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The effects of three different temperatures on the total lipid and total fatty acid amounts of *Acanthoscelides obtectus* Say, 1931 (Coleoptera: Bruchidae) adults

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Abstract
Age and sex dependent changes of total lipid and total fatty acid amounts in *A. obtectus* Say, 1931 (Coleoptera: Bruchidae) adults under three different temperatures were examined. The experiments were carried out under laboratory conditions at 15, 20 and 30±2 ºC and % 65±5 relative humidity. Total lipid amounts in all temperatures decreased in parallel with the increase in age and temperature in both sexes. Total fatty acid amounts increased in parallel with the increase in temperature in general. On the other hand, total fatty acid amounts first increased and then decreased with age.

Keywords: *Acanthoscelides obtectus,* lipid, fatty acids, temperature, age, sex

1. Introduction

Insects, which have a wide place in the world of living beings, are poikilothermic living beings. Thus, temperature plays an important role on their metabolic activities because of their effect on enzymes. Activities of insects such as nutrition, metabolism, developmental speed, longevity and reproduction vary significantly depending on temperature [1]. Pre-adult development of insects and the degrees of temperature they need to carry on their adult lives can vary depending on species. This is one of the most important adaptations they have in order to be able to survive under varying climatic conditions. Thanks to this adaptation, insects can change their geographical distributions and behaviors and they can react quickly to changing climate and new climatic conditions. As in other living beings, extremely low or extremely high temperatures cause important physiological reactions in insects. Insects need basic nutrients such as protein, carbohydrates and lipid in order to continue their growth, development and reproduction activities. For this reason, researches about these basic nutrients in insects have always been interesting because the information about these play an important role in understanding very different metabolic functions of these, the continuity of homeostasis in animals and the metabolic associations of basic nutrients [11]. Lipids have important functions in living beings. Since some lipids include both hydrophobic and hydrophilic parts, they operate as constructional component in the structure of organelle membrane in the cell membrane and eukaryote cells. Since lipids can store too much energy without causing an increase in the body weight, they function as the store and transport form of the metabolic fuel in acquiring energy [10]. In migrating insects, lipids are used as the primary source of energy since they give more energy. Glycolipids and lipoproteins proteins form with carbohydrates and proteins play a role in cells' recognizing each other as the element of cell surface, in the emergence of cellular specificity and tissue immunity. Lipids function as protective cloth over the surfaces of a great number of living beings and as transporters in transporting some products in the blood [4].

Bean seed weevil *A. obtectus* is a pest that causes very big yield losses both qualitatively and quantitatively during the storage of beans. In order to prevent the damages in both fields and storages caused by seed weevils, producers and storage owners generally fight these pests chemically. Recently, as a result of the increase in ecological awareness, integrated struggle methods that can be an alternative for chemical fight have begun to be used. Success in biological fight depends on determining the strategies and timing of the fight. This state occurs by knowing the biological features of the pest and the biological control agent to be used against this pest. In the fight with *A. obtectus*, first the biology of the pest and the environmental factors that affect the biological features should be known.

For this reason, the purpose of this study is to assess the age and sex dependent change of total lipid and total fatty acids of *A. obtectus* adults raised under three different temperatures. It is expected that the data obtained will fill in the blanks about the lipid mechanism in insects and contribute to the determination of strategies and the timing with *A. obtectus*.

2. Material and Methods

A storage pest, *A. obtectus*, was used in the experiments. The experiments were carried out under laboratory conditions at 15, 20 and 30±2 ºC and % 65±5 relative humidity. The method applied by Sönmez and Gülel [18] and Sönmez *et al.* [19] was followed for drying the stocks and determining the ages of the pest. The adults which reached the intended age were grouped in sexes and put in the deep freezer of -80 °C. 40 individuals from each sex were collected for each age group. Folch *et al.* [5] and Moss *et al.* [12] s methods were used to determine total lipid and total fatty acid amounts.

2.1. Statistical Analyses

SPSS 15.0 package program was used to assess the data statistically. In the statistical assessment of *A. obtectus* adults' total lipid and total fatty acid percentages depending on three different temperatures and ages, one way variance analysis (ANOVA) was used to compare more than two groups. Significance levels of the results of this test were assessed by using 'Tukey' test. Independent samples t-test was used for the comparison of groups of two. 0.05 reliability limit was taken as the basis in the assessments ($p<0.05$).

3. Results

Temperature, age and sex dependent total lipid and total fatty acid amounts (%) in *A. obtectus* were examined in this study. Since sufficient number of 12 day old adults was not found for analysis at 20 and 30 ºC, analyses were not made at this age group.

Table 1: Total lipid amounts (%) of *Acanthoscelides obtectus* adults

Total Lipit Amounts (%)									
Temperatures									
Age (Day)	15 °C			20 °C			30 °C		
	¹Mean±S.D			¹Mean±S.D			¹Mean±S.D		
	Female	Male	*	Female	Male	*	Female	Male	*
0	14.47±0.15 Aa	15.67±0.40 De	p<0.05	15.71±0.30 Aa	14.38±0.51 De	p<0.05	15.54±1.24 Aa	16.72±1.72 De	p>0.05
4	13.62±0.26 Ab	14.41±0.80 Def	p>0.05	14.46±0.55 Aa	13.39±0.77 DEe	p>0.05	10.93±0.34 Bb	12.73±0.33 Ef	p>0.05
8	11.52±0.15 Ad	12.66±0.26 Df	p<0.05	11.13±0.20 Ab	11.32±0.43 Ef	p>0.05	7.48±0.34 Bc	8.57±0.63 Fg	p>0.05
12	11.96±0.35 c	10.23±0.35 g	p<0.05						

¹ Means of three replicates, each with 40 individuals.
Within the same line, same sex/different age groups followed by the same capital letters are not statistically different, $p>0.05$

Within the same line different sexes/same age groups followed by the same lower case letter are not statistically different, $p>0.05$

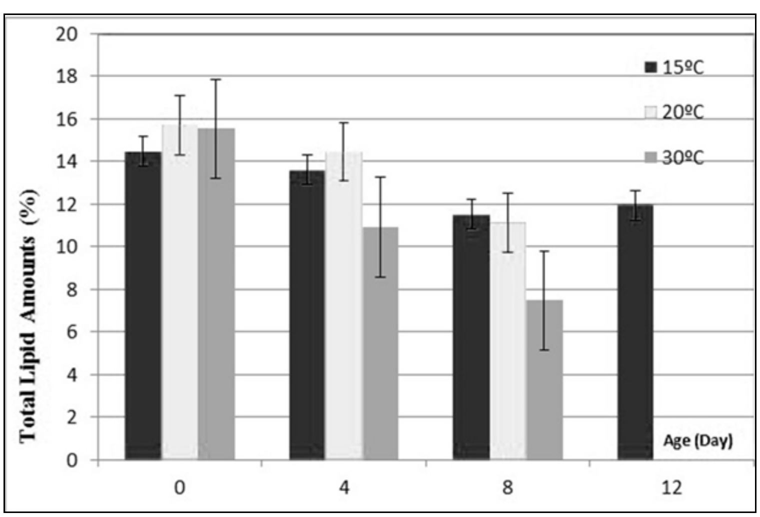

Fig 1: Total lipid amounts (%) in female adults

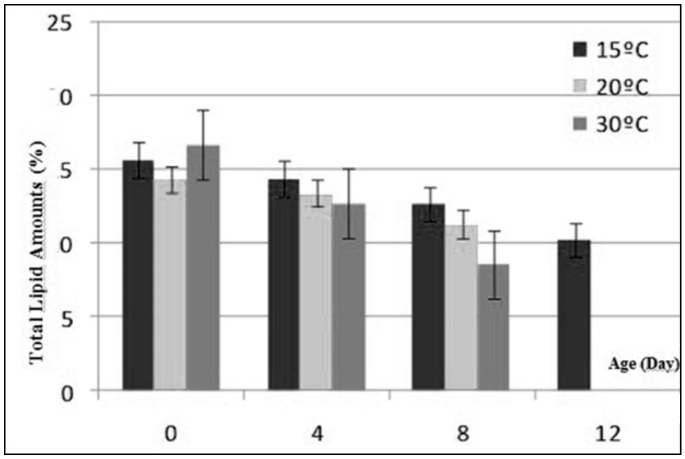

Fig 2: Total lipid amounts (%) in male adults

Table 2: Total fatty acid amounts (%) of *Acanthoscelides obtectus* adults

Total Fatty Acids Amounts (%)									
	Temperatures								
Age (Day)	15 °C			20 °C			30 °C		
	[1]Mean±S.D			[1]Mean±S.D			[1]Mean±S.D		
	Female	Male	*	Female	Male	*	Female	Male	*
0	1.15±0.04 Bc	1.00±0.05 Eg	p>0.05	1.11±0.13 Bc	0.95±0.05 Ef	p>0.05	3.49±0.10 Ac	3.61±0.11 Df	p>0.05
4	4.20±0.10 Ba	2.04±0.17 Ef	P<0.05	3.02±0.03 Ca	2.39±0.11 Ee	P<0.05	8.03±0.13 Aa	6.54±0.08 De	P<0.05
8	4.81±0.08 Aa	3.28±0.22 De	P<0.05	2.06±0.05 Bb	0.99±0.13 Ef	P<0.05	4.65±0.21 Ab	2.33±0.21 Dg	P<0.05
12	2.59±0.13 b	1.93±0.16 f	P<0.05						

[1] Means of three replicates, each with 40 individuals.
Within the same line, same sex/different age groups followed by the same capital letters are not statistically different, $p>0.05$

Within the same line different sexes/same age groups followed by the same lower case letter are not statistically different, $p>0.05$

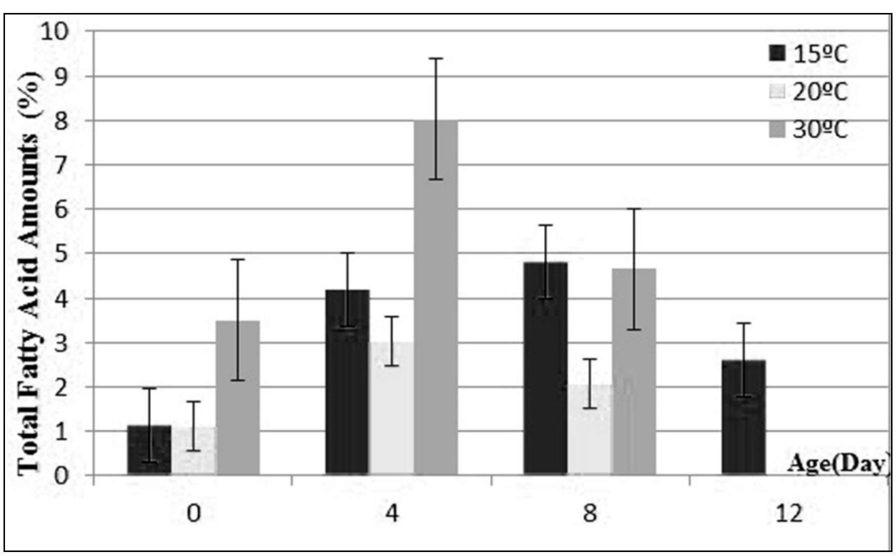

Fig 3: Total fatty acid amounts (%) in female adults

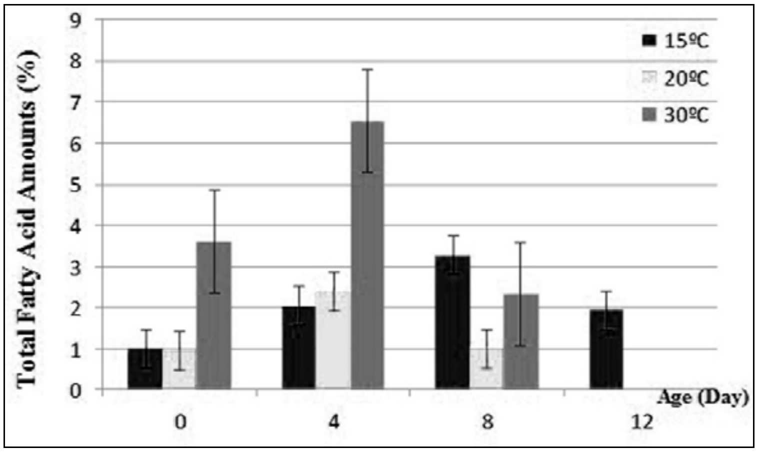

Fig 4: Total fatty acid amounts (%) in male adults

3.1. Age dependent changes of total lipid and total fatty acid percentages per individual in *A. obtectus* Adults

There were age and sex dependent changes in total lipid and total fatty acid percentages per individual in *A. obtectus* adults (Table 1 and 2, Figure 1, 2, 3 and 4). As can be seen, total lipid percentages per individual decreased as age increased in both females and males (Table 1, Figure 1 and 2). Total per individual fatty acid percentage at 20 and 30 ºC in both sexes decreased as age increased in both females and males (Table 2, Figure 3 and 4). Differences between total lipid percentages of different age groups at the same temperatures and for the same sexes were found to be significant (p<0.05).

At 15 ºC, there are differences between total fatty acid percentages in different age groups of the same sex. In addition, total fatty acid percentages per individual in both sexes at different temperatures first increased and then decreased as ages increased. Differences between total fatty acid percentages in different age groups of the same sex at 20 and 30 ºC were statistically significant.

3.2. Temperature dependent changes of total lipid and total fatty acid percentages per individual in *A. obtectus* Adults

Changes between per individual total lipid percentages of adults of the same age under there different temperatures were different from each other (Table 1, Figure 1 and 2). Total lipid percentages per individual at 0 day old males and females were significant at 15 and 20 ºC. Lipid percentages of 4 and 8 day old females were significantly different at 30 ºC were significantly different than those of the females at other temperatures. Difference between per individual total lipid percentages of 4 day old males at 15 and 20 ºC was significant. Per individual total lipid percentages of 8 day old males and females decreased as temperature increased. Difference between per individual total lipid percentages of 8 day old males was significant at three different temperatures. Difference between per individual total lipid percentages of 12 day old males and females was significant.

It can be seen that per individual total fatty acid percentages of *A. obtectus* adults changed depending on the temperature and sex and in general the changes between per individual total fatty acid percentages of the same ages at three different temperatures were different from each other. In both males and females, the differences between the percentages of 30 ºC and other temperatures were significant. Difference between per individual total fatty acid percentages of 4 day old females was significant at three different temperatures. In males, the differences between the percentages of 30 ºC and other temperatures were significant. Differences between the percentages of 20 ºC and other temperatures were significant for 8 day old females and males.

4. Discussion

As can be seen from the literature discussed, this study which is about the age dependent changes between the total lipid and total fatty acid percentages of *A. obtectus* adults at three different temperatures is the first study to address this subject. According to the results, total lipid and total fatty acid percentages were affected by temperature, age and sex.

The longevity of *A. obtectus* adults is between 7 and 14 days depending on the temperature and humidity [24]. Long temperatures are known to extend the larval development period and adult life of insects. In our study, *A. obtectus* adults' lengths of life varied depending on the temperature. It was found that at 15 ºC, a great majority of adults lived for 12 days on average and died in mass after 12nd day while at 20 and 30 ºC lived for 8 days on average and started to die in mass after 8th day. All experiments were repeated three times with 40 individuals. For this reason, since there weren't sufficient number of 12-day-old adults at 20 and 30 ºC for analysis, the analysis of 12-day-old adults was made only at 15 ºC. At all temperatures studied, total lipid percentages decreased almost at the same level in both sexes as age increased. This result is in parallel with the results of the study Wigthman [22] conducted with *Callosobruchus maculatus*. These ages dependent differences in total lipid amounts may be because adults use too much lipid for different activities at the studied ages while they cannot synthesize lipid sufficiently since they cannot feed at all or rarely feed on pollens and nectars for their short term energy needs after they become adults. According to our results, although total lipid amount decreases as age increases, adults have a significant amount of energy during their lives. This result may be because although adults are not fed, they store enough lipid in pre-adult period as suggested by Tucic *et al.* [20, 21] and Seslija *et al.* [16, 17].

It has been found that *A. obtectus* adults can breed within the first 24 hour of adult life and start oviposition within the next 24 hours. Parsons and Credland [15] (cited from Pankanin-Franckzy [14], stated that the most suitable activity and development temperature was 30 ºC for *A. obtectus* and maximum egg laying time was 3-5 days after becoming adult while Howe and Currie [7] stated that it was 2-3 days later. According to our results, the decrease in the total lipid amount of adults at a specific temperature occurred at 30 ºC at most. This result may be because the living being uses maximum level of lipid at this temperature. Similarly, Howe and Currie [8] and Parsons and Credland [15] stated that the reason for the maximum activity they found at 30 ºC was because of maximum lipid use.

Metabolic activities in insects are known to differ according to age, sex, temperature and type of food. In our study, temperature dependent differences were found in the per individual total lipid percentages of individuals of the same age. Total lipid percentages per individual were affected by temperature in all age groups except for age 0. There is no significant difference between per individual total lipid percentages of 0-year-old adults of a specific age under three different temperatures. When it is taken into consideration that *A. obtectus* adults can breed within the first 24 hour of adult life and start oviposition within the next 24 hours, the fact that there is no significant difference between per individual total lipid percentages of 0-year-old adults under different temperatures is not surprising. This result shows that the lipids stored at pre-adult period are not used very much in the first day of adult life. On the other hand, there are significant differences between the per individual total lipid percentages of 4 and 8-day old males and females at specified temperatures.

Adult life of *A. obtectus* lasts between 7 and 14 days. During this period, adults' activity varies depending on the temperature. During the active periods, females are busy with egg-laying while males are busy with breeding activities. In both sexes, there are significant differences between per individual total lipid percentages at periods when activities are intense. It is known that temperature increases enzymatic activity positively within specific limits and *A. obtectus* adults are less active at temperatures lower than 20 ºC. In our study, per individual total lipid percentage was found to decrease at 4 and 8 day old adults as temperature increased. This result shows that lipids are used excessively in adults at active period for different purposes. The effect of temperature on the per individual total lipid percentage of adults differ depending on sexes. Hamed [7] found that male *A. obtectus* adults were less affected of temperature when compared with females. In our study, it was found that per individual total lipid percentage of different ages of females at 30 ºC was found to be less than that of males. This result may be because females used more lipid than males at high temperatures.

As can be seen from Table 1, Figure 1 and 2, in general males have more total lipid percentage than females. Despite this, the differences between total lipid percentages of females and males at all temperatures studied are mostly insignificant. The fact that females use lipids too much for egg supplementary nutrient during oogenesis may be important in their having lower degrees of total lipid percentage. Total lipid percentage was found to be higher in male *Lertha sheppardi* when compared with females and *C. maculatus* females were found to include more total lipid and triglyceride than males [3]. While the results of *L. sheppardi* study were in parallel with the results of our study, our results were not in parallel with *C. maculatus* [13] study. This result may be because of different lipid synthesizing and using degrees of each species.

As can be seen from Table 2, Figure 3 and 4, although total fatty acid percentages were quite low in the first day of adult life in all studies temperatures, they reached the highest level on the 4th day of adult life and then began to decrease. In both sexes, the highest increase in total fatty acid percentage on the fourth day was at 30 ºC at most in both sexes. Taking into consideration that the most suitable developmental temperature for *A. obtectus* is 30 ºC and the most suitable egg-laying time is 3-5 days after becoming adult, the increase in total fatty acid level on the 4th day can be assumed to occur to meet the necessary fatty acids for the synthesis of sex hormones in both sexes [8]. Age dependent changes in total fatty acid percentages at a specific temperature are in parallel with the results of the studies conducted with *Anthonomus grandis* [6], *Tenebrio molitor* [9] and *Melanogryllus desertus* [2]. Total fatty acid amount in *A. grandis* adults was found to be very little in the first four days of adult life and increase with age, while the lipid amount in ovarium of *T. molitor* females was found to increase on the first three days of adult life and then to decrease. *M. desertus* females were found to have different fatty acid amounts in phospholipid and triglyceride fractions depending on the age.

According to our results, there are temperature dependent per individual total fatty acid percentage differences between adults of the same age. As can be seen from Table 2, the differences between temperature dependent per individual total fatty acid percentages at a specific age of *A. obtectus* adults are significant. per individual total fatty acid percentage in adults of the same sex at all temperatures is more in females than males and the differences between the two sexes are significant. Lipids synthesized at lipid tissue during oogenesis are known to be sent to ovariums and be stored there in order to be used at embryogenesis. Ximenes *et al.* [23] found that fatty acids in *C. maculatus* were used in egg production in females. The result of our study showing that females had more total fatty acid amount than males maybe because they need more fatty acids for ovarium development and egg production.

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