

ORIGINAL ARTICLE

## Energy budget changes in response to desiccation stress in *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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### Abstract

Humidity is probably the most important abiotic factor influencing life cycles, distribution, survival, and population dynamics of stored product pests. Although most of these pests can complete their life cycles in any given relative humidity, their prolonged development time, as well as decreased emergence rate and fecundity, have been well documented in several previous studies. In the present study, we evaluated the changes in energetic substances (lipids, soluble carbohydrates, glycogen, and proteins) accumulated in different life stages of larvae and adults of *Tribolium castaneum* in response to different relative humidity levels (5, 12, 22, 30, 45, and 65%). The results showed that young larvae were more susceptible to low relative humidity levels and desiccation stress. Larvae tended to accumulate higher proportions of lipids during earlier stages while their energy content shifted towards proteins with an increase in their age. Adult beetles experienced a significant decrease in their protein content immediately after they initiated reproduction. The importance of these fluctuations in the biology of the red flour beetles was discussed in detail.

**Keywords:** age, energy, protein, relative humidity, *Tribolium castaneum*

## Introduction

The red flour beetle, *Tribolium castaneum* (Herbst), is a common pest of processing plants and indoor storage facilities (Arthur 2000). This pest is one of the most important pests of stored products. This beetle attacks materials of both animal and plant origin, seriously infesting raw stored grain, cereal processing facilities, warehouses, retail stores and home pantries (Zettler 1991; Talukder and Howse 1995; Zettler and Arthur 1997; Collins 1998; Haubruge and Arnaud 2001). In addition to direct damage, stored products infested by *T. castaneum* may contain insect fragments, benzoquinones, discarded skins, as well as individuals of different life stages (Baur 1984).

The moisture content of stored products has critical importance in the survival, development, and

reproduction of stored-product insects (Subramanyam and Hagstrum 1993). Therefore, a variety of morphological, physiological, and behavioral mechanisms have been evolved by these insects for maintaining water balance (Arbogast 2003). It has been generally accepted that the magnitude of the attraction of stored product pests to wheat flour highly depends on the moisture content of the flour (Willis and Roth 1950). Relative humidity affects the distribution of insect pests in stored products (Arbogast 2003). The suitable range of relative humidity, where insects reveal the longest survival period, has been estimated to be between 60% and 98% for *T. castaneum* (Khan 1983). Generally, when organisms live in suboptimal environments (such as high temperature or low relative humidity),

they can not deal with stress in terms of metabolic resources, leading to a decrease in their storage components (carbohydrates, lipids, and proteins) (De Coen *et al.* 2001; Smolders *et al.* 2004; Verslycke *et al.* 2004). To test this hypothesis, for the first time, we evaluated precisely the energy substances (used as a stress indicator) of *T. castaneum* larvae and adults under suboptimal conditions. The amount of energy reserves in this insect (under optimal or stress conditions) has not been previously researched. We used a recently developed biochemical method (Foray *et al.* 2012) (similar to Ahsaei *et al.* 2013; Tabadkani *et al.* 2013) by which both total and individual energy substances can be quantified at the individual level. Simultaneously, we analyzed the energy substances of three and four-time points of two different stages of development, larva and adult, respectively, to elucidate any change in energy composition of *T. castaneum* within a generation.

## Materials and Methods

### Insect rearing and exposure to desiccation stress

A stock colony of *T. castaneum* was established in a growth chamber in the insect physiology laboratory, the University of Tehran at 27–30°C and 70–75% relative humidity in continuous darkness. To prepare an appropriate diet for the beetles, 1 kg wheat flour plus 50 g yeast powder were mixed uniformly in a jar (9.5 × 7.5 cm). Equal numbers of male and female adult beetles ( $n = 50$ ) ( $F_0$ ) were released into the jar for oviposition. After 24 h, all adults were removed and the media were incubated in the growth chamber until the appearance of 1st instar larvae. Those larvae which hatched within 24 h were recorded, in other words they were >0 and <24 h 1st instar larvae. Also, equal numbers of male and female adult beetles ( $n = 50$ ) ( $F_0$ ) were released into the jar for oviposition. After 24 h, all adults were removed and the media were incubated in the growth chamber for the next 28–35 days when the adults of the first generation ( $F_1$ ) appeared. The  $F_1$  generation insects were used for the experiments. These adults were adults which emerged within 24 h, in other words they were >0 and <24 h adults. The adults (equal numbers of >0 and <24 h male and female adults,  $n = 40$ ) and 1st instar larvae ( $n = 40$ ) were transferred to Petri dishes (35 mm in diameter) with different relative humidities and 4 g appropriate diet for each Petri dish. The relative humidity (5, 12, 22, 30, 45, and 65%) was maintained and controlled using saturated solutions (NaOH, lithium chloride, potassium acetate, magnesium chloride, potassium carbonate, and sodium nitrite), respectively (Greenspan 1977; Labuza *et al.* 1985) in glass desiccation chambers at  $30 \pm 2^\circ\text{C}$  3 days

prior to the Petri dishes containing insects were placed in the desiccation chambers. Sampling was conducted from insects exposed to desiccation stress in three and four-time points of two different stages of development, larva, and adult, respectively. In the study on the effect of age on energy reserves, 30% relative humidity was used for different life stages of *T. castaneum* larvae and adults. Three replicates were considered for all assays.

### Sample preparation

The prepared samples (one insect per each microtube) were placed separately in 1.5-ml microtubes containing 180  $\mu\text{l}$  of aqueous lysis buffer solution [100 mM  $\text{KH}_2\text{PO}_4$ , 1 mM dithiothreitol, and 1 mM ethylenediaminetetraacetic acid, pH 7.4] and homogenized using a plastic micropestle for 1 min. The contents of lipid, glycogen, water-soluble carbohydrates and proteins were quantitatively determined using a series of biochemical tests originally developed by Foray *et al.* (2012) with slight modifications as follows.

### Protein assay

Protein contents of both larvae and adults of *T. castaneum* were quantified using the method of Lowry *et al.* (1951) with bovine serum albumin as the standard. The dilution-series of bovine serum albumin was 0.1, 0.3, 0.6, 0.8, 1, and 1.2  $\text{mg} \cdot \text{ml}^{-1}$ . A stock of Lowry Reagent D was made up of Lowry Reagents A [2% (w/v)  $\text{Na}_2\text{CO}_3$  in 0.1 N NaOH], B [0.5% (w/v)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ ] and C [1% (w/v) NaK Tartrate tetrahydrate] at a 48 : 1 : 1 ratio, respectively. Also, this assay employs Folin-Ciocalteu phenol reagent. Fifty  $\mu\text{l}$  of the protein extract was added to individual 1.5 ml microtubes, followed by 950  $\mu\text{l}$  of Lowry Reagent D by mixing. After incubation of samples for 10 min at room temperature, 100  $\mu\text{l}$  of the Folin-Ciocalteu phenol reagent was added to each microtube. The plate was homogenized, incubated at room temperature for 30 min and its absorbance was measured spectrophotometrically at 630 nm.

### Glycogen and soluble carbohydrate assay

Total carbohydrates were dissolved by adding 20  $\mu\text{l}$  of sodium sulfate solution to 180  $\mu\text{l}$  homogenate to reach a final concentration of 20% sodium sulfate (van Handel 1965; van Handel and Day 1988). This solution was then mixed with 1,500  $\mu\text{l}$  of a chloroform-methanol solution (1 : 2 v/v) to solubilize the total lipids, as well as the water-soluble carbohydrates (van Handel 1965; van Handel and Day 1988). After vigorous agitating, each sample was centrifuged for 15 min at  $180 \times g$  and  $4^\circ\text{C}$  to remove glycogen from the supernatant. The resultant supernatant was transferred to a new tube for subsequent analysis. The pellet was also

kept for further determination of the glycogen content. All carbohydrates, including glycogen and the soluble carbohydrates, were quantified using the colorimetric method based on anthrone reagent and D-glucose as the standard (van Handel 1965) [see Foray *et al.* (2012) for more details]. The dilution-series of the standard was 0.1, 0.3, 0.6, 0.8, 1, and 1.2 mg · ml<sup>-1</sup>. The absorbance was measured spectrophotometrically at 625 nm.

### Lipid assay

The total lipid content was determined using vanillin reagent (van Handel 1985) with triolein as the standard. The dilution-series of the standard was 0.3, 0.6, 0.8, 1, 1.2, and 1.4 mg · ml<sup>-1</sup>. One hundred microliters of the supernatants, from the centrifuged chloroform-methanol solution (see above), were transferred to new borosilicate microplate wells and heated until complete solvent evaporation. Ten microliters of 98% sulfuric acid were then added to each well and the complex was incubated at 90°C for 2 min in a water bath. After cooling the microplate on ice, 190 µl of vanillin reagent was added to each well. The plate was homogenized, incubated at room temperature for 15 min and its absorbance was measured spectrophotometrically at 525 nm.

Components of energy reserves (lipids, proteins, soluble carbohydrates, and glycogens) were transformed to their corresponding energetic equivalents. The equivalents of these reserves are 39,500 mJ · mg<sup>-1</sup> for lipids, 24,000 mJ · mg<sup>-1</sup> for proteins, and 17,500 mJ · mg<sup>-1</sup> for carbohydrates (Gnaiger 1983).

### Statistical analyses

All data were analyzed using SPSS computer software (Version 17.1). To clarify any statistical differences, the average values of energy components in water-stressed insects in larval and adult stages were exposed to the analysis of variance (one-way ANOVA) ( $p < 0.05$ ) with a Tukey-HSD *post-hoc* test to compare specific differences. For statistical analysis of the quantification of energy substances in three different life stages of the larvae of *T. castaneum* in six different relative humidity levels, a two-way ANOVA (a two-factor factorial design) was conducted with both age and relative humidity as factors in the model. The statistical analysis was the outcome of the interaction effect between relative humidity and age.

## Results

### Effect of age on energy substances

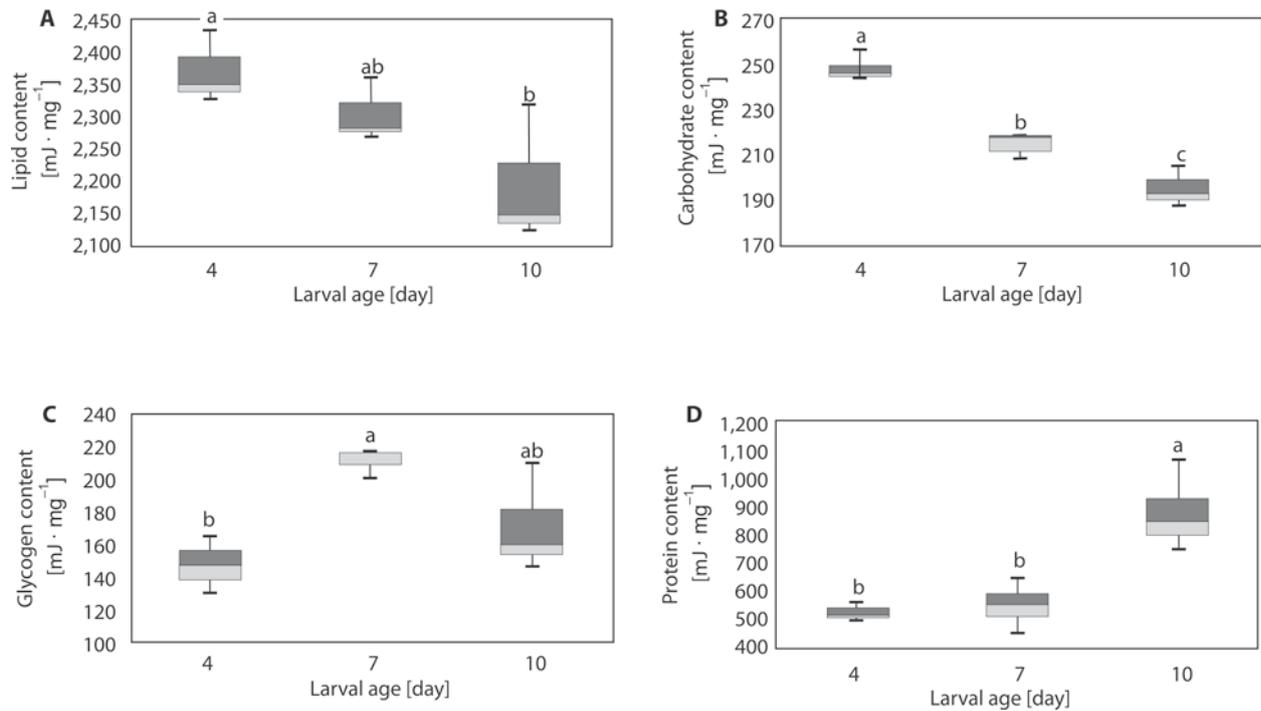
The results showed significant differences in the average content of all energetic substances (lipids, soluble

carbohydrates, glycogen, and proteins) accumulated during different life stages of *T. castaneum* larvae in the average of different relative humidity levels (30%) (Fig. 1). The largest amounts of lipids and soluble carbohydrates were observed in the first-time point of larvae followed by the second and third-time points of larvae, respectively (Fig. 1A) (one-way ANOVA,  $F_{2,8} = 4.07$ ,  $p = 0.076$  for lipids and  $F_{2,8} = 46.39$ ,  $p = 0.00$ ) (Fig. 1A and B). The glycogen content was the greatest in the second-time point of larvae followed by the third and first-time points of larvae, respectively (one-way ANOVA,  $F_{2,8} = 6.59$ ,  $p = 0.031$ ) (Fig. 1C). The third-time point of larvae accumulated, in contrast, significantly larger amounts of proteins compared to the two other life stages (one-way ANOVA,  $F_{2,8} = 9.29$ ,  $p = 0.015$ ). The protein content of the first and second-time points of larvae was not statistically different (Fig. 1D). Although the larvae accumulated different levels of energetic fractions at each age (see Fig. 1), the total content of energetic substances, calculated by summing the individual content of all fractions (i.e. lipids, carbohydrates, glycogen, and proteins), the difference between the three studied life stages was not statistically different (one-way ANOVA,  $F_{2,8} = 0.88$ ,  $p = 0.46$ ) (Fig. 2).

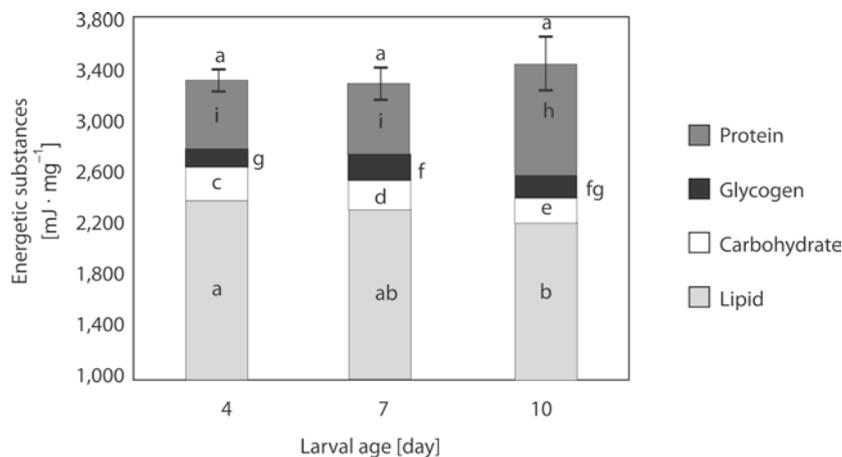
The amounts of lipids, carbohydrates, and glycogen increased as a result of an increase in the age of adult beetles (30% relative humidity). Although there was a significant difference in the contents of these substances between different ages (one-way ANOVA,  $F_{3,11} = 5.76$ ,  $p = 0.021$  for lipids,  $F_{3,11} = 16.45$ ,  $p = 0.001$  for soluble carbohydrates, and  $F_{3,11} = 27.8$ ,  $p = 0.00$  for glycogen), no specific trend was observed in the changes of these fractions in response to increased beetle age (Fig. 3). The protein content of the first-time point of adults was significantly greater than that of the three other studied stages. From the 7th day onward, a statistically constant amount of protein was detected in adult beetles (one-way ANOVA,  $F_{3,11} = 22.63$ ,  $p = 0.00$ ). The overall content of energetic substances peaked in the first-time point of adult beetles but experienced a significant decrease in the second-time point of beetles. From this time onward, the total content of energetic fractions was slightly increased (one-way ANOVA,  $F_{3,11} = 5.66$ ,  $p = 0.022$ ) (Fig. 4).

### Effect of relative humidity on energy substances

The results of quantification of energy substances in three different life stages of the larvae of *T. castaneum* in six different relative humidity levels are summarized in Tables 1 and 2 [mJ per weight (mg) of the larva (or adult) and mg substance per weight (mg) of the larva (or adult)]. As Table 1 shows, the interaction effect of relative humidity and age on the energy content



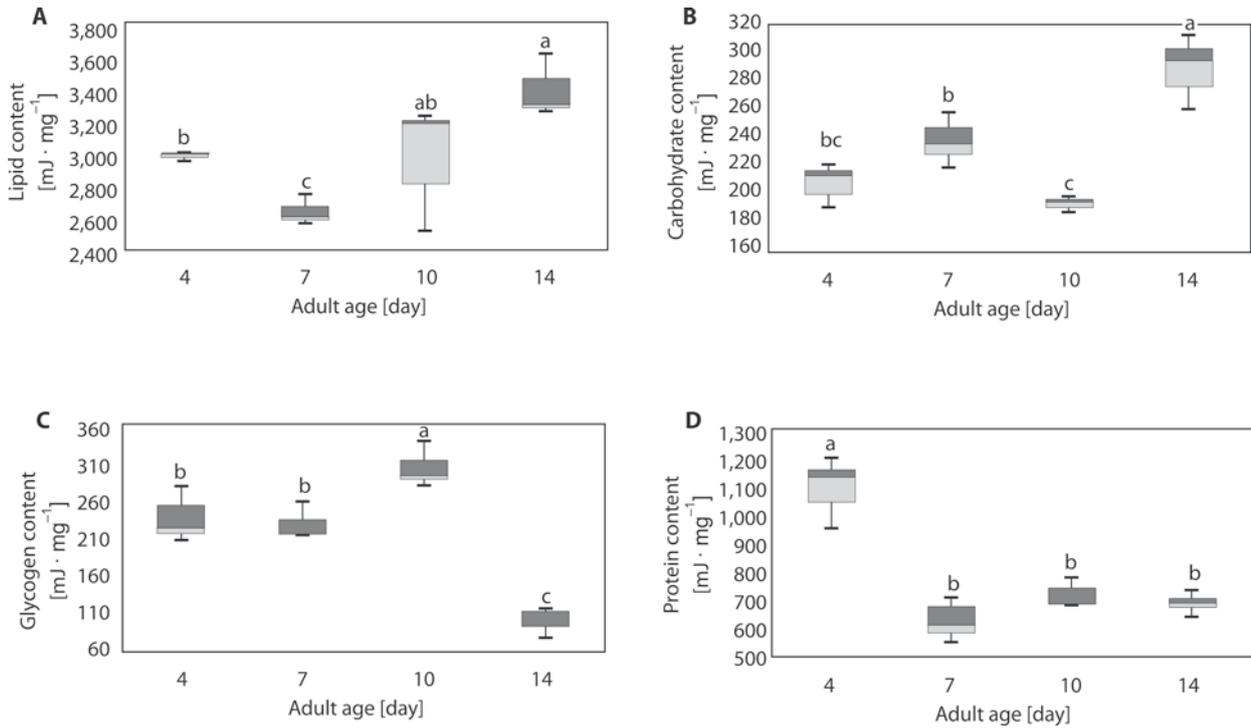
**Fig. 1.** Box plots of average energy equivalents of (A) lipids, (B) carbohydrates, (C) glycogen, (D) proteins in the larvae of *Tribolium castaneum* at different ages and the average of different relative humidity levels (30%); similar letters indicate non-significant differences between different ages ( $p < 0.05$ , 5% significance level)



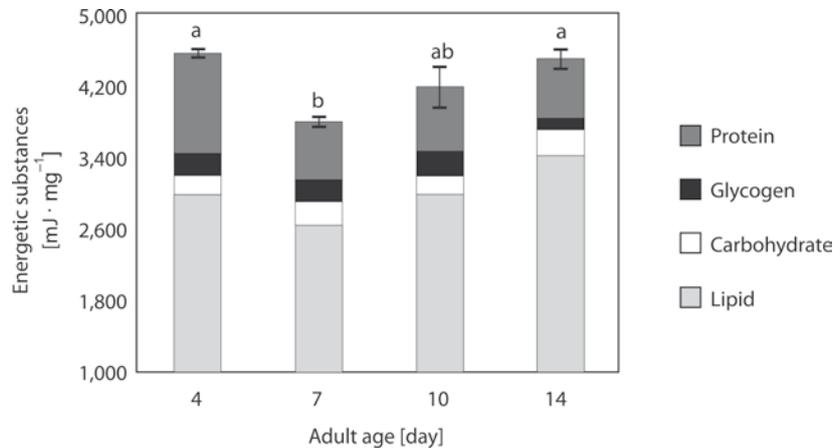
**Fig. 2.** The total contents of energetic substances (mean  $\pm$  SE) at humidity levels (5, 12, 22, 30, 45, and 65%) calculated by summing the individual contents of all fractions (i.e. lipids, carbohydrates, glycogen, and proteins) in different larval life stages in *Tribolium castaneum*; similar letters indicate non-significant differences between different stages ( $p < 0.05$ , 5% significance level) ( $n = 3$ )

showed a significant difference (two-way ANOVA,  $p < 0.05$ ). Despite these statistical differences, no specific trend was observed in energetic substances in response to relative humidity. The largest amounts of lipids and soluble carbohydrates were observed in 4-day-old larvae with a relative humidity of 45% followed by 4-day-old larvae with a relative humidity of 5%, respectively (two-way ANOVA,  $F_{10,53} = 6.39$ ,  $p = 0.000$  for lipids and  $F_{10,53} = 212.87$ ,  $p = 0.00$  for soluble carbohydrates). No specific trend was observed in the amount

of lipids at different ages and relative humidities. But the amount of soluble carbohydrates in 4-day-old larvae (5% relative humidity) was significantly different between various ages and relative humidities. The glycogen content was the greatest in 10-day-old larvae, 30% relative humidity (two-way ANOVA,  $F_{10,53} = 45.66$ ,  $p = 0.000$ ). The 4- and 10-day-old larvae, 65% relative humidity, had significantly more proteins compared to 7-day-old larvae and other relative humidities (two-way ANOVA,  $F_{10,53} = 3.87$ ,  $p = 0.001$ ).



**Fig. 3.** Box plots of average energy equivalents of (A) lipids, (B) carbohydrates, (C) glycogen, and (D) proteins in the adults of *Tribolium castaneum* at different ages and the average of different relative humidity levels (30%); similar letters indicate non-significant differences between different ages ( $p < 0.05$ , 5% significance level)



**Fig. 4.** The total contents of energetic substances (mean  $\pm$  SE) at humidity levels (5, 12, 22, 30, 45, and 65%) calculated by summing the individual contents of all fractions (i.e. lipids, carbohydrates, glycogen, and proteins) in different adult life stages in *Tribolium castaneum*; similar letters indicate non-significant differences between different stages ( $p < 0.05$ , 5% significance level) ( $n = 3$ )

A comparison of total energy, calculated as the sum of energetic equivalents of all four energetic substances, revealed that the first and second-time points of larvae accumulated more energy in high relative humidity (45% and 65%) (one-way ANOVA,  $F_{5,17} = 5.25$ , and  $F_{5,17} = 3.59$  for the first and second-time points of larvae, respectively,  $p = 0.00$ ). In the third-time point of larvae, however, no statistical difference was observed in overall energy accumulated in the larval bodies in different relative humidities (one-way ANOVA,

$F_{5,17} = 1.42$ ,  $p = 0.00$ ). In contrast to the larval stage, the total energy of adult beetles maximized in lower relative humidities (an exception was the third-time point of beetles that accumulated higher rates of energy in higher humidities) (Table 3).

As in the larval stage, although significant differences were observed in energy components of each life stage in different relative humidities, no specific trend was found between changes in relative humidity and energy accumulation of *T. castaneum* adults.

**Table 1.** The average values (mean  $\pm$  SE) ( $\text{ml} \cdot \text{mg}^{-1}$ ) obtained for energetic substances (lipids, soluble carbohydrates, glycogen, and proteins) of three different life stages of *Tribolium castaneum* larvae feeding on wheat flour under six different humidity conditions

Energy source	Age [day]	Relative humidity [%]					
		5	12	22	30	45	65
Lipid	4	2,376 $\pm$ 64.4 abc*	2,234 $\pm$ 30.0 bcd	2,191 $\pm$ 29.3 bcd	2,240 $\pm$ 377.9 bcd	2,690 $\pm$ 121.9 a	2,451 $\pm$ 68.6 abc
	7	2,136 $\pm$ 80.2 cd	2,373 $\pm$ 129.2 abc	2,087 $\pm$ 18.5 cd	2,321 $\pm$ 126.8 bcd	2,430 $\pm$ 213.9 abc	2,439 $\pm$ 104 abc
	10	2,181 $\pm$ 128.4 bcd	2,163 $\pm$ 188.7 bcd	2,597 $\pm$ 34.1 ab	1,874 $\pm$ 49.1 d	2,098 $\pm$ 83 cd	2,263 $\pm$ 67.1 bcd
Carbohydrate	4	292.2 $\pm$ 9.2 a	249.8 $\pm$ 1.7 cd	253.3 $\pm$ 15.4 c	212.5 $\pm$ 7.14 fg	196.1 $\pm$ 7.62 hi	276.5 $\pm$ 8.56 b
	7	247 $\pm$ 2.39 cde	220.9 $\pm$ 10.5 f	242.3 $\pm$ 18.9 de	203.1 $\pm$ 9.60 gh	185.6 $\pm$ 7.17 jk	173.3 $\pm$ 6.36 l
	10	177.1 $\pm$ 1.4 kl	194.6 $\pm$ 9.2 hij	238.7 $\pm$ 17.9 e	193.4 $\pm$ 1.92 ij	174.2 $\pm$ 12.2 l	174.6 $\pm$ 11.1 l
Glycogen	4	199.1 $\pm$ 9.5 bc	246.7 $\pm$ 21.1 ab	196.0 $\pm$ 15.8 bc	67.1 $\pm$ 5.56 f	77.9 $\pm$ 18.5 ef	86.8 $\pm$ 9.42 ef
	7	262.5 $\pm$ 38.3 cd	262.3 $\pm$ 25.5 c	177.4 $\pm$ 31.9 c	125.2 $\pm$ 26.7 de	188.4 $\pm$ 26.1 c	245.2 $\pm$ 12.2 ab
	10	167.4 $\pm$ 7.1 cd	177.0 $\pm$ 16.1 c	192.3 $\pm$ 6.9 c	267.1 $\pm$ 15.6 a	115.1 $\pm$ 16.7 ef	96.9 $\pm$ 2.46 ef
Protein	4	376.8 $\pm$ 15.3 f	358.7 $\pm$ 17.4 f	363.0 $\pm$ 49.9 f	361.1 $\pm$ 18.8 f	652.3 $\pm$ 73.1 bcdef	1,059 $\pm$ 25.7 a
	7	602.9 $\pm$ 63.6 cdef	551.8 $\pm$ 13.2 def	342.5 $\pm$ 43.4 f	463.6 $\pm$ 41.3 ef	712.0 $\pm$ 101 bcde	712 $\pm$ 101 bcde
	10	711.4 $\pm$ 93.3 bcde	934.9 $\pm$ 7.6 abc	720.6 $\pm$ 30.7 bcde	833.1 $\pm$ 253.4 abcd	967.1 $\pm$ 183.3 ab	1,071.6 $\pm$ 10.5 a

\*similar letters indicate non significant differences between different stages and humidities in each energy source using Tukey's test (two-way ANOVA,  $p < 0.05$ , 5% significance level)

**Table 2.** The average values (mean  $\pm$  SE) ( $\text{mg} \cdot \text{mg}^{-1}$ ) obtained for energetic substances (lipids, soluble carbohydrates, glycogen, and proteins) of three different life stages of *Tribolium castaneum* larvae feeding on wheat flour under six different humidity conditions

Energy source	Age [day]	Relative humidity [%]					
		5	12	22	30	45	65
Lipid	4	0.060 $\pm$ 0.0010 a*	0.056 $\pm$ 0.0007 a	0.055 $\pm$ 0.0007 a	0.056 $\pm$ 0.0090 a	0.068 $\pm$ 0.0030 a	0.062 $\pm$ 0.0010 a
	7	0.054 $\pm$ 0.0020 a	0.060 $\pm$ 0.0030 a	0.052 $\pm$ 0.0004 a	0.058 $\pm$ 0.0030 a	0.061 $\pm$ 0.0050 a	0.061 $\pm$ 0.0020 a
	10	0.055 $\pm$ 0.0030 bc	0.054 $\pm$ 0.0040 bc	0.065 $\pm$ 0.0008 a	0.047 $\pm$ 0.0010 c	0.053 $\pm$ 0.0020 bc	0.057 $\pm$ 0.0010 b
Carbohydrate	4	0.016 $\pm$ 0.0005 a	0.014 $\pm$ 0.0000 b	0.014 $\pm$ 0.0008 b	0.012 $\pm$ 0.0004 c	0.011 $\pm$ 0.0004 c	0.015 $\pm$ 0.0004 ab
	7	0.014 $\pm$ 0.0001 a	0.012 $\pm$ 0.0005 ab	0.013 $\pm$ 0.0010 a	0.011 $\pm$ 0.0005 bc	0.010 $\pm$ 0.0003 c	0.009 $\pm$ 0.0003 c
	10	0.010 $\pm$ 0.0007 b	0.011 $\pm$ 0.0005 b	0.013 $\pm$ 0.0009 a	0.011 $\pm$ 0.0001 b	0.009 $\pm$ 0.0006 b	0.009 $\pm$ 0.0005 b

**Table 2.** The average values (mean  $\pm$  SE) ( $\text{mg} \cdot \text{mg}^{-1}$ ) obtained for energetic substances (lipids, soluble carbohydrates, glycogen, and proteins) of three different life stages of *Tribolium castaneum* larvae feeding on wheat flour under six different humidity conditions – continuation

Energy source	Age [day]	Relative humidity [%]					
		5	12	22	30	45	65
Glycogen	4	0.011 $\pm$ 0.0005 b	0.014 $\pm$ 0.0010 a	0.011 $\pm$ 0.0008 b	0.003 $\pm$ 0.0002 c	0.004 $\pm$ 0.0009 c	0.004 $\pm$ 0.0004 c
	7	0.015 $\pm$ 0.0020 a	0.014 $\pm$ 0.0010 a	0.010 $\pm$ 0.0010 ab	0.007 $\pm$ 0.0010 b	0.010 $\pm$ 0.0010 ab	0.014 $\pm$ 0.0006 a
	10	0.009 $\pm$ 0.0003 ab	0.010 $\pm$ 0.0009 ab	0.010 $\pm$ 0.0003 ab	0.015 $\pm$ 0.0060 a	0.006 $\pm$ 0.0008 ab	0.005 $\pm$ 0.0001 b
Protein	4	0.015 $\pm$ 0.0006 c	0.014 $\pm$ 0.0006 c	0.015 $\pm$ 0.0020 c	0.015 $\pm$ 0.0007 c	0.027 $\pm$ 0.0030 b	0.044 $\pm$ 0.0010 a
	7	0.025 $\pm$ 0.0020 ab	0.022 $\pm$ 0.0005 abc	0.014 $\pm$ 0.0010 c	0.019 $\pm$ 0.0010 bc	0.029 $\pm$ 0.0040 a	0.029 $\pm$ 0.0040 a
	10	0.029 $\pm$ 0.0030 a	0.038 $\pm$ 0.0003 a	0.030 $\pm$ 0.0010 a	0.034 $\pm$ 0.0100 a	0.040 $\pm$ 0.0070 a	0.044 $\pm$ 0.0004 a

\*similar letters indicate non significant differences between different stages and humidities in each row using Tukey's test ( $p < 0.05$ , 5% significance level)

**Table 3.** The total energy (mean  $\pm$  SE) ( $\text{mJ} \cdot \text{mg}^{-1}$ ) accumulated in larval and adult stages of *Tribolium castaneum* under six different relative humidities calculated as sum of the energetic equivalents of lipids, carbohydrates, glycogen, and proteins

Life stage	Age [day]	Relative humidity [%]					
		5	12	22	30	45	65
Larvae	4	3,235 $\pm$ 85 bc*	3,089 $\pm$ 30 bc	3,003 $\pm$ 47 c	2,881 $\pm$ 385 c	3,617 $\pm$ 60 ab	3,874 $\pm$ 79 a
	7	3,248 $\pm$ 170 ab	3,408 $\pm$ 156 a	2,849 $\pm$ 53 b	3,113 $\pm$ 163 ab	3,516 $\pm$ 179 a	3,570 $\pm$ 90 a
	10	3,237 $\pm$ 218 a	3,470 $\pm$ 204 a	3,749 $\pm$ 58 a	3,167 $\pm$ 222 a	3,355 $\pm$ 143 a	3,606 $\pm$ 72 a
Adults	4	4,865 $\pm$ 316 a	4,854 $\pm$ 41 a	4,346 $\pm$ 195 a	4,540 $\pm$ 234 a	4,393 $\pm$ 181 a	4,273 $\pm$ 101 a
	7	3,500 $\pm$ 129 abc	5,036 $\pm$ 198 a	3,799 $\pm$ 422 ab	4,122 $\pm$ 292 a	3,044 $\pm$ 27 bc	2,865 $\pm$ 82 c
	10	4,547 $\pm$ 206 ab	3,447 $\pm$ 342 b	3,479 $\pm$ 147 b	3,811 $\pm$ 324 b	5,268 $\pm$ 585 a	4,468 $\pm$ 268 ab
	14	4,459 $\pm$ 185 a	4,610 $\pm$ 203 a	4,672 $\pm$ 368 a	4,422 $\pm$ 256 a	4,390 $\pm$ 590 a	4,116 $\pm$ 220 a

\*similar letters indicate non significant differences between different stages and humidities in each row using Tukey's test ( $p < 0.05$ , 5% significance level)

## Discussion

In this study, a recently developed biochemical method was applied to evaluate the energy accumulation changes of larvae and adults of *T. castaneum*, in response to changes in their age and relative humidity of their rearing environment. In both larval and adult stages, lipids were the most important fractions (68.9% and 70.9% of the total energy reserves in larvae and adults, respectively) followed by proteins (19.1% and 18.3% of total energy). This means that lipids were quantitatively the most important energy sources in *T. castaneum*. Studies on other insects also showed that lipids were the major component of energy substances representing more than 50% of the total dry weight (Arrese and Soulages 2010). Storage of energy in lipids has some advantages to insects because lipids provide almost two times more metabolic water and almost eight times more energy than carbohydrates (Perez-Mendoza *et al.* 1999; Davidowitz *et al.* 2003).

Humidity is probably the most important abiotic factor affecting the distribution, life cycles, survival, and population dynamics of insects in warehouses (Sinha 1973; Flinn and Hagstrum 1990). Although it has been suggested that both larval and pupal stages of *Tribolium* beetles are eventually completed in a wide range of relative humidity (0–85%), prolonged larval development time is a well-known effect of living under low humidity conditions (Howe 1956; Howe 1960; Chaudhry and Kapoor 1968; Imura and Nakakita 1984; White 1987). We found no specific trend between changes in relative humidity and individual energy accumulation of *T. castaneum* larvae. By considering the total energy, it was revealed that young larvae accumulated significantly higher rates of energetic substances in higher relative humidities, while older larvae showed no difference in energy accumulation under different relative humidities. These findings imply that young larvae are more susceptible to desiccation stress and have less energy accumulation under inappropriate humidities. Older stages are more resistant to desiccation and changes in the relative humidity of their environment. The inability to accumulate energetic substances by larvae may subsequently lead to decreased overall fitness because many other biological characteristics of adult insects are strongly affected by the nutritional status during pre-adult stages (Dmitriew and Rowe 2011). There is clear evidence that insects experiencing unbalanced nutrition during larval stages show lower fecundity (Awmack and Leather 2002), increased susceptibility to pathogens (Lord 2010), and smaller body size (Arrese and Soulages 2010; Dmitriew and Rowe 2011) during maturation. Additionally, it is believed that organisms living in suboptimal environments suffer stress in terms of metabolic resources and undergo

a decrease in their storage components (De Coen *et al.* 2001; Smolders *et al.* 2004; Verslycke *et al.* 2004). So, it is not surprising that larvae of *T. castaneum* reared under suboptimal humidity conditions accumulate fewer energetic substances compared to those living in appropriate humidity.

Although proteins are typically stored to be used in morphogenesis, metamorphosis, and reproduction (Brown 1980), storage of lipids is essential because they are the most important reserve used by insects to meet their energy needs during diapause and starvation. Furthermore, they provide energy for developing embryos, and supply fuel during prolonged periods of flight (Arrese and Soulages 2010). Additionally, lipids are the precursors for biosynthesis of many biologically important molecules, such as eicosanoids, pheromones, phospholipids, and waxes (Arrese and Soulages 2010). More importantly, lipids provide a more efficient source of metabolic water upon oxidation than glycogen. Therefore, the accumulation of energy in lipids seems to be essential for insects such as *T. castaneum* that live in extremely dry environments with no access to water. Lipid and protein metabolism are similar under starvation or desiccation stress in *Drosophila* and in some cases desiccated flies appeared to synthesize proteins and lipids (Marron *et al.* 2003). The limited data suggest that most insects depend on lipid metabolism when exposed to desiccation stress (Loveridge and Bursell 1975; Nicolson 1980; Nicolson 1990). Marron *et al.* (2003) suggested that metabolic water derived from lipids may compensate for respiratory losses and cuticular transpiration. As Table 1 shows, the larvae of *T. castaneum* retain nearly constant rates of lipids in different relative humidities. However, its protein content is significantly reduced as a result of rearing under low relative humidity conditions, implying that the cost associated with desiccation stress is reflected in protein content, but not in lipid content. Both are essential for normal development of the larvae.

Quantification of energetic substances in different life stages of *T. castaneum* larvae revealed that although the total energy was not statistically different between the three studied life stages, the older larvae experienced a significant decrease in their lipid and soluble carbohydrate contents. These decreases were accompanied by a significant increase in the protein content of the larvae implying that with age, the destination of energy accumulation is shifted from lipids towards proteins. This probably should provide enough proteins needed for morphogenesis and metamorphosis in the adult. The energy accumulation of adult beetles did not show a definite pattern during their first 14-day period except for proteins that peaked in the first-time point of beetles and then, reached a constant rate during further stages. The total energy of adults maximized in the first-time point of beetles, and was

followed by a significant decrease in the second-time point of beetles. There was a slightly increasing trend during further stages. The higher rates of proteins and total energy during the first days after adult emergence can be explained by the fact that their larvae had accumulated high levels of proteins during their last developmental days and that the beetles had not yet undergone morphogenesis and metamorphosis.

Our current study suggests that the larvae of *T. castaneum* change the pathway of energy accumulation from lipids to proteins probably due to their need for protein for metamorphosis. Additionally, the young larvae accumulate lower contents of energetic substances in low relative humidities. Since the larvae living in low humidity conditions, fail to reserve enough energetic substances during their pre-adult stage, it is expected that their survival and fitness is affected negatively by desiccation stress. It will be useful for future research to analyze the energetic substances in the pupal stage of *T. castaneum*.

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## References

- Ahsaei S.M., Tabadkani S.M., Hosseinaveh V., Allahyari H., Bigham M. 2013. Differential accumulation of energy by the different colour morphs of the pea aphid mirrors their ecological adaptations. *European Journal of Entomology* 110 (2): 241–245. DOI: 10.14411/eje.2013.035
- Arbogast R.T. 2003. Humidity response of adult male *Oryzaephilus surinamensis* (Coleoptera: Cucujidae) with special reference to the effect of carbon dioxide. *Environmental Entomology* 32 (2): 264–269. DOI: <https://doi.org/10.1603/0046-225X-32.2.264>
- Arrese E.L., Soulages J.L. 2010. Insect fat body: energy, metabolism, and regulation. *Annual Review of Entomology* 55: 207–225. DOI: 10.1146/annurev-ento-112408-085356
- Arthur F.H. 2000. Impact of accumulated food on survival of *Tribolium castaneum* on concrete treated with cyfluthrin wettable powder. *Journal of Stored Product Research* 36 (1): 15–23. DOI: [https://doi.org/10.1016/S0022-474X\(99\)00022-3](https://doi.org/10.1016/S0022-474X(99)00022-3)
- Awmack C.S., Leather S.R. 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* 47: 817–844. DOI: 10.1146/annurev.ento.47.091201.145300
- Baur F.J. 1984. Introductory background information. p. 1–14. In: “Insect Management for Food Storage and Processing” (F.J. Baur, ed.). American Association of Cereal Chemists, St. Paul, Minnesota, USA, 384 pp.
- Brown J.J. 1980. Haemolymph protein reserves of diapausing and nondiapausing codling moth larvae, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *Journal of Insect Physiology* 26 (7): 487–491. DOI: 10.1016/0022-1910(80)90121-3
- Chaudhry H.S., Kapoor R.P.D. 1968. Effect of relative humidity on the developmental stages of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Annals of the Entomological Society of America* 61 (2): 290–292. DOI: <https://doi.org/10.1093/aesa/61.2.290>
- Collins P.J. 1998. Inheritance of resistance to pyrethroid insecticides in *Tribolium castaneum* (Herbst). *Journal of Stored Product Research* 34: 395–401. DOI: [https://doi.org/10.1016/S0022-474X\(98\)00020-4](https://doi.org/10.1016/S0022-474X(98)00020-4)
- Davidowitz G., D’Amico L.J., Nijhout H.F. 2003. Critical weight in the development of insect body size. *Evolution and Development* 5 (2): 188–197. DOI: 10.1046/j.1525-142-X.2003.03026.x
- De Coen W.M., Janssen C.R., Giesy J.P. 2001. Biomarker applications in ecotoxicology: bridging the gap between toxicology and ecology. p. 13–25. In: “New Microbiotests for Routine Toxicity Screening and Biomonitoring” (G. Persoone, C. Janssen, W. De Coen, eds.). Kluwer Academic Press, Dordrecht, Netherlands, 550 pp.
- Dmitriew C., Rowe L. 2011. The effects of larval nutrition on reproductive performance in a food-limited adult environment. *PLoS ONE* 6 (3): e17399. DOI: <https://doi.org/10.1371/journal.pone.0017399>
- Flinn P.W., Hagstrum D.W. 1990. Simulations comparing the effectiveness of various stored grain management practices used to control of *Rhyzopertha dominica*. *Environmental Entomology* 19 (3): 725–729. DOI: 10.1093/ee/19.3.725
- Foray V., Pelisson P.F., Bel-Venner M.C., Desouhant E., Venner S., Menu F., Giron D., Rey B. 2012. A handbook for uncovering the complete energetic budget in insects: the van Handel’s method (1985) revisited. *Physiological Entomology* 37 (3): 295–302. DOI: <https://doi.org/10.1111/j.1365-3032.2012.00831.x>
- Gnaiger E. 1983. Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. p. 337–345. In: “Polarographic Oxygen Sensors, Aquatic and Physiological Applications” (E. Gnaiger, H. Forstner, eds.). Springer, Berlin, Germany, 370 pp.
- Greenspan L. 1977. Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards – A. Physics and Chemistry* 81 (1): 89–96. DOI: 10.6028/jres.081A.011
- Howe R.W. 1956. The effect of temperature and humidity on the rate of development and mortality of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Annals of Applied Biology* 44 (2): 356–368. DOI: <https://doi.org/10.1111/j.1744-7348.1956.tb02128.x>
- Howe R.W. 1960. The effect of temperature and humidity on the rate of development and mortality of *Tribolium confusum* Duval (Coleoptera: Tenebrionidae). *Annals of Applied Biology* 48 (2): 363–376. DOI: <https://doi.org/10.1111/j.1744-7348.1960.tb03532.x>
- Haubruege E., Arnaud L. 2001. Fitness consequences of malathion-specific resistance in red flour beetle (Coleoptera: Tenebrionidae) and selection for resistance in the absence of malathion. *Journal of Economic Entomology* 94 (2): 552–557. DOI: <https://doi.org/10.1603/0022-0493-94.2.552>
- Imura O., Nakakita H. 1984. The effect of temperature and relative humidity on the development of *Tribolium freemani* Hinton (Coleoptera: Tenebrionidae). *Journal of Stored Product Research* 20 (2): 87–95. DOI: [https://doi.org/10.1016/0022-474X\(84\)90014-6](https://doi.org/10.1016/0022-474X(84)90014-6)
- Khan M.A. 1983. Effect of relative humidity on adults of 10 different species of stored product beetles. *Journal of Applied Entomology* 95 (1–5): 217–227. DOI: <https://doi.org/10.1111/j.1439-0418.1983.tb02635.x>
- Labuza T.P., Kaanane A., Chen Y. 1985. Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. *Journal of Food Science* 50 (2): 385–392. DOI: <https://doi.org/10.1111/j.1365-2621.1985.tb13409.x>
- Lord J.C. 2010. Dietary stress increases the susceptibility of *Tribolium castaneum* to *Beauveria bassiana*. *Journal of Economic Entomology* 103 (5): 1542–1546. DOI: <https://doi.org/10.1603/EC09311>

- Loveridge J.P., Bursell E. 1975. Studies on the water relations of adult locusts (Orthoptera: Acrididae). 1. Respiration and the production of metabolic water. *Bulletin of Entomological Research* 65 (1): 13–20. DOI: <https://doi.org/10.1017/S0007485300005708>
- Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193 (1): 267–275.
- Marron M.T., Markow T.A., Kain K.J., Gibbs A.G. 2003. Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. *Journal of Insect Physiology* 49 (3): 261–270. DOI: [https://doi.org/10.1016/S0022-1910\(02\)00287-1](https://doi.org/10.1016/S0022-1910(02)00287-1)
- Nicolson S.W. 1980. Water balance and osmoregulation in *Onymacris plana*, a tenebrionid beetle from the Namib Desert. *Journal of Insect Physiology* 26: 315–320. DOI: [https://doi.org/10.1016/0022-1910\(80\)90140-7](https://doi.org/10.1016/0022-1910(80)90140-7).
- Nicolson S.W. 1990. Water relations of the Namib tenebrionid beetles. p. 173–178. In: “Namib Ecology: 25 Years of Namib Research” (M.K. Seely, ed.). Transvaal Museum Monograph 7, Pretoria, South Africa, 230 pp.
- Perez-Mendoza J., Dover B.A., Hagstrum D.W., Hopkins T.L. 1999. Effect of crowding, food deprivation, and diet on flight initiation and lipid reserves of the lesser grain borer, *Rhyzopertha dominica*. *Entomologia Experimentalis et Applicata* 91 (2): 317–26. DOI: <https://doi.org/10.1046/j.1570-7458.1999.00498.x>
- Sinha R.N. 1973. Interrelations of physical, chemical and biological variables in the deterioration of stored grains. p. 15–47. In: “Grain Storage Part of System” (R.N. Sinha, W.E. Muvi, eds.). AVI Publishing, Westport, CT, 481.
- Smolders R., Bervoets L., De Coen W., Blust R. 2004. Cellular energy allocation in *Zebra mussels* exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. *Environmental Pollution* 129 (1): 99–112. DOI: <https://doi.org/10.1016/j.envpol.2003.09.027>
- Subramanyam A.M., Hagstrum D.W. 1993. Predicting development times of six stored-product moth species (Lepidoptera: Pyralidae) in relation to temperature, relative humidity, and diet. *European Journal of Entomology* 90: 51–64.
- Tabadkani S.M., Ahsaei S.M., Hosseininaveh V., Nozari J. 2013. Food stress prompts dispersal behavior in apterous pea aphids: Do activated aphids incur energy loss? *Physiology and Behavior* 110–111: 221–225. DOI: <https://doi.org/10.1016/j.physbeh.2012.12.004>
- Talukder F.A., Howse P.E. 1995. Evaluation of *Aphanamixis polystachya* as repellants, antifeedants, toxicants and protectants in storage against *Tribolium castaneum* (Herbst). *Journal of Stored Product Research* 31 (1): 55–61. DOI: [https://doi.org/10.1016/0022-474X\(94\)00036-S](https://doi.org/10.1016/0022-474X(94)00036-S)
- Van Handel E. 1965. Microseparation of glycogen, sugars, and lipids. *Analytical Biochemistry* 11 (2): 266–271. DOI: [https://doi.org/10.1016/0003-2697\(65\)90014-X](https://doi.org/10.1016/0003-2697(65)90014-X)
- Van Handel E. 1985. Rapid determination of total lipids in mosquitoes. *Journal of the American Mosquito Control Association* 1: 302–304.
- Van Handel E., Day J. 1988. Assay of lipids, glycogen and sugars in individual mosquitoes: correlations with wing length in field collected *Aedes vexans*. *Journal of the American Mosquito Control Association* 4: 549–550.
- Verslycke T., Ghekiere A., Janssen C.R. 2004. Seasonal and spatial patterns in cellular energy allocation in the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) of the Scheldt estuary (The Netherlands). *Journal of Experimental Marine Biology and Ecology* 306: 245–267. DOI: <https://doi.org/10.1016/j.jembe.2004.01.014>
- White G.G. 1987. Effects of temperature and humidity on the rust-red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), in wheat grain. *Australian Journal of Zoology* 35: 43–59. DOI: 10.1071/ZO9870043
- Willis E., Roth L. 1950. Humidity reactions of *Tribolium castaneum* (Herbst). *Journal of Experimental Zoology* 115 (3): 561–587. DOI: <https://doi.org/10.1002/jez.1401150308>
- Zettler J.L. 1991. Pesticide resistance in *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) from flour mills in the USA. *Journal of Economic Entomology* 84 (3): 763–767. DOI: 10.1093/jee/84.3.763
- Zettler J.L., Arthur F.H. 1997. Dose-response tests on red flour beetle and confused flour beetle (Coleoptera: Tenebrionidae) collected from flour mills in the United States. *Journal of Economic Entomology* 90 (5): 1157–1162. DOI: 10.1093/jee/90.5.1157