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# No detectable impact of chronic oral lactic acid exposure on honey bee health: Insights from survival, lactate accumulation and head transcriptome

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A R T I C L E I N F O

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A B S T R A C T

Natural acids such as oxalic, formic or lactic acids are used as alternative treatments against *Varroa destructor*, the parasitic mite of honey bees (*Apis mellifera*). Lactic acid has recently been shown to impair mites' grip skills through local action after exposure of adult honey bees. However, little is known about the lethal and sublethal effects of lactic acid on honey bees. In this work, we investigated the effects of chronic oral exposure to lactic acid through a contaminated diet on age-controlled worker bees. We monitored survival under artificial conditions, quantified lactate levels in various worker organs (the digestive tract, the thorax, the fat body, the head and the haemolymph) and analysed the transcriptome of the workers' heads. Our results indicate that consuming lactic acid at residual concentration (1.5 mg/mL) did not impact the survival. No lactate accumulation was detected in any of the honey bee organs analysed. Furthermore, transcriptomic analysis on the bees' heads revealed no differences in gene expression. While further research on sublethal effects is still needed, this work provides one of the first reports on the off-target effects of lactic acid on honey bee health.

## 1. Introduction

Over the past two decades, growing concerns have emerged about the effects of pesticides on pollinators. Even at sublethal concentrations, these chemicals can alter various insect and parasite behaviours such as food foraging, choice of mating partner or pheromonal communication (Colin et al., 2020; Desneux et al., 2007; Simon-Delso et al., 2015). Such sublethal effects may represent drawbacks for hosts, especially with prolonged exposure (Rondeau et al., 2015). This issue is particularly important for treatments against *Varroa destructor*, a major threat to *Apis mellifera*. This parasite weakens colonies by feeding on bees and spreading lethal viruses (Martin and Brettell, 2019; Piou et al., 2022, 2024). Moreover, persistent miticide residues stored in hive products can impair honey bees' olfactory memory and locomotion (Charreton et al., 2015; Gashout et al., 2020) while mite populations are increasingly developing resistances (Hernández-Rodríguez et al., 2021).

Because of such resistance in parasites and sublethal effects on honey bees, alternative solutions to synthetic chemical treatments against *V. destructor* were developed like organic acids, namely oxalic, formic or lactic acids (Eguaras et al., 2003; Maggi et al., 2016; Vilarem et al., 2021). For lactic acid treatments, the only information available from the field remains about its laborious way of application and its miticide effect in hive (Charrière et al., 2004; Imdorf, 1989; Kraus and Berg, 1994). Laboratory studies have demonstrated that lactic acid can impair *V. destructor* grip ability, both through direct application and when applied to honey bees carrying mites (Vilarem et al., 2023a, 2023b, 2024). However, despite these promising findings, the potential off-target effects of lactic acid remain underexplored (Genath et al., 2020, 2021; Papežíková et al., 2017). A rigorous ecotoxicological assessment of lactic acid is essential to fully understands its sublethal

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impacts on non-target organisms like *A. mellifera*, the host of *V. destructor* (Benito-Murcia et al., 2022; Gashout et al., 2018; Higes et al., 2020). While lactic acid is ubiquitous in the honey bee environment through food and the metabolism of lactate at the cellular level (Brooks et al., 2022; Hölscher et al., 2008; Strachecka et al., 2019), the effects of its use in treatment on bees remain poorly characterized. Lactic acid treatment typically involves spraying a 150 mg/mL lactic acid solution onto hives two to three times to achieve effective mite control. This application can expose honey bees to additional concentrations of lactic acid through oral ingestion of residues in honey and topical contact *via* the cuticle, potentially interfering with biological processes. As a matter of fact, exchanges of lactate between glial cells and neurons play a key role in the way of providing supply during high-energy demand implied by neuronal activity in brain cells of Drosophila (González-Gutiérrez et al., 2020; Volkenhoff et al., 2015). Moreover, disruption of lactate metabolism in glia or neurons impacts their memory and survival (Frame et al., 2023). Although direct data on lactate role in the brain of *A. mellifera* are lacking, this organ holds a key role in the maintenance of good health for honey bees and is often challenged by chemicals (Zhi-Xiang et al., 2024). Therefore, investigating the sublethal effects of lactate on the bee head, a central organ for treating environmental stimuli and regulating honey bee behaviours (Wu et al., 2017) is of prime interest.

In this study, we investigated the lethal and sublethal effects of chronic oral exposure to lactic acid in honey bees (Fig. 1). We hypothesized that such prolonged ingestion would disrupt lactate metabolism within glial cells or neurons, thereby impairing memory and reducing survival like in Drosophila (Frame et al., 2023). Additionally, we expected elevated level of lactate in specific tissues or haemolymph, leading to acidosis, similar to the effects observed with oral exposure to oxalic acid (Rademacher et al., 2017). We tested three lactic acid concentrations based on previous studies: the concentration sprayed in hive against mites (150 mg/mL) (Kraus and Berg, 1994) along with the residual concentration (1.5 mg/mL) measured in honey after this sprayed administration (Bogdanov et al., 1998). We also included an intermediate lactic acid concentration of 25 mg/mL, which in our previous study resulted in reduced grip for mites (Vilarem et al., 2023b). Once the survival curves established, we selected the sublethal duration of seven days for chronic oral administration of food contaminated with lactic acid. After the chronic exposure we measured lactate concentration in honey bees' organs and haemolymph to check for acidosis (Fig. 1). Besides, we used transcriptomic as a tool to investigate sublethal effects on the head, a crucial body part in the preservation of health for honey bees when challenged by chemicals.

## 2. Material and methods

All our studies were conducted according to the European ethics laws for scientific research currently in force. The experiments were led in 2023 during spring, summer, and autumn using honey bees from three Buckfast colonies provided by ADA Occitanie (France). The colonies were maintained on the University campus (Albi, France). They were only treated for a month the previous year with oxalic acid. No treatment was applied during the experiments or during the previous six months. Infestation rates were monitored weekly throughout the experiments, with an average of 1 % observed.

### 2.1. Acid preparation

Lactic acid was purchased from Thermoscientific, USA. Dilutions of lactic acid (90 %) (CAS no. 50-21-5) were made with a 50 % (w/v) sucrose solution. All stock solutions were kept at 4°C. Final concentrations were 1.5, 25 and 150 mg/mL of lactic acid. Note that 150 mg/mL was chosen because it is the standard concentration used by beekeepers to control *V. destructor* (Kraus and Berg, 1994). In addition, 25 mg/mL serves as an intermediate level: under laboratory conditions, it has been shown to induce mites' fall (Vilarem et al., 2023a). Lastly, 1.5 mg/mL reflects the concentration detected in honey following a hive spray treatment with lactic acid at 150 mg/mL (Bogdanov et al., 1998).

### 2.2. Honey bees sampling

Brood frames containing newly emerged adults from three different hives were brought back to the laboratory and placed in an incubator set to 28°C and 60 % relative humidity (RH). They remained under these conditions for two hours, allowing the bees to emerge fully. Newly emerged bees were identified using a water-based marker (Posca™) and put back to their respective colonies. Seven days later, groups of ten marked bees were taken to the laboratory and kept in experimental cages (Pain type: 10.5 × 7.5 × 11.5 cm) in an incubator (28°C, 60 % RH) to conduct experiments detailed in Table 1. It should be noted that 7-day-old bees were selected because they are in a stable developmental window characterized by consistent behavioural roles, stable physiological profiles and mature learning abilities. These factors make them

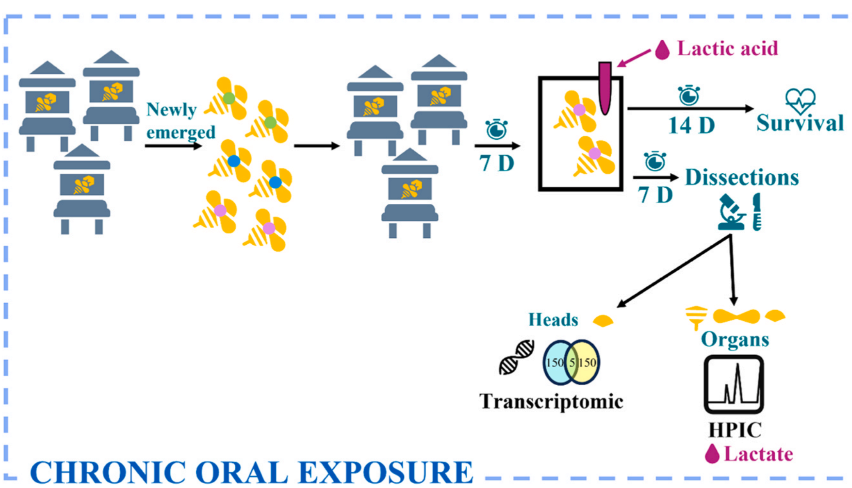


**Fig. 1.** Schematic diagram of the experimental design for chronic oral exposure of *Apis mellifera* to lactic acid. Newly emerged bees (N = 300/colony) were collected and marked from three different hives, then reinserted in their respective colonies. After seven days, marked workers (N = 30/condition) were collected again and transferred into experimental cages, where they were provided with either a sucrose solution (control group) or a lactic acid contaminated solution at concentrations of 1.5, 25 or 150 mg/mL. The exposure lasted 14 days (14D) in the case of survival assessment or 7 days (7D) for organ analysis with HPIC (High-Performance Ionic Chromatography) and transcriptomic.

**Table 1**
Overview of the sample size for each experiment.

| Experiments | Conditions | N/ hive | Number of hives | Total |
|---|---|---|---|---|
| **Survival experiment** | Control (50 % sucrose solution) | 10 honey bees | 3 | 30 honey bees |
| | 1.5 mg/mL of lactic acid (0.01 mol/L) | 10 honey bees | 3 | 30 honey bees |
| | 25 mg/mL of lactic acid (0.27 mol/L) | 10 honey bees | 3 | 30 honey bees |
| | 150 mg/mL of lactic acid (1.66 mol/L) | 10 honey bees | 3 | 30 honey bees |
| **Dosage HPIC organs** | Control | 5 honey bees | 3 | 15 honey bees |
| | Treated with lactic acid (1.5 mg/mL) | 5 honey bees | 3 | 15 honey bees |
| **Dosage HPIC haemolymph** | Control | 10 honey bees | 3 | 30 honey bees |
| | Treated with lactic acid (1.5 mg/mL) | 10 honey bees | 3 | 30 honey bees |
| **RNA seq** | Control | 6 honey bees | 3 | 18 honey bees |
| | Treated with lactic acid (1.5 mg/mL) | 6 honey bees | 3 | 18 honey bees |

homogeneous organisms for toxicological, cognitive and transcriptomic experiments (Li et al., 2019; Ray and Ferneyhough, 1997; Wheeler et al., 2015).

Regarding the presentation of the statistics and results, note that for HPIC dosage, organs were pooled by 5; for haemolymph honey bees were pooled by 10; for RNA seq heads were pooled by 3.

### 2.3. Survival experiment

*Treatment* – Each cage containing ten honey bees was supplied with two gravity feeders: one filled with water and the other one with a 50 % (w/v) sucrose solution. The sucrose solution was either contaminated with lactic acid at final concentrations of 1.5, 25, 150 mg/mL or left uncontaminated as a control group. According to the standard methods for toxicology research in honey bees, feeders were replaced once daily throughout the exposure period (Medrzycki et al., 2013).

*Survival* – The chronic oral exposure lasted fourteen days in an incubator (28°C, 60 % RH) with three replicates per condition (Table 1). Dead bees were retrieved daily and counted. Water and food consumptions were weighed every day for the duration of the experiment and divided by the number of alive bees. Natural evaporation was considered and retrieved from the measured consumption of food and water (Figure A). Note that the lethal concentration ($LC_{50}$) is defined as the concentration of lactic acid that causes 50 % mortality in honey bee population under controlled conditions (Desneux et al., 2007). In contrast, a sublethal concentration refers to a level of lactic acid that does not result in immediate death but can lead to a measurable biological effect in honey bees (Desneux et al., 2007). To ensure a more cautious approach than the sublethal definition, we chose to take the day before any death events for the sublethal experiment.

### 2.4. Organ and haemolymph dosage of lactic acid with high-performance ionic chromatography (HPIC)

*Treatment* – The first mortality events in honey bees exposed to 1.5 mg/mL of lactic acid occurred on day 8 of the chronic oral exposure (Fig. 2). Therefore, to assess sublethal effects, we initiated the experiment on day 7, prior to any mortality occurrences (Fig. 1). Each cage, containing ten honey bees, was equipped with two gravity feeders: one filled with water and the other one with a 50 % (w/v) sucrose solution. The sucrose solution was either contaminated with lactic acid at a final concentration of 1.5 mg/mL or left uncontaminated as a control group.

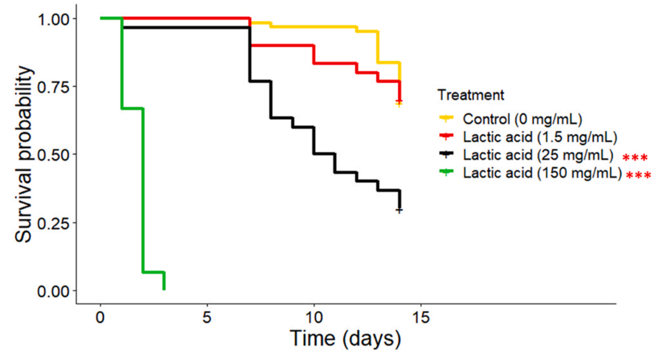


**Fig. 2.** Survival probability of caged honey bees during chronic oral exposure to lactic acid (1.5, 25 & 150 mg/mL) or sucrose solution (control) for 14 days, N = 30/treatment, *** indicates *p*-value< 0.0001. The survival probability of honey bees fed with 150 mg/mL lactic acid was significantly reduced (Kaplan-Meier *p*-value<0.0001). However, food intake was also measured and indicated that honey bees avoided consumption at this concentration (Figure A). Similarly, at 25 mg/mL, survival probability also decreased significantly likely due to reduced food intake rather than direct toxicity (Kaplan-Meier *p*-value<0.0001 & Figure A).

*Dissection and purification* – After a seven-day chronic oral exposure to 1.5 mg/mL of lactic acid or a 50 % (w/v) sucrose solution (Fig. 1), honey bees were lethally cooled at 4°C for 30 min and dissected on ice. Haemolymph was collected through the antennae and pooled in groups of ten per tube (Borsuk et al., 2017). Digestive tract was pulled out, head and thorax were cleaved with a scalpel. Lastly, fat body were dissected from the abdomen (Carreck et al., 2013). Five organs were pooled together in 1 mL of ultrapure water and kept on ice (Table 1). Tissues were disrupted with a TissueLyser II (Qiagen, Germany) and three iron beads (3 mm) for 1 min at 30 Hz. A filtration step (0.2 µm) was followed, and each tube was stored at −20°C until further analysis.

*HPIC analysis* – Each sample was unfrozen, centrifugated and filtered (0.22 µm PTFE syringe filter) prior to analysis of lactate concentrations by high-performance ionic chromatography with AS11-HC-4 µm column, solvent: KOH, gradient from 1 mM to 44 mM (Dionex Ics-5000+, Thermo Fisher Scientific Inc., Waltham, MA, USA) following standard procedures with conductometric cell and UV absorption at 194 nm. Results are presented per honey bee for an easier comparison.

### 2.5. RNA sequencing

*Treatment* – After seven days of chronic oral exposure to 1.5 mg/mL lactic acid or a 50 % (w/v) sucrose solution for the control group (Fig. 1), honey bees were directly lethally frozen at −80°C (Carreck et al., 2013; Evans et al., 2013).

*Dissection* – Their heads were cleaved with a scalpel, weighed, cut in half, immersed in RNAlater® (Thermo fisher scientific, USA) and stored at −80°C. Pool of three heads were gathered by tube (Table 1).

*RNA extraction* – Tissue disruption was led with a TissueLyser II (Qiagen, Germany) and three iron beads (3 mm) for 1 min at 30 Hz in the lysate buffer Qiagen. Total RNA was then extracted with RNeasy Midi Kit® (Qiagen, Germany), following the manufacturer protocol.

*Quality check & RNA sequencing* – RNA quality was checked using a BioAnalyzer (Agilent 2100) according to manufacturer standard procedures. Samples were sent to GeT-PlaGe platform for final quality control, RNA concentration and purity were determined using a ND-8000 Spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Integrity of RNA was checked with a Fragment Analyzer (Agilent Technologies, Santa Clara, USA), using the RNA Standard Sensitivity Kit. RNA-seq paired-end libraries were prepared according to Illumina protocol with some adjustments, using the TruSeq Stranded mRNA library prep Kit (Illumina, San Diego, USA). Libraries were equimolarly pooled and RNA sequencing was then performed on one S4 lane of the Illumina

NovaSeq™ 6000 instrument (Illumina, San Diego, USA), using the NovaSeq 6000 S4 v1.5 Reagent Kit (300 cycles), and a paired-end 2 × 150 pb strategy. This generated approximately 50 M reads per sample.

*Bioinformatic analysis pipeline* – All samples were analysed separately through the quality control, mapping and quantification steps through the nf-core (Ewels et al., 2020) RNA-Seq pipeline (version 3.12) (Patel et al., 2024). Briefly, raw sequences filtered with fastp, mapped to the *Apis mellifera* genome sequence Amel HAv3.1 (Genbank: GCA_003254395.2) using STAR and RSEM were used to generate the expression matrix. The quality of the reads was assessed at different steps of the pipe-line with FastQC, QualiMap and SAMtools.

### *2.6. Statistical analyses*

The statistical analysis of survival and lactate quantification were carried out using standard methods on R software (version 4.0.5). Survival probability was analysed over fourteen days through a Kaplan-Meier method with the survival and survminer packages (Alboukadel et al., 2022; Therneau, 2023). For lactate quantification in each organ, as the data did not meet the assumptions for parametric tests, results were presented as boxplots and analysed using Wilcoxon rank tests to assess the differences between treated and control bees.

Exploratory analysis of RNA-seq data was first performed using a Principal Component Analysis (PCA) on pseudo-counts (log2 transformed counts) with R software. Differential expression analysis was then performed using edgeR (Robinson et al., 2010) standard approach: A Generalized Linear Model (GLM) with two fixed effects for the treatment and the hive (blocking factor) was estimated. Only the treatment effect was assessed during test. All *p*-values were corrected for multiple testing using the Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) that controls the False Discovery Rate (FDR). PLS-DA was also performed using the mixOmics package (version 6.26.0) from R/Bioconductor (Rohart et al., 2017). Its predictive performance was assessed with the perf function, using leave-one-out cross-validation. Given the sample size, K-fold cross-validation is not considered appropriate.

## 3. Results

### *3.1. Longevity of caged honey bees after chronic oral exposure to lactic acid*

We administered lactic acid to honey bees *via* feeding at three different concentrations (1.5, 25, 150 mg/mL). Honey bees exposed to 150 mg/mL showed a significant reduction in survival probability (Fig. 2, Kaplan-Meier *p*-value<0.0001). However, food intake data indicated that honey bees refused contaminated feed at this concentration (Figure A). At 25 mg/mL, survival also declined significantly, again probably due to a reduced consumption of food (Fig. 2, Kaplan-Meier *p*-value<0.0001 & Figure A). Thus, starvation rather than direct toxicity seems to explain the mortality at the two higher concentrations (25, 150 mg/mL) preventing accurate lactic acid exposure. In contrast, honey bees fed 1.5 mg/mL of lactic acid showed survival rates comparable to controls (sucrose solution) over a 14-day period (Fig. 2, Kaplan-Meier *p*-value= 0.87) and their food consumption did not differ significantly from controls.

### *3.2. No lactate acidosis in honey bee organs or haemolymph after lactic acid chronic oral exposure*

In the survival experiment, no honey bees fed 1.5 mg/mL of lactic acid (treated group) died before day 8, thus sublethal effects were investigated on day 7. After seven days of chronic oral exposure to lactic acid, the median quantity of lactate measured in the digestive tract was 2.399 µg/bee, which was not statistically different from the control group at 2.738 µg/bee (Fig. 3A, Wilcoxon rank test, W = 4, *p*-value = 1). This suggests that chronic oral exposure to lactic acid does not significantly alter lactate levels in the digestive tract of honey bees.

There was also no significant difference in the thorax with 0.638 µg/bee for the treated group and 0.548 µg/bee for the control group (Fig. 3B, Wilcoxon rank test, W = 3, *p*-value = 0.7). Although the Wilcoxon test only compared six points and a slight increase was observed in both the fat body and the head after treatment, the differences between the two groups do not appear to be statistically significant. Indeed, we measured 0.072 µg/bee of lactate in the fat body when treated and 0.052 µg/bee in the control group (Fig. 3C, Wilcoxon rank test, W = 3, *p*-value = 0.7). Furthermore, the median quantity of lactate measured in heads when treated was 0.091 µg/bee and for control bees 0.061 µg/bee (Fig. 3D, Wilcoxon rank test, W = 2, *p*-value = 0.4). These results indicate that chronic oral exposure to lactic acid does not seem to alter lactate concentrations in the thorax, fat body or head of honey bees.

Lastly, after seven days of oral chronic exposure to lactic acid, the median lactate quantity measured in the haemolymph (0.047 mg/mL) was not statistically different from the control group with 0.062 mg/mL (Fig. 4, Wilcoxon rank test, W = 4, *p*-value = 1). This demonstrates that chronic oral exposure to lactic acid does not systematically increases lactate levels in the haemolymph of honey bees.

### *3.3. Transcriptomic analysis revealed no changes in gene expression after lactic acid chronic oral exposure in honey bee heads*

Sequencing the RNA samples generated an average of 50 million reads of paired end sequences with 150 bp length, aligned to *A. mellifera* reference genome (Amel HAv3.1-Genbank assembly accession GCA_003254395.2 (Wallberg et al., 2019)).

For the PCA (Principal Component Analysis) in Fig. 5A, two PCs are sufficient to explain a large proportion of the variance and the projection of the individuals does not show a strong condition effect. Over the 11,643 genes expressed in bee head, the differential analysis displayed on the volcano plot highlights that only 1 gene transcript has a significant lower expression in the treated group compared to the control group (Fig. 5B, NB GLM adjusted *p*-value = 0.021). This gene, *LOC113219138,* is a non-coding RNA not identified for *A. mellifera*. However, a blast analysis showed potential mapping with the Asian honey bee, *A. cerana* for an “integrin”. To further support these conclusions, PLS-DA was applied, and its misclassification error rate was estimated using leave-one-out cross-validation. The resulting model showed poor predictive performance (with error rates ranging from 33 % to over 50 %), confirming the absence of a clear relationship between the treatment and gene expression profiles.

## 4. Discussion

This study represents the first investigation into the lethal and sublethal effects of chronic oral exposure to lactic acid in honey bees. We used age-controlled honey bees, as lactate concentrations vary along life of bees (Strachecka et al., 2019). We hypothesized that lactic acid exposure would elevate lactate levels in the digestive tract triggering dysbiosis (Paris et al., 2020). Meanwhile in the thorax we anticipated flight impairment due to muscles soreness and mitochondrial oxidative stress (Strachecka et al., 2019). Lastly in the head, we anticipated that an increase in lactate could enhance memory consolidation (Ho et al., 2024; Sun et al., 2017). Our findings revealed that after three days of administering a 150 mg/mL lactic acid solution (corresponding to the treatment in hive), all caged honey bees were dead. However, the direct toxicity of the organic acid was not conclusively involved, as we measured daily food consumption and observed that honey bees likely died from starvation (Figure A). It is consistent with our previous study showing that honey bees tend to avoid lactic acid at 150 mg/mL as it seems repulsive (Vilarem et al., 2024). While this avoidance could

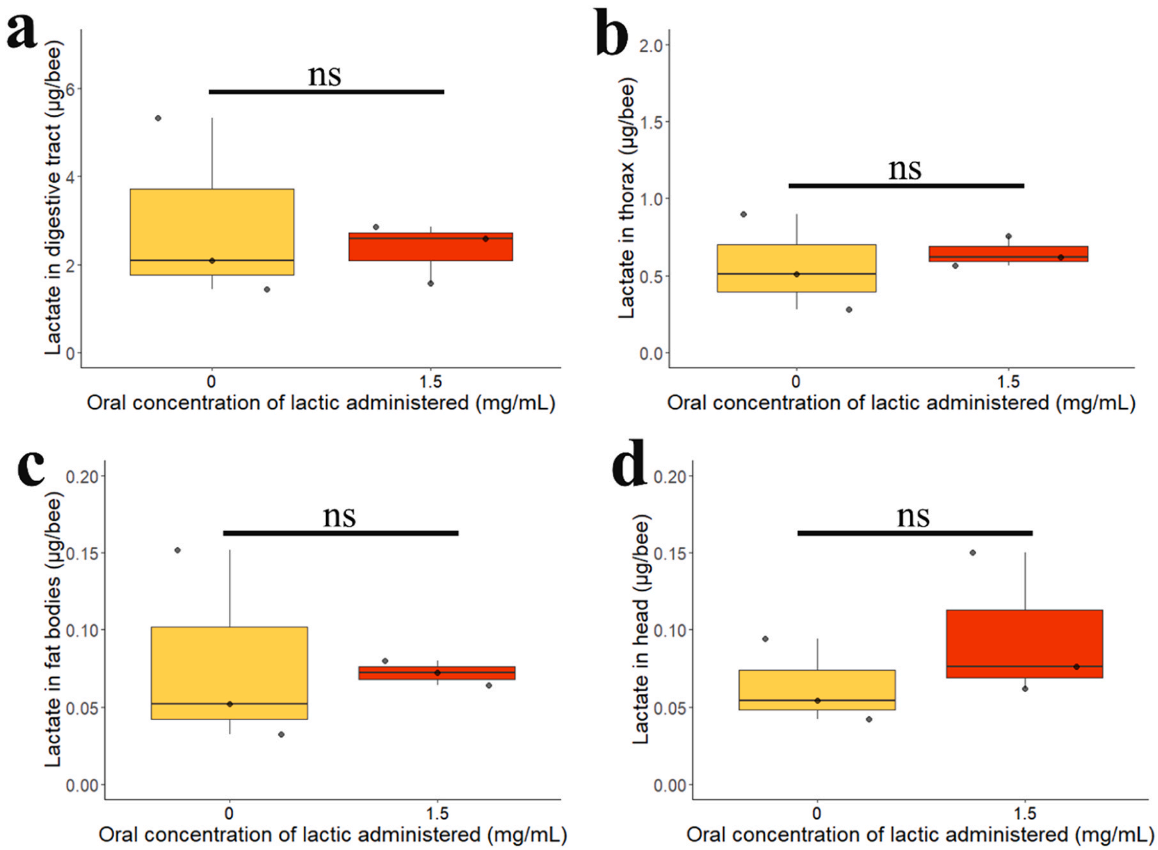


**Fig. 3.** Lactate quantities in honey bee organs following chronic oral exposure to lactic acid (treated group) or a sucrose solution (control group). Lactate levels were measured in (a) the digestive tract, (b) the thorax, (c) the fat bodies and (d) the head of honey bees after 7 days of oral exposure to either a sucrose solution or a diet contaminated with 1.5 mg/mL of lactic acid. Each dot represents a pool of 5 organs. “ns” stands for non-significant.

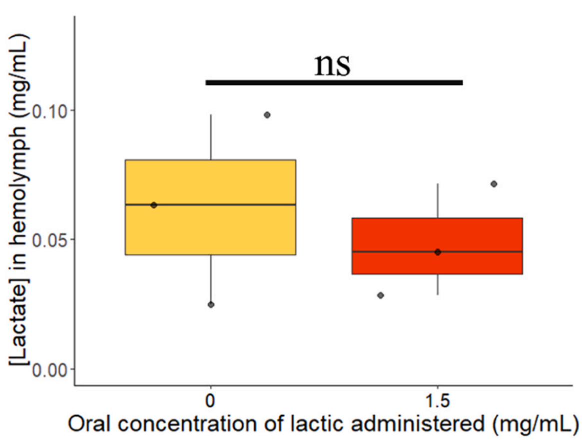


**Fig. 4.** Concentration of lactate in honey bee haemolymph after chronic oral exposure to 1.5 mg/mL lactic acid (treated group) or a sucrose solution (control group). Each dot represents a pool of 10 bees. “ns” stands for non-significant.

disturb the chemical communication within the hive (Bortolotti and Costa, 2014), it can also serve as a protective mechanism, preventing over ingestion of high lactic acid concentrations. Besides, we found no lethal effects when lactic acid was ingested at residual concentration (1.5 mg/mL) which is the most plausible scenario in hives after the sprayed treatment at 150 mg/mL (Bogdanov et al., 1998). These results align with field observations, indicating that lactic acid treatments do not notably impact honey bee survival (Domatskaya et al., 2020; Kraus and Berg, 1994). Even though no significant reduction of survival was observed in the 1.5 mg/mL treated group compared to controls, we chose to examine sublethal effects in the absence of mortality.

A key parameter to consider is that lactic acid dissolved in water undergoes deprotonation, forming lactate ion. Given that Hymenoptera haemolymph has a pH measured around 6.7, the lactate form prevails within the honey bees’ body (Matthews, 2017; Phypers and Pierce, 2006). Therefore, exposure to lactic acid could lead to elevated internal concentrations of lactate which in turn could affect essential metabolic pathways. Indeed, lactate balance preservation within the body remains crucial. For instance, lactate concentration among other glucose precursors regulates the rate of gluconeogenesis, a pathway generator of ATP, one of the most important factor in the lifespan of an organism (Berg et al., 2015; Tresguerres, 2016). In honey bees, the Cori-cycle occurs when muscles need energy, glucose and lactate are interconverted between muscles and the fat body (Strachecka et al., 2019). This cycle is essential for keeping steady glucose levels during high energy demand periods like flights (Schippers et al., 2010).

Besides, elevated lactate concentrations can indicate an exceeding of the anaerobic threshold with the following development of metabolic acidosis. To assess this risk, we checked lactate concentrations in the haemolymph and various organs of both treated and untreated honey bees to determine the potential of lactate acidosis but also to identify organs where lactate accumulation could occur and become prejudicial (Li et al., 2022). As demonstrated by previous studies (Kwong and Moran, 2016; Strachecka et al., 2019) and in Figure B, lactate is distributed throughout the honey bee’s tissues, with the highest concentration in the digestive tract, followed by the thorax, head and fat body, underlying its ubiquitous nature. However, we did not find a significant lactate increase when treated with lactic acid, suggesting that lactate homeostasis across the body is tightly regulated and limiting the risk of lactate burst. In the bee organs, the measurement was performed

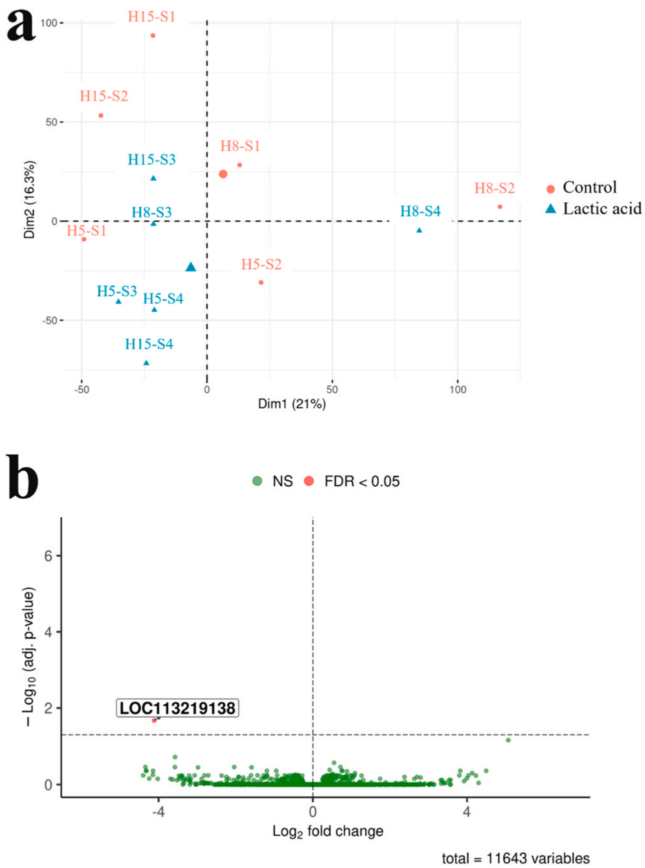


**Fig. 5.** Differential analysis of head gene expressions in honey bee workers: comparison between control and lactic acid groups (1.5 mg/mL) following chronic oral exposure. (a) PCA (Principal Component Analysis) with control group in red and exposed to lactic acid group in blue. “H” stands for the hive number and “S” for the sample number. Each dot represents a hive with 3 heads pulled. Barycenters are shown as a bigger triangle for exposed to lactic acid and a bigger dot for control groups. (b) Volcano plot illustrating the differential gene expression profiles in the heads of honey bee workers exposed to lactic acid compared to controls. Green dots indicate the non-DEGs. The red dot represents the down regulated gene (FDR < 0.05) in the head of honey bee workers exposed to lactic acid. “NS” stands for non-significant and “FDR” for false discovery rate.

randomly on individuals fed *ad libitum*. However, future studies should explore the kinetics of lactate absorption and assimilation following ingestion. In other organic acids such as ascorbic acid, a recovery phase in the haemolymph has been documented (Harz et al., 2010). More studies are needed to investigate the role of lactate and its involvement in honey bee metabolism. Nevertheless, our results suggest a homeostatic modulation of lactate, consistent with the regulation of a native molecule with an already effective cellular machinery (Brooks et al., 2022).

Additionally, honey bees are exposed to multiple environmental contaminants, including pesticides. Some studies have demonstrated impacts on bee lactate metabolism (Almasri et al., 2021). For instance, certain fungicides have been shown to inhibit mitochondrial respiration and ATP synthesis in honey bees, directly compromising oxidative metabolism and thus triggering increased lactate production (Nicodemo et al., 2020). On one hand, co-exposure to lactic acid and these pesticides could overwhelm honey bees’ physiological regulation (Kang et al., 2025). The resulting accumulation of lactate in tissues could provoke acidosis (Rademacher et al., 2017), muscle soreness during flight (Strachecka et al., 2019), and energy inefficiency, leading to metabolic disruptions that warrant further investigations. On the other hand, probiotic supplementation in hives, containing lactic acid bacteria and lactic acid, showed interesting results. In some cases, it helped increase hive populations or boost honey production (Maggi et al., 2013; Patruica and Hutu, 2013). In others, it reduced the presence of pathogens like *N. ceranae*, demonstrating the potential benefits of lactic acid in multifactorial environments (Maggi et al., 2013; Vásquez et al., 2012).

Despite the lack of significant difference, lactate level increased slightly in honey bee heads after chronic oral exposure to lactic acid. Lactate is considered as a signalling molecule, and it is particularly important for biological processes like brain executive functions (Sun et al., 2017). For instance, experimental evidence underlines the role of lactate in cognitive function not only as a metabolic substrate for neurons but also as a signalling molecule driving synaptic plasticity in humans, mice and Drosophila (Calì et al., 2019; González-Gutiérrez et al., 2020; Ho et al., 2024; Yang et al., 2014). Therefore, an increase in lactate levels in honey bee brain, specifically within neurons or glial cells, could enhance long-term memory. This is because lactate generated by glial cells fuels neurons during memory consolidation, a mechanism well-established in Drosophila and others organisms (Bajaffer et al., 2022; Dembitskaya et al., 2022). Moreover, lactate produced by glial cells serves as a metabolic substrate for mushroom body neurons, which are crucial for olfactory learning and memory in bees. Chronic oral supplementation of lactate *via* lactic acid treatment could potentially enhance learning and memory in honey bees (Alberini et al., 2018; Basu et al., 2024; Ho et al., 2024). Nevertheless, to our knowledge there is no study about the role of lactate in honey bee memory (Menzel, 2021; Popov and Szyszka, 2020). To check possible side effects post-treatment, we ran a differential transcriptomic analysis on bees’ heads. The lack of difference between treated and control showed that the residual concentration seemed well managed physiologically by honey bees. Additionally, no modifications from potential pathways related to the lactate homeostasis were detected (Magistretti and Allaman, 2018; Mason, 2017; Wang et al., 2019). It is important to underline that in contrast to other treatments against *V. destructor*, lactic acid did not seem to alter the regulation of the detoxification genes as demonstrated for tau-fluvalinate or formic acid (Gashout et al., 2018; Li et al., 2019), nor memory-related genes like formic acid or thymol (Gashout et al., 2020).

## 5. Conclusion

In this study, we examined the lethal and sublethal effects of chronic oral exposure to lactic acid in honey bees. We hypothesized that prolonged ingestion might disrupt lactate metabolism in glial cells or neurons, potentially impairing memory and reducing survival, as observed in other insect species. However, we did not detect any significant changes in gene expression in the bees’ heads. Notably, the highest concentration of lactic acid led to lethal effects presumably due to starvation, which might ultimately alter colony functions and justify further investigation. Additionally, no increase in lactate levels was detected in tissues or haemolymph. This suggests that acidosis did not occur, unlike what is seen with oxalic acid oral exposure. Yet, the risks associated with co-exposure to pesticides in the field should be addressed to prevent potential acidosis and its metabolic consequences for honey bees. While our study focused on chronic oral exposure, known to trigger sublethal effects in several other treatments, exploring the combination of chronic oral and topic exposures could provide complementary insights. Our encouraging results call for future studies to better characterize the impact of lactic acid treatment on bee colonies, including their dynamics and behaviours.

## CRediT authorship contribution statement

**Caroline Vilarem:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Vincent Piou:** Writing – review & editing, Investigation. **Lucie Bouly:** Writing – review & editing. **Frédéric Julien:** Writing – review & editing, Methodology, Investigation. **Rachel Fourdin:** Writing – review & editing, Methodology, Investigation. **Nathalie Vialaneix:** Writing – review & editing, Methodology, Investigation. **Matthias Zytnicki:** Writing – review & editing, Methodology, Investigation. **Angélique Vétillard:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

## Compliance with ethical standards

Ethical standards were respected.

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## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Vincent Piou reports financial support was provided by The French Agency for Ecological Transition. Caroline Vilarem reports financial support was provided by M2I Biocontrol. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### *Patents*

Results from this article may be part of a patent.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118807.

## Data availability

Data will be made available on request.

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