FATTY ACIDS AND AMINO ACIDS IN THE FAT BODY OF BUMBLEBEE *Bombus terrestris* (L.) IN DIAPAUSING AND NON-DIAPAUSING QUEENS

Monika Fliszkiewicz, Zdzisław Wilkaniec

Department of Useful Insects Breeding, Faculty of Animal Science A. Cieszkowski Agricultural University of Poznań. E-mail: monef@au.poznan.pl

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S u m m a r y

In a closed culture, young inseminated bumblebee queens have been observed not to fall into the state of diapause, which leads to losses in rearing. One of the conditions necessary for the inseminated queens to pass into a state of hibernation is the accumulation of an appropriate quantity of fat body. The present study was aimed at comparing the dry matter content of the fat body as well as its amino acid and fatty acid composition in diapausing and non-diapausing bumblebee queens from artificial culture. The obtained results demonstrated that the diapausing queens were characterized by a higher mean content of dry matter in the fat body and a higher content of fat in dry matter as compared to non-diapausing ones. No differences were observed, however, in the contents of fatty acids in the fat body. In contrast, contents of amino acids in the fat body appeared to differ between both groups of queens examined, yet only in the case of serine, cystine, glycine and alanine.

Keywords: large earth bumblebee, *Bombus terrestris* (L.), diapause, fat body.

INTRODUCTION

Bumblebees are insects used on an increasingly wider scale for pollination of various plant species, especially in glasshouses and plastic-covered greenhouses. Since their usage has appeared advantageous, by the beginning of the last century, trials were undertaken to rear these insects. Colonies of bumblebees to be used as pollinators have been reared in a number of countries. Various aspects of rearing methods have been described by Beekman et al. (1998a), Bowers (1986), Eijnde et al. (1991), Plowright and Jay (1966), Pridal and Hofbauer (1996), Tasei and Aupinel (1994), Biliński (1976, 1981, 2002) as well as many others.

A variety of methods of bumblebee rearing have been elaborated to date, of which the most effective and widely applied these days is the laboratory method. According to Biliński (2002), the leaders in the production of bumblebee colonies using this method are currently the Netherlands, Belgium and Israel. Large companies in those countries deal with rearing and exporting bumblebee colonies. In a number of centres located world-wide, work is underway on improving rearing methods. In a closed culture, young inseminated queens have been observed not to fall into the state of diapause, which leads to losses in rearing (Biliński 2002). One of the conditions necessary for the inseminated queens to pass into the state of hibernation, both in natural and culture conditions, is the accumulation of an appropriate quantity of fat body (Dylewska 1996, Biliński 2002) allowing them to survive the period of diapause. The fat body of insects is generated from cellular material of mesodermal sacs. In most insects, including bumblebees, it constitutes two separate layers: a wall layer that adheres to walls of the body,

and a visceral layer coating the alimentary tract and other internal organs (Klimaszewski 1996). The major function of the fat body, especially of its wall layer, is the accumulation of storage nutrients used at various stages of life.

Intensive growth of the fat body is typical of insects having a period of diapause in their development. It is always preceded by intense feeding that enables the maximum accumulation of feed reserves in the body. Young bumblebee queens accumulate, mainly in the abdomen, considerable feed reserves in the form of a fat body as early as in the first days of their life (Dylewska 1996). In the period of diapause, those reserves are utilized almost completely. Hence, reserves accumulated in the fat body must be large enough to survive the entire period of diapause. In the culture described in this study, although young queens had free access to a large quantity of feed they did not fall into the state of diapause. Therefore, the objective of this study was to compare dry matter of fat body and its fat content as well as the amino acid and fatty acid composition of fat in diapausing and non-diapausing queens.

MATERIAL AND METHODS

The experimental material were queens of the large earth bumblebee *Bombus terrestris* (L.) originating from a laboratory culture. In total, 100 queens were analysed for dry matter content of fat body, its fat content and fatty acid composition. Amino acids occurring in the fat body were determined as well.

In the study, the fat body pudding abdominal rings of bumblebee queens were sampled for analyses by scraping them from abdomens previously devoid of internal organs. The dry matter of the fat body was determined with the gravimetric method. To this end, grains of sand were poured onto the bottom of weighing bottles with a ground-in stopper, and a thin glass rod was fixed in it. The bottles were numbered, transferred to a dryer and dried at a temperature of 105°C for one hour. After that time, they were again weighed and transferred to an exsiccator, in which calcium chloride was earlier fixed to eliminate potential humidity. Successively obtained fat body from the prepared queens was put into the bottles, which were then weighed again. The fat body was then ground in the bottles with sand using a glass rod and the weighing bottles were transferred to a dryer at a temp. of 105°C for 8 hours. Next, they were taken out of the dryer, kept in an exsiccator for 20 min and weighed. Mass differences enabled the calculation of dry matter of fat body for individual bumblebee queens.

The fat content of the fat body was determined with the extraction method. To this end, 3 ml of petroleum benzene was poured into each weighing bottle with the dried fat body, and were then thoroughly stirred with the glass rod and closed with a stopper. The bottles were left for 24 h, mixing the solution several times. Next, the fat extract from above the sand was gently decanted to Erlenmayer flasks. A portion of 3 ml of ether was poured twice to each bottle and the extract was decanted again. The ether remaining in the bottles was evaporated under an extractor and, making sure there is no ether left, they were again fixed in a dryer and dried at a temp. of 105°C for 1 hour. Next, the bottles were successively transferred to the exsiccator for 20 min and weighed. Mass differences enabled the calculation of the fat content of the fat body.

In order to determine the fatty acids in the fat body, the fat extract from 5 subsequent queens was collected to an Erlenmayer flask, then evaporated and frozen. Next, the samples (in total 20 samples) were dissolved in 1 ml of heptane and subjected to the process of trans-

Table 1

Qualitative composition of fatty acids determined in the fat body of bumblebee queens.

Types of fatty acids determined		
monounsaturated (MUFA)	polyunsaturated (PUFA)	saturated (SFA)
14:1 myristoleic	16:2 hexa decadienoic	8:0 caprylic
16:1 palmitoleic	18:2ϖ 6 linoleic	12:0 lauric
18:1ϖ 9 oleic	18:3ϖ 3 linolenic	14:0 myristic
18:1ϖ 7 oleic	20:2 eicosadienoic	16:0 palmitic
20:1 gadoleic		18:0 stearic
22:1 erucic		20:0 arachidic
24:1 nervonic		22:0 behenic

esterification with sodium methanol (Wąsowicz 1984). The methyl esters of fatty acids obtained were analysed on a Hewlett Packard 5890 gas chromatograph, using a capillary column INNOWAX (30 m x 0.25 mm x 0.2 μm) and the following analytical conditions:

1. Carrier gas - helium
2. Detector's temp. - 240°C
3. Injector's temp. - 230°C
4. Programmed furnace temp.:
   initial - 60°C,
   increase - 12°C/min,
   final - 200°C kept for 20 min.

Identification of acids was carried out based on retention times of standards (a kit by SUPELCO). This method was used to determine fatty acids, which – according to generally adopted principles – were divided into monounsaturated (MUFA), polyunsaturated (PUFA) and saturated fatty acids (SFA). The composition of fatty acids is presented in Table 1.

This part of the study, as well as assays of the amino acid composition of the fat body were commissioned at the Institute of Technology of Food of Plant Origin, Faculty of Food Technology, University of Agriculture in Poznań, which possesses the equipment necessary for such analyses.

The amino acid composition was determined in dry matter of the fat body, after first having extracted fat from it. A single sample was the material obtained from 5 subsequently analysed bumblebee queens, both in the diapausing and non-diapausing groups. A total of 20 samples were analysed. Separation of individual amino acids was carried out on an automatic MIKROTECHNA Praha AAAT-339 amino acids analyser. A hydrolysate of 17 amino acids by Pierce in 0.1 μ HCl was used as a standard. Next, acid hydrolysis was carried out with 6N HCl for 24 h at a temp. of 108-110°C.

In order to analyse the dry matter of the fat body and its fat content in diapausing and non-diapausing bumblebee queens, mean values were compared with the t-test. Mean contents of fatty acids and amino acids were compared with one-way analysis of variance. All calculations were made with Statgraphis plus, ver. 4.1 software.

RESULTS

1. Dry matter content of fat body

The data presented in Table 2 indicate that the mean content of dry matter in the fat body is higher in the diapausing bumblebee queens. The empirical value of the t-test is higher than the t value read out from the table at a significance level of $\alpha = 0.05$, hence the differences between the mean are significant. The mean content of dry matter in the fat body of the diapausing

Table 2

Comparison of dry matter content of the fat body in bumblebee queens (g).

Diapausing queens	Non-diapausing queens
min = 0.0022	min = 0.0024
max = 0.0926	max = 0.0564
$\bar{x}$ = 0.0213	$\bar{x}$ = 0.0158
$t_{emp.(\alpha = 0.0440)} = 2.035 > t_{tab.(\alpha = 0.05)} = 1.97$	

Table 3

Comparison of fat content of dry matter in the fat body of bumblebee queens (g).

Diapausing queens	Non-diapausing queens
min = 0.0031	min = 0.0014
max = 0.0943	max = 0.0721
$\bar{x}$ = 0.0225	$\bar{x}$ = 0.0123
$t_{emp.(\alpha = 0.0092)} = 2.645 > t_{tab.(\alpha = 0.01)} = 2.60$	

Table 4

Comparison of the contents of particular groups of fatty acids in the fat of the fat body of the analysed bumblebee queens.

Group of queens	Groups of fatty acids [%]		
	Monounsaturated fatty acids (MUFA)	Polyunsaturated fatty acids (PUFA)	Saturated fatty acids (SFA)
Diapausing queens	min = 64.05	min = 1.23	min = 17.35
	max = 77.76	max = 12.88	max = 22.95
	$\bar{x}$ = 74.20	$\bar{x}$ = 4.94	$\bar{x}$ = 19.57
Non-diapausing queens	min = 56.86	min = 1.12	min = 17.15
	max = 77.83	max = 6.23	max = 40.76
	$\bar{x}$ = 70.53	$\bar{x}$ = 3.39	$\bar{x}$ = 23.93

queens is significantly higher compared to that reported for the non-diapausing queens.

2. Fat content of dry matter of the fat body

The data collected in Table 3 demonstrate that the mean content of fat in the dry matter of the fat body is higher in the diapausing than in the non-diapausing bumblebee queens. The empirical value of the t-test is higher than the t value read out from the table at a significance level of $\alpha = 0.01$, which indicates a highly significant difference between the values compared. The mean contents of fat in the dry matter of the fat body of the diapausing queens are significantly higher than those in the non-diapausing queens.

3. Fatty acid analysis in fat

The contents of particular groups of fatty acids in fat of diapausing and non-diapausing queens are presented in Table 4.

Table 5

Comparison of the contents of monounsaturated fatty acids (MUFA) in the fat body of bumblebee queens [%].

Diapausing queens	Non-diapausing queens
min = 64.05	min = 56.86
max = 77.76	max = 77.83
$\bar{x}$ = 74.20	$\bar{x}$ = 70.53
$t_{emp.(\alpha = 0.2278)} = 1.261 > t_{tab.(\alpha = 0.05)} = 1.97$	

Table 6

Comparison of the contents of polyunsaturated fatty acids (PUFA) in the fat body of bumblebee queens.

Diapausing queens	Non-diapausing queens
min = 1.23	min = 1.12
max = 12.88	max = 6.23
$\bar{x}$ = 4.94	$\bar{x}$ = 3.39
$t_{emp.(\alpha = 0.3073)} = 1.059 > t_{tab.(\alpha = 0.05)} = 1.97$	

In bumblebee queens, the fat of the fat body consists mostly of monounsaturated fatty acids, which constitute 74.20% in the diapausing queens and 70.5% in the non-diapausing ones, on average. The lowest concentrations were observed for polyunsaturated acids, i.e. 4.94% and 3.39% in the diapausing and non--diapausing bumblebee queens, respectively. In turn, the mean content of saturated fatty acids reached 19.57% and 23.93% for the respective groups of queens. In order to determine statistically significant differences in the contents of particular groups of fatty acids in the fat of the fat body, statistical values of the t-test were calculated for each group of fatty acids in the bumblebee queens analysed and are presented in Tables 5, 6 and 7.

The results collated in Table 5 show that the mean content of monounsaturated fatty acids (MUFA) in the fat body of the diapausing bumblebee queens is higher than that reported for the non-diapausing queens. The empirical value of the t-test is lower than the t value read out from the table at a significance level of $\alpha = 0.05$, which points to significant differences between the means. According to assumptions of the t-test, lack of significant differences between both groups of bumblebee queens was assumed in terms of the mean content of monounsaturated fatty acids.

The data presented in Table 6 demonstrate that the mean content of polyunsaturated fatty acids (PUFA) in the fat body of the diapausing bumblebee queens was higher than the mean content of respective acids in the fat body of the non-diapausing queens. The empirical value of the t-test is lower than the t value read out from the table at a significance level of $\alpha = 0.05$, and thus indicates a significant difference between the means. According to assumptions of the t-test, a lack of significant differences between mean values of this trait was assumed in both groups of bumblebee queens examined.

The results presented in Table 7 demonstrate that the mean content of saturated

Table 7

Comparison of the contents of saturated fatty acids (SFA) in the fat body of bumblebee queens.

Diapausing queens	Non-diapausing queens
min = 17.35	min = 17.15
max = 22.95	max = 40.76
$\bar{x}$ = 19.57	$\bar{x}$ = 23.93
$t_{emp.(\alpha = 0.1323)} = 1.598 > t_{tab.(\alpha = 0.05)} = 1.97$	

fatty acids (SFA) in the fat body of the diapausing bumblebee queens was lower than the content of respective fatty acids in the fat body of the non-diapausing queens. The empirical value of the t-test is lower than the t value read out from the table at a significance level of $\alpha = 0.05$, which indicates that the mean values are significantly different. According to the assumptions of the t-test, a lack of significant differences was assumed between the mean contents of saturated fatty acids in both groups of bumblebee queens examined.

4. Amino acid analysis in the fat body of bumblebee queens examined

In order to depict the results obtained for amino acid composition, distribution of mean values is presented in Figure 1.

The one-way analysis of variance carried out for each amino acid determined demonstrated significant differences in the contents of serine, cystine, glycine and alanine. Higher contents of serine, cystine and alanine were reported in the diapausing queens. In contrast, the non-diapausing queens were characterized by a signifi-

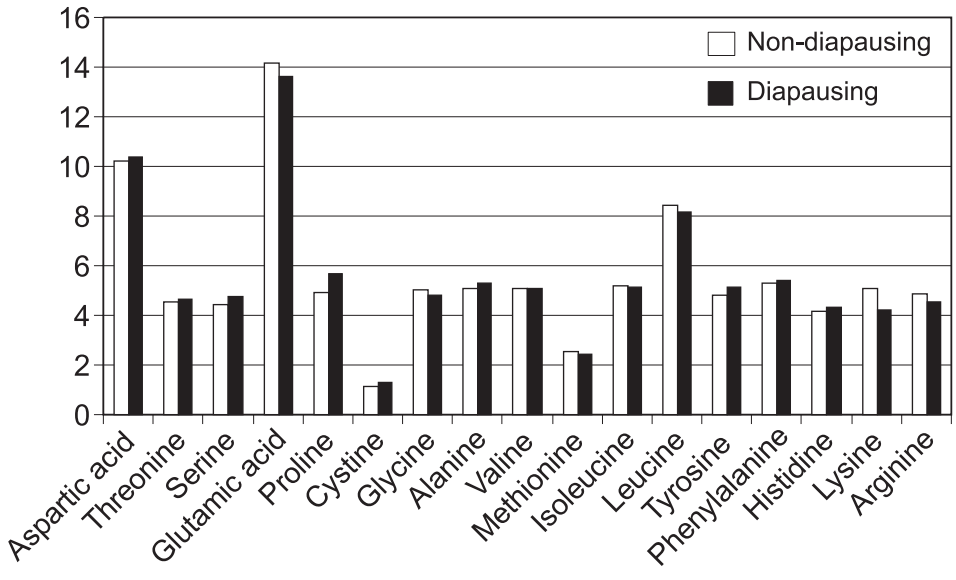

Fig. 1. Mean content of amino acids in the fat body after fat extraction in diapausing and non-diapausing queens (in g/100g of total protein).

cantly higher content of glycine in their fat body. However, only in the case of serine were the differences highly statistically significant.

DISCUSSION

In the investigations of the fat body, use was made of queens of the large earth bumblebee, since it is the most commonly applied species both in rearing as well as in commercial pollination of crops (Ruijter and Richards 1997, Manino et al. 1994). Therefore, studies aimed at searching for causes of not falling into diapause by large earth bumblebee females can be of high practical value and can affect the improvement of rearing methods of those insects.

The fat body is strongly linked with the circulatory system of insects and serves a variety of metabolic functions. That organ develops especially intensively in bumblebee queens preparing for the survival of the period of diapause, since during winter sleep it is the only source of storage nutrients. In addition, it is the main organ responsible for indirect metabolism of insects (Price 1973, Wyatt 1975), as it synthesizes and accumulates feed reserves in the form of fats, carbohydrates and proteins. The fat body also plays a significant role in detoxication processes and hormone metabolism (Sujak 1984).

It constitutes the largest fat deposit in an insect's body. Its structure enables the maximum access to hemolymph. As the main storage tissue, it possesses a system of circulation operating on the basis of diffusion and is well located both for released and absorbed metabolites (Keeley 1985).

Previous studies by the authors of this paper show that bumblebee queens that fell into the state of diapause had substantially more fat body than non-diapausing ones (Fliszkiewicz 2002). The fat content of the fat body was also statistically significantly higher in the diapausing queens than in those not falling into diapause. Thus, both its content and composition may be factors determining the survival of adult insects in the period of diapause as well as their preparation for the survival of such a hard period.

The fat body is mainly composed of triglycerides and fatty acids, predominated by palmitic, stearic, oleic, linolic and linolenic acids (Stephen and Gilbert 1969). As shown by the author's previous research, in the fat body of the bumblebee queens examined, the highest concentrations were reported for the following fatty acids: palmitic, palmitoleic, stearic, oleic and myristic acids. Nevertheless, differences in the contents of individual fatty acids between the diapausing and non-diapausing queens appeared to be statistically insignificant.

Pelt-Verkuil (1979) claims, however, that the role of the fat body in insects, especially in the period of diapause, has not been explicitly recognized yet. Likewise, Writz (1973), who also studied the fat body in *Hymenoptera*, claims that despite numerous research reports, data on this organ in hymenopteran insects are still insufficient and fragmentary. Hence, studies addressing this problem require undertaking further research into the role and changes occurring in the fat body of diapausing insects.

CONCLUSIONS

1. A significantly higher content of dry matter in the fat body, as well as its fat content in the diapausing queens as compared to the queens not falling into diapause, indicate that both of these factors do not affect the diapause of bumblebees.
2. A lack of differences in the contents of fatty acids, both monounsaturated, polyunsaturated and saturated ones, in the fat body of the diapausing and

non-diapausing bumblebee queens indicates that this factor cannot affect falling into the state of diapause by bumblebee queens.

3. Amongst the amino acids determined in the fat body of bumblebee queens, of key significance to the process of falling into the state of diapause are serine, glycine and alanine, since only in the case of these amino acids were quantitative differences observed between the compared groups of bumblebee queens.

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REFERENCES

Beekman M., Stratum P., Lingeman R., Stratum P. (1998a) – Diapause survival and post diapause performance in bumblee qeens (*Bombus terrestris*). *Entomologia Experimentalis et Applicata*. 89 (3) : 207-214.

Bilinski M. (1976) – Chów trzmieli w izolatorach. *Pszczeln. Zesz. Nauk.* 20: 41-68.

Biliński M. (1981) – Zasady chowu i wykorzystania trzmieli. *Pr. Inst. Sad.* Ser. F, Instrukcje Upowszechnieniowe, Skierniewice.

Biliński M. (2002) – Bionomia chów i wykorzystanie trzmiela ziemnego *Bombus Terrestris* (Linnaeus, 1758) do zapylania upraw szklarniowych. *Zesz. Nauk*. ISiK.

Bowers M.A.(1986) – Resource availability and timing of reproduction in bumble bee colonies (*Hymenoptera*, *Apidea*). *Anvironmental Entomology*. 15 : 750-755.

Dylewska M. (1996) – Nasze trzmiele. Ośrodek Doradztwa Rolniczego, APW w Karniowicach.

Eijnde J., Ruijter A., Steen J. (1991) – Method for rearing *Bombus terrestris* continuously and the production of bumblebee colonies for pollination purposes. *Acta Horticulturae*. 288: 154-158.

Fliszkiewicz M. (2002) – Causes of the lack of diapause in bumble bee females (*Bombus* Latr., *Apoidea*). *J. apic. Sci.* vol. 46 No. 1: 31-40.

Keeley L.L. (1985) – Physiology and biochemistry of fat body. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology. *Pergamon Press*, Oxford, England, 3(6): 211-249.

Klimaszewski S.M. (1996) – Zarys entomologii ogólnej. Skrypty Uniwersytetu Śląskiego 520. *Wydawnictwo Uniwersytetu Śląskiego*, Katowice.

Manino A., Marletto F., Porporato M., Allais L. (1994) – Research on the rearing of bumblebees in artificial nests. *Ethology Ecology and Evolution*. 0 (SPEC. ISSUE 3): 95-99.

Pelt-Verkuil E. (1979) – Hormonal control of lysosomal activity in the larval fat body of Calliphora erythrocephala Meigen. *Duth Efficiency Bureau-Pinjocker*. Leiden.

Plowright R.C., Jay S.C. (1966) – Rearing bumble bee colonies in captivity. *J. apic. Res*. 5 : 155-165.

Price G.M. (1973) – Protein and nucleic acid metabolism in insect fat body. *Biol. Rev.* 48: 333-375.

Pridal A., Hofbauer J., (1996) – Laboratory rearing and nutrition of young queens of bumblebee (*Bombus terrestris* L.) from emergency to diapause. Sbornik Vedeckych Praci Vyzkumny Ustav Picnicarsky spol. s. r. o. Troubsko. 14 : 125-131; Ba.

Ruijter A., Richards K.W. (1997) – Commercial bumblebee rearing and its implications. Proceedings of the 7th International Symposium on Pollination, Lethbridge, Alberta, Canada, 23-28 June 1996. 261-269; Bd.

Stephen W.F.Jr., Gilbert L.I. (1969) – Fatty acid biosynthesis in the silkmoth, Hyalophora cecropia. *J. Insect Physiol.* 15: 1833-1854.

Sujak P. (1984) – Metabolizm tlenowy ciała tłuszczowego w cyklu życiowym owadów. Seria Biologia 30, *Wydawnictwo Naukowe UAM* w Poznaniu.

Tasei J.N., Aupinel P. (1994) – Effect of photoperiodic regimes on the oviposition of artificially overwintered *Bombus terrestris* L. queens and the production of sexuals. *J. apic. Res*. 33 (1) : 15-20.

Wąsowicz E. (1984) – Szybka metoda oznaczania kwasu erukowego w nasionach rzepaku za pomocą chromatografii gazowej. Przemysł Spożywczy 38: 353-355.

Writz P. (1973) – Differentiation in the honeybee larva. A histological, electron – microscopical and physiological study of caste induction in *Apis mellifera mellifera*. *LandbHoogesh Wageningen*. 73-75:1-155.

Wyatt G. R. (1975) – Regulation of protein and carbohydrate metabolism in insect fat body. *Verh. Dtsch. Zool. Ges*. 67: 209-226.

KWASY TŁUSZCZOWE I AMINOKWASY W CIELE TŁUSZCZOWYM MATEK TRZMIELA *Bombus terrestris* (L.) ZAPADAJĄCYCH I NIEZAPADAJĄCYCH W STAN DIAPAUZY

Fliszkiewicz M., Wilkaniec Z.

Streszczenie

W hodowli zamkniętej trzmieli obserwuje się zjawisko niezapadania w stan diapauzy młodych unasienionych matek, co prowadzi do strat w hodowli. Jednym z warunków przejścia unasienionych matek w stan hibernacji jest zmagazynowanie przez nie odpowiedniej ilości ciała tłuszczowego. Celem niniejszej pracy było porównanie suchej masy ciała tłuszczowego, a także jego składu aminokwasowego i kwasów tłuszczowych u diapauzujących i niediapauzujących matek trzmielich pozyskanych z hodowli. Na podstawie uzyskanych wyników stwierdzono, że matki zapadające w stan diapauzy charakteryzowały się większą średnią zawartością suchej masy w ciele tłuszczowym i zawartego w niej tłuszczu, aniżeli matki niediapauzujące. Nie stwierdzono natomiast różnic w ilości kwasów tłuszczowych w ciele tłuszczowym. Natomiast stwierdzono różnice zawartości aminokwasów w ciele tłuszczowym obydwu grup matek. Jednakże wystąpiły one jedynie w przypadku seryny, cystyny, glicyny i alaniny.

Słowa kluczowe: trzmiel ziemny, *Bombus terrestris* (L.), diapauza, ciało tłuszczowe.