

Original papers

Composition of fatty acids in the *Varroa destructor* mites and their hosts, *Apis mellifera* drone-prepupae

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ABSTRACT. The fatty acid (FA) profile of lipids extracted from the *Varroa destructor* parasitic mite and its host, drone prepupae of *Apis mellifera,* was determined by gas chromatography (GC). The percentages of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were generally similar in parasites and their hosts. Fatty acids were arranged in the following descending order based on their content: MUFAs (ca. 52–55%), SFAs (ca. 41%) and PUFAs (ca. 3%). The predominant fatty acids were oleic acid (46% in mites, 44% in prepupae) and palmitic acid (23% and 30%, respectively). *Varroa* parasites differed from their hosts in the quantity of individual FAs and in their FA profiles. Three PUFAs noted in the host were not observed in parasitic mites, whereas the presence of C21:0, C24:0 and C22:1 FAs was reported in mites, but not in drones.

Key words: fatty acids, lipids, *Varroa destructor*, *Apis mellifera*, drone brood, parasitic mite

Introduction

Fatty acids (FA) play numerous roles in living organisms. They are the main source of energy and the building blocks of cell membranes. In arthropods, FAs are the precursors for the synthesis of waxes, growth regulators, defensive and semiochemical compounds [1,2]. The latter function is observed in host-parasite relationships where FAs act as kairomones that enable the parasite to find and establish contact with a suitable host. The kairomonic effect of FAs and their derivatives in promoting *Varroa destructor* invasions in honey bees (*Apis mellifera*) has been discussed in numerous studies. In addition to free FAs and their esters, a similar role is played by FA reduction products: aldehydes, alcohols and hydrocarbons [3–7]. High-molecular-weight compounds, mainly the parasite's dimethyl alkanes, which change brood recognition pheromones, can prompt nurse bees to open infected brood cells [8] where *V. destructor* mites develop and reproduce [9]. Hemolymph compounds ingested by mites from brood are the only source of nutrients that satisfy the parasite's life needs [9,10], therefore, the proximate composition of the body's building blocks can be expected to be similar in the host and the parasite. The proximate composition, including the lipid composition of *V. destructor* females, was determined in our previous study [11]. This paper complements our previous findings and supplies new data about the fatty acid profile of lipids extracted from the analyzed parasite. This study also compares the FA composition of lipids extracted from infected drone prepupae with that of mature *V. destructor* females parasitized them. The results of the study will expand our knowledge of metabolic connections in parasitic interactions between *Varroa* mites and honey bees. Such connections were previously noted in protein metabolism [12]. The presence of connections in lipid metabolism was suggested by studies of cuticular steroids and hydrocarbons in

bees and parasites [13–16]. They are also signified by molecular mimicry which protects mites against removal from cells and weakens the nest-cleaning behavior of adult bees [6]. This study aims to determine the presence of metabolic connections between the parasite and the host based on the fatty acid composition of lipids isolated from *V. destructor* and its host, *A. mellifera* drone prepupae.

Materials and Methods

Biological material. The experimental material consisted of mature female mites and drone prepupae from five *A. mellifera carnica* colonies naturally infected with *V. destructor.* The material was collected in early July 2013 from an apiary situated 20 km from Gdańsk (N 54,192722; E 18,519556). *V. destructor* mites were isolated from drone brood cells immediately after transport to the laboratory (within approximately 1.5 h). Drone prepupae infected by the parasites were collected simultaneously. Three pooled mite samples (n=150 each) were randomly collected from the entire mite population (n=540). They were rinsed four times in 30 mL of distilled water for 10 minutes with light shaking to remove any impurities. Three pooled samples of drone prepupae (n=12 each) were randomly selected from the entire population of the *Varroa* infected drone prepupae (n=370). They were gently rinsed with cool distilled water in a mesh sieve to remove impurities. Rinsed impurities were discarded. Mites and drone prepupae were dried on filter paper, weighed and stored at –70°C.

Analytical procedure. *Total lipid extraction.* Lipids were extracted from pooled samples of mites and prepupae by the method described by Fochl et al. [17]. The samples were ground in a porcelain mortar in an ice bath by combining 50 mg of frozen material with 1 mL solution of chloroform/methanol (2:1, v/v) with the addition of 0.005% butylated hydroxytoluene (BHT). After 15 minutes, 15 mL of the extraction mixture and chloroform were gradually added, and the homogenate was passed through filter paper. The retained sediment was rinsed several times with the extraction mixture. The filtrate was combined with of 0.1 M aqueous solution of potassium chloride (1/3 of the filtrate's volume), it was stirred and left to stand at 2°C for 12 hours. The top two of the three formed layers were discarded. The bottom layer was passed through filter paper which was then rinsed three times with chloroform. The filtrate was evaporated in a rotary vacuum evaporator at 40°C. Lipids were dissolved in 1 mL of chloroform, the lipid solution was evaporated in a nitrogen atmosphere and stored in tight glass containers.

Analysis of fatty acid composition. Fatty acids were converted to methyl esters (FAMEs) in accordance with EN: ISO 5509 [18]. FAMEs were separated based on the length of the hydrocarbon chain, the degree of FA unsaturation and the position of double bonds, by high-resolution gas chromatography (HR-GC) in the Hewlett Packard 5890 Series II GC system equipped with a split/splitless injector and a flame-ionization detector (FID), on the Rtx 2330 chromatography column (105 m x 0.25 mm) (Restek, Bellefonte, Pennsylvania, USA). Helium was the carrier gas with the flow rate of 0.65 mL/min. Separation temperature was set at: 180°C (20 min) to 210°C in the column, in steps of 1.5°C/min, and maintained for 80 min, 250°C in the detector, and 250°C in the injector. A qualitative and quantitative analysis of chromatograms was carried out by comparing FAME retention times in the evaluated samples with the retention times of FAME standards (Supelco 37 Component FAME Mix, Supelco Bellefonte, Pennsylvania, USA; Larodan Fine Chemicals, Malmö, Sweden). FA percentages were normalized with the use of internal standards and adjustment coefficients (to convert the percentage of peak area to the percentage of component weight) according to PN-EN ISO 5508 [19]. Each sample was analyzed in triplicate .

The results were processed statistically by the t-test for independent samples in the Statistica 9.0 PL application (StatSoft Polska).

Results and Discussion

The quantity of lipids and their fatty acid composition are largely determined by living conditions and diet [1,2]. According to Garadev et al. [10], lipids and carbohydrates are a vital source of energy for *V. destructor.* Fatty acids and their metabolites are important semiochemical compounds for arthropods [20]. In recent research, much attention has been paid to volatile compounds: alcohols, esters and hydrocarbons which play an important role in bee colonies and in host-parasite relations [14,15,21–24].

The lipids extracted from the drone prepupaes and mites parasitizing them were composed of minimum 24 fatty acids with chain length of C8:0 to

Table 1. Fatty acid composition (%) of total lipids extracted from *V. destructo*r and *A. mellifera* drone prepupae

Fatty acid		***V. destructor*** **(mean* ± SD)**	***Apis mellifera* drone (mean* ± SD)**	***P***
Caprylic	8:0	ND	0.15 ± 0.01	
Lauric	12:0	0.12 ± 0.02^{a} **	0.25 ± 0.02^{b}	p=0.0015
Myristic	14:0	0.95 ± 0.14^{a}	1.17 ± 0.07^{a}	p=0.5000
Pentadecylic	15:0	0.29 ± 0.04^{a}	0.59 ± 0.03^{a}	p=0.0013
Palmitic	16:0	23.34 ± 1.30^{a}	29,29 ± 0.65^{a}	p=0.0325
Margaric	17:0	0.39 ± 0.05^{a}	0.51 ±0.03^{b}	p=0.0092
Stearic	18:0	13.56 ± 1.63^{a}	8.49 ± 0.99^{b}	p=0.0026
Arachidic	20:0	1.06 ± 0.32^{a}	0.37 ± 0.06^{b}	p=0.0107
Heneicosylic	21:0	0.06 ± 0.01	ND***	
Behenic	22:0	0.92 ± 0.09^{a}	0.39 ± 0.06^{b}	p=0.0005
Lignoceric	24:0	0.78 ± 0.07	ND	
Total saturated fatty acids (SFA)		**41.47**	**41.21**	
Myristoleic	14:1	0.08 ± 0.02^{a}	0.22 ± 0.05^{b}	p=0.0046
Pentadecenoic	15:1	0.33 ± 0.05^{a}	0.40 ± 0.05^{a}	p=0.0921
Palmitoleic	16:1 9c	2.72 ± 0.38^{a}	5.24 ± 0.26^{b}	p=0.0011
Heptadecaenoic	17:1	0.93 ± 0.06^{a}	4.12 ± 0.24^{b}	p=0.0002
Elaidic	18:1 9t	0.80 ± 0.05^{a}	0.79 ± 0.07^{a}	p=0.4446
Oleic	18:1 9c	46.09 ± 2.62^{a}	43.81 ± 2.38^{a}	p=0.2039
Gondoic	20:1	0.06 ± 0.01^{a}	0.30 ± 0.05^{b}	p=0.0005
Erucic	22:1	0.53 ± 0.09	ND	
Total monounsaturated fatty acids (MUFA)		**52.54**	**54.88**	
Linoleic	18:2 (n-6)	0.96 ± 0.07^{a}	0.34 ± 0.20^{b}	p=0.0007
α-Linolenic	18:3 (n-3)	2.07 ± 0.17^{a}	0.76 ± 0.41^{b}	p=0.0004
γ-Linolenic	18:3 (n-6)	ND	0.24 ± 0.07	
Stearidonic	18:4	ND	0.28 ± 0.05	
Eicosadienoic	20:2	ND	1.21 ± 0.26	
Total polyunsaturated fatty acids (PUFA)		**3.03**	**2.83**	
Unrecognized fatty acids		**2.96**	**1.08**	

*Averages of three replicates of *V. destructor* (n=150 each) and of *A. mellifera* drone prepupae (n=12 each); **Means with the same letter in each row do not differ significantly from one another, P<0.05; *** ND – not detected

C24:0, 20 of which were identified in *V. destructor* and 21 – in *A. mellifera*. The identified fatty acids were divided into three groups: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) (Table 1). No significant differences in the percentages of FAs representing the three analyzed groups were observed between parasitic mites and drone prepupae. The major difference was noted in the MUFA content of lipids which was approximately 2% lower in *V. destructor* than in drone prepupae (Table 1). The qualitative composition of FAs was also similar in specific FAs groups in drone prepupae and parasitic mites. Certain differences were noted in the group of PUFAs, where three fatty acids were identified in the host, but not in the parasite. By contrast, two long-chain SFAs (C21 and C24) and one MUFA (C22:1) were determined in *V. destructor,* but not in *A. mellifera*. It is also worthwhile noting that the content of specific FAs from all groups differed considerably between the parasite and the host (Table 1).

A quantitative analysis of FAs in lipids extracted from both species revealed a predominance of MUFAs which accounted for more than 50% of all FAs. The second most abundant group were SFAs, whereas PUFAs were determined in the smallest quantity. Our findings were not identical to those reported by Finke [25] who observed that unlike in other insects, bee brood lipids are composed mostly of SFAs (51.75%) and MUFAs (46.25%), and contain very small amounts of PUFAs (around 2%). Short-chain SFAs (≤ C17) had a smaller share of lipids extracted from parasites than from drone prepupae, whereas SFAs with chains longer than C18 had a greater share of the lipids extracted from the *Varroa* mites. C21:0 and C24:0 SFAs were not identified in lipids isolated from drone prepupae (Table 1). The above could be attributed to the fact that mites live in a more stable environment than bees. Mites can spend their entire life inside a beehive in almost stable temperatures, therefore, their lipids could be characterized by a higher melting temperature and a higher number of long-chain SFAs in comparison with bees. Parasites leave the beehive only when they prey on foragers and adult drones [9]. Palmitic acid was the most prevalent SFA, and it accounted for 23.34% and 29.29% of total FAs on average in parasites and drone prepupae, respectively. Stearic acid was the second predominant SFA, and it was significantly more abundant in the lipids of parasites than prepupae. High concentrations of palmitic and stearic acids in parasite lipids can contribute to the reproductive success of mites. Recent research revealed that C16:0, C18:0, C18:1 and C18:2 fatty acids and their ethyl esters constitute sex pheromones of *V. destructor* females [26]. SFAs with an odd number of carbon atoms represented only 0.81% and 1.1% of total FAs in mites and drone prepupae, respectively (Table 1).

The predominant FAs from both species were arranged in the following descending order: oleic acid (C18:1 9c), palmitic acid (C16:0), stearic acid (C18:0) and palmitoleic (C16:1 9c). Le Conte et al. [5] analyzed hexane extracts from the surface of 8-day-old larvae of *A. mellifera* drones to determine the highest content of C18:3 methyl esters and 50% lower concentrations of C18:0 and C16:0. Our study analyzed lipids from whole body extracts of prepupae, where cuticular lipids were only one of many components, therefore, the cited data cannot be compared with our findings. Our findings corroborate the results of an earlier study investigating bee brood. According to Van der Vorst et al. [27], the above fatty acids have a 70% share of the FA pool in the lipids of bee larvae.

MUFAs with an even number of homologous series carbon atoms (C16 – C22) had a high share of FAs. They were represented mainly by oleic acid, followed by palmitoleic acid (C16:1 9c) whose content was approximately 17-fold lower in mites and 8-fold lower in drone prepupae in comparison with C18:1 9c. In drone prepupae, MUFAs were characterized by a high percentage of heptadecenoic acid (C17:1) with an odd number of carbon atoms (Table 1). The above observation was surprising because heptadecenoic acid was not identified in lipids extracted from developing worker bee brood [27].

PUFAs play important vital physiological functions [1]. They are the building blocks of cell membranes which are responsible for membrane fluidity [28]. In vertebrates and in insects, PUFAs can be a source of prostaglandins and eicosanoids which regulate immunity, inflammatory responses and development [29,30]. PUFAs had a similar share of the FA pool in *V. destructor* and *A. mellifera*. The predominant PUFA was α-linolenic acid which accounted for approximately 68% and 27% of total PUFAs in mites and drone prepupae, respectively. The only other PUFA in *V. destructor* was linoleic acid, but its content was half lower in comparison with linolenic acid. In drone prepupae, the remaining PUFAs were γ-linolenic acid, stearidonic acid (C18:4) and eicosadienoic acid (C20:2), none of which were found in mites (Table 1). De Renobeles et al. [31] observed that 15 insect species belonging to four orders were capable of *de novo* synthesis of linoleic acid. For most insects, however, diet is the only source of PUFAs [22]. Some insects are supplied with essential fatty acids from dietary MUFAs with the participation of enzymes from the microbiota that colonize their gastrointestinal system. Selected insects, such as the house cricket and the American cockroach, have their own desaturation enzymes (Δ12 desaturases) and enzymes that participate in the elongation of C18 acids [29]. There is a general absence of knowledge about the presence of such enzymes and the processes they catalyze in *V. destructor*. The content of C18:3 and C18:2 fatty acids is much higher in mites than in prepupae, but it does not exclude the possibility of desaturation processes in mites. Detailed analyses are required to confirm the above hypothesis. Dietary pollen is a rich source of

unsaturated fatty acids for bees [32,33], and prepupae lipids are characterized by a more diverse PUFA profile than parasite lipids. The impact on *V. destructor* infections on the host's FA profile remains unknown, and it will be investigated in a follow-up study. Changes were observed in the lipids of *A. mellifera* infected by *Nosema apis.* Changes in the percentages of C18 fatty acids – an increase in the content of C18 PUFAs and a decrease in the concentrations of C18:0 and C18:1 fatty acids – were noted in worker bees infected by the above protozoan [34].

Conclusions

As expected, the FA profile of lipids in *V. destructor* mites infected the prepupae of honeybee drones is similar to the FA profile of the host's lipids which constitute the main source of FAs for parasitic mites. However, significant and numerous differences in the content of specific FAs in the Varroa-mite and drone-prepupae lipids point to metabolic differences between the parasite and the host. The content of C18 FAs was significantly higher in mites than in drone prepupae, which could suggest that those fatty acids are particularly valuable for the parasite. Stearic acid is an important sex hormone for mature *Varroa* females. The concentrations of C18:1, C18:2 and C18:3 (n-3) unsaturated fatty acids were higher in mites, which could imply that those FAs are not only extracted from the host's lipids, but that the parasite may be capable of desaturating or synthesizing those compounds *de novo*. Desaturation and synthesis reactions were not investigated in parasitic mites to date. Our knowledge about the presence and properties of FA-catalyzing enzymes should be expanded to effectively control parasitic infections based on inhibition of FA metabolic processes in the parasite.

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