International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

ISSN 2347-2677
IJFBS 2016; 3(4): 90-96
Received: 16-05-2016
Accepted: 17-06-2016

Evrim Sönmez
Sinop University, Faculty of Education, Department of Science Teaching, Sinop.

Dilek Güvenç
Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Clinical Sciences, Pharmacology-Toxicology, Samsun.

Adem Gülel
Ondokuz Mayıs University, Faculty of Science and Letters, Department of Biology, Samsun.

Correspondence:
Evrim Sönmez
Sinop University, Faculty of Education, Department of Science Teaching, Sinop.

The changes in the types and amounts of fatty acids of adult *Acanthoscelides obtectus* (Coleoptera: Bruchidae) in terms of age and sex

Evrim Sönmez, Dilek Güvenç and Adem Gülel

Abstract
This study examined the changes in the types and amounts of fatty acids of 0, 4 and 8 day-old adult *Acanthoscelides obtectus* bred at 30 ˚C in terms of age and sex. Tests were conducted under 30±2 ˚C temperature and % 60±5 moist. The types and amounts of the fatty acids of adults were determined with gas chromatography. 11 fatty acids were found in 0, 4 and 8 day-old adult *Acanthoscelides obtectus* breeded at 30 ˚C as lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1n9c), linolelaidic acid (C18:2n6t), linoleic acid (C18:2n6c), linolenic+cis-11-eicosenoic acid (C18:3n3+C20:1) and cis-11,14-eicosadienoic acid (C20:2). Of these 11 fatty acids, oleic and palmitic acids were determined as dominant fatty acids. While the types of fatty acids found in *A. obtectus* adults remained stable in general, their contents were found to change in terms of sex and age.

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

1. Introduction
Insects can store their primary energy source fatty acids in adipose tissue in very large amounts. Many kinds of insects need lipids in activities such as sexual maturity, egg production and energy source in long distance flights [5, 16, 51, 68]. Lipids can be found in many forms in living creatures. In insects, fat tissue is the synthesis area of many of the proteins in hemolymph and the primary storage area of triglycerides which include 90% of lipids [3, 18, 23, 41, 47, 59]. Composition and amount of fatty acids are influenced by several factors, such as temperature, moisture, light, nutrition, reproduction, sex, developmental time and age [18, 27, 28, 33, 38, 45]. Recently, various researchers have found through studies conducted with *Lyctus planicollis*, *Tenebrio molitor*, *Dermestes maculatus*, *Mobosteira lobulifera*, *Photinus pyralis*, *Magicicada septendecim, Ostrinia nubilalis* of different ordos that insects can synthesize some saturated, unsaturated and oversaturated fatty acids [1, 2, 4, 15, 16, 17, 19, 31, 39, 45, 72, 75].

Bean weevil *Acanthoscelides obtectus* is a pest which causes very big yield losses both qualitatively and quantitatively during the storage of dry beans. In order to prevent the harm caused by weevils both in the field and in stores, producers and store owners generally fight with chemicals against these pests [37, 54, 63, 64, 65, 67]. Focusing on chemicals in pest control has caused the emergence of many problems in time on contrary to what was expected, due to the harms chemical cause on both people and other living beings directly or through food chain [50, 63, 64]. Recently, with the increases in ecologic awareness, integrated fight methods have begun to be used as an alternative to chemical fight [6].

Success in biological fight depends on the determination of fighting strategies and timing. This is provided with knowing the biological properties of both the pest and the biological control agent to be used against this pest. In fight with *A. obtectus,* first of all the biology of the pest and the environmental factors which influence the biological properties of the pest should be known. Thus, the purpose of the study was to assess the changes in types and amounts of the total fat, total fatty acids and fatty acids in adult *A. obtectus* bred at 30 ˚C in terms of age and sex. It is expected that the data obtained will contribute to fill in the gaps about lipid metabolism in insects and to determine the strategies and timing in the fight against *A. obtectus.*

2. Material and Method

In the tests, *A. obtectus,* which is a warehouse pest, was used. Experiments were conducted under 30±2 ˚C temperature and %60±5 moist. The tests were started with setting up stock cultures of the pest. In setting up the stocks, the method applied by Sönmez and Gülel [73] was used. On the day the beetle matured, they were considered as 0-age. After adults began to come out in stocks, age groups of 0, 4 and 8 were formed and the adults were taken into petri plates and they were kept under the same conditions until they reached the specified age. Before the adults that reached the desired age were put in -80 °C deep freezer, they were grouped in terms of their sexes. 40 beetles of each sex for each age group were collected. Folch [21], Moss *et al*. [48]'s method was used in the determination of total fat and fatty acid amounts. The types and amounts of the fatty acids of adults were determined with gas chromatography.

2.1 Lipid and Fatty Acid Analysis

Insects were transferred to a glass homogenizer containing 5 mL choloroform-methanol (2:1; v/v) for extraction of the total lipid using the method of Folch *et* al [21]. After centrifugation at 35.000 r.p.m. for 5 min, the choloroform-methanol phase removed and lipid extracts were dried under a stream N_2. The lipid samples were processed for fatty acid analyses following the methods described by Moss *et al*. [48]. 10 ml 6% KOH was used in saponification procedure of fats, while 3 ml BF3-CH3OH solution was used to form the volatile methyl ester of fatty acids (FAMEs).

Compounds were identified using gas chromotagrapy (GC).

2.2 Gas Chromatography Analysis

The fatty acid methyl esters were analyzed with a SHIMADZU 17A GC-FID and a fused silica capillary column (SP 2380, 60 m x 0.25 mm id, 0.2 µm film; Supelco, Supelco Park, PA). A splitt injection (1:25) of 1 µl was used. Inlet temperature of injection column was 250 ˚C. Nitrogen carrier gas total flow rate was 39 ml/min.

The oven condition was as follows: 90 ˚C for 7 min, 4 ˚C increase/ min up to 220 ˚C, without waiting 2 ˚C increase min/ up to 250 ˚C, and 250 ˚C for 10 min. Total analysis time was 64.50 min and temperature of FID dedector was 260 ˚C. Identification of fatty acid methyl esters was determined by comparison of their retention times with those of standards provided from Supelco. The percentage of each peak area for each fatty acid was calculated using Shimadzu Software Program in each treatment.

2.3 Statistical analyses

SPSS 15.0 package program was used for the statistical assessment of the data. For the statistical assessment of the total fat, total fatty acid and fatty acid percentages of adult *A. obtectus* in terms of three different temperatures and age, one way variance analysis (ANOVA) was used to compare groups of more than two. The significance levels of the results obtained from this test were assessed with "Tukey" test. "Independent Samples t-test" was used for the comparison of paired groups. 0.05 reliability limit was taken as the basis in assessments.

3. Results

11 fatty acids were found in 0, 4 and 8 day-old adult *A. obtectus* bred at 30 ˚C as lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linolelaidic acid, linoleic acid, linolenic+cis-11-eicosenoic acid and cis-11,14-eicosadienoic acid. Of these 11 fatty acids, oleic and palmitic acids were determined as dominant fatty acids. Lauric and myristic acids were not found in 8-day-old females. In addition, palmitoleic acid was not found in 0-day-old males while lauric, myristic and palmitoleic acid were not found in 8-day-old males.

While the differences between the lauric, linolelaidic and linolenic acid percentages of females of different age groups are significant, they are insignificant for males. The differences between palmitic, palmitoleic, oleic and cis-11, 14 eicosadienoic acid percentages are insignificant in males and significant in females. While the differences between myristic acid percentages were significant in both sexes, the differences between linoleic acid+cis-11 eicosenoic acid percentages were insignificant.

Table 3.1: Fatty acid composition (%) of 0 day old *Acanthoscelides obtectus* adults ¹ Means of three replicates, each with 40 individuals

Fatty acids	Female ¹Mean±S.D			Male ¹Mean±S.D		
	0 day old	4 days old	8 days old	0 day old	4 days old	8 days old
C12:0	2.49±0.41 Aa	0.41±0.01 Ab	---	3.04±0.45 Ca	1.98±0.85 Cb	---
C14:0	1.19±0.17 Aa	0.32±0.02 Bb	---	1.61±0.12 Ca	0.95±0.41 Cb	---
C16:0	17.56±0.81 Aa	15.62±0.19 Ab	14.05±0.09 Ac	17.05±0.54 Ca	13.81±0.80 Db	13.38±1.02 Dc
C16:1	0.60±0.15 A	0.39±0.07 Ab	0.41±0.06 A	---	0.39±0.07 b	---
C18:0	9.99±1.24 Aa	6.74±0.18 Ab	8.08±1.84 Ac	8.91±2.58 Ca	10.36±1.41 Cb	8.84±0.05 Cc
C18:1n9c	50.07±1.83 Aa	52.49±0.80 Ab	49.92±0.80 Ac	44.12±0.81 CDa	50.88±1.84 CEb	54.28±2.52 Ec
C18:2n6t	0.52±0.19 Aa	0.95±0.30 Ab	2.24±0.44 Bc	0.60±0.30 Ca	1.61±0.56 Cb	0.67±0.02 Cc
C18:2n6c	9.81±0.44 Aa	11.25±0.48 Bb	15.87±0.77 Bc	12.73±1.99 Ca	11.35±0.48 Cd	14.62±0.22 Cc
C18:3n3+C20:1	6.78±0.31 Aa	8.60±0.45 Ab	6.47±0.27 Ac	4.06±0.44 Ce	5.03±0.12 Cf	5.92±1.24 Cc
C20:2	0.96±0.23 Aa	1.08±0.14 Ab	2.93±1.76 Ac	7.55±2.50 Ca	1.54±0.57 Db	1.95±0.20 Dc

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, $p>0.05$

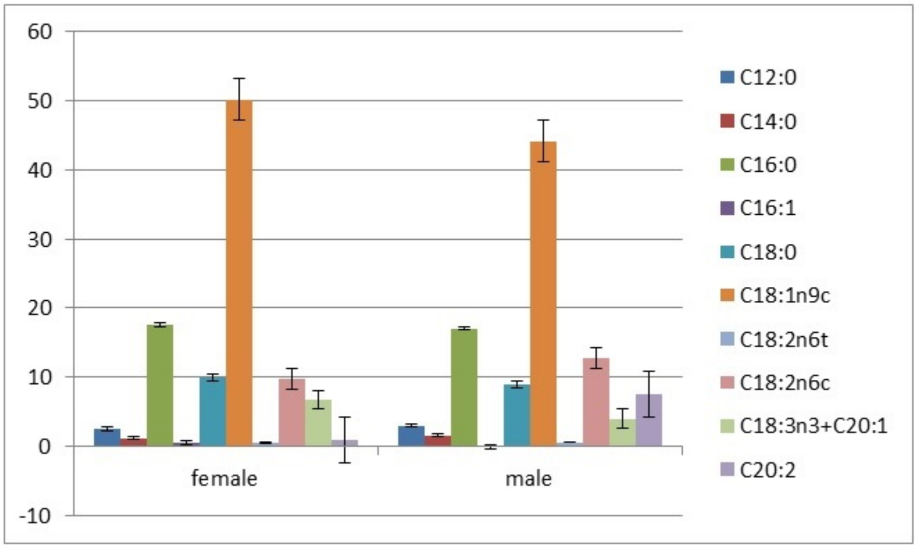

Fig 3.1: Fatty acid percentages (%) of 0 day old *Acanthoscelides obtectus* adults

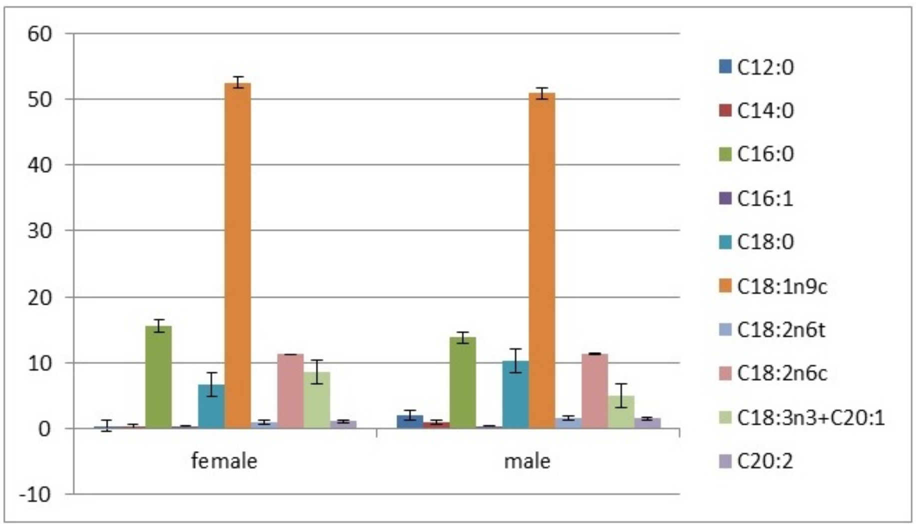

Fig 3.2: Fatty acid percentages (%) of 4 days old *Acanthoscelides obtectus* adults

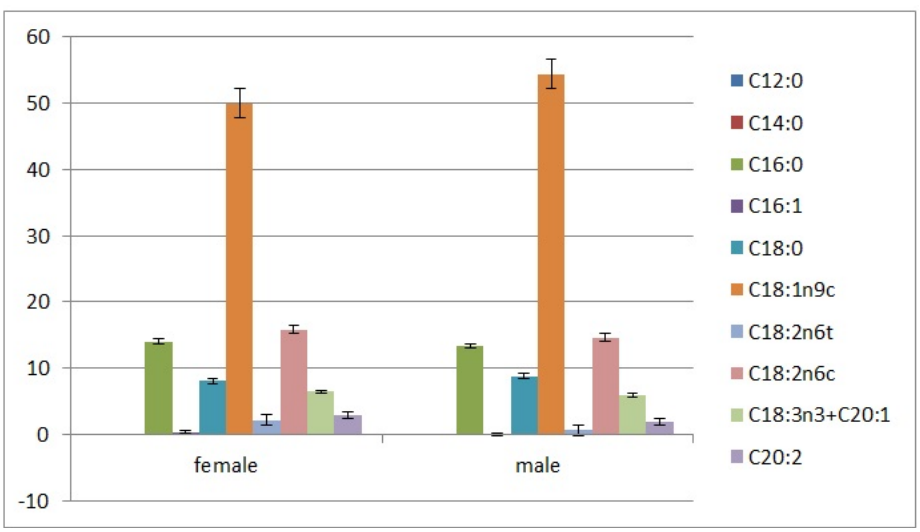

Fig 3.3: Fatty acid percentages (%) of 8 days old *Acanthoscelides obtectus* adults

4. Discussion

Insects can change their fatty acid compositions based on environmental conditions and their physiological needs in order to be able to survive and continue living [5, 8, 11, 13, 18, 23, 31, 35, 39, 47, 61, 62]. The fact that the amounts and types of the fatty acids found in this study vary in terms of age and sex bring to mind that amounts of these fatty acids may have been organized by *A. obtectus* adults based on their states of being used. The only study on fatty acids of *A. obtectus* is the one conducted about the fatty acid compound on the cuticula of adults by Golebiowski *et al*. [24]. In this study, it was found that there were hydrocarbons, aldehydes, methyl and ethyl fatty acid esters, triacylglycerols, free fatty acids, alcohol and sterols in the cuticula of adults and oleic, linoleic, palmitic and stearic acids were found to be dominant fatty acids.

It was found that adult *A. obtectus* could breed within the first 24 hours of their adult lives and that they started oviposition in the next 24 hours [58, 43, 44, 60]. Parsons and Credland [58], (cited from Pankanin-Franckzy [57]) stated the most suitable activity and development temperature for *A. obtectus* was 30 ºC and the maximum egg-laying time was 3-5 days after becoming adults while Howe and Currie [36] stated this time as 2-3 days later. Adult life lasts 7-14 days in *A. obtectus* adults. In their active periods, females are busy with laying eggs while males are busy with breeding activities. In our study, lauric and myristic acid were not found in 0 and 8 days old females and males, while palmitoleic acid was not found in 0 and 8 days old males. Haubert *et al*. [30] found that fatty acids differed in terms of temperature, age and species in three different Collembola species, Ogg *et al*. [56] stated that some fatty acid amounts differed in terms of species and sex in five different *Diabrotica spp*. species. In our study, the differences between some fatty acids in terms of age and sex are in line with the results of other researchers. The changes in the amounts of fatty acids and some fatty acids being seen only in a certain sex and certain age brings to mind that *A. obtectus* adults can regulate their fatty acid metabolism according to their changing needs.

A great number of researchers have found that the amount and types of fatty acids in insects differ depending on species, age, developmental stage, tissue, nourishment and environmental factors [2, 7, 16, 25, 26, 32, 33, 35, 52, 55, 61].

In our study, 11 different fatty acid types were found in *A. obtectus* for the first time. Of these fatty acids, oleic and palmitic acids are dominant fatty acids. The remaining fatty acids found are caprylic acid, capric acid, lauric acid, myristic acid, stearic acid, palmitoleic acid, linolelaidic acid, linoleic acid, linolenic acid+cis11-eicosanoic acid, cis11, 14-eicosadienoic acid, cis13,16-docosadienoic acid and cis-5, 8, 11, 14, 17-eicosapentaenoic acid. It is known that the type and amount of fatty acid in a species change according to the body parts of the insects, metabolic needs and tissues before and during adult period [26, 71, 72]. In studies with *T. molitor,* the dominant fatty acids have been found as palmitic and stearic acid in the ovaries of females, as palmitic, oleic and linoleic acid [39] in the adipose tissue and palmitic acid in the cuticula [12]. Although the fatty acid types of insects resemble each other, dominant fatty acids may differ according to tissues. For example, dominant fatty acids were found as palmitic and oleic acid in *S. oryzae* and *S. Zeamais* [76], oleic and palmitic acid in *C. maculatus* [53], oleic and palmitic acid in *D. Maculatus* [15], oleic and palmitic acid in *A. grandis* [27], oleic, palmitic and linoleic acid in *R. dominica* [61], palmitic, oleic, stearic and linoleic acid in *C. togata globicollis* [32] and palmitic, stearic, oleic and linoleic acid in *P. pyralis* [2]. Although the number of dominant fatty acids in different species of the same ordo changes, the fact that oleic and palmitic acids are dominant in all the studied species shows that there is an evolutionary association in the fatty acid metabolism in insects. The dominant acids found in *A. obtectus* in our study are in parallel with the findings of other researchers. According to our results, the acid percentages of oleic and palmitic acids, which were the dominant fatty acids in both sexes studied, were found to change depending on the age. However, the difference between the percentages of a dominant fatty acid in males and females of the same age was found to be insignificant (p>0.05). The amount of oleic acid, which is one of the dominant fatty acids, changes according to age, temperature and sex. These differences, which appear in the percentages of oleic and palmitic acids as a result of age, may have been caused by the different degrees of these two dominant acids used by adults. Our results about the oleic acids are in parallel with the results of Haubert *et al*. [30] who conducted a study on the age dependent results of oleic acid with three Collembola species. Oleic acid plays an important role in females in laying eggs and in *T. molitor* females, oleic acid amount in ovarium was found to increase in sexual maturity [39]. Bozkuş [11] found that palmitic and oleic acid decreased at 30 ºC depending on the age in *M. desertus* females, an Orthoptera species. In our study, the decrease in the amount of palmitic acid at 30 ºC depending on the age is similar to the result found in Orthoptera species. This result may be caused by the similar palmitic acid metabolism in the females of two species belonging to two different ordos. This similarity is important in terms of presenting the associations between species.

The age-dependent changes in oleic acid amount may be resulting from the overuse of oleic acid for the supplementary nutrient in oogenesis egg or by the synthesis of linoleic acid from oleic acid as put forward by Renobales *et al* [66] and Blomquist *et al* [9]. Nurullahoğlu *et al* [52] claimed that the difference in the amount of oleic acid between two sexes in *Apanteles galleria* may have resulted from the use of oleic acid in females for the formation of egg or by the transformation of oleic acid into linoleic acid with this purpose. Ogg *et al* [56] reported that the amount of dominant fatty acids in *D. longicornis* changed depending on the sex, and the females had more palmitic and linoleic acid and less oleic acid than males. In our study, the dominant fatty acid amount differs between sexes. The amount of oleic acid in females increasing in the first four days and then decreasing may be due to the overuse of this acid at oogenesis since maximum egg-laying activity takes place on the 3rd-5th days and in addition, as a result of oleic acid being transformed into linoleic acids as stated by Haubert *et al*. [30]. In males, the change at 30 ºC in 3-5 days of age group in which the maximum activity is highest, is more remarkable. Considering that the males have the potential to mate all the time, it can be assumed that they probably use oleic acid as a source of energy in mating activities and thus they increase the synthesis of this acid [44]. Evans [20] found that although saturated fatty acids were constant during metamorphosis, unsaturated fatty acids were found to decrease. Çakmak *et al*. [17] found that saturated and unsaturated fatty acids in *L. sheppardi* differed in terms of the developmental stages of the insect; however, they found that the amounts of saturated and unsaturated fatty acids in adults did not differ. In our study, it was found that the amounts of saturated, unsaturated and multi unsaturated fatty acid amounts in *A. obtectus* adults differed according to age and sex. While unsaturated fatty acids increased in females at ages they were most active, they decreased in males. This result shows that the amount of unsaturated fatty acid used in both sexes in the most active ages are different. This difference may have resulted especially from the different needs in gametogenesis. Linoleic acid in *Labidura riparia* was found to be in large amounts at the start of vitellogenesis and it was found to decrease extremely during vitellogenesis [68]. Murata and Tojo [49] found that unsaturated fatty acids increased during the ovarian development of females in *Spodoptera litura*. The changes in saturated and unsaturated fatty acids of *A. obtectus* are in parallel with the results of other researchers. During our study, some fatty acids, for example caprylic acid, capric acid, cis-13, 16- docosadienoic acid and cis-5, 8, 11, 14, 17-eicosapentaenoic acid were found generally in males. While caprylic, capric and cis-13, 16- docosadienoic acid are found only in males, cis-5, 8, 11, 14, 17-eicosapentaenoic acid was found in both sexes. The fact that these fatty acids are sometimes found in one of the sexes, but not in another, shows that the fatty acid metabolism of insects differ depending on the sex. This change can be provided by the regulation of fatty acid synthesis as much as needed in a given sex. In general, since these fatty acids are used by insects during direct defense and in communication activities by sexes or since they are the fatty acids used in the formation of pheromones, it is natural for them to be found in very small quantities [10, 13, 29, 72, 74]. Since *A. obtectus* does not need too much defense in warehouses as a warehouse pest, it is probably used in the formation of substances such as pheromone or those similar to pheromone in the communication between the specified fatty acids. Thus, it is thought that the smaller number of these fatty acids won't cause a problem in the insect. The findings of Meinwald and Eisner [46] and Smith and Grula [69] that caprylic acid is used in secretions about defense in insects, and the findings of Koidsumi [40], that capric and caprylic acid are used for defense against fungus in *Bombyx mori* in cuticula confirm our thoughts. Lauric, myristic, palmitoleic and linolelaidic acids were found in *A. obtectus,* although is small amounts. The amount of each of these acids differs between sexes, based on the age. Linoleic and linoleic acids and their long chain metabolytes are the precessors of eicosanoids which play an important role in the insect physiology. Eicosanoids were stated to have an influence on reproduction, cellular immunity and thermoregulatory [11, 14, 33, 70, 72]. Insects can turn 18 C multi unsaturated fatty acids in to 20 C multi unsaturated fatty acids through biochemical methods [72]. The small amount of main fatty acids since the eicosanoids which have an important role in insects may have not caused a problem by insect metabolism. Thus, there are less fatty acids when compared with fatty acids in question.

The differences between the 18 and 20 C multiple unsaturated fatty acids differ bring to mind that the amounts of these fatty acids may have been organized according to the state of being used by the insect.

5. Conclusion

Fatty acid composition is not constant in insects. These have the ability to change fatty acid compositions based on environmental conditions and needs in order to be able to survive [8, 11, 13, 18, 31, 35, 39, 62].

6. Acknowledgements

This study was summarized in my doctoral thesis and was supported by Ondokuz Mayıs University Scientific Research Projects Commission. I would thank Prof. Dr. Abdurrahman Aksoy and his team for supports in Gas Chromatpgraphy analysis, OMU, Faculty of Veterinary Medicine, Department of Clinical Sciences, Pharmacology-Toxicology, Samsun.

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