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Different diets affecting biology, physiology and cold tolerance of *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

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ABSTRACT

Biology, some physiological processes and cold hardiness of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) on ten diets (barley, corn, millet, rice, rye, sorghum, triticale, wheat groundnut, and walnut) were studied under laboratory conditions (33 ± 1 °C with 65 ± 5% RH, 14L: 10D). According to the results, the insects reared on triticale had shortest development time, and the highest fecundity and fertility. By contrast, the longest development time, and the lowest fecundity and fertility were on groundnut. The survival rates ranged from 40 to 87% with the lowest values observed on groundnut. *T. granarium* larvae fed on triticale and millet had the highest amylolytic activity. In contrast, groundnut-fed larvae possessed the lowest amylolytic activity. The highest and lowest proteolytic activity was on rice and millet, respectively. Different diets had a significant effect on larval energy reserves (total body sugars, glycogen, lipid, and protein), which were at the highest levels on triticale and rye, and lowest levels on sorghum. The highest level of trehalose was on triticale, groundnut and rye, and the lowest level was on barley and sorghum. The supercooling point (SCP) of larvae reared on triticale was −20.6, which was significantly lower than on the other diets. Exposures to −5 and −10 °C/24 h were somewhat endured by larvae fed on triticale, rye, walnut, and groundnut whilst exposure to −20 °C/24 h caused 100% mortality in these food groups. These results suggest that larval food quality can affect biological and physiological characteristics and influence the supercooling point and cold hardiness of *T. granarium*.
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1. Introduction

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is an important pest of grain, cereal and other stored products throughout the world (Burges, 2008; Borzoui et al., 2015). The larvae of *T. granarium* cause serious damage by loss of carbohydrate, crude fat and protein contents of stored products. Besides the physical damage, the skins and barbed hairs of the larvae cause allergenic diseases (Ahmedani et al., 2009; Borzoui et al., 2015). Control of *T. granarium* populations has been principally achieved through the use of chemical insecticides. However, the use of most insecticides for the control of pests causes damages to human health and the environment (Hagstrum and Subramanyam, 1996; Finkelman et al., 2006). For development of more environmentally-benign alternatives for control of *T. granarium*, it is essential to understand the biological aspects and nutritional physiology of the pest in response to feeding on different host diets.

Insects perform differently when feeding on different diets. Thus, diet can be considered a key factor that potentially affects all life-history components of pests (Naya et al., 2007; Borzoui and Naseri, 2016). Carbohydrates and proteins are particularly important dietary nutrients (Simpson and Raubenheimer, 2012), and although they have identical caloric value, from an insect's perspective, they are functionally very different (Thompson, 2000; Thompson et al., 2002; Behmer and Joern, 2012). Food nutritional levels and proteinaceous inhibitors often affect diet suitability and resistance to insect herbivores (Chen et al., 2008). Many insects have evolved effective strategies to combat the inadequacy of nutrients and inhibitors in their food (Bede et al., 2007). A key step in building links between nutrition and insect performance is clarifying how nutritional challenges affect the expression of digestive enzymes.

Nutritional regulations in insects represent the integrated outcome of a highly complex set of interacting processes (Simpson and Raubenheimer, 1999). Comparative physiological analyses

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often seek to test the hypothesis of correlation between an independent variable, such as dietary carbohydrate and protein, and variations in amylolytic and proteolytic activity (Kotkar et al., 2009; Karasov et al., 2011). The interaction of the nutrient content of diet and digestive abilities to assimilate these nutrients determines the energy levels of insect (Chen et al., 2009). Several studies confirmed that energy storage is critical for survival in low temperature (Li et al., 2000, 2002).

The insects' ability to survive in low temperature varied according to their cryoprotectants (Andreadis and Athanassiou, 2017). On the other hand, energy storage of insects, which affected supercooling points and levels of low-molecular-weight carbohydrates acting as cryoprotectants, closely correlate with the nutritive quality of host plants (Zvereva, 2002). For many years, the use of extreme temperatures, particularly low temperatures, has been extensively used for pest control in storage facilities (Donahaye et al., 1995; Imai and Harada, 2006; Abdelghany et al., 2010; Arthur et al., 2015). However, the information about lethal temperatures for *T. granarium* throughout their distribution area is poor.

Borzoui et al. (2015) reported that different diets differ in their nutrient levels, affect biology and digestive physiology of *T. granarium*. Also, Athanassiou et al. (2016) investigated population growth of *T. granarium* on different commodities. As a review, Wilches et al. (2016) investigated diapause and tolerance to extreme temperatures in dermestids (Coleoptera) such as *T. granarium*.

Although life table of *T. granarium* was studied on different diets, but we additionally tested a wider range of diets as well as examined the effects of different diets on energy reserves, supercooling capacity, and the accumulation of cryoprotectants of *T. granarium* larvae. We hypothesized that these larval diets might contribute to the energy reserves, supercooling capacity and accumulation of cryoprotectants of *T. granarium* larvae. Five aspects were examined in this study: (1) changes in the biology of *T. granarium* that fed on different diets as larvae, (2) changes in energy reserves of *T. granarium* larvae that fed on different diets (3) whether the supercooling capacity and cold hardiness of *T. granarium* larvae would be enhanced after larval feeding on suitable diets or not, (4) to determine the most cold-hardy larvae after feeding on suitable and unsuitable diets, and (5) changes in cryoprotectants content of *T. granarium* larvae that fed on different diets.

2. Materials and methods

2.1. Food sources

Different grains including barley (*Hordeum vulgare* L.; cultivar Yousof), corn (*Zea mays* L.; hybrid Simax), pearl millet (*Pennisetum glaucum* (L.) R. Br.), rice (*Oryza sativa* L.; cultivar Khazar), rye (*Secale cereal* L.; population IRAN IX), sorghum (*Sorghum bicolor* (L.); cultivar Titan), triticale (X Triticosecale Wittmack), and wheat (*Triticum aestivum* L.; cultivar Bam) along with groundnut (*Arachis hypogaea* L.) and walnut (*Juglans regia* L.), were provided from the Plant and Seed Improvement Research Institute (Karaj, Iran). The tested seeds were broken, and then used for the experiments. Moisture content of different grains was measured using the hot air oven method (AOAC, 1984) and varied from 10.6% to 13.2%.

Crude protein concentration of the different diets was measured using the Kjeldahl method (Kjeldahl, 1883).

Starch concentration of the different diets was measured with iodine reagent using starch as a standard (Bernfeld, 1955). Briefly, each diet was powdered thoroughly, and then 200 mg of each diet was homogenized by adding 35 ml of distilled water, and the mixture heated for 10 min in boiling water (90 °C). To determine the amount of starch in different diets, 100 μl of each sample was mixed with 2.5 ml of iodine reagent (0.02% I_2 and 0.2% KI). The amount of starch was determined spectrophotometrically at 580 nm.

2.2. Insect rearing

A laboratory colony was started using *T. granarium* larvae collected from stored rice seeds from Karaj, Iran. The larvae were reared on different diets in a controlled environmental chamber at 33 ± 1 °C with 65 ± 5% RH (by using saturated salt solution) and a photoperiod of 14:10 (L:D) h, for one generation, as described by Borzoui et al. (2015). The second generation was used for the experiments.

2.3. Life history variables

One hundred newly emerged first instar *T. granarium* larvae (within 24 h) were individually transferred into Petri dishes (diameter 6 cm, depth 1 cm), containing 0.2 g of each diet. The Petri dishes were placed in a growth chamber that was set at the above described standard rearing conditions and visited daily. The duration of immature stages (from egg to adult emergence), their survival and adult longevity were recorded. Five replicates with 15 newly emerged fifth instar larvae were weighted on each diet.

To determine daily realized fecundity ($n = 15–45$ of adult pairs), newly emerged adults (one male and one female) were transferred to plastic tubes (diameter 2 cm, height 5 cm) containing 1 g of each host diet. The females were daily transferred to the new tubes with food provided. The eggs were maintained for 15 days to estimate the percentage of hatched eggs (fertility) ($n = 15–45$).

2.4. Digestive enzyme activity in larvae

For enzyme assays, newly molted last instar larvae (within 24 h) were chilled on ice, the posterior and anterior tips of the larvae were removed in 0.15 M NaCl, and the gut was removed from one end. One hundred midguts were homogenized at 4 °C for 3 min in an ice-cold solution of 10 mM NaCl in a pre-cooled homogenizer (Teflon pestle, 0.1 mm clearance). The homogenate was centrifuged at 12,000 *g* for 15 min at 4 °C and the supernatant was stored at −70 °C until use.

The amylolytic activity of extracts was assayed according to the method described by Bernfeld (1955) with some modifications described by Mohammadzadeh et al. (2013). Starch (0.1% w/v) dissolved in 20 mM Tris–HCl (pH 8.0) was used as substrate. In brief, a quantity of 20 μl of the enzyme preparation was incubated with 500 μl of 20 mM Tris–HCl (pH 8.0) at 37 °C. The enzymatic reaction was started by the addition of 40 μl of soluble starch and stopped 30 min later by adding 100 μL of dinitrosalicylic acid (DNS) reagent (Coughlan and Moloney, 1988). Precipitation was caused by cooling (4 °C for 10 min) and the solution was centrifuged at 12,000 *g* for 5 min and the absorbance was read spectrophotometrically at 540 nm. All determinations were done in five replicates. Each five replicate assay included blanks in which the changes in absorbance were recorded without the enzyme extract.

Total protease assay was done according to the methods of Elpidina et al. (2001), and Gatehouse et al. (1999), with slight modification. A typical 100 μl assay consisted of 10 μl of midgut homogenate, 40 μl of glycine-NaOH buffer (pH 10) and 50 μl of 2% azocasein substrate. The reaction was incubated for 60 min at 37 °C and then was stopped by adding 100 μl of 30% trichloroacetic acid, held at 4 °C for 30 min and then centrifuged at 10,000 *g* for 15 min. The supernatant was dissolved in an equal volume of 1.0 M NaOH before recording the absorbance at 405 nm. Blanks, in which TCA

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