrate contents were determined using anthrone reagent and D-glucose as the standard, using the colorimetric method (van Handel, 1965). Vanillin reagent and cholesterol were used for measuring total lipid content as the standard (van Handel, 1985b). From centrifuged chloroformmethanol solution (100 μL) was transferred into new microtubes and heated. Ten μL of 98% sulphuric acid was added to each microtube and then incubated at 90°C for 120s. Then, Vanillin reagent was added to each ice pre-cooled microtube. After 15 min incubation at room temperature, absorbance was determined at 540 nm. The different energy reserves (lipids, proteins, carbohydrates,and glycogen) were converted into energetic equivalents. The energy equivalents of these reserves are 39,500 mJ mg^{-1} for lipids, 24,000 mJ mg^{-1} for proteins and 17,500 mJ mg^{-1} for carbohydrates (Gnaiger, 1983).

2.4 Data analysis

Data were analyzed using SPSS software, Version 15 (SPSS Inc., Chicago, IL). Duncan tests were used to compare the average energy contents of the bugs with different diets.

3 Results

Total available energy calculated as the sum of the energy contents of lipids, carbohydrates, glycogen, and proteins did not significantly differ in the third ($F_{2,11} = 4.841$, $P > 0.05$) and fourth ($F_{2,11} = 4.001$, $P > 0.05$) nymphal instars fed on natural and artificial diets (Fig. 1). Among the energy reservoirs, only total lipid storage ($F_{2,8} = 4.874$, $P > 0.05$) and ($F_{2,8} = 4.474$, $P > 0.05$) in third and fourth starved nymphal instars was significantly different from that in the bugs fed on the natural diet (Fig. 2, 3). But there wasn't significant effects on protein ($F_{2,8} = 0.109$, $P > 0.05$) and ($F_{2,8} = 2.495$, $P > 0.05$), glycogen ($F_{2,8} = 1.584$, $P > 0.05$) and ($F_{2,8} = 0.146$, $P > 0.05$) or carbohydrate storage ($F_{2,8} = 0.846$, $P > 0.05$) and ($F_{2,8} = 1.288$, $P > 0.05$) in third and fourth nymphal instars for different diets (Fig. 2, 3). In both of third and fourth instars, the most important energy reserve was lipid (48.72% ,54.02% , 63.86% for starvation, artificial and natural diet, repectively in third instar) and (62.45% , 67.88% , 70.92% for starvation, artificial and natural diet, repectively in fourth instar) that there was significant difference under variety of diets. The soluble carbohydrates content was (22.53% , 21.43% , 17.34% for starvation, artificial and natural diet, repectively in third instar) and (16.04% , 14% , 12.88% for starvation, artificial and natural diet, repectively in fourth instar) .The proteins contentwas (3.58% ,3.58% , 2.86% for starvation, artificial and natural diet, repectively in third instar) and (3.41% , 2.93% , 2.82% for starvation, artificial and natural diet, repectively in fourth instar). Also, Glycogen content was (25.17% , 20.97% , 15.94% for starvation, artificial and natural diet, repectively in third instar) and (18.10% , 15.19% , 13.38% for starvation, artificial and natural diet, repectively in fourth instar).

4 Discussion

The total available energy calculated as the sum of the energy content of lipids, carbohydrates, glycogen, and proteins did not differ statistically between the three diets (Fig. 1). Nymphal diet availability had significant effects on lipid storage between starvation and natural diet, but not protein, glycogen or carbohydrate storage at nymphs in *P. maculiventris* (Fig. 2, 3). Across diets, there was a significant effect of nymphal diet only on lipid content. Across all diets, lipid was stored in greater amounts than proteins or carbohydrates in *P. maculiventris*. It seems that, predator bugs use stored fat for exposure to adverse conditions and in sugar and protein levels are not changed. Also, feeding rate of predator bugs from artificial diet is lower than natural diet and It looks, they were under similar conditions of starvation.

Although the function of nymphal lipid stores in *P. maculiventris* is unknown, carrying over significant lipid reserves, but not carbohydrate or protein reserves, suggests an important function for nymphal lipid reserves. In insects and most other animals, Lipid is the primary stored nutrient, Because of its high-energy content (Downer and Matthews, 1976). For insects to store energy, It is advantageous in the form of lipids because lipids provide almost eight times more energy per unit weight than carbohydrates and two times more metabolic water (Downer and Mathews, 1976; Chapman, 1998; Perez-Mendoza et al., 1999). Lipids generally accumulate during pre-adult stages and they are the major energy reserves for non-feeding or starved insects (Urs and Hopkins, 1973; Lim and Lee, 1981). The use of lipids and carbohydrates as energy sources for locomotion and flight is well documented for different insect orders, including Hemiptera, Orthoptera, Diptera, Lepidoptera, Hymenoptera etc. (Hansford and Johnson, 1975; Martin and Lieb, 1979). Lipids and carbohydrates are also most important resources used, as energy sources during starvation (Newton, 1954; Lim and Lee, 1981; Wheeler and Buck, 1992; Perez-Mendoza et al., 1999; Olson et al., 2000; Satake et al., 2000; Rivero and West, 2002).

Survey of energy content in prediapause and diapausing adult of *Eurygaster integriceps* (Hemiptera:Scutelleridae) showed prediapause sunn pest bugs accumulate lipids for their metabolic needs include dispersal and reproduction during diapause and post-diapause functions (Amiri et al., 2013).

In the grasshoppers *Schistocerca americana*, individuals fed high nutrient content diets were contained a significantly greater amount of lipid stores at adult eclosion, but not carbohydrate or protein stores than individuals fed low nutrient content diets (Hahn, 2005). In the both of grasshoppers *Schistocerca gregaria* and *Locusta migratoria* accumulate significant lipid contents in adulthood for use in dispersal, and both have been shown to accumulate during larval feeding some of these lipid contents (Zanotto et al., 1993; Pener et al., 1997; Raubenheimer and Simpson, 1997; Simpson et al., 2002). Larvae of *L. migratoria* forced to fed diets with an extremely high carbohydrate-to-protein ratio accumulate significant fat reserves and survive starvation in early adulthood better than individuals fed from protein-based diets (Raubenheimerand & Simpson, 1997). The capable of mobilizing the fat body lipid reserves is the reason for the decrease in fat body lipid starvation in *Oxya japonica* (Orthoptera: Acrididae) (Lim and Lee, 1981). Also, Jutsum (1975) showed in male of *Locusta migratoria migratorioides*, starvation promotes a change in haemolymph lipid concentration which is alonged with a decrease in fat body lipid content.

A significant loss of lipid reserves occurred in the lesser grain borer, *Rhyzopertha dominica* from two strains when they were deprived of food for more than 48 h (Perez-Mendoza et al., 1999). A 50% loss in lipid reserves occurred after 72 h of starvation in both strains (Perez-Mendoza et al., 1999). Both lipid reserves and flight initiation decreased rapidly as starvation period increased (Perez-Mendoza et al., 1999). The results offer that lower available energy resources lead the low flight initiation of the lesser grain borer, *Rhyzopertha dominica* deprived of food for more than 48 h (Perez-Mendoza et al., 1999). Carbohydrate and lipid levels reduced with food deprivation in cockroaches by depletion of available reserves (Downer, 1985).

It seems, a bet hedging strategy for ameliorating the effects of unpredictability in future food quality may be reason for accumulating greater lipid contents during periods of excess (Boggs, 1981; Philippi and Seger, 1989; Rogers et al., 1993; Hopper, 1999). Even though the artificial diet supported development in *P. maculiventris*, it is clear that even the best growth diet was less suitable than the natural diet.

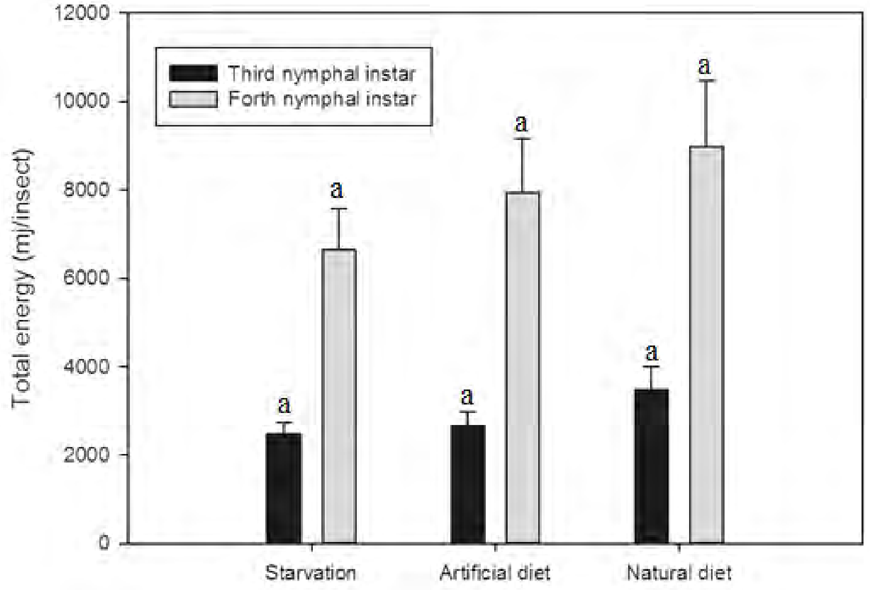

Fig. 1 Total energy content (mean±SE) of the carbohydrate, glycogen, lipid and protein reserves of third and fourth nymphal instars were compared at the same time using the Duncan test; Similar letters indicate non-significant differences between variety of foods.

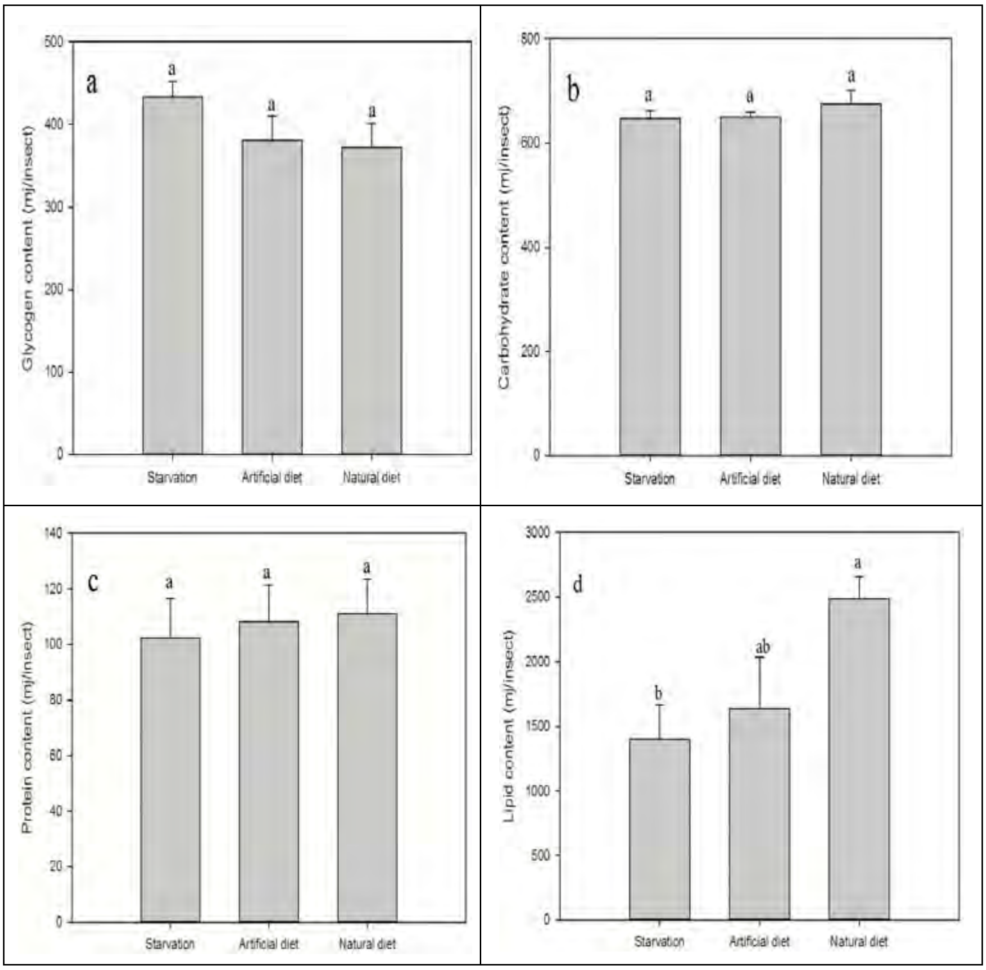

Fig. 2 Average energy equivalents (mean±SE) of the glycogen (a), carbohydrates (b), proteins (c), and lipids (d) in third nymphal instars were compared at the same time using the Duncan test; Similar letters indicate non-significant differences between variety of diets.

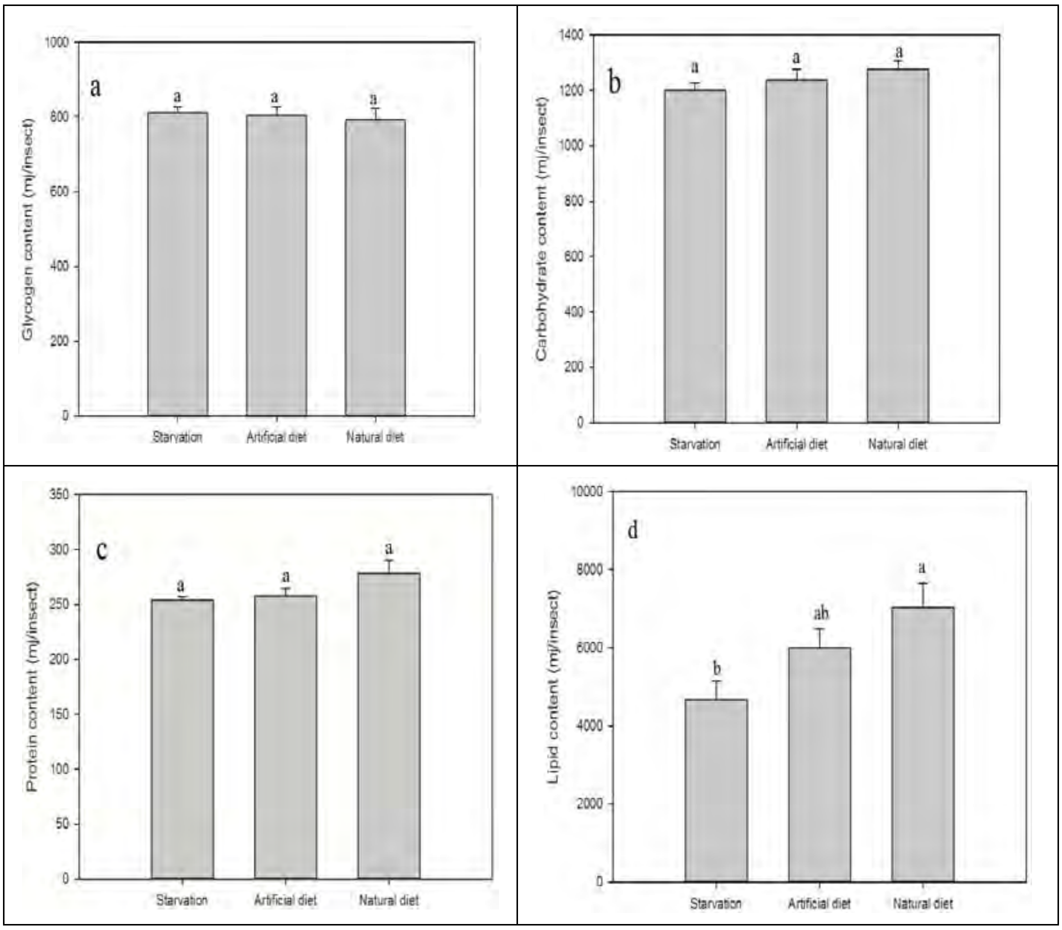

Fig. 3 Average energy equivalents (mean±SE) of the glycogen (a), carbohydrates (b), proteins (c), and lipids (d) in fourth nymphal instars were compared at the same time using the Duncan test; Similar letters indicate non-significant differences between variety of diets.

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