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ADMINISTRATION OF ACETATE AND LACTATE AS POTENTIAL BRAIN–GUT MODULATORS IN *DROSOPHILA MELANOGASTER*

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Dissertação apresentada à Universidade Estadual Paulista (UNESP), Instituto de Biociências de Botucatu, campus de Botucatu para obtenção do título de mestre em Biotecnologia.

Área de concentração: Biotecnologia
Orientadora: Profa. Dra. Marina Piacenti da Silva

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IMPACTO POTENCIAL DESTA PESQUISA

Com o avanço das pesquisas acerca das doenças neurodegenerativas e a identificação dos múltiplos fatores envolvidos em sua progressão, a modulação da microbiota intestinal tem se consolidado como fundamental no tratamento destas condições. Diante de limitações financeiras, temporais e éticas associadas aos modelos tradicionais de experimentação animal, novas abordagens têm sido exploradas. Neste contexto, o uso de moscas *Drosophila melanogaster* destaca-se por permitir a investigação dos mecanismos moleculares fundamentais a patologias neurológicas, ao mesmo tempo em que viabiliza a implementação de um modelo experimental *in vivo* de baixo custo, e com alto potencial de aplicação em regiões com menor investimento em pesquisa e grande demanda por soluções científicas de impacto social local.

POTENTIAL IMPACT OF THIS RESEARCH

With the advancement of research on neurodegenerative diseases and the identification of multiple factors involved in their progression, modulation of the intestinal microbiota has become established as a fundamental strategy in the treatment of these conditions. In light of the financial, temporal, and ethical limitations associated with traditional animal experimentation models, new approaches have been increasingly explored. In this context, the use of *Drosophila melanogaster* stands out for enabling the investigation of molecular mechanisms fundamental to neurological pathologies, while also making it possible to implement a low-cost *in vivo* experimental model with high potential for application in regions with lower research investment and a strong demand for locally impactful scientific solutions.

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RESUMO

Inúmeros fatores ambientais têm contribuído para o aumento no número de casos de doenças associadas ao eixo intestino-cérebro. Além da influência genética, o manejo da microbiota intestinal e dos metabólitos produzidos por ela têm se destacado como abordagens promissoras no tratamento de doenças neurológicas, inclusive em modelos vivos como *Drosophila melanogaster*. Moléculas produzidas pelas bactérias do intestino podem ser transportadas até o cérebro, agravando o quadro clínico através da neuroinflamação, alteração da microbiota residente ou ativação das células gliais. O objetivo deste trabalho foi verificar se a administração dos sais acetato e lactato, principais metabólitos produzidos pelas bactérias *Acetobacter* e *Lactobacillus,* respectivamente, influenciavam no tempo de vida, capacidade locomotora, perfil da microbiota intestinal, e morfologia das células gliais de *Drosophila*. Verificou-se uma toxicidade dose-dependente maior para o acetato do que do lactato conforme o aumento da concentração dos sais. Os testes de escalada não demonstraram correlação entre a diluição dos sais e a capacidade locomotora. Identificou-se a presença de *Lactobacillus* no intestino das moscas que receberam lactato, mas não nos grupos controle. Em suma, a administração a longo prazo dos sais influenciou o tempo de vida, mas não a capacidade locomotora. A análise do perfil da microbiota e da morfologia glial não permitiu conclusões quanto ao uso destes sais em distúrbios do eixo intestino-cérebro. Apesar dessas limitações, o estudo contribui ao estabelecer parâmetros iniciais de concentração e resposta biológica para o uso desses metabólitos em *Drosophila*, fornecendo uma base experimental para investigações futuras sobre o efeito de metabólitos bacterianos na comunicação intestino-cérebro.

Palavras-chave: *Drosophila melanogaster*; eixo intestino-cérebro; microbiota; acetato; lactato.

ABSTRACT

Numerous environmental factors have contributed to the increasing number of cases of diseases associated with the gut-brain axis. In addition to genetic influence, modulation of the gut microbiota and its metabolites have emerged as a promising approach for the treatment of neurological diseases, including in *in vivo* models such as *Drosophila melanogaster*. Molecules produced by gut bacteria can be transported to the brain, aggravating the clinical condition through neuroinflammation, alteration of the resident microbiota, or activation of glial cells. The aim of this study was to evaluate whether the administration of acetate and lactate salts, the main metabolites produced by *Acetobacter* and *Lactobacillus* bacteria, respectively, influenced lifespan, locomotor capacity, gut microbiota profile, and glial cell morphology in *Drosophila*. A greater dose-dependent toxicity was observed for acetate compared with lactate as salt concentration increased. Climbing assays did not demonstrate a correlation between salt dilution and locomotor capacity. The presence of Lactobacillus was identified in the intestines of flies treated with lactate, but not in the control groups. In summary, long-term administration of the salts influenced lifespan, but not locomotor capacity. The analysis of microbiota profile and glial morphology did not allow conclusions regarding the use of these salts in gut-brain axis disorders. Despite these limitations, this study establishes initial concentration and biological response parameters for the use of these metabolites in Drosophila, providing an experimental foundation for future studies investigating the role of bacterial metabolites in gut–brain communication.

Keywords: *Drosophila melanogaster*; gut-brain axis; microbiota; acetate; lactate.

Summary

Introduction

The implementation of *Drosophila melanogaster* as a model for investigating gut–brain communication is an emergent field of research. Several factors influence the composition of gut bacteria population such as lifestyle, eating habits, chronical stress, and social interactions (Buffington *et al.*, 2016; Valles-Colomer *et al.*, 2023). Despite harboring a simpler microbiota compared to humans, fruit flies can support the early stages of clinical research in humans.

The microbiota of this invertebrate comprises *Acetobacter* and *Lactobacillus* genera, bacteria with distinct symbiotic interactions with fruit flies (Dodge *et al.*, 2023). The microbiota can be modified to protect the patient against neurodegeneration, but rather than administering isolated species such as *Lactobacillus* (Kleerebezem *et al.*, 2010), the answer may lie in modeling a proper gut environment where beneficial bacteria can thrive. Although more studies on gut-brain-microbiota communication are needed to establish a solid therapy for central nervous system disorders, focusing on multiple–organ strategies would offer valuable correlations (Grenham *et al.*, 2011).

The administration of Short-Chain Fatty Acids (SCFAs) in the food of wild *Drosophila* could cause glial changes similar to those observed in models of neurodegenerative diseases (Colombo *et al.*, 2021). Therefore, it would be helpful to understand whether this activation is directly caused by acetate and lactate—SCFAs commonly found in the gut—or whether *Acetobacter* and *Lactobacillus* influence the neurodegenerative process indirectly. Identifying a common link between multiple causes of neurodegenerative processes is key to establishing its correlation with metabolites found in the gut and maintaining brain health (Carter *et al.*, 2019).

The goal of this project was to evaluate whether the administration of lactate and acetate salts in the food of *Drosophila* would affect its lifespan and climbing performance. The secondary goal was to investigate the presence of *Acetobacter* and *Lactobacillus* in the gut of the flies changes according to the administration of each salt.

This manuscript is organized into two chapters. Chapter I provides a brief introduction to the topic and reviews the key literature that motivated the investigation of *Drosophila* as a model in gut–brain axis studies. A more detailed contextualization of this topic can be found in our previously published paper (Alves *et al.*, 2025). Chapter II describes the experiments that were carried out and their respective results.

CHAPTER I
Theoretical Background

Gut Microbiota and Neurological Diseases

The interplay between diet, microbiota, and the intestinal epithelium offers valuable insights into brain health, as the gastrointestinal tract engages in a complex bidirectional communication with the nervous system through an intricate network of signaling pathways (Makdissi; Parsons; Di Cara, 2023). Neurological processes significantly influence the composition and relative abundance of diverse bacterial genera. *Lactobacillus rhamnosus* is an indirect reductor of the inhibitory neurotransmitter GABA, leading to reduction in anxiety and depression (Bravo *et al.*, 2011). In contrast, psychological stress enriches mice gut commensal *L. murinus*—a indole-3-acetate (IAA) producer—, also contributing to intestinal secretory cells loss (Wei *et al.*, 2024).

Patients with Alzheimer's harbor increased *Bacteroidetes* and decreased *Firmicutes* and *Bifidobacterium* in the microbiome (Vogt *et al.*, 2017), reinforcing the notion of a stereotypical gut disease–specific microbiota profile. The microbiota also modulates the immune response, which indirectly influences hormone levels, neurotransmitter metabolism, neuronal signaling (Morais; Schreiber; Mazmanian, 2021), and the integrity of the blood-brain barrier (Fung; Olson; Hsiao, 2017). However, the mechanisms through which the intestinal host–microbiota interactions remotely alter brain physiology remains unclear(Fung; Olson; Hsiao, 2017).

Genera such as *Lactobacillus* shows an intrinsic positive metabolic interaction with *Acetobacter* strains (Dodge *et al.*, 2023), a group equally reduced in neurodegenerative diseases (Liu *et al.*, 2023). The interaction between these groups encourages new studies on the influence that bacterial metabolites might have on neurological diseases, especially given their diversity, which tends to decline with age and diseases progression (Kong; Jiang; Luo, 2018; Lynn *et al.*, 2022) (Figure 1).

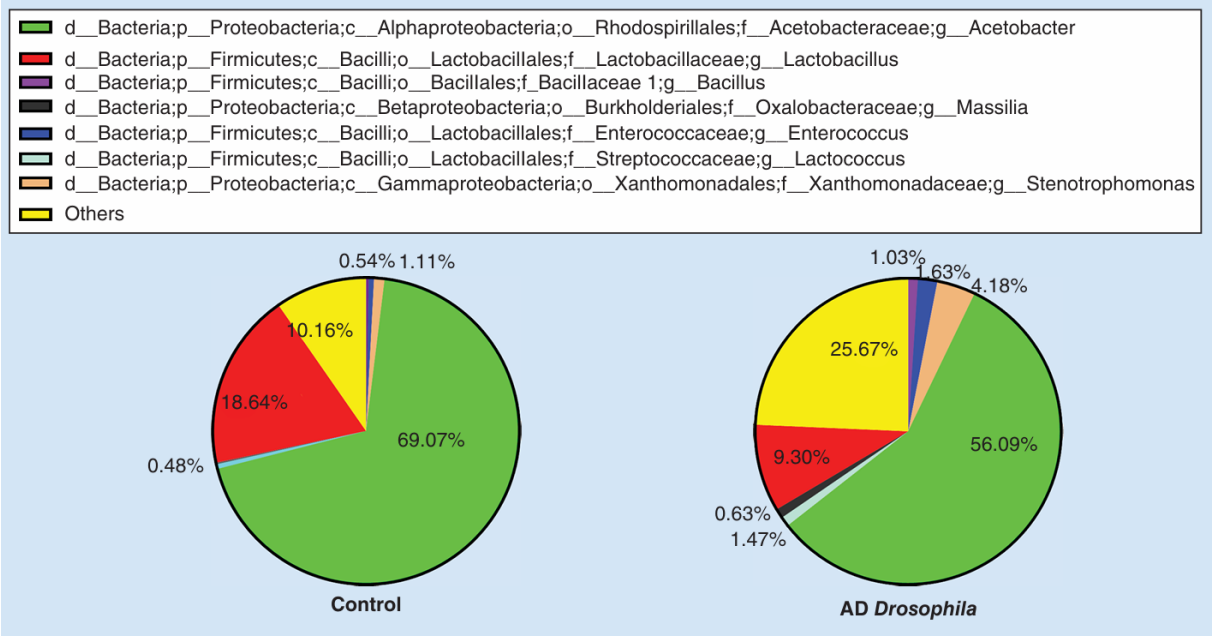


Figure 1. Bacteria microbiota profile in Control and Alzheimer's disease-model flies. Source: Kong; Jiang; Luo, 2018 (Kong; Jiang; Luo, 2018).

Acetobacter is a group of gram-negative aerobic acetic acid producers that belong to Pseudomonadota phylum, formerly known as Proteobacteria (Oren; Garrity, 2021). Similarly to *Lactobacillus*, its members present a rod-shaped morphology, but differs in length and color under Gram staining, with *Lactobacillus* appearing longer and purple, while *Acetobacter* is shorter and pinkish (Figure 2).

The microbiota can be remodeled or improved to protect the patient against neurodegeneration process, however, along with administering isolated species such as *Lactobacillus* (Kleerebezem *et al.*, 2010), the answer may lay on cultivating a proper gut environment in which good bacteria can grow; even social interactions seem to play a role in microbiome-associated diseases (Valles-Colomer *et al.*, 2023). Although more studies on brain-intestine-microbiota communication are needed to establish a solid therapy for Central Nervous System (CNS) disorders, multidisciplinary approaches to this topic offer valuable and fruitful correlations (Grenham *et al.*, 2011).

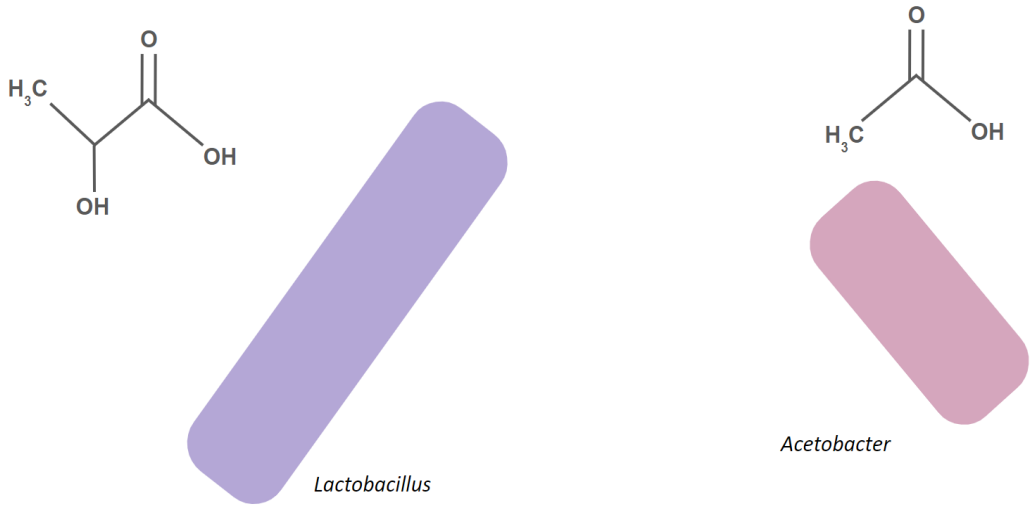


Figure 2. *Lactobacillus plantarum* and lactic acid molecule (left). *Acetobacter pomorum* and acetic acid molecule (right). Source: The Author.

Drosophila **as a Gut-brain Axis Model**

The presence of few bacteria species in the microbiome of *Drosophila melanogaster* and the established methods to implement it as a germ-free model (Li *et al.*, 2024) supports its use in gnotobiotic studies, providing a controlled interaction between host and known microorganisms. With a relatively simple microbiota (Marra *et al.*, 2021), *D. melanogaster* harbors microbial communities of 2 to 30 species, that both in laboratory and field are represented by two phyla: *Proteobacteria* and *Firmicutes*. The most consistently associated species across different studies is a community of several lactic and acetic acid bacteria that reflects the fermentative substrates on which flies feed (Arias-Rojas; Iatsenko, 2022).

The proportion of *Acetobacter* and *Lactobacillus* is decreased in most frequent neurological deficits, such as AD and Parkinson's disease (Kong; Jiang; Luo, 2018; Liu *et al.*, 2023). Species such as *Acetobacter fabarum* and *Lactobacillus brevis* assist in *Drosophila* nutrition processes (Sommer; Newell, 2019), while microbiota-derived acetate activates intestinal innate immunity (Jugder; Kamareddine; Watnick, 2021) and some species may disrupt the circadian cycle (Zhang *et al.*, 2023).

Drosophila's metabolism is highly adaptive. When glycolysis is insufficient, its glial cells can switch to using fatty acids to fuel neuronal metabolism (McMullen *et al.*, 2023), suggesting the contribution of glial cells in the gut-brain axis as either intermediaries in neurodegeneration or in nutrient processing. Furthermore, *L. plantarum* and its by-product, lactic acid, contributes to the accumulation of Reactive Oxygen Species (ROS)(Iatsenko;

Boquete; Lemaitre, 2018). In short, gut microbiota-derived molecules may contribute to or signal processes occurring in the brain.

The intestine of *Drosophila melanogaster* exhibits well-conserved molecular aspects compared to humans (Apidianakis; Rahme, 2011) and distinct pH zones (Sapre *et al.*, 2020), supporting its use in gut-related studies (Dodge *et al.*, 2023; Iatsenko; Boquete; Lemaitre, 2018; Silva *et al.*, 2020). The gastrointestinal tract of flies is commonly divided into the foregut, midgut, and hindgut, with the midgut housing gastric acid-producing copper cells (Miguel-Aliaga; Jasper; Lemaitre, 2018) (Figure 3). Antimicrobial peptides are also produced by the gut epithelium (Vodovar *et al.*, 2005) and fat body (Buchon *et al.*, 2009) of flies, while the gut mucus layer and the chitin-based peritrophic matrix act as filters for pathogenic microorganisms (Apidianakis; Rahme, 2011; Vodovar *et al.*, 2005).

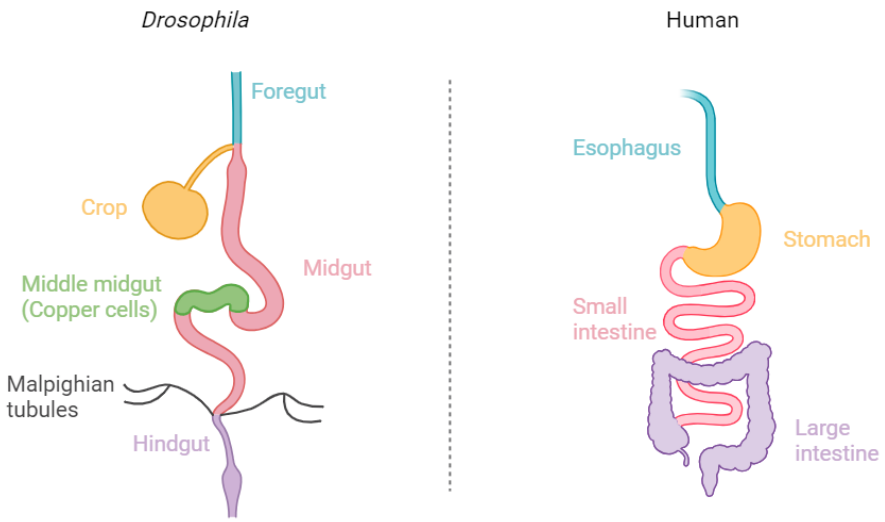


Figure 3. The human analogous intestine regions in *Drosophila*, differentiated by Malpighian tubule presence in flies. Iron/Copper cells region is found in a low pH region (green). Source: The Author. Created with https://BioRender.com.

Drosophila as a Model in Neuroscience

The nervous system of the *Drosophila melanogaster* fly maintains a high degree of complexity, composed of multiple neuronal and glial subtypes that share cellular, genetic, and functional characteristics with their mammalian counterpart (Salazar *et al.*, 2022). Fruit flies are widely used as a model in neurodegeneration experiments (Kitani-Morii *et al.*, 2021), such as climbing assays (Ferreiro *et al.*, 2018; Rahmani *et al.*, 2022), gastric motility (Rydbom *et al.*, 2021), and memory assays (Gil-Martí *et al.*, 2023). Physiological disorders can be measured by sleep monitoring (Vaccaro *et al.*, 2020), transcriptome profiling (Liu *et al.*, 2023; Marsh *et al.*, 2016; Zhang *et al.*, 2023), lifespan assays (McMullen *et al.*, 2023; Vaccaro *et al.*, 2020),

bacterial quantitative Polymerase Chain Reaction (PCR) (Trébuchet *et al.*, 2019; Zhang *et al.*, 2023), and microglia metabolic alterations (Huang *et al.*, 2023; Marsh *et al.*, 2016) and development (Stork; Bernardos; Freeman, 2012). Furthermore, *Drosophila* flies serve as both axenic and gnotobiotic models (Brummel *et al.*, 2004; Steven *et al.*, 2021).

Several cell types contribute to immune-like responses in the fly CNS. The glial cells are classified into four categories based on their cell bodies position and form: cortical glia, neuropil glia, peripheral glia, and surface glia (Freeman; Doherty, 2006; Yildirim *et al.*, 2019). These categories hold morphological and functional similarities with their human counterparts.

In mammals, neuroinflammation is intricately associated with microglial activation (Johnson *et al.*, 2020), which causes the cell to undergo a morphological transformation from a slender, ramified form to a more rounded shape with fewer extensions (Loh *et al.*, 2024). Similar to mammalian microglia, the surface and neuropil glia in flies, specifically the ensheathing glia, perform macrophage-like functions (Freeman; Doherty, 2006), suggesting a role in Aβ or tau engulfment (Sheng *et al.*, 2023). A summary of each glial subtype is presented in Table 1.

Several cell types of the digestive tract are regulated by the brain. Serotonergic enterochromaffin cells—a type of human gut epithelium cell—were indicated to modulate sensory nerves via serotonin receptors and synaptic connections (Bellono *et al.*, 2017). Remarkably, some enterochromaffin subtypes are also found in *Drosophila* (Guo; Lv; Xi, 2022), suggesting conserved gut–brain communication pathways in both humans and flies.

Table 1. Types of glial cells in the adult *Drosophila* CNS and their functions.

Glial type	Function	Position
Astrocyte-like	Ionic and neurotransmitters homeostasis	Outside the neuropil (cell bodies) and inside (extended processes)
Ensheathing	Phagocytosis of debris	Between neuropil surface and cortex cells
Cortex	Trophic support to neurons	CNS cortex
Subperineural	Chemo-protection and selective transport of nutrients	CNS periphery
Perineural	Chemo-protection, selective transport of nutrients, and barrier physical support	Covering the entire nervous system

Source: The Author.

In summary, an increasing number of studies have employed *Drosophila melanogaster* as a model organism for neurodegenerative disorders, including tauopathies such as

Alzheimer's and Parkinson's diseases. The relatively simple gut microbiota, short life cycle, and ease of genetic manipulation make *Drosophila* a valuable tool for investigating how diet can modulate microbial composition and, consequently, how microbiota-derived metabolites influence on neurological health. Although the complexity of the physiological systems of invertebrates differs from that of mammals, comparative studies have provided important insights into how diet and other indirect factors affect neuronal function and diseases progression. Furthermore, intestinal dysbiosis, immune activation, and oxidative stress can exacerbate neurodegeneration through conserved signaling pathways. Therefore, it could serve as a tractable system to dissect the molecular mechanisms mediating gut-brain communication, including the role of microbial–derived metabolites on brain health and therapeutic interventions.

CHAPTER II
Experimental Procedures

INTRODUCTION

The cases of neurological diseases has notably increased in recent years, with some of these complication projected to triple by 2050 (Scheltens *et al.*, 2021), causing not only suffering to family, friends and caregivers (Beata *et al.*, 2023), but deeply consequences to health systems. For instance, the etiology of such diseases is shown to be influenced by the gastrointestinal tract and its resident microorganisms (Pluta *et al.*, 2020; Vogt *et al.*, 2017). The modulation of the microorganisms found in the gut has been presented as a new path to treat these complications, since the molecules produced by the microbiota can reach neurons and immune cells in the central nervous system and influence them in several ways (Huang *et al.*, 2023; Mayneris-Perxachs *et al.*, 2022).

In addition to murine models, *Drosophila melanogaster* is an inexpensive, genetically modulable and easily reproducible model (Jennings, 2011) currently used in microbiota-related research (Kong; Jiang; Luo, 2018; Rydbom *et al.*, 2021; Tan *et al.*, 2020). The flies possess common gut residents with humans, such as the bacteria genera *Acetobacter* and *Lactobacillus* (Simhadri *et al.*, 2017), producers of acetic acid and lactic acid, respectively. In fact, lactate is transported from glial cells to neurons and utilized by the latter in the tricarboxylic acid cycle (Volkenhoff *et al.*, 2015; Xu *et al.*, 2023), playing a fundamental role in disrupted energy metabolism. In addition, the molecular and cellular conserved aspects of *Drosophila* support its use in intestinal epithelium (Apidianakis; Rahme, 2011) and brain-gut communication (Kitani-Morii *et al.*, 2021; Makdissi; Parsons; Di Cara, 2023) research, encouraging new diagnosis insights from an interorgan perspective.

Microglia is a class of glial cell of the central nervous system that participates in the engulfment of toxic proteins and neuroprotection in both zebrafish (Hassan-Abdi *et al.*, 2019) and mammals (Freeman; Doherty, 2006; Yildirim *et al.*, 2019). Since the *Drosophila* counterparts—neuropil, cortex, and ensheathing glia (Doherty *et al.*, 2009; Freeman; Doherty, 2006)—also play a role in the phagocytosis of pathogenic proteins, the activation of ensheathing glia is believed to be crucial for elucidating the pathways involved in neuroprotection and toxicity.

Therefore, exploring the toxicity of lactate and acetate in a complex organism would promote a better understanding on how bacteria by-products modulate the lifespan, locomotion and homeostatic aspects of *Drosophila*. These insights could foster more effective and comprehensive treatments for neurological diseases associated with the gut–brain axis.

MATERIALS AND METHODS

Workflow of Experiments

The workflow of the study is shown in Figure 4.

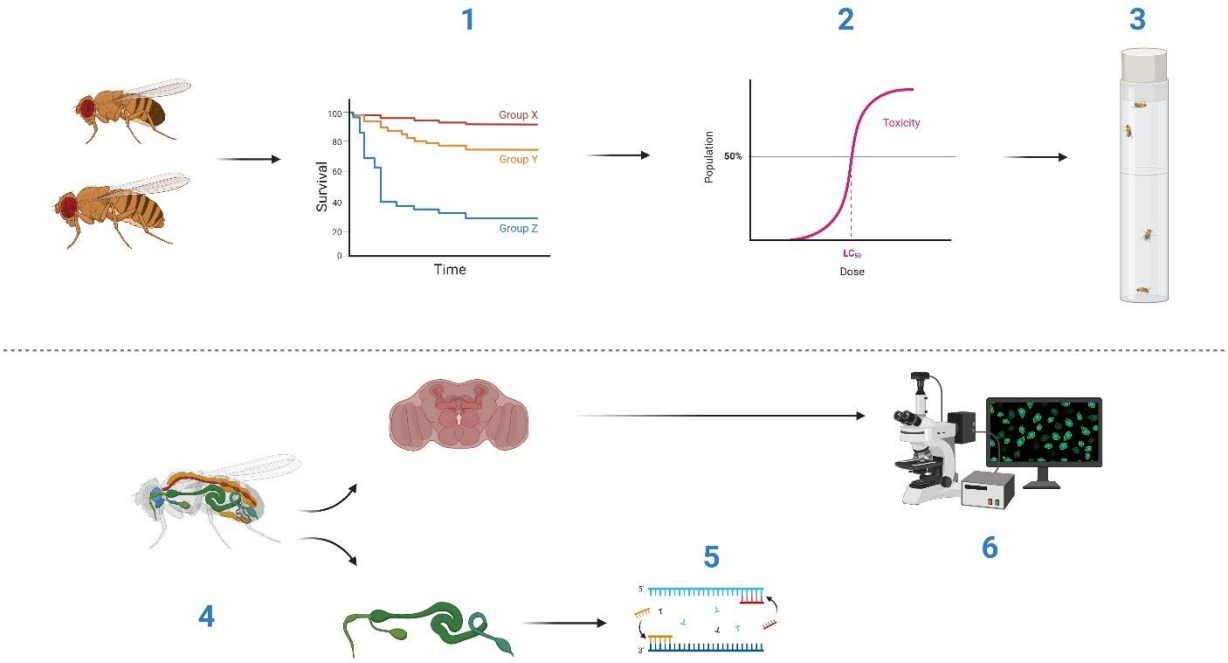


Figure 4. Experimental workflow. **1**. Lifespan assay; **2.** median lethal concentration; **3**. negative geotaxis; **4**. *Drosophila* dissection; 5. Polymerase Chain Reaction Assays (PCR); **6**. Confocal microscopy measurements. Source: The Author.

Flies Strains and Culturing

Drosophila melanogaster Canton-S wild-type models were kindly provided by Prof. Karen C. M. de Morais, from IB-UNESP (Rio Claro), and maintained at 25°C on a 12:12 h light/dark cycle and 40-60% relative humidity, with each vial containing 10 mL of standard medium. The culture medium was prepared to a final volume of 800 mL consisted of molasses (25.5 g), agar (8.5 g), sucrose (12.75 g), glucose (25.5 g), cornmeal (12.75 g), wheat germ (8.5 g), soy flour (8.5 g), baker's yeast (29.75 g), propionic acid (4.5 mL), and nipagin (0.85 g). All experiments used male flies.

Lethal Concentration and Benchmark Dose

To model the effect of metabolites produced by *Acetobacter* and *Lactobacillus* on the fly brain, sodium acetate and sodium lactate were administered. Similar to the stock group, the

flies exposed to the salts were maintained at 25°C on a 12:12 h light/dark cycle and 40-60% relative humidity. For reaching the highest beneficial salt concentration and less effect on lifespan, six concentration groups were formed: control (no salts), and saline dilutions of 1:1, 1:2, 1:10, 1:100, and 1:1000 (Holsopple *et al.*, 2023). For acetate, the 1:15 and 1:50 dilutions were additionally evaluated in the Lethal Concentration (LC_{50}) assay to optimize the dose-response curve. The 1:1 dilution corresponded to 465 g/L, the most concentrated solution of sodium acetate (ThermoFisher, CAS 127-09-3). This species subsequently undergoes dissociation, yielding sodium and acetate ions(Devineni *et al.*, 2019). Sodium DL-lactate (ThermoFisher, CAS 72-17-3) was used as lactate source, with the highest concentration of 771 g/L. Both salts were added to the food during cooking process at 60°C, before solidification occurred. These values correspond to the maximum solubility of the compounds. For all tests mortality at first 7 days of age was used, unless otherwise stated.

Experimental replicates were pooled by dose for binomial dose–response modeling, allowing the combined estimation of LD_{10}, LD_{50}, benchmark dose (BMD), and its lower confidence limit (BMDL) (Davis; Gift; Zhao, 2011). Generalized linear models (GLMs) with logit, probit, and complementary log-log link functions were evaluated, as well as nonlinear models from the log-logistic, lognormal, and Weibull families. Model selection was based on the Akaike Information Criterion (AIC), with the model presenting the lowest value being selected. Abbott's correction was applied when control mortality exceeded the 5% threshold. All analyses were performed using R (version 2026.01.2+418). Lethal dose (LD_{50}) and lethal-concentration (LC_{50}) were used as synonyms when referring to BMD, although conceptually the LC_{50} was implemented because flies ingested the compounds.

Lifespan Assay

Lifespan measurements were performed stocking 20 to 30 Canton-S male flies per vial in triplicates (Brummel *et al.*, 2004; Holsopple *et al.*, 2023). Every 2–3 days the number of dead flies was registered (Vaccaro *et al.*, 2020). Flies that escaped or died during transfer were censored from the data. For longevity, log-rank Mantel-Cox test was performed with GraphPad Prism 8, set for survival curve algorithm (Brummel *et al.*, 2004). Equality between groups was tested using a log-rank test, $p < 0.05$ is assumed to be of significant difference (Kong; Jiang; Luo, 2018; Linford *et al.*, 2013). Flies were counted as dead if they fail to move (e.g., leg movements) in response to taps on the side of the vials (Vaccaro *et al.*, 2020). The age-specific

survivorship at age x (S_x) is determined by dividing the number of individuals alive at age x (N_x) by the total number of flies in the experiment (N_0), or $S_x = \frac{N_x}{N_0}$.

Negative Geotaxis Assay

The neuromotor activity was assessed with the negative geotaxis assay, as described (Coulom; Birman, 2004). Firstly, approximately 30 males were anesthetized and placed in an empty glass vial (length, 15 cm; diameter, 2.5 cm). After the recovery from CO_2 exposure (approximately 30 minutes), flies were gently tapped to the bottom of the column. Flies that reached the 10 cm mark of the column under 1 minute were counted. Three trials were performed in each experiment. The scores are the mean of the numbers of flies at the top (n_{top}), expressed as percentages of the total number of flies (n_{tot}). The test was performed once a week, for six weeks or until all flies died. For each experiment, a performance index (PI), defined as n_{top} / n_{tot}, was calculated as an adaptation of the literature (Coulom; Birman, 2004; Vaccaro *et al.*, 2020).

PCR of *Acetobacter* and *Lactobacillus* in the Gut

Gut microbiota was assessed by PCR to determine whether the relative abundance of *Acetobacter* and *Lactobacillus* species varied according to salt treatment. Before the PCR steps, the genomic DNA extraction occurred as described in Appendix I. The primers for *Lactobacillus* genus detection were LacF (5′CGATGAGTGCTAGGTGTTGGA-3′) and LacR (5′-CAAGATGTCAAGACCTGGTAAG-3), 186 bp (Vaccalluzzo *et al.*, 2023), whereas for *Acetobacter* PASTEU-F (5′-TCAAGTCCTCATGGCCCTTATG-3′) and PASTEU-R (5'-TCG AGTTGCAGAGTGCAATCC-3′), 130 bp amplicon, were used (Mokeev *et al.*, 2021). The control primers F (5'-ACTACGTGCCAGCAGCC-3′) and R (5'-GGACTACCAGGGTATCTAATC-3′) were used to set a reference, since *Drosophila* do not harbor a core microbiota as defined as bacterial species of mammals or social insects (Miguel-Aliaga; Jasper; Lemaitre, 2018).

The cycling conditions included an initial holding phase at 95°C for 45 sec followed by 30 cycles at 94°C for 45 s, test temperature for 45 s, and 72°C for 45 s. The reaction was then held at 72°C for 1 min and stopped at 15°C. To ensure amplification specificity, a melting curve analysis was performed considering the test temperatures of 48, 49.7 and 52°C. Gel electrophoresis was performed on a 1.5% agarose gel in TBE buffer using a mini-gel system at 90 V for 1 h.

For analysis of the electrophoresis gel, ImageJ version 1.54 was used. First, the image was converted to 8-bit grayscale. Background intensity was then determined using the Rectangle tool via Analyze – Set measurements – Mean Gray Value. After obtaining the background value using Analyze – Measure, it was subtracted from the image using Process – Math – Subtract. The image colors were subsequentially inverted using Edit – Invert. Gel bands were selected using the Rectangle tool, followed by Analyze – Gel – Select First Lane. Once the lane was defined, intensity profiles were generated using Analyze – Gels – Plot Lanes. The area of each intensity peak was calculated by drawing a straight baseline at the bottom of the band, selecting the peak with the Wand tool, and applying Analyze – Gels – Label peaks.

Glia Phenotypes According to Salt Administration

The morphology of ensheathing glial cells and its potential alterations in activation profiles associated with the salts administration was evaluated using the Rumpel protein immunofluorescence (Yildirim *et al.*, 2022), which could be associated with increased cytosolic area (Colombo *et al.*, 2021). The assay was performed using the monoclonal anti-Rumpelstiltskin antibody (10C3, DSHB, 1:100) and Fluoroshield with DAPI. Brains were processed as described in Appendix II. Images were acquired with a Leica TCS SPE confocal microscope.

Fluorescence intensity was quantified using ImageJ. Image stacks were first converted into a single projection using Image - Stacks - Z Project - Sum Slices. The resulting image was then processed with a Gaussian blur filter using Process - Filters - Gaussian Blur to reduce noise. A threshold was subsequently applied using Image - Adjust - Threshold to define the region of interest. The brain region was selected using the Wand Tool and added to the ROI Manager using Analyze - Tools - ROI Manager - Add. Background regions were selected using rectangular selection tools and added to the ROI Manager. The original image measurements were obtained using Analyze - Measure. The resulting values were exported to a spreadsheet for further analysis. Mean background intensity was calculated and used to correct fluorescence values according to the following equation: corrected fluorescence intensity = integrated density - (area × mean background intensity).

RESULTS

Higher Doses of Acetate Result in Greater Mortality Than Lactate

The administration of acetate and lactate salts was intended to modulate the lower representation of *Acetobacter* and *Lactobacillus* genus found in flies with impaired gut–brain communication (Kong; Jiang; Luo, 2018). For this, in this study with Canton-S strain, the continuous ingestion of compounds and the effects on lifespan were evaluated in an age-dependent manner. The Figure 5 presents the survival according to the dose of each compound.

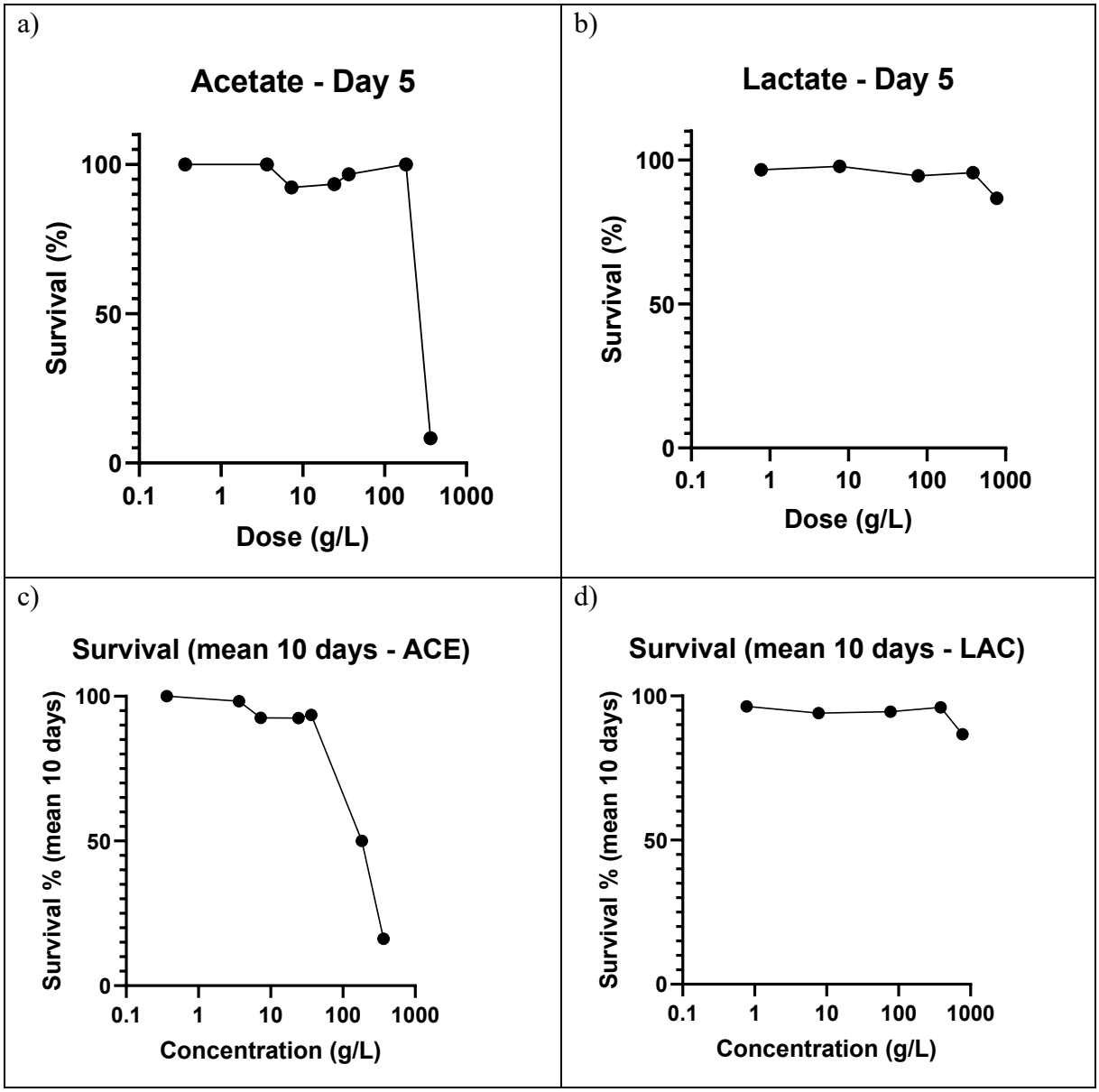


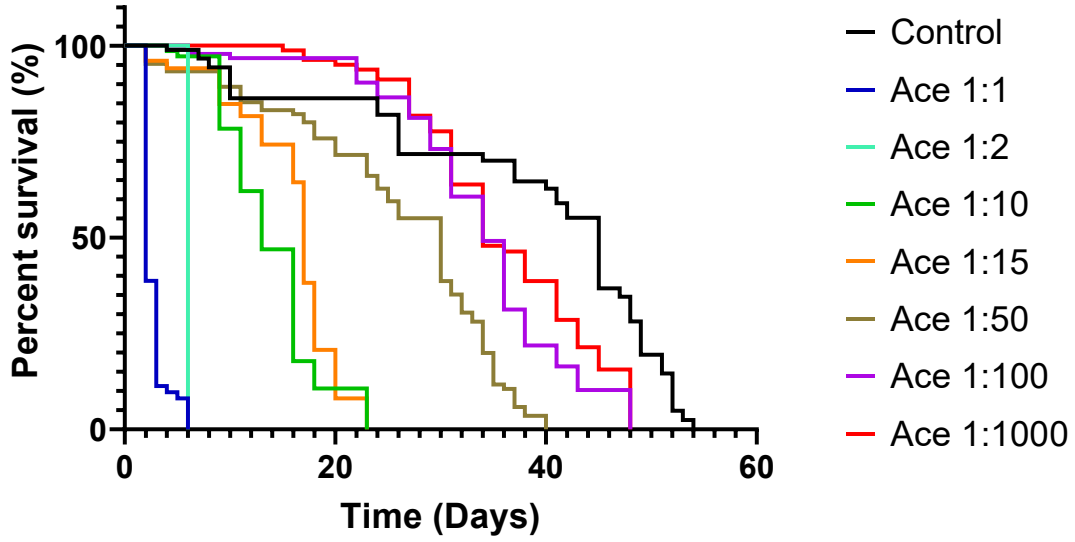
Figure 5. Log dose-response curve for LC_{50} estimation of flies exposed to acetate (a) and lactate (b) at 5 days old. (c) represents acetate and (d) lactate dose-response curve considering values among 10 days old. Source: The Author.

Mortality at 5 days old was higher in the acetate treatment than in lactate at the highest concentrations. At lower concentrations, mortality was minimal and did not demonstrate a clear dose-response correlation. Therefore, survival data was also ordered as the mean over 10 days (Figure 5c and Figure 5d). The survival rate for acetate exposure during this period declined according to higher doses, especially at 465.0 g/L (1:1) and 232.5 g/L (1:2) dilutions. For lactate, only exposure to the 771 g/L (1:1) dilution resulted in a reduced survival rate. However, the lower survival for lactate was not as pronounced as the reduction observed for acetate.

To obtain reference dose values for salts, the statistical evaluation using the benchmark dose (BMD) and median LC_{50} analysis were performed. More information about the methods used is provided in Appendix III. For acetate, the LC_{50} was estimated at 1:3.73 (124.6 g/L) and the benchmark dose at 1:6.42 (72.4 g/L). For lactate, LC estimation showed marked instability and substantial extrapolation beyond the experimental range 1:1 × 16.6 (12,823.6 g/L); therefore, toxicological interpretation of this salt was primarily based on the benchmark dose approach, which was estimated at 1:1.01 (764.0 g/L).

To evaluate the long-term effects of salt intake, lifespan was measured. Mortality of Canton-S flies according to sodium acetate and lactate ingestion is illustrated at Figure 6.

a)



b)

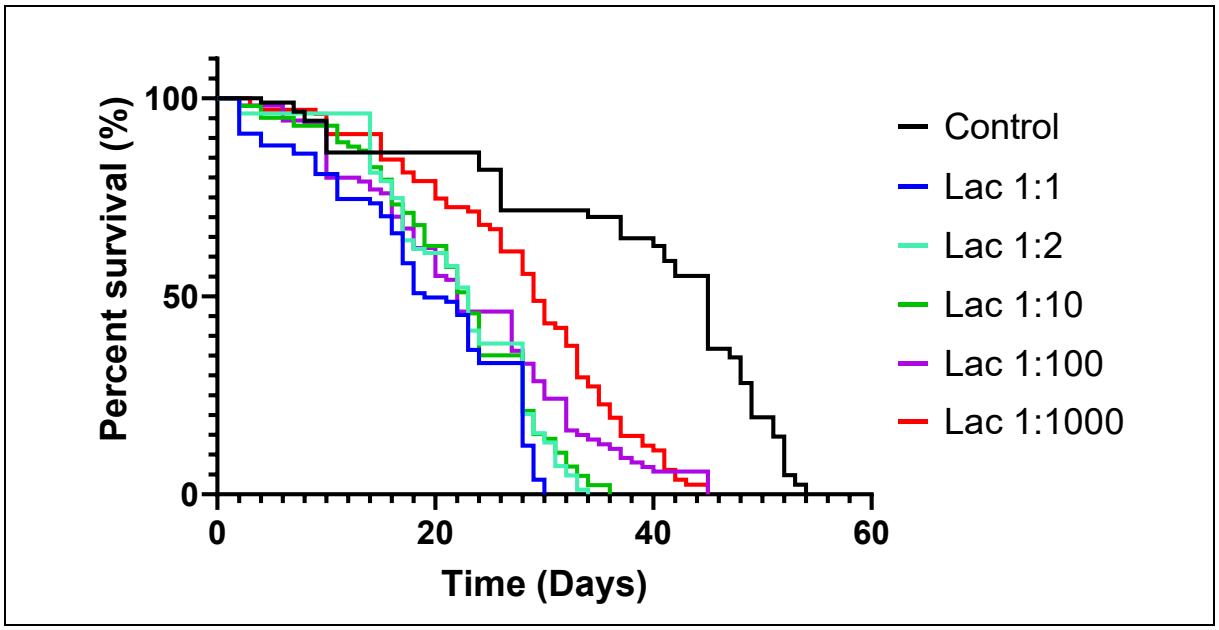


Figure 6. Survival rates of *Drosophila* in response to acetate (a) and lactate (b) exposure at different food dilutions. Source: The Author

For acetate, Mantel–Cox pairwise comparisons were all significant except between 1:100 and 1:1000 ($p = 0.1389$). For lactate, no significant differences were observed between 1:2 and 1:10 ($p = 0.711$), 1:2 and 1:100 ($p = 0.0603$), and 1:10 and 1:100 ($p = 0.0791$). All other comparisons were significant. We hypothesize that the reduced survival in the control group at approximately 20 days of age is a genotype-dependent phenomenon, as described (Qiu; Xiao; Meldrum Robertson, 2017).

As acetate and lactate differ in composition, we also compared the compounds based on their effects on lifespan and climbing performance, rather than solely on matching concentrations. For assessing this, we arranged the data according to the respective BMD of each salt: 1:10 for acetate (46.5 g/L); and 1:1 for lactate (771.0 g/L). Subsequently, the effects of low-dose administration were also investigated, using 1:100 and 1:1000 dilutions for both salts. Figure 7 shows the survival curves obtained for the comparison across these different experimental conditions.

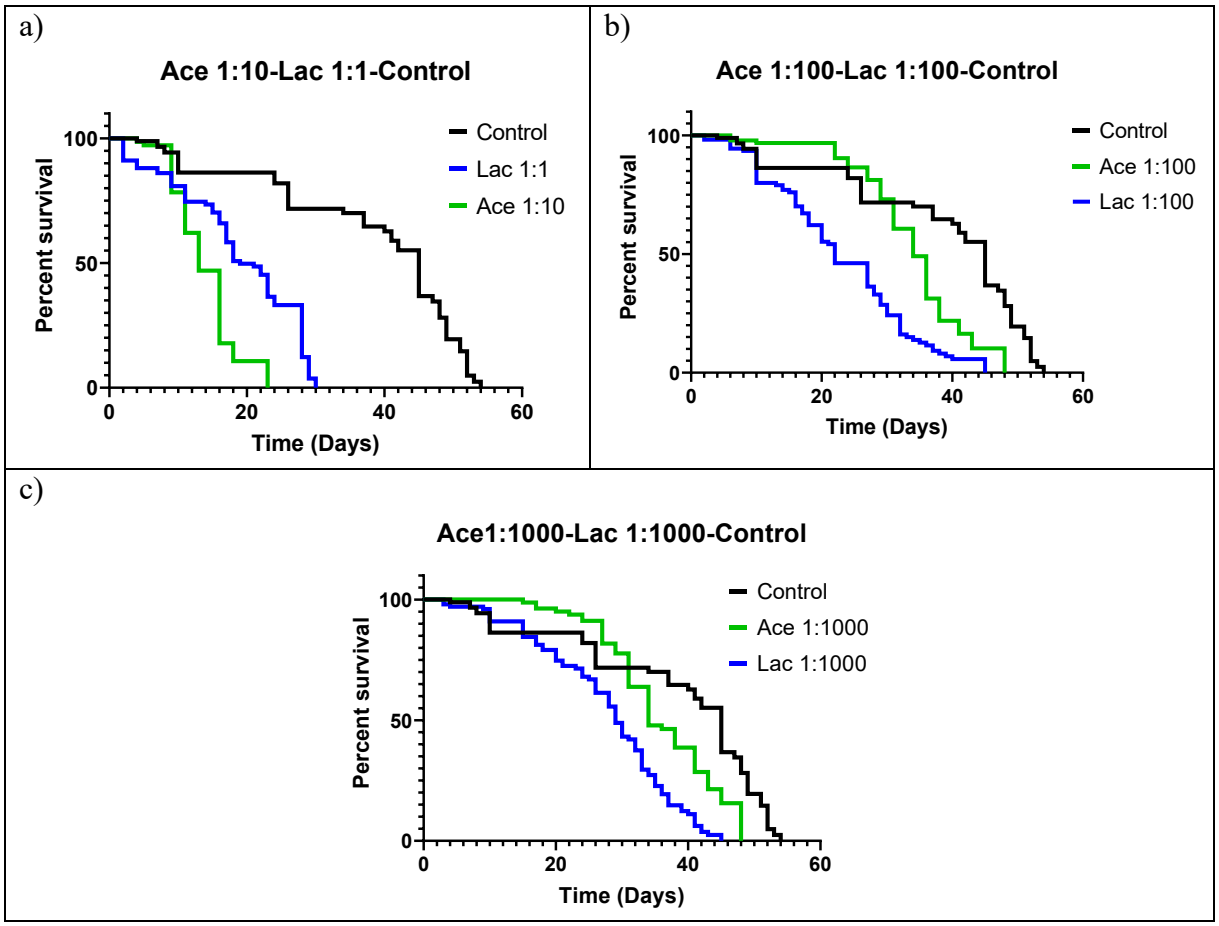


Figure 7. Survival rates of Canton-S between acetate 1:10 and lactate 1:1 (a); between 1:100 dilutions (b); and 1:1000 dilutions (c). Source: The Author.

Interestingly, when normalized by the BMD, the comparable same concentration of the two compounds leads to a shorter lifespan for acetate (Figure 7a), but when only the dilution is considered, acetate slightly improves lifespan (Figure 7b and Figure 7c).

The Effects of Acetate and Lactate on Climbing Performance Were Inconsistent

A temporal plot of the climbing data is presented in Figure 8. Flies exposed to acetate at a 1:1 dilution died within the first few days, and the climbing performance of the control group (black line) at Week 3 was abnormal; both datasets were therefore excluded from analysis. Inconsistent significance of climbing performances was observed across different salt dilutions. The Tukey test was applied to assess pairwise differences. The p-value between acetate 1:2 and 1:10 was 0.0365, while between acetate 1:10 and 1:1000 p=0.0096. For the comparisons involving lactate, the p-value between the control group and lactate 1:2 was 0.0145, and between the control group and lactate 1:1000 it was 0.0019. (Figure 8c and Figure 8d). Statistical significance was denoted using as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

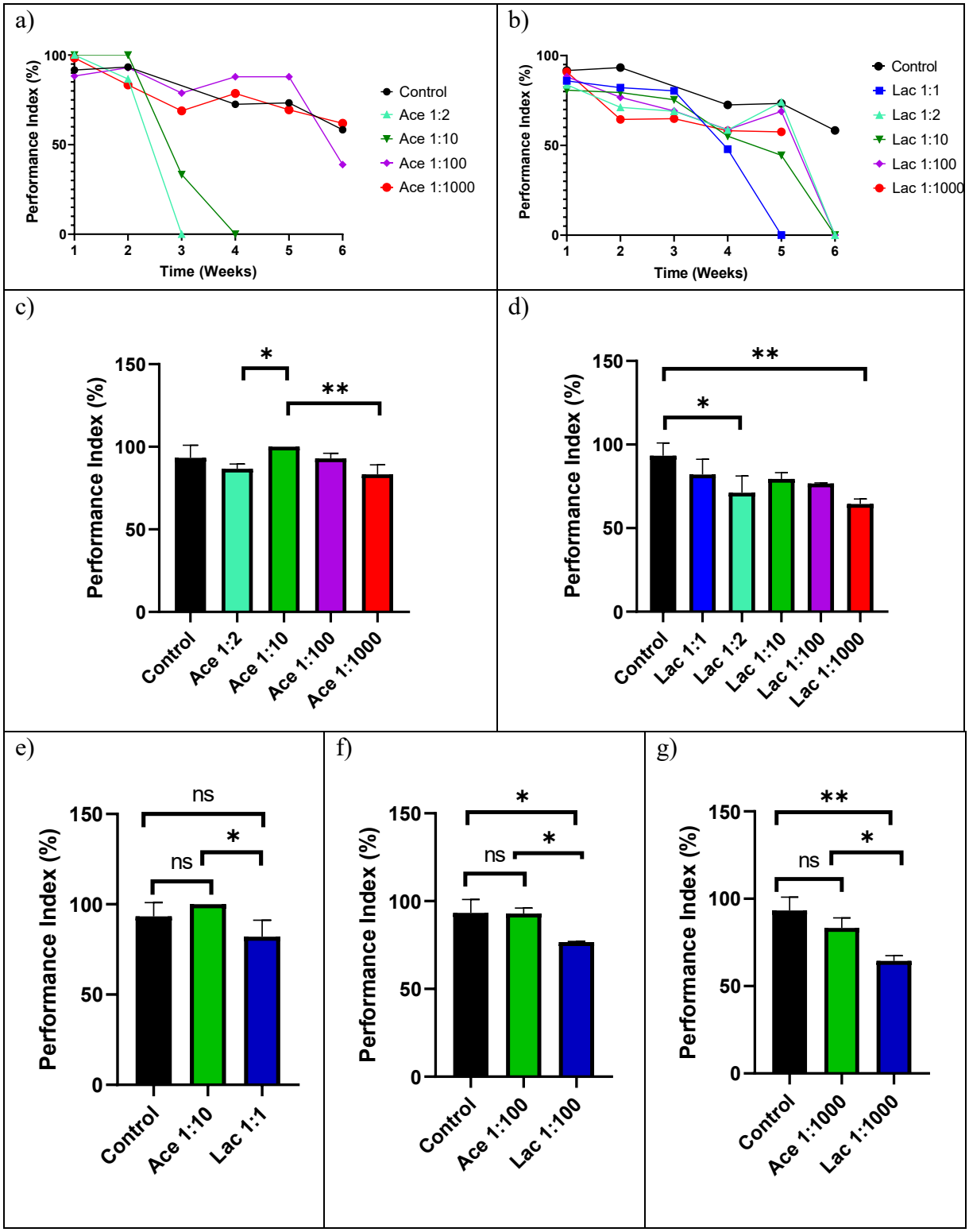


Figure 8. Climbing performance of flies exposed to acetate and lactate according to weeks (a, b); at 2 weeks old (c, d); at comparable BMD values (e); and at comparable dilutions (f, g). Data are presented as the mean, with error bars representing the standard deviation (SD). Source: The Author.

Considering the lifespan and climbing data, a 1:100 dilution of both salts was chosen for further investigation, as higher concentrations would lead to increased mortality in the flies

(Figure 6a and Figure 6b), whereas the 1:1000 dilution showed significant difference in climbing performance (Figure 8c and Figure 8d).

Lactobacillus Presence on Flies Fed with Salts

The gut microbiota composition was assessed by PCR, evaluating whether acetate and lactate administration could disrupt the bacteria profile. For that, approximately 30 intestines were dissected from flies exposed to each of the salts at 1:100 dilution for 5 days. The genomic DNA quality is presented in Table 2.

Table 2. DNA concentration and quality.

Sample (N of intestines)	Concentration (ng/µl)	A260/A280	A260/A230
Control (N = 28)	33	1.941	2.207
Acetate 1:100 (N = 27)	56.5	1.687	1.413
Lactate 1:100 (N = 27)	16.3	2	3.705

Source: The Author.

The group that received acetate showed higher DNA concentration and appropriate A260/A280 and A260/A230 ratios, suggesting low levels of contamination. In contrast, gut samples from control and lactate-exposed flies did not yield DNA of satisfactory quality. To further evaluate DNA integrity and primer specificity, agarose gel electrophoresis was performed. The results are shown in Figure 9, except for acetate-exposed flies that results were not consistent.

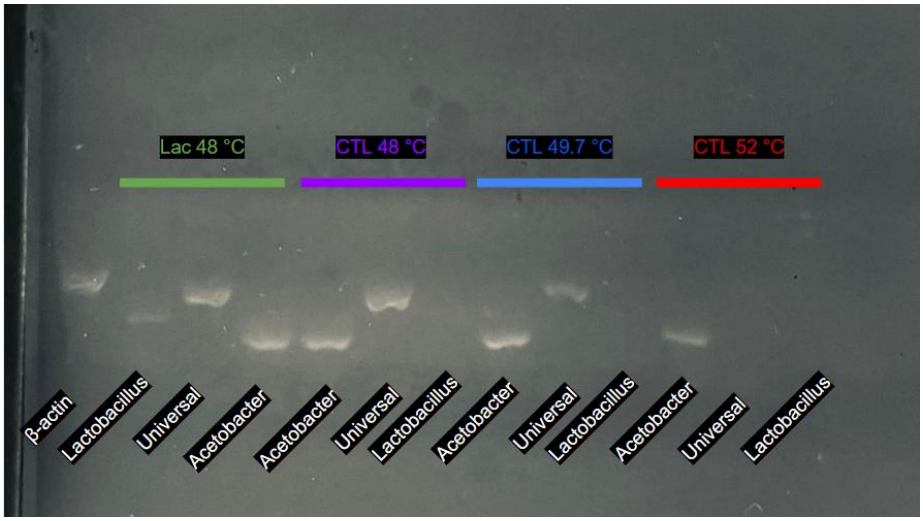


Figure 9. Gel electrophoresis of bacteria groups (*Lactobacillus*, *Acetobacter*, and universal primer) in dissected *Drosophila* intestines under control and lactate diets. Source: The Author.

PCR amplification targeting bacterial markers was used to assess the presence of selected bacterial groups in dissected *Drosophila* gut under lactate administration. Amplification with *Acetobacter*-specific primers produced detectable bands in several samples, but higher *Lactobacillus*-specific band intensity only in flies exposed to a lactate-supplemented diet, even though the 1:100 dilution was applied. Universal bacterial primers produced bands of consistent size across all samples, while amplification of β-actin served as a control for DNA extraction and template quality. Amplification band was more intense at an annealing temperature of 48°C.

To verify the differences, band intensities were quantitatively analyzed using ImageJ, as shown in Table 3 (percent intensity relative to overall fluorescence) and Figure 10 (fluorescence relative to β-actin).

Table 3. DNA concentration and quality.

Sample	Area (a.u.)	Percent intensity (a.u.)
β-actin	1770.284	5.286
Lactate 1:100 48°C – Universal	4444.882	13.271
Lactate 1:100 48°C – *Acetobacter*	5931.953	17.711
Lactate 1:100 48°C – *Lactobacillus*	1042.648	3.113
Control 48°C – Universal	6986.782	20.860
Control 48°C – *Acetobacter*	4976.569	14.859
Control 48°C – *Lactobacillus*	-	-
Control 49.7°C – Universal	998.527	2.981
Control 49.7°C – *Acetobacter*	6514.468	19.450
Control 49.7°C – *Lactobacillus*	-	-
Control 52°C – Universal	-	-
Control 52°C – *Acetobacter*	826.820	2.469
Control 52°C – *Lactobacillus*	-	-

Source: The Author.

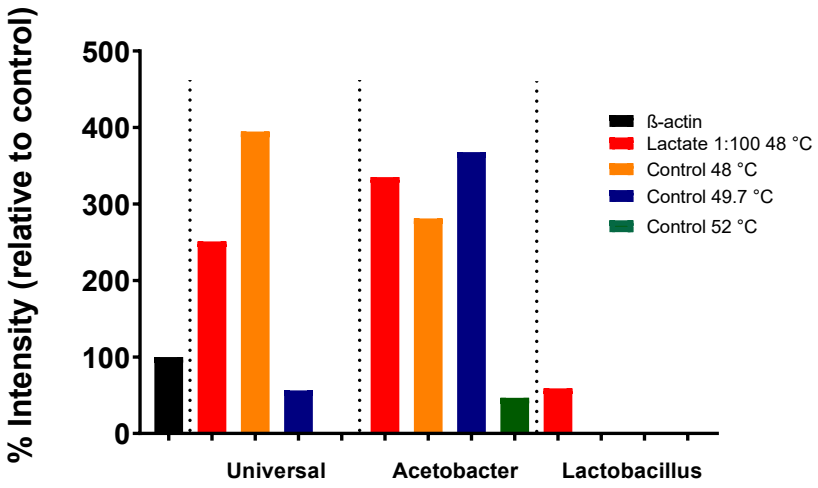


Figure 10. Effects of Temperature and Lactate Ingestion on Band Relative Intensity (%) Compared to Control (β-actin). Source: The Author.

Ensheathing Glia Immunofluorescence Assay

The immunofluorescence assay was performed for Canton-S flies labeled with anti-Rumpel as a tentative to verify the implication of the salts on brain morphology. Fortunately, the fluorescence detected in the green channel (488 nm) was remarkably specific for control groups not exposed to salts (Figure 11a and Figure 11b).

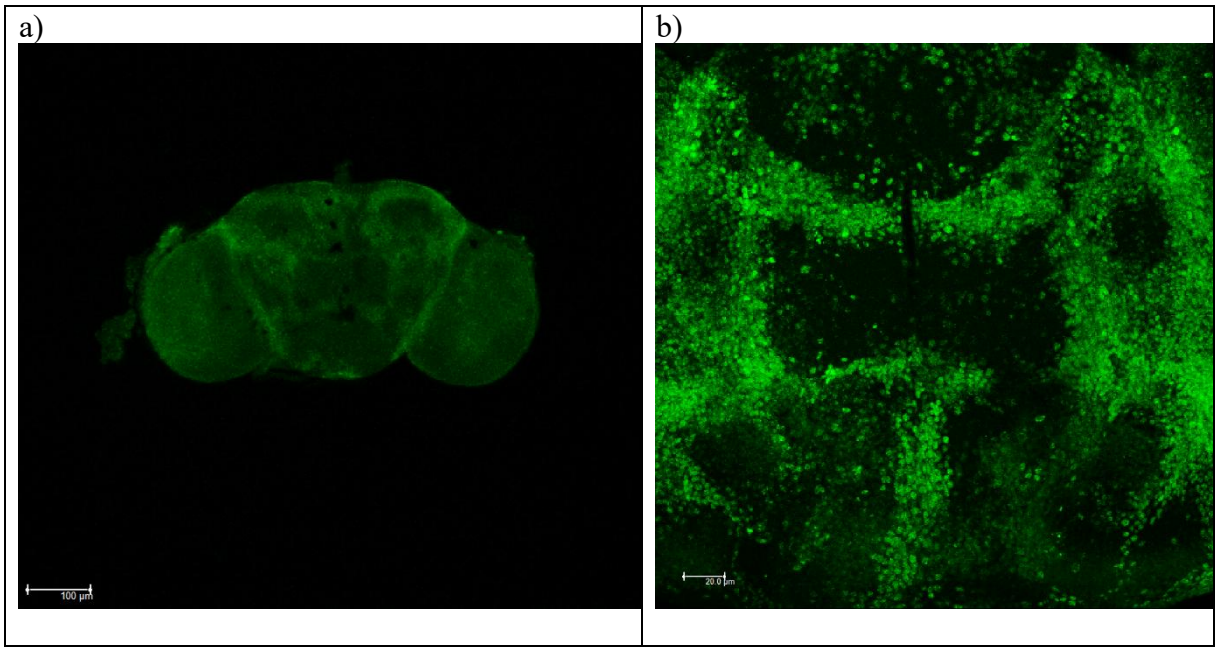


Figure 11. Confocal microscopy of (a) a 7-day-old Canton-S brain labeled with rumpel that did not receive salt treatment, imaged at 10× magnification (scale bar: 100 µm). (b) A 40× magnification of the same brain (scale bar: 20 µm). Source: The author.

Although the immunofluorescence protocol was properly optimized for our lab routine (Appendix II) and ensheathing glia labeling for flies was similar to the literature (Yildirim *et al.*, 2022), the image analysis for Canton-S flies that received acetate and lactate at 1:100 dilution were not consistent. Due to the absence of proper images of treatment groups for comparison, the fluorescence values calculated using ImageJ were not shown.

DISCUSSION

This study examined whether the compounds acetate and lactate influence lifespan and climbing ability of *Drosophila melanogaster* models. Following dietary supplementation with these metabolites, behavioral assays, and preliminary brain immunofluorescence imaging and PCR analyses were performed to explore potential links between these compounds administration and gut–brain disruptions.

We found that higher concentrations of acetate mixed into the food of flies were associated with a dose-dependent reduction in lifespan, indicating a toxic effect at elevated levels. In contrast, lactate supplementation did not produce comparable toxicity across the tested concentrations. Furthermore, assessment via the negative geotaxis assay revealed no significant differences in most of the locomotor performance comparisons between acetate or lactate.

While the differences in PCR suggest a possible enrichment of *Lactobacillus* in the intestines of flies exposed to dietary lactate, the assay based on electrophoresis gel does not allow precise quantification, and therefore these observations should be interpreted only as a rough indication of differences in bacterial abundance.

Green fluorescence from anti-Rumpelstiltskin labeling was tested, expected to selectively mark neuropil-associated glial cells (Yildirim *et al.*, 2022). Although the specific staining of glial cells was attained, no comparisons between glial phenotype under the administration of salts were completed. Considering the lifespan and climbing results, a more cautious examination of glia fluorescence intensity according to each acetate and lactate concentration is recommended, as flies could require different doses of these compounds to reach the same level of ensheathing glia activation, considering our BMD models.

Although no improvement was observed in the climbing parameters on salts ingestion, lifespan differences were detected upon supplementation with the salts. We hypothesize this difference will be fundamental to future investigations on the implication acetate or lactate would have on glial cells. Such studies could support the use of these compounds on diseases associated with the gut–brain axis.

CONCLUSION

We conclude lactate and acetate affect the lifespan of *Drosophila* in a dose-dependent manner, but not climbing performance. The preliminary analysis of the gut microbiota indicates that lactate ingestion may modulate the abundance of *Lactobacillus*.

STUDY LIMITATIONS

In respect to the lifespan assay, additional replicates should be performed to assure the dose–response mortality for each salt concentration is maintained, as well as survival curves with a positive control. This group should ingest sodium chloride to ensure that mortality is associated with acetate and lactate, rather than with sodium itself. The negative geotaxis test could be enhanced by measuring climbing only once during the lifetime of flies, by avoiding the transfer of flies using CO_2 anesthesia, or by restocking the dead flies in the vial every week, reducing the effect that body concussions or decreasing sample size could have on the performance index.

The gut microbiota investigation by PCR on groups that received acetate could not be finished and further analysis should be made. Additional replicates for each primer would also

ensure that lactate administration is properly associated with higher *Lactobacillus* presence, as well as for the acetate–*Acetobacter* pair. A more robust technique, such as quantitative PCR (qPCR) is required to adequately compare the presence of each bacterial group in the gut samples. Immunofluorescence analysis comparing ensheathing glial fluorescence in acetate and lactate treated flies could provide insights into glial activation by each salt.

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Appendix I

Protocolo - Extração de DNA genômico de *Drosophila melanogaster* - Karen C. M. de Morais, IB-UNESP (Rio Claro)

Materiais

1. Pipetas (p20, p200 e p1000)
2. Ponteiras
3. Descarte (para fenol e comum)
4. Microtubos de 1,5 mL
5. Centrífuga
6. Falcon
7. Gelo
8. Banho seco
9. Rack termostável

Reagentes

01. Tampão A (armazenar em 4°C)
    a. 10 mM Tris, pH 8 – Estoque 1 M
    b. 60 mM NaCl – Estoque 1 M
    c. 10 mM EDTA – Estoque 0,5 M
    d. 300 µM espermidina – Estoque 0,1 M
    e. 200 µg/mL Proteinase K
    f. Completar com água destilada

02. Tampão B (armazenar em 4°C)
    a. 200 mM Tris, pH 8
    b. 30 mM EDTA
    c. 2% SDS – Estoque 10%
    d. 200 µg/mL Proteinase K

e. Completar com água destilada

03.RNAse A
04.Etanol 100%
05.Etanol 70%
06.Acetato de sódio 3 M
07.Fenol, pH 8
08.Clorofórmio
OBS: Preparar o tampão A e B sem a proteinase K, deixar para pipetar a enzima direto na amostra para não correr o risco dela perder sua atividade.

Métodos

OBS: Antes de iniciar o ensaio, verificar se TODOS os reagentes estão preparados. Só iniciar o ensaio quando todos os tampões e reagentes estiverem preparados, para que não haja problemas durante o ensaio, evitando que reagentes, amostras e tempo sejam desperdiçados
O protocolo possui três etapas: Preparação da amostra, extração com Fenol-clorofórmio e precipitação

Etapa 1: Preparação da amostra

01. Coletar 30 abdomens de moscas
02. Adicionar à amostra 250 µL do Tampão A (adicionar Proteinase K - 2,5 µL)
03. Macerar a amostra utilizando o pistilo no cooler (-20°C)
04. Adicionar 250 µL do Tampão B (adicionar Proteinase K - 2,5 µL)
05. Colocar no gelo/rack termostável por 5 min
06. Incubar à 45 min à 65°C
07. Resfriar à amostra em gelo por 5 min
08. Adicionar RNAse A (10 µL)
09. Incubar por 30 min à 37°C (Se possível, utilizar dois banhos, caso não haja disponibilidade, esperar o banho chegar à 37°C)
10. Centrifugar à 8000 rpm por 4 min a 4°C
11. Transferir o sobrenadante para um tubo novo. Tomar cuidado para não transferir sujeira. (Prestar atenção no volume transferido)

Etapa 2: Extração com Fenol-clorofórmio

12. Adicionar à amostra o mesmo volume de fenol-clorofórmio (fenol-clorofórmio 1:1). Ex: Amostra 300 µL; Fenol 150 µL; Clorofórmio 150 µL
13. Inverter a amostra gentilmente para misturar
14. Centrifugar por 8 min à 12000 rpm a 4°C (Centrifugar mais se necessário)
15. A amostra ficará bifásica. Transferir o sobrenadante para um tubo novo. Tomar cuidado para não transferir sujeira. ANOTAR o volume transferido.
16. Adicionar o mesmo volume de clorofórmio e inverter
17. Centrifugar por 8 min à 12000 rpm a 4°C
18. Na capela de fluxo, transferir o sobrenadante (DNA) para um tubo novo. Tomar cuidado para não transferir sujeira (RNA). ANOTAR o volume transferido.

Etapa 3: Precipitação com Etanol

19. Adicionar 0,1 do volume ANOTADO de acetato de sódio
20. Adicionar 2,5 do volume ANOTADO de Etanol 100%
21. Inverter gentilmente e colocar no freezer (-20°C) e deixar overnight (ou cerca de 2h no *SpeedVac*)
22. Centrifugar por 22 min à 4°C à 12000 rpm.
23. Descartar quase todo sobrenadante, tomando cuidado para não descartar o pellet. Dica: sempre colocar a ponteira contra o pitoco do tubo, evitando tocar no pellet
24. Adicionar 700 µL de etanol 70%
25. Centrifugar por 12 min à 4°C à 12000 rpm. Ao final, será possível observar um pellet.
26. Retirar todo o sobrenadante, se necessário, dar um spin e retirar o restante.
27. Deixar secando overnight (quanto mais seco, melhor para diluir)
28. Ressuspender em 20 µL de água autoclavada
29. Dar um spin na amostra novamente e transferir para outro microtubo
30. Quantificar

Appendix II

PROTOCOLO PARA PREPARAÇÃO DE CÉREBROS DE DROSOPHILA PARA IMUNOFLUORESCÊNCIA

Materiais

- PBS 1X
- Cerca de 5 cérebros de Drosophila dissecados em PBS 1X gelado (colocar a placa de dissecação acima do gelo artificial);
- Pinças de dissecação finas;
- PBST (0,5% Triton X-100)
- PBST + BSA 2%
- Eppendorfs de fundo cônico
- Agitador de kline (que caiba na geladeira)

Método

1. Disseque os cérebros e os coloque em um eppendorf com cerca de 400 µL de paraformaldeído (PFA) a 4%. Use as pinças de dissecação (com cuidado para não danificá-los) um uma pipeta com a ponteira cortada. Conte 30 minutos.

2. Após 30 minutos de incubação, tire apenas o PFA com uma pipeta e adicione 400 µL de PBST (0,5% Triton X-100). Após 15 minutos, retire o PBST e adicione 400 µL de PBST novos. Faça isso por 6 vezes, num total de 1,5 horas. Para não sugar os cérebros nesta e nas etapas seguintes, sempre retire o material colocando o eppendorf contra a luz.

3. Retire o excesso de PBST e adicione 400 µL de PBST + BSA 2%. Deixe o eppendorf em pé no agitador a 4 °C overnight.

4. Lave o excesso de PBST + BSA 2% com a solução de PBST por 6 vezes e adicione o anticorpo primário na diluição desejada. É importante que o anticorpo esteja suspenso também em PBST + BSA 2%. Deixe o eppendorf em pé no agitador a 4 °C overnight.

5. Lave o excesso de anticorpo primário com a solução de PBST por 6 vezes e adicione o anticorpo secundário fluorescente na diluição desejada. É importante que o anticorpo esteja suspenso também em PBST + BSA 2%. Deixe o eppendorf em pé no agitador a 4 °C overnight.

6. Lave o excesso de anticorpo secundário fluorescente com a solução de PBST por 6 vezes.

7. Com uma pipeta de ponteira cortada, transfira os cérebros para a área central de uma lâmina de microscopia limpa. Seque o excesso de PBST ao redor dos cérebros, com cuidado para não removê-los ou deixar fiapos de papel na lâmina. Posicione-os como preferir enquanto estão na gota de PBST, depois de secos eles não podem ser movidos.

8. Coloque uma gota de Fluoroshield with DAPI nos cérebros. Com cuidado e com a ajuda de uma pinça, deixe cair lentamente uma lamínula limpa em cima dos cérebros. Colocar a lamínula lentamente e de forma ondulatória evita a formação de bolhas. Não mexa depois de colocada.

9. Com cuidado para não mexer a lamínula e sem excessos para facilitar a visualização, passe esmalte incolor ao redor da lamínula, selando-a para impedir que o tecido seque. Identifique a lâmina e a deixe o esmalte secando por algumas horas no escuro. Envolva a lâmina com papel alumínio e armazene a 4 °C.

Appendix III

1. Cálculo de DL50 e BMD10% para Acetato

Tabela 1:Mortalidade das moscas expostas ao acetato.

Dose	Réplicas	N total	D1	D2	D3	D4	D5	D6	D7	Mortes totais	Vivos	Mortalidade (%)
0,0000 g/L	3	95	0	3	0	1	0	0	2	0	95	0,00
0,4650 g/L	3	63	0	0	0	0	0	0	0	0	63	0,00
4,6500 g/L	3	63	0	0	0	0	0	1	0	0	63	0,00
9,3000 g/L	3	90	0	5	0	2	0	0	0	1	89	1,11
31,0000 g/L	3	90	0	4	0	2	0	0	0	0	90	0,00
46,5000 g/L	3	60	0	0	0	1	1	0	0	0	60	0,00
232,5000 g/L	3	60	0	0	0	0	0	60	0	60	0	100,00
465,0000 g/L	3	60	38	17	0	0	0	5	0	60	0	100,00

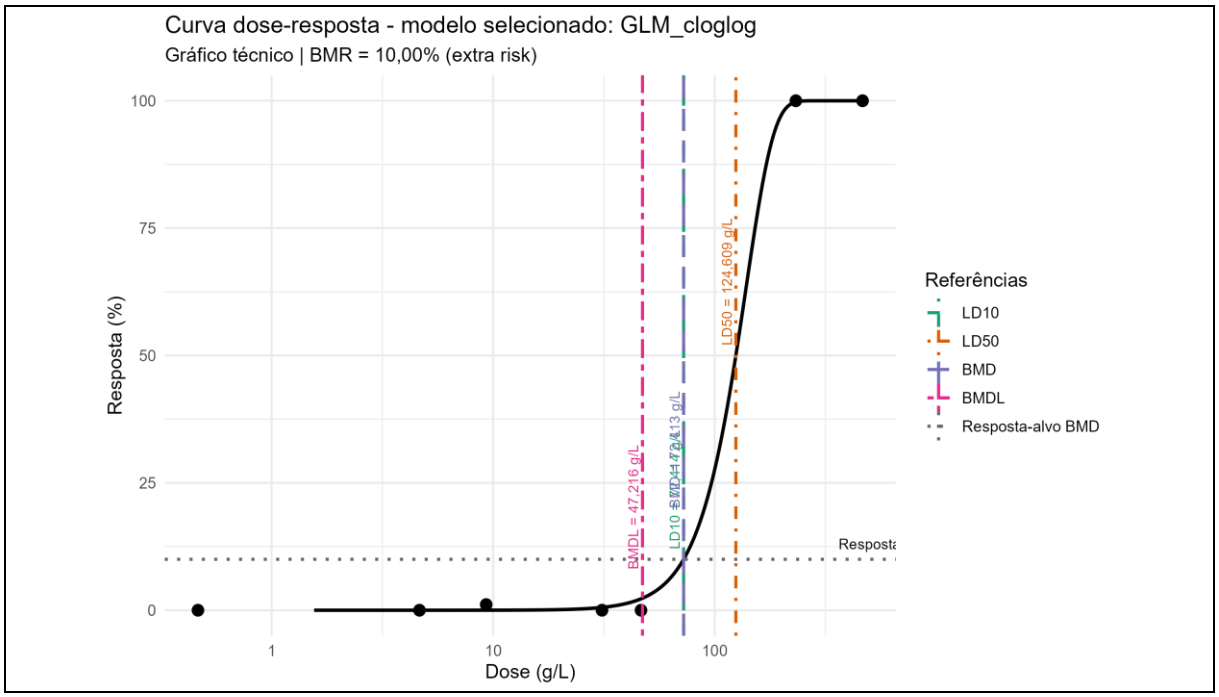

Figura 1: Curva de dose–resposta do acetato com determinação da BMD (Benchmark Dose), definida como a dose associada a uma alteração pré-especificada na resposta em relação ao controle.

O modelo selecionado pelo menor AIC foi GLM_cloglog. A LD10 estimada foi 72,4135 g/L (IC95%: 49,7202 a 93,4832 g/L). A LD50 estimada foi 124,6091 g/L (IC95%: 98,0061 a 153,8459 g/L). Para BMR = 10,00% (extra risk), a resposta basal prevista pelo modelo foi

0,00% e a resposta-alvo correspondente foi 10,00%. A dose estimada para atingir esse efeito foi BMD = 72,4135 g/L e o limite inferior unilateral de 95% foi BMDL = 47,2163 g/L.

2. Cálculo de DL50 e BMD10% para Lactato

Tabela 1: Mortalidade de moscas expostas ao lactato.

Dose	Réplicas	N total	D1	D2	D3	D4	D5	D6	D7	Mortes totais	Vivos	Mortalidade (%)
0,0000 g/L	3	95	0	3	0	1	0	0	2	0	95	0,00
0,7710 g/L	3	88	0	0	2	1	0	0	0	0	88	0,00
7,7100 g/L	3	90	0	2	0	0	0	4	0	0	90	0,00
77,1000 g/L	3	90	0	2	0	3	0	0	2	1	89	1,11
771,0000 g/L	3	90	0	9	0	3	0	0	2	9	81	10,00

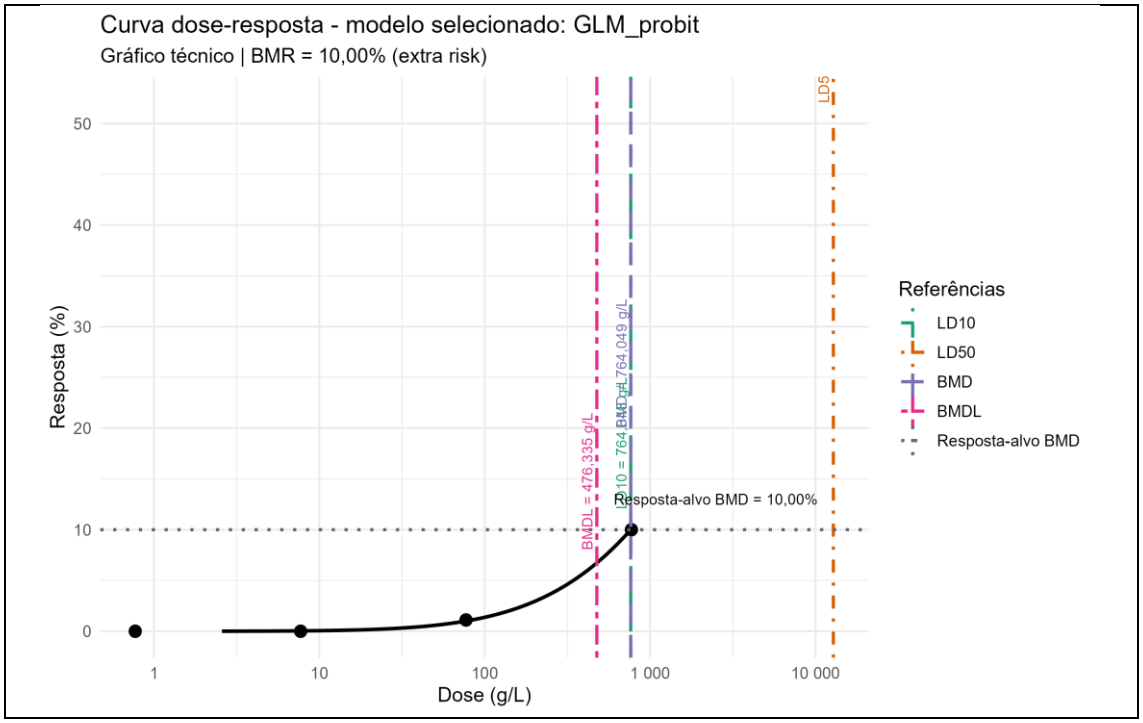

Figura 2: Curva de dose–resposta do lactato com determinação da BMD (Benchmark Dose), definida como a dose associada a uma alteração pré-especificada na resposta em relação ao controle.

O modelo selecionado pelo menor AIC foi GLM_probit. A LD10 estimada foi 764,0484 g/L (IC95%: 353,3399 a 4157,8542 g/L). A LD50 estimada foi 12823,6257 g/L (IC95%:

2857,8935 a 17494411,8662 g/L). Para BMR = 10,00% (extra risk), a resposta basal prevista pelo modelo foi 0,00% e a resposta-alvo correspondente foi 10,00%. A dose estimada para atingir esse efeito foi BMD = 764,0486 g/L e o limite inferior unilateral de 95% foi BMDL = 476,3346 g/L.