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Fatty acid profile of *Apis mellifera* workers in the face of an immune challenge

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Abstract – Fatty acids (FAs) are important in metabolism and in the maintenance of *Apis mellifera* colonies. Our objective was to identify the variation in FA composition of the bodies of *A. mellifera* worker bees after immunological challenges with sterile injections and *Paenibacillus larvae* vegetative cells. The expression of the hymenoptaecin and abaecin genes was studied and the FAs were identified and quantified. After a challenge with *P. larvae*, the immune system was activated and abaecin and hymenoptaecin peptide genes were overexpressed. Also, the content of short-chain FAs such as caprylic, capric, and tridecylic acids increased significantly; on the contrary, oleic acid decreased. To the best of our knowledge, this is the first analysis of changes in the FA profile of honey bee workers after immune stimulation with *P. larvae*. Health status may be a key factor that alters FA composition. More research about the interaction with other stressors affecting the survival of honey bee colonies is needed.

fatty acids / *Apis mellifera* / immune challenge / *Paenibacillus larvae* / abaecin / hymenoptaecin

Abbreviations

FAs Fatty acids

TAGs Triglycerides

CH Cuticular hydrocarbons

BHT Butylated hydroxytoluene

FAMES Methyl esters of fatty acids

GC-FID Gas chromatography with flame ionization detection

SFAs Saturated fatty acids

MUFAs Monounsaturated fatty acids

PUFAs Polyunsaturated fatty acids

1. INTRODUCTION

According to recent reports, an estimated 101 million honey bee colonies worldwide (Shahbandeh 2023) are commercially exploited to provide pollination services and extract products derived from the colony, like wax, propolis, and honey. However, losses and depopulation of *Apis mellifera* colonies lately recorded in different regions (Maggi et al. 2016; Potts et al. 2016; Requier et al. 2015) have been attributed to various risk factors such

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as pathogens, agrochemical exposure, habitat loss and fragmentation, poor beekeeping practices, and malnutrition. Nowadays, there is a consensus that the interaction between pathogens and other environmental stress factors would act synergistically in bee populations (Potts et al. 2016). Therefore, understanding how each one of these factors modifies individuals' physiology and metabolism could help explain the causes of the global honey bee colony decline.

In *A. mellifera*, fatty acids (FAs) are obtained from pollen, which is the principal determinant of FA body composition in bees (Wright et al. 2018). Pollen consumption and lipid gain have been linked to honey bees' health enhancement (Corby-Harris et al. 2021) by increasing survival (Annoscia et al. 2017) and reducing susceptibility to insecticides (Crone and Grozinger 2021).

The FA composition of an individual is given by several abiotic and biotic factors, such as diet (Arien et al. 2015), seasonality (Castaños et al. 2023), age of development (Ghosh et al. 2016, 2021; Martin et al. 2019), temperature (Giri et al. 2018), and sanitary conditions (Roberts 1968; Nelson et al. 1971; Zalewski et al. 2016; Gołębiowski et al. 2020) and results from the balance between synthesis, accumulation, and degradation processes (Kaczmarek and Boguś 2021). Changes produced by such factors can directly affect the physical properties of an insect's constituent lipids, like membrane fluidity, and alter metabolism and energy production (Giri et al. 2018).

FAs play an essential role in the physiology and metabolism of insects as chemical precursors of pheromones, secondary metabolites (Slessor et al. 2005; Howard and Blomquist 2005; Kaczmarek and Boguś 2021), and energy demand (Arrese and Soulages 2010; Kaczmarek and Boguś 2021). Moreover, they are also mobilized to the hemolymph during an immune challenge (Mullen and Goldsworthy 2003) associated with the synthesis and activity of lipid transport proteins (Dettloff et al. 2001; Mullen and Goldsworthy 2003; Cheon et al. 2006; Lourenço et al. 2009; Zdybicka-Barabas and Cytryńska 2013).

Despite its importance and connection with the health of bees, a limited amount of research has explored the linkage between the health status of honey bees and their FA composition. Roberts (1968) examined the impact of *Nosema apis* infections on the FA profile of worker bees, while Nelson et al. (1971) investigated how virus infections affect FAs. Additionally, Zalewski et al. (2016) focused on analyzing these compounds in bee larvae parasitized by *Varroa destructor*. Therefore, their results set an encouraging precedent for further exploring this relationship.

In the present work, we studied the fatty acid (FA) profile in worker bees after different immunological challenges, including *P. larvae* injections. To our knowledge, this is the first work that studied the composition of FAs in immunostimulated adult bees and expands the knowledge about possible mechanisms of lipid metabolism regarding the health status of honey bee colonies.

2. MATERIALS AND METHODS

2.1. Biological material

Experimental apiary of a hybrid *Apis mellifera mellifera* × *Apis mellifera ligustica* (Porrini et al. 2020), belonging to the Research Institute in Production, Health, and Environment (IIPROSAM-CONICET-UNMdP) located at 25 km from Mar del Plata (38° 10' 06" S; 57° 38' 10" W), in optimal sanitary conditions was used.

Paenibacillus larvae strain isolated from larvae affected by American foulbrood from an Argentine apiary belonging to our strain collection was used (IIPROSAM-CONICET-UNMdP). Bacterial isolate has been previously identified as ERIC-I by biochemical and molecular analysis (Fernández et al. 2014) and maintained in MYPGP agar (Mueller-Hinton broth, yeast extract, glucose, K₂HPO₄, and sodium pyruvate agar). Vegetative cells from 48-h fresh cultures were suspended in sterile distilled water (0.5 McFarland scale) for use.

2.2. Experimental design and bee microinjection procedure

Capped comb with brood in the last stages of development of a single colony was transferred to the laboratory and kept under controlled conditions at 32–34 °C and 60% RH. Newly emerged bees (less than 24-h-old) were collected from this comb and placed in three experimental cages (a, b, and c) with 200 same-age individuals each. Those bees were fed with 63.6% w/w powdered sugar, 27.3% w/w glucose, and 4.5% v/w feed supplement (Promotor L[®]) for 10 days. Bees received fresh food daily, and their consumption was recorded simultaneously. The mortality and amount of food consumed per live bee per day were calculated.

Ten-day-old bees from the three cages (a, b, and c) were split randomly into four treatment groups. The first group remained without treatment (control), bees of the second group received cutaneous microinjection (stick), the third group was injected with 1 µl/bee of sterile distilled water (water-injected), and the fourth group was inoculated with 1 µl/bee of a *P. larvae* suspension at 0.5 McFarland scale (PL-injected). Microinjection was performed in the abdominal cavity between the second and third sternites of the bee using a sterile glass microcapillary connected to a micropipette, using the method from Randolt et al. (2008) adapted. Thereby, bees were grouped in special cages according to the treatment received and kept at 29–30 °C with a food supply for 24 h. Three cages (replicates) for each treatment ($N=12$, 3 cages per treatment) containing 25 bees each were used. After 24 h, food intake and bees' mortality were measured, and the surviving bees were quick-frozen and preserved at –80 °C for gene expression and at –18 °C for the analytical determination of FAs.

2.3. Gene expression

2.3.1. RNA extraction, DNase treatment, and reverse transcription

Total RNA from individual adult bees was isolated using TRI reagent (BioBasic) according to the manufacturer's protocol. DNase I

Amplification Grade (Invitrogen) was used to degrade genomic DNA carried over in the RNA extraction by digestion at 37 °C for 30 min. The quantity of the resulting RNA was determined in a Rotor Gene 6000 cycler (Qiagen) using a QuantiT[™] RiboGreen RNA Assay Kit (Invitrogen). Complementary DNA (cDNA) was synthesized using a reaction mixture containing 1 µg of total RNA, random hexamers (12 ng/µl), and Moloney murine leukemia virus reverse transcriptase (Invitrogen) according to the procedures suggested by the manufacturer. Negative controls omitting either the RNA or the reverse transcriptase were included during reverse transcription.

2.3.2. Primers

Primers for qPCR (synthesized by Eurofins MWG Operon, USA) used in PCR protocols, specific annealing temperature, and PCR product melting temperature are summarized in Table I.

2.3.3. RT-qPCR

To determine whether injection treatment has an effect on the expression of worker bees' immune-related genes, levels of abaecin and hymenoptaecin mRNA were quantified. RT-qPCR reactions were run with a KAPA HRM FAST qPCR kit, with EvaGreen as an intercalating dye (KAPA Biosystems, USA) and 1 µl of cDNA sample in a final volume of 20 µl. Amplification and detection of the specific products were carried out using an Analytic Jena qTOWER³Q thermocycler (Germany), with the following amplification conditions: 2 min at 95 °C, 40 cycles of 20 s at 95 °C, 205 s at specific annealing temperature, and 30 s at 72 °C. After amplification, a melting curve analysis resulted in a single product-specific melting curve. Negative controls for cDNA synthesis and qPCR procedures were included in all cases. The amplification efficiency was determined for each gene using tenfold dilutions of cDNA. *Apis mellifera* β-actin gene was used as a reference/

Table 1 Primer sequences, primer pair annealing temperature, PCR product melting temperature, and references

Primer	5'- 3' sequence	At (°C)	Tm (°C)	Reference
β-Actin-fw	ATGCCAACACTGTCCTTTCTGG	60	83.2	Yang and Cox-Foster (2005)
β-Actin-rv	GACCCACCAATCCATACGGA			
Hymenoptaecin-fw	CTCTTCTGTGCCGTTGCATA	60	83	Evans et al. (2006)
Hymenoptaecin-rv	GCGTCTCCTGTCATTCCATT			
Abaecin-fw	AGATCTGCACACTCGAGGTCTG	58	85	Yang and Cox-Foster (2005)
Abaecin-rv	TCGGATTGAATGGTCCCTGA			

At Annealing temperature, *Tm* Melting temperature

housekeeping gene. Relative expression values were calculated by the ddCT method (Livak and Schmittgen 2001). In all assays, standard curves for each gene had a slope from -3.2 to -3.5 , and the standard deviation between duplicates was <0.167 in all cases, as recommended for qPCR (Bustin 2002).

2.4. Total lipid extraction and fatty acid quantification

Total lipids were extracted from individual bees using the method from Folch et al. (1957) adapted. First, the bee's midgut was removed to avoid contamination with diet lipids. Each sample was weighed and homogenized with 1 ml of extraction mixture (chloroform/methanol solution 2:1 v/v; Biopack) with the addition of 0.005% w/v butylated hydroxytoluene (BHT). Then, 2 ml of the mixture was added and vortexed for 15 s. The homogenate was filtered, and the retained sediment was washed with 1 ml of extraction mixture. One milliliter of 1% w/v aqueous sodium chloride (NaCl; Biopack) solution (1/4 of the volume of the filtrate) was added to the resulting filtered liquid. Mixtures were stored at 4 °C for 12 h. Subsequently, the upper liquid layer was removed and the lower layer containing the lipids was evaporated under a stream of nitrogen gas. Finally, the total mass of lipids extracted was measured by weight difference.

Methyl esters of FAs (FAMES) were obtained according to the official AOCS method Ce 2-66/Ch 2-91. One milliliter of

hydrochloric acid in methanol solution (1% v/v; Biopack) was added to each tube with fat and vortexed for 15 s. Samples were left at 44 °C for 24 h, and then, 1 ml of hexane (purity $>98\%$; Merck) was added. FAMES were separated according to their chain length and double bond position using gas chromatography with a flame ionization detector (GC-FID) Agilent, HP 6890.

2.5. Statistical analyses

To test bee survival during the 10 days of confinement, survival curves were made with the Kaplan-Meier method and compared with the log-rank method. To evaluate food consumption by bees over 10 days, a generalized linear mixed model (GLMM) was used by fitting the fixed effects (time) and random effects (cages a, b, and c). Bees' mortality and food consumption at 24 h post-challenges were also analyzed using a χ^2 contingency test and variance analysis (ANOVA), respectively. Relative gene expression data were analyzed with a generalized linear model (GLM) with gamma distribution. A permutational multivariate analysis of variance (PERMANOVA) and a principal component analysis were used to analyze the FA profile in bees from all groups. The percentage of FAs extracted was compared using ANOVA. All statistical analyses and graphics were performed using the R 4.2.1 software (Team 2016).

3. RESULTS

3.1. Food consumption and mortality

Survival and food consumption of bees confined for 10 days were analyzed. The survival probability on the tenth day was greater than 0.9, and there were no significant differences among the survival curves (log-rank test: $\chi^2=5$, $df=1$, $p=0.1$; Figure 1A). Analysis of food intake showed that only during the first day did the bees consume less food; then, the consumption increased and kept constant for

the remaining days of confinement (GLMM: $F=5.132$, $p=0.001$; Figure 1B).

ANOVA showed no differences among treatment groups concerning the amount of food consumed by bees 24 h post-challenges ($F_{(3,8)}=1.647$, $p=0.254$). On the contrary, the mortality was statistically significant ($\chi^2=18.715$, $df=3$, $p=0.0003$). All immunological challenges caused higher bee mortality than the control, but there was no difference in mortality among the different injection treatments. Comparisons among the treatments' lethality 24 h post-challenges are shown in Table II.

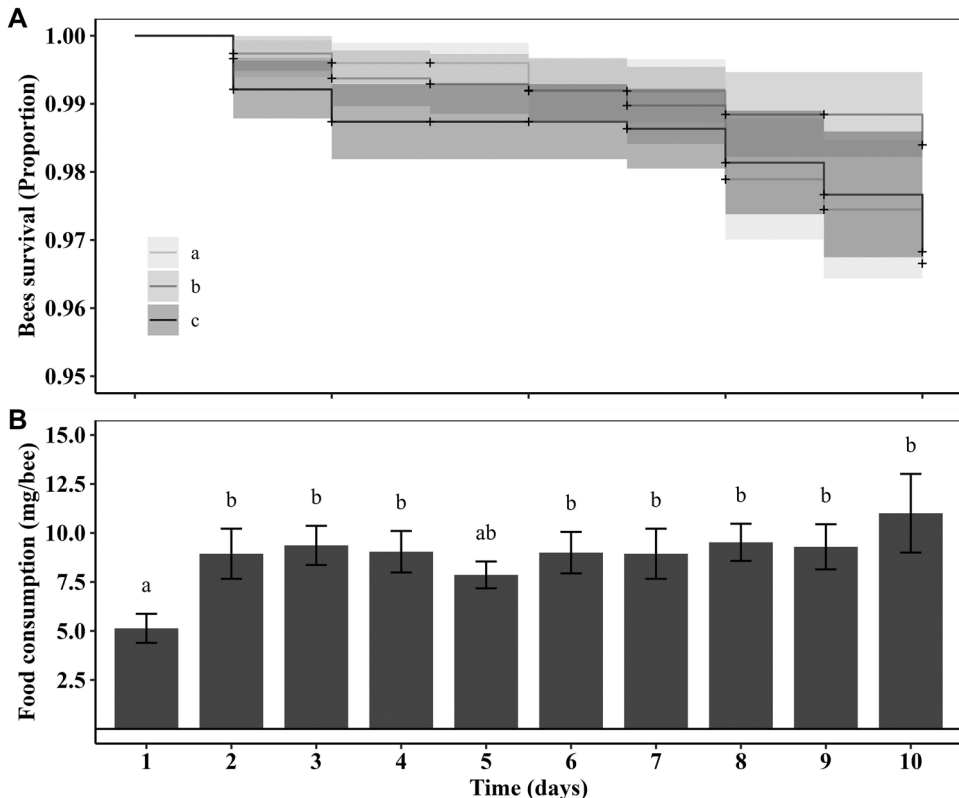


Figure 1. **A** Bee survival curves (Kaplan-Meier/log-rank test method). **B** Food consumption of bees confined for 10 days. Three replicates (a-b-c) with 200 bees each were used. Different letters indicate significant differences among mean amounts of food consumed per bee per day (Tukey method).

Table II Number of live and dead bees after 24 h post-challenges

Bees	Control	Stick	Water-injected	PL-injected
Alive	75	58	62	66
Dead	0	17	13	9
Control		Chi ² = 16.984, df= 1, p<0.001	Chi ² = 12.128, df= 1, p<0.001	Chi ² = 7.565, df= 1, p=0.005
Stick			Chi ² =0.375, df= 1, p=0.540	Chi ² =2.279, df= 1, p=0.131
Water-injected				Chi ² =0.479, df= 1, p=0.488
PL-injected				

Chi-square test comparing the mortality of four different treatments

Bee groups: without treatment (control), cutaneous microinjection (stick), sterile water injection (water-injected), and *P. larvae* suspension injection (PL-injected)

3.2. Expression of genes associated with immune responses in worker bees after challenges

Gene expression of the antimicrobial peptides abaecin and hymenoptaecin was studied to determine whether bees (*n* = 24, six bees per treatment) were immune-stimulated by the treatments. The analysis showed that bees injected

with bacterial suspension had a higher relative expression of both genes concerning bees of the other treatment groups (Figure 2). In addition, the abaecin gene was overexpressed in bees from stick groups (*t* = 3.10, *df* = 20, *p* = 0.005) and down-expressed in water-injected bees (*t* = -3.6, *df* = 20, *p* = 0.001) compared to the control bee groups (Figure 2A, C). For its part, hymenoptaecin was overexpressed in bees from stick

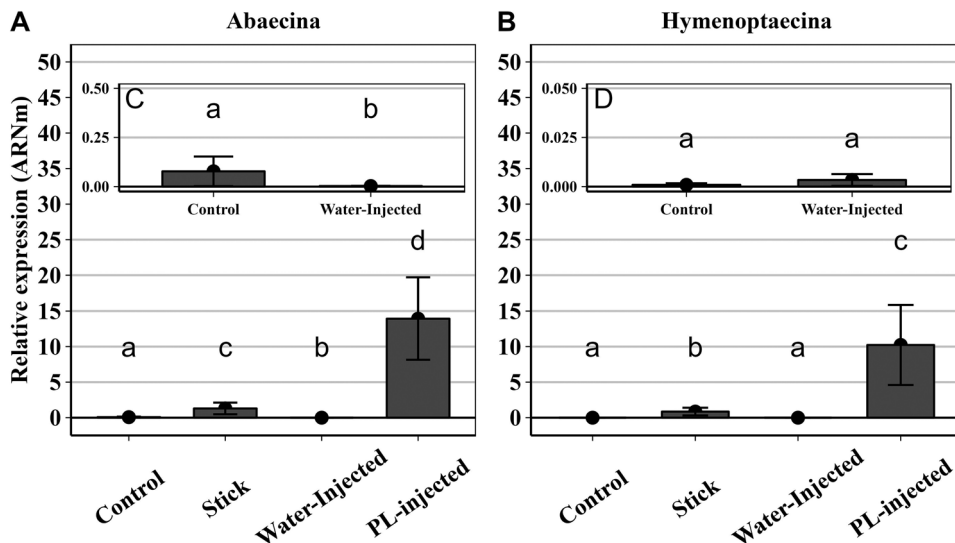


Figure 2. Relative mRNA expression levels of abaecin (A) and hymenoptaecin (B) in immune-challenged bees. Upscaling of data of abaecin expression in control and water-injected groups (C) and upscaling of data of hymenoptaecin expression in control and water-injected groups (D). Bee groups: without treatment (control), cutaneous microinjection (Stick), sterile water injection (water-injected), and *P. larvae* suspension injection (PL-injected). Different letters indicate statistically significant differences (GLMgamma, *p* < 0.05).

groups ($t=6.43$, $df=20$, $p=2.80e-6$) and water-injected bees ($t=-5.20$, $df=20$, $p=4.31e-05$) compared to control bees (Figure 2B, D).

3.3. Effects of immune challenges on total lipids and fatty acid composition

Analysis of the lipid content of bees from each treatment group showed no statistically significant differences among the mean amount of fat extracted from each group ($F_{(3,61)}=0.778$, $p=0.515$, Figure 3).

Multivariate analysis showed that the FA profile of bees was significantly affected by treatments (PERMANOVA: $F=1.565$; $R^2=0.071$; $p=0.044$), but the pairwise comparisons between them were not significantly different (control vs. PL-injected, $p=0.091$; control vs. stick, $p=0.248$; control vs. water-injected, $p=0.216$; PL-injected vs. stick, $p=0.216$; PL-injected vs. water-injected, $p=0.303$; stick vs. water-injected, $p=0.763$). The PCA showed a clear separation between control and PL-injected bee groups and two principal components that captured 44.2% of the total variability

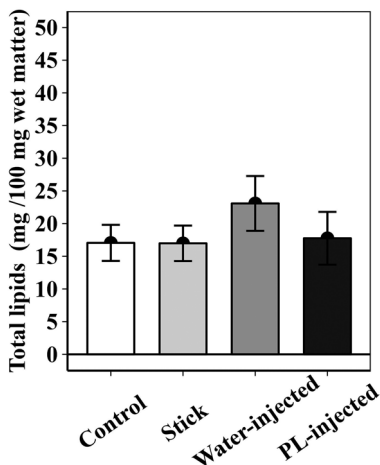


Figure 3. Total lipids extracted per treatment group expressed in $\text{mg}\cdot 100\text{ mg}^{-1}$ of wet matter. Means and standard deviations are represented. Bee groups: without treatment (control), cutaneous microinjection (stick), sterile water injection (water-injected), and *P. larvae* suspension injection (PL-injected). Non-significant differences were found (ANOVA, Tukey, $p<0.05$).

(CP1 = 28%; CP2 = 16.2%). The compounds that most influenced CP1 were oleic, tridecylic, and caprylic acids. In particular, bees of the control group displayed high levels of oleic acid and bees of the group injected with *P. larvae* showed high levels of tridecylic and caprylic acids. A separation between the stick and water-injected groups was not observed (Figure 4).

The percentage of specific FAs recognized in the bee body after the different challenges is shown in Table III. Also, FAs were grouped into saturated (SFAs), monounsaturated (MUFAs), and polyunsaturated (PUFAs) acids. A total of 13 FAs (C8 to C20) were identified in the sampled bees. Regarding the individual FAs, a significantly increased percentage of caprylic (ANOVA: $F_{(3,46)}=3.53$, $p=0.022$, Tukey: control vs PL-injected, $p=0.011$), capric (ANOVA: $F_{(3,60)}=3.96$, $p=0.012$, Tukey: control vs PL-injected, $p=0.033$), and tridecylic acids (ANOVA: $F_{(3,60)}=2.95$, $p=0.039$, Tukey: control vs PL-injected, $p=0.079$) was found in the group of bees injected with *P. larvae* compared to those of the control group. Contrarily, the percentage of oleic acid decreased significantly in the group of bees injected with bacterial suspension compared with the control (ANOVA: $F_{(3,61)}=2.79$, $p=0.047$, Tukey: control vs PL-injected, $p=0.030$). On the other hand, bees injected with *P. larvae* suspension showed a higher percentage of SFAs than those non-injected bees ($F_{(3,61)}=2.76$, $p=0.030$), while no differences were recorded in the levels of MUFAs ($F_{(3,61)}=2.39$; $p=0.076$) and PUFAs ($F_{(3,61)}=0.21$, $p=0.884$). No differences among the percentages of FAs from bees of the control, water-injected, and stick groups were detected (all $p>0.05$).

4. DISCUSSION

The change in the lipid and FA composition of organisms after exposure to parasites and pathogens can be related both to the metabolism of the host to ensure its development (Van der Meer-Janssen et al. 2010) and to the metabolism of the organism itself in the immunological defense

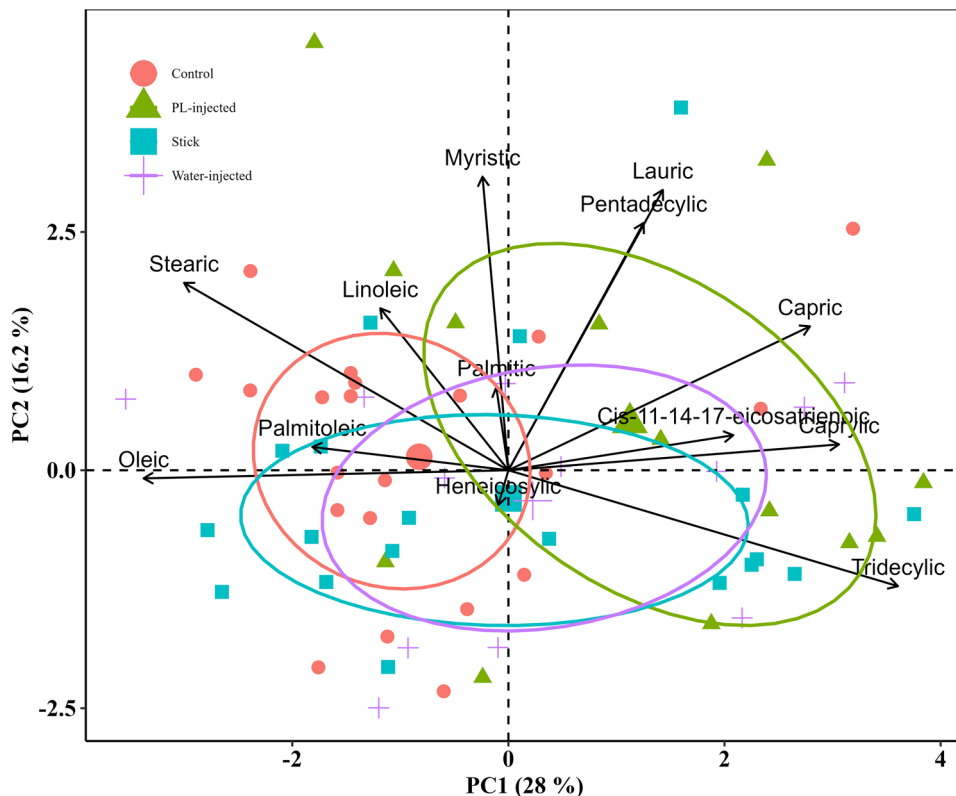


Figure 4. Biplot formed by the first two principal components (PC1 and PC2) corresponding to the analysis of principal components of the fatty acid composition of 10-day-old bees after immunostimulation treatments. Each point corresponds to each individual bee (color and shape depending on treatment). The arrows represent the intensity of the correlation between each major component fatty acid. Numbers in parentheses indicate the percentage of total variance captured by each principal component.

against diseases (Mullen and Goldsworthy 2003; Cheon et al. 2006; Arrese and Soulares 2010; Toprak 2020).

In the present work, the effect of different immune challenges in honey bees on the FA composition was evaluated. Activation of the individual immune system due to the challenges was verified by studying the relative gene expression of the antimicrobial peptides (AMPs) abaecin and hymenoptaecin. Results confirmed immune response activation in *P. larvae*-injected worker bees where the abaecin and hymenoptaecin genes were upregulated regarding the control, stick, and water-injected bees.

Our results also showed that the total lipid was not modified by either of the immune

challenges done to honey bees. The same 13 FAs (C8 to C20) were found in all bees regardless of the treatment received. Despite this, quantitative changes in the percentage of FA groups and individual compounds were detected after immunostimulation treatment with *P. larvae*.

Honey bees are capable of endogenously synthesizing most of the SFA and MUFA, so their composition is not strongly affected by the diet (Robinson and Nation 1970). However, *A. mellifera* bees are unable to synthesize PUFA from the ω 3 and ω 6 series as the acids α -linolenic (C18:3) and linoleic (C18:2), respectively, which must be acquired through pollen intake (Plettner et al. 1998; Arien et al. 2015). Here, 10-day-old bees from all four treatment groups showed around

Table III Fatty acid composition (expressed as a % w/w of the total lipids) extracted from 10-day-old worker bees after challenges (means \pm SD)

RT		Fatty acid	Control	Stick	Water-injected	PL-injected
6.22	8:0	Caprylic	0.115 \pm 0.05 a	0.167 \pm 0.09 ab	0.153 \pm 0.07 ab	0.220 \pm 0.09 b
8.46	10:0	Capric	0.142 \pm 0.07 a	0.132 \pm 0.05 a	0.170 \pm 0.04 ab	0.184 \pm 0.08 b
10.52	12:0	Lauric	0.140 \pm 0.08 a	0.152 \pm 0.08 a	0.119 \pm 0.03 a	0.146 \pm 0.05 a
12.00	13:0	Tridecylic	12.322 \pm 3.21 a	15.244 \pm 3.19 ab	14.320 \pm 4.52 ab	16.060 \pm 3.36 b
12.88	14:0	Myristic	0.308 \pm 0.07 a	0.306 \pm 0.08 a	0.304 \pm 0.09 a	0.321 \pm 0.11 a
14.41	15:0	Pentadecylic	0.341 \pm 0.09 a	0.333 \pm 0.10 a	0.350 \pm 0.11 a	0.340 \pm 0.09 a
15.81	16:0	Palmitic	6.667 \pm 1.08 a	6.506 \pm 1.03 a	6.252 \pm 0.53 a	7.097 \pm 1.05 a
19.32	18:0	Stearic	7.426 \pm 0.54 a	7.444 \pm 0.83 a	7.597 \pm 0.69 a	7.184 \pm 0.80 a
26.15	21:0	Heneicosylic	2.04 \pm 0.825 a	1.327 \pm 0.50 a	1.401 \pm 0.74 a	1.760 \pm 1.04 a
		SFAs	29.502 \pm 2.99 a	31.61 \pm 2.86 ab	30.662 \pm 4.08 ab	33.341 \pm 3.39 b
16.46	16:1	Palmitoleic	5.763 \pm 0.452 a	5.713 \pm 0.59 a	6.002 \pm 0.54 a	5.685 \pm 0.59 a
19.92	18:1	Oleic	58.324 \pm 2.71 a	56.152 \pm 2.73 a	57.047 \pm 3.87 a	54.775 \pm 2.85 b
		MUFAs	64.088 \pm 3.03 a	61.866 \pm 3.06 a	63.049 \pm 4.10 a	60.461 \pm 3.05 a
21.07	18:2	Linoleic	6.026 \pm 1.54 a	5.92 \pm 1.62 a	5.841 \pm 1.77 a	5.570 \pm 1.46 a
26.82	20:3	Eicosatrienoic	0.312 \pm 0.38 a	0.443 \pm 0.351 a	0.447 \pm 0.37 a	0.475 \pm 0.41 a
		PUFAs	6.338 \pm 1.69 a	6.286 \pm 2.32 a	6.288 \pm 1.95 a	6.223 \pm 1.68 a

Means with at least one letter in common are not significantly different

The second column shows the number of carbon atoms and the number of the double bond of the carbon chain

RT retention time (min) in chromatography analysis, SFAs total saturated fatty acids, MUFAs total monounsaturated fatty acids, PUFAs total polyunsaturated fatty acids

60% MUFAs and 30% SFAs, similar to those reported in previous works (Martin et al. 2019). However, the bees here showed average levels of PUFAs of 6.5%, which represents relatively low amounts in 10-day-old bees. Commonly, PUFA levels in *A. mellifera* increase from 1% in pupae to 5% in newly emerged bees and 18% in older worker bees (nurse and forager), and this increment is attributed to pollen consumption by bees (Arien et al. 2015; Martin et al. 2019). Although the food supplied to the bees during the assay lacked FAs, the percentage of PUFAs detected in the treated individuals could be due to the newly emerged bees (< 24 h) consuming honey and pollen of the brood box in the first hours of emergence before being caged. In this way, the individuals obtained and maintained a level of PUFAs similar to those found in recently emerged bees in nature.

We highlight that bee's challenge by *P. larvae* suspension injections affected the total profile

and amount of specific FAs. Particularly, modifications were observed in the short- or medium-chain FAs, caprylic (C8:0), capric (C10:0), and tridecylic (C13:0) acids, whose percentages increased in *P. larvae*-challenged bees by 91, 29, and 30% compared to untreated bees, respectively. From unsaturated long-chain FAs, the only one affected by the *P. larvae* injection was oleic acid (C18:1), decreasing by 6% with respect to control bees. No effects on FA amount were found in the bee groups treated with cutaneous injection (stick) or injection with sterile water (water-injected). Lipids and FA composition in the body of insects can be modified in response to pathogen infections and immunological challenges (Angelo et al. 2013; Gołębowski et al. 2020), and, in honey bees, some studies have linked health conditions and FA body composition. Roberts (1968) and Nelson et al. (1971) have studied the effects of *N. apis* and virus infections on the FA composition in adult

bees, respectively; while Zalewski et al. (2016) oriented their research to study these compounds on bee prepupae parasitized by *V. destructor*. Nevertheless, the results of those reports have been conflicting. Oleic acid (C18:1) increased in bees affected by *N. apis*, while it decreased in those infected by viruses. On the other hand, palmitic (C16:0), linoleic (C18:2), and linolenic (C18:3) acids increased in response to *N. apis* infections and decreased against viruses (Roberts 1968; Nelson et al. 1971). In contrast, worker prepupae infested with *V. destructor* showed lower amounts of palmitic (C16:0) and higher levels of oleic (C18:1) acids than the uninfected ones (Zalewski et al. 2016). However, methodological differences between the aforementioned works and the present study and dissimilarity in the pathogens used as immunological challenges prevent an unequivocal comparison of the results. Regardless, these results together are evidence to support the idea that pathogens are able to modify the FA profile of honey bees.

Our results suggest that the FA profile was modified in worker bees after immunostimulation with *P. larvae* but the reason and possible mechanisms involved are unknown. One possibility, proposed by Gołębiowski et al. (2020), is the mobilization of FAs from the fat body to the hemolymph to act as antimicrobial agents. The antimicrobial activity of certain FAs such as lauric (C12:0), tridecyl (C13:0), palmitoleic (C16:1), and linoleic (C18:2) acids has been demonstrated against *P. larvae* (Feldlaufer et al. 1993). Another possible cause of change in the composition of the FA reserve is their mobilization to act in the synthesis of pheromones and cuticular chemical signals related to the health status of the individuals (Richard et al. 2008, 2012; Hernández López et al. 2017; McAfee et al. 2018).

The composition of FAs in honey bees is crucial for cognitive development and foraging (Arien et al. 2015), maintenance of colonies during winter (Castaños et al. 2023), the transition from nurse to forager activities at the beginning of spring (Toth et al. 2005), and understanding the effect of immunological challenges. For that, FA composition has become of great importance, and

more studies are required to have a clear picture of the relationships between immune and lipidic pathways. This is the first report about the modification of the FA profile in adult bees immunostimulated with injections of *P. larvae* suspensions. We consider it as a starting point for future research that deepens the knowledge of the role of FAs in honey bee health.

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AUTHOR CONTRIBUTION

ED developed the study idea, managed the honey bee colonies, collected data and samples, analyzed the samples in the laboratory, statistically analyzed the data, and wrote the manuscript. PMG managed the honey bee colonies, collected data and samples, and analyzed the samples in the laboratory. SQ, MPM, MC, and SKM analyzed the samples in the laboratory. LBG and ND direct the investigation. All authors read and approved the final manuscript.

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DATA AVAILABILITY

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Ethics approval This is an observational study, and no ethical approval is required.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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