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Gut Microbiota Dynamics and Phenotypic Changes Induced by Tetracycline in *Drosophila melanogaster*

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ABSTRACT

The gut microbiota plays a crucial role in both physiological and pathological processes in humans and animals. Antibiotics, designed to combat bacterial infections, can induce alterations in the composition and abundance of the gut microbiota over prolonged exposure. This study addresses the limited understanding of the connections between gut microbiota and phenotypic profiles of metazoan species. We investigated the impact of early-life exposure to tetracycline in wild-type *D. melanogaster*, which were fed a standard diet, comparing them to a control group not exposed to tetracycline. The primary objective was to examine the consequences of early-life tetracycline exposure on gut microbiota and its implications for phenotypic profiles, including survival, locomotor activity, and reproduction in adult flies. Results revealed a significant reduction in lactic acid bacteria in adult flies exposed to tetracycline. However, tetracycline exhibited no interference with fly development, allowing them to maintain a normal lifespan. In adult flies, tetracycline significantly decreased the lifespan on day 35 at a concentration of 10 µg/mL and reduced locomotion on day 27 at concentrations of 0.1 µg/mL and 10 µg/mL. Remarkably, tetracycline did not impact the reproductive capabilities of the flies. This study demonstrates that while tetracycline led to a decline in lactic acid bacteria, locomotion, and lifespan in adult flies, it did not disrupt their development or reproductive processes.

Keywords: Tetracycline, gut microbiota, phenotypic profile, *Drosophila melanogaster*.

INTRODUCTION

The gut microbiota constitutes a complex assembly of microorganisms that reside within the digestive tracts of both humans and animals (Quigley, 2013). This microbial community plays a pivotal role in preserving intestinal physiology and health and actively protects the host against pathogens through the synthesis of diverse antimicrobial substances (Mills *et al*., 2019). Furthermore, it engages in multiple digestive and metabolic pathways (Rothschild *et al.*, 2018) while concurrently influencing the growth and differentiation of enterocytes (Zhang *et al*., 2015). The gut microbiota also transforms host-derived components such as bile acids and nutrients into various metabolites that affect the host's intestinal environment and immunological balance (Tafesh-Edwards & Eleftherianos, 2023). The composition of the gut microbiota changes during early development, starting at the moment of host birth. Factors influencing the gut microbiota composition have long-term implications for health and disease risk as individuals transition into adulthood (Nicholson *et al*., 2012).

One factor that disrupts the equilibrium of the gut microbiota is exposure to antibiotics (Lu & Lu, 2019; Sun *et al*., 2018), which is associated with various human disorders, including inflammatory bowel disease, obesity, cardiovascular disease, and neurological disorders (Gebrayel *et al.*, 2022). Numerous studies have underscored the significance of different bacterial types within the host body, contributing to various aspects, such as development (Buchon *et al*., 2009; Storelli *et al*., 2011) and lifespan (Gilbert *et al*., 2018; Sommer & Backhed, 2013). However, establishing the causative role of the gut microbiota in phenotypic profiles presents challenges. Additionally, discerning the relationship between changes in the gut microbiota composition and phenotypic

profiles, such as survival, locomotion, and reproduction, is inherently intricate.

To date, studies investigating the relationship between gut microbiota and phenotypic profiles have predominantly focused on invertebrate model organisms (Clark & Walker, 2018; Kinross, *et al.* 2011; Vuong *et al*., 2017). In *Metaphire guillemi*, alterations in several bacterial types, such as *Proteobacteria* (*Pseudomonadota*) and *Firmicutes* (*Bacillota*), have been observed (Chao *et al*., 2020). Furthermore, in *D melanogaster*, an elevation in the *Firmicutes/Bacteroidetes* ratio has been noted, exhibiting obesogenic effects (Yu *et al*., 2020). An increased population of *Gammaproteobacteria* in the gut microbiota of *D. melanogaster* has been associated with an increased production of proinflammatory cytokines and a shorter lifespan (Clark *et al*., 2015). Additionally, a change in wing shape has been observed in *D. nigrosparsa*, but it does not affect the movement of larvae or adult flies (Weiland *et al.*, 2022). Some of these studies used antibiotics to investigate their effects on gut microbiota composition.

Currently, the use of antibiotics continues to escalate (Klein *et al*., 2018; Kummerer, 2009). Antibiotics, which are used to eradicate or impede the growth of microorganisms in humans and animals, are commonly administered orally and can potentially influence the gut microbiota (de Vries *et al*., 2011; Sapkota *et al*., 2008). This practice extends beyond the adult population to include children (Vaz *et al*., 2014; WHO, 2018). Children typically receive antibiotics when they become unwell, and the long-term consequences of antibiotic exposure in early life remain unclear.

In this study, *D. melanogaster* was chosen as the model organism because of its similarities in gut microbiota composition with mammals. The mammalian digestive tract is comprised of four main phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* (Vajro *et al*., 2013). *D. melanogaster* is dominated by *Proteobacteria* and *Firmicutes* (Lu & Lu, 2019), making it a suitable platform for investigating gut homeostasis and disease development (Charroux & Royet, 2012). Various studies have employed *D. melanogaster* as a model organism to understand the development of the gut microbiota (Guo *et al*., 2023; Yu *et al*., 2020). While existing studies strongly affirm the substantial role of the gut microbiota in survival, there is a lack of research exploring the impact of antibiotic administration during the early life stages on phenotypic profiles (such as survival, locomotor abilities, and reproduction) in genetically unaltered individuals receiving a standard diet. This study reports that exposure to the antibiotic tetracycline during the early life stages of *D. melanogaster* disrupts its phenotypic profile later in life, which is most likely associated with alterations in the gut microbiota.

MATERIALS AND METHODS

Chemicals

The tetracycline utilized in this study was in the form of tetracycline hydrochloride (TCH, CAS RN: 64-75-5, $C_{22}H_{24}N_2O_8$·HCl, purity 98.03%), procured from PhytoTech Labs Inc (USA). The stock solution was prepared by changing the medium throughout the experiment. The details of tetracycline hydrochloride, including its CAS RN and purity, were obtained from a previous study (Guo *et al*., 2023).

Preparation of Model Organism

D. melanogaster wild-type (Oregon R, OR) was acquired from the Laboratory of Host Defense and Response at Kanazawa University, Japan and has been consistently maintained at the Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, Hasanuddin University, Indonesia. The flies were cultured on solid media containing sugar, agar powder, corn flour, yeast, and propionic acid dissolved in distilled water. The cultivation conditions involved maintaining the flies at 25°C with a light-dark cycle of 12 hours each (Liling *et al.*, 2021; Rumata *et al*., 2023). Temporary anesthesia through CO_2 exposure was employed for sex identification, transfer, and random grouping of flies, as outlined in the study by Guo *et al*. (2023).

Preparation of Growth Media Containing TCH

The stock solution was prepared at a concentration of 1000 µg/mL (Guo *et al*., 2023). A stock solution was freshly prepared for each change in the medium. The TCH stock solution was diluted in sterile distilled water to prepare a series of working solutions at concentrations of 10 µg/mL and 0.1 µg/mL. Subsequently, 50 µL of the stock solution and working solution were mixed into 5 mL of growth media (5 replicate culture tubes per treatment group).

Survival Analysis

Survival analysis was used to quantify the lifespan of *D. melanogaster*, which served as a parameter to elucidate the lifecycle dynamics of flies. This analytical approach enables the identification of factors that influence the

deceleration or extension of fly lifespan (Landis *et al.*, 2020). The experimental design comprised four groups, including three groups with treated with TCH concentrations of (0.001 µg/mL, 0.1 µg/mL, and 10 µg/mL) and one control group without TCH. Each vial contained 10 second-instar larvae. TCH administration commenced at the larval stage and continued seamlessly throughout the life cycle of the flies. Every 2-3 days, the flies were transferred to a new medium and daily observations were conducted, including the enumeration of deceased flies (Guo *et al*., 2023).

Negative Geotaxis Analysis

Negative geotaxis analysis was employed to evaluate movement abnormalities in flies. The outcome of a negative geotaxis test, indicating deficient or impaired behavior in response to gravity, can provide insights into specific conditions or issues in the fly population under examination (Ali *et al*., 2011). Four groups, including three groups with TCH concentrations of (0.001 µg/mL, 0.1 µg/mL, and 10 µg/mL) and one control group without TCH were examined in these studies. The flies were then transferred to empty vials and sterilized with 70% alcohol. The flies were allowed a 1-minute adaptation period before the test commenced. The procedural steps involved a rapid tap of the vial containing the flies on the table, followed by a 10-second interval during which the number of flies crossing the 8 cm mark was counted. A resting period of 1 min was introduced between each trial to ensure consistency and accuracy of the assessment (Ali *et al*., 2011; Jaya *et al.*, 2021; Syamsidi *et al.*, 2023).

Reproduction Analysis

Reproductive analysis serves as an assay designed to investigate morphological changes and discern the conditions influencing the reproductive output of flies (Boulétreau-Merle *et al.*, 1982; Markow, 2015). This analytical approach involved the introduction of three female and three male flies into vials containing the treatment media. The vials were organized into three groups with tetracycline hydrochloride (TCH) concentrations of 0.001 µg/mL, 0.1 µg/mL, and 10 µg/mL, respectively, along with a control group without TCH. Subsequently, the flies were allowed to mate for a duration of five days, after which all flies were removed from the vials. Quantification was performed for the number of pupae and flies in each experimental group was quantified. It is essential to note that virgin female flies were used in this test, and the maintenance of virginity in female flies was considered viable within–5-7 hours of emergence from the pupal stage (Ashburner & Roote, 2007). This approach facilitated a focused examination of the reproductive aspects under the influence of varying TCH concentrations.

Quantitative analysis of the bacteria

Colony-forming unit (CFU) analysis is a crucial method for quantifying viable bacterial populations in the fly gut (Lee *et al.*, 2019). This analytical approach is instrumental in elucidating dynamic interactions between flies and bacteria (Lee *et al*., 2019; Leftwich *et al*., 2017; Nikolopoulos *et al*., 2023; Pais *et al*., 2018). The determination of CFU (Figure 1) involved the utilization of five flies in each treatment groups, namely TCH concentration of (0.001 µg/mL, 0.1 µg/mL, and 10 µg/mL) and control group without TCH. Subsequently, the flies were sterilized with 70% ethanol for 20-30 seconds to mitigate potential contamination from the fly surface. Following sterilization, the flies were rinsed three times with sterile water and subsequently homogenized in phosphate-buffered saline (PBS). The resulting homogenate was appropriately diluted and plated on de Mann-Rogosa-Sharpe agar (MRSA) medium for 24 h to facilitate the quantification of colony-forming units (Siva-Jothy *et al*., 2018). This approach allows the assessment of bacterial populations within the fly gut and provides valuable insights into the complex interplay between flies and their associated bacteria.

Data Analysis

For the analysis of survival data, the log-rank test was used to assess statistical significance, and the resultant data were graphically represented using a Kaplan-Meier plot. Furthermore, one-way Analysis of Variance (ANOVA) was utilized not only in the survival analysis, but also in other investigations, including reproduction and colony-forming units (CFU). For the analysis of negative geotaxis, a Two-way ANOVA was applied. Following the ANOVA tests, a post hoc analysis was conducted using the Tukey honest significant difference (HSD) test. This post-hoc test allowed for the identification of significant differences between groups. The results of these statistical analyses are visually presented using bar graphs. All statistical analyses were performed using GraphPad Prism® 9.

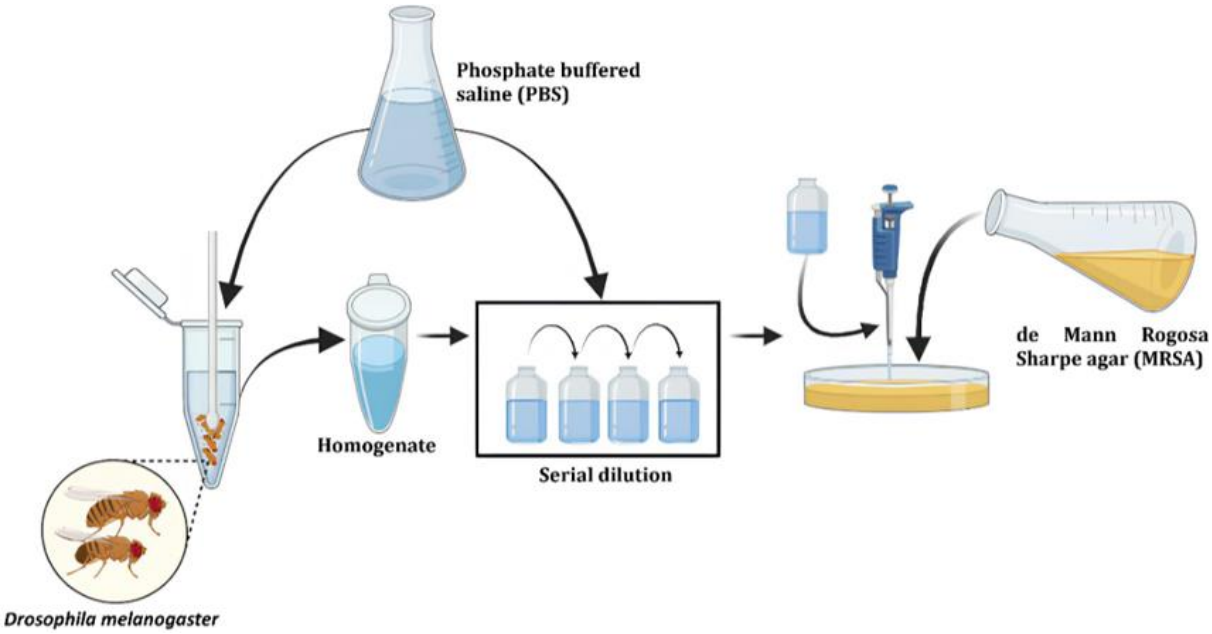

Figure 1. The pour plate method employed for colony-forming unit (CFU) analysis in Drosophila melanogaster.

RESULTS AND DISCUSSION

Continuous exposure to tetracycline hydrochloride (TCH) did not affect the developmental stages of *Drosophila melanogaster*.

To investigate the effect of TCH on the developmental stages of *Drosophila melanogaster*, second instar larvae were exposed to TCH. The objective of this study was to assess whether TCH influences the progression of larvae into pupae and ultimately into adult flies. This experimental design aimed to provide insight into the potential effects of TCH on the developmental processes of *D. melanogaster* throughout its lifecycle. Exposure of second-instar larvae to tetracycline hydrochloride (TCH) had no discernible effect on the developmental process, as evidenced by the normal progression of larvae into pupae and pupae into flies. Developmental stages proceeded without any observable abnormalities or toxic effects (Figure 2A). Quantitative analysis (Figures 2B and 2C) further supported these observations, revealing no statistically significant differences between the control group, which was not exposed to TCH, and the experimental groups subjected to TCH at concentrations of 0.001 µg/mL, 0.1 µg/mL, and 10 µg/mL. This consistency across groups suggests that TCH, at the tested concentrations, did not induce notable alterations in the developmental trajectory of *D. melanogaster* from larvae to pupae, and subsequently into adult flies. These results confirmed the absence of developmental toxicity following TCH exposure in this experimental context.

Adverse effects of prolonged TCH exposure on adult Drosophila lifespan

The initial observations indicated that the normal development of *D. melanogaster* larvae into pupae and adult flies was unaffected by TCH. Subsequent experiments were conducted to further explore the potential long-term effects of TCH on the lifespan of *D. melanogaster*. The adult flies that emerged from larvae previously treated with TCH were subjected to prolonged exposure to antibiotics. The objective of this study was to test the hypothesis that TCH might influence the entire lifespan of *D. melanogaster*, even after completion of the developmental stages. This study assessed the cumulative and delayed effects of TCH on the overall longevity of adult flies. Comprehensive analysis of the effects of TCH exposure on the lifespan of *D. melanogaster* revealed a clear concentration-dependent effect. The continuous exposure to TCH early in the life of adult flies resulted in a reduction in the lifespan (Figure 3A).

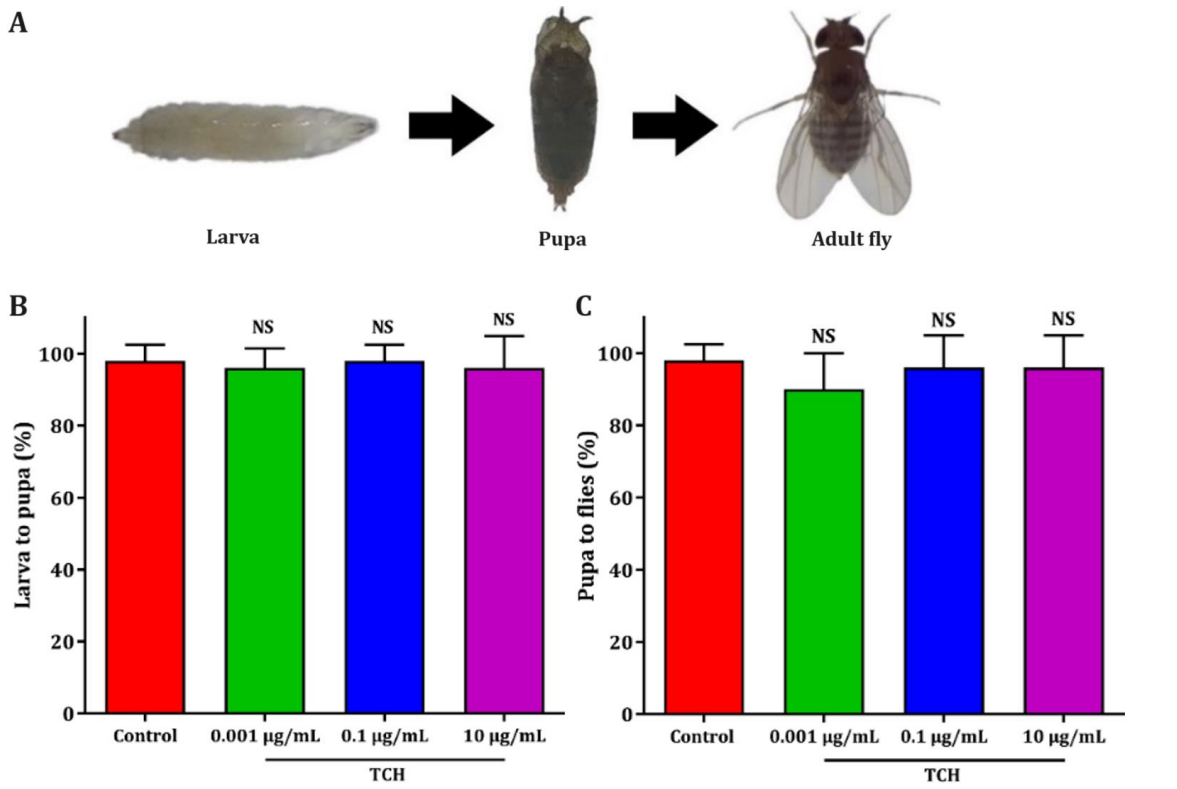

Figure 2. Prolonged exposure of *Drosophila melanogaster* to tetracycline hydrochloride (TCH) did not induce any discernible effects on its developmental stages. Larval transformation into adult flies (A), larval development into pupae (B), and pupal development into adult flies (C). NS: Not significant.

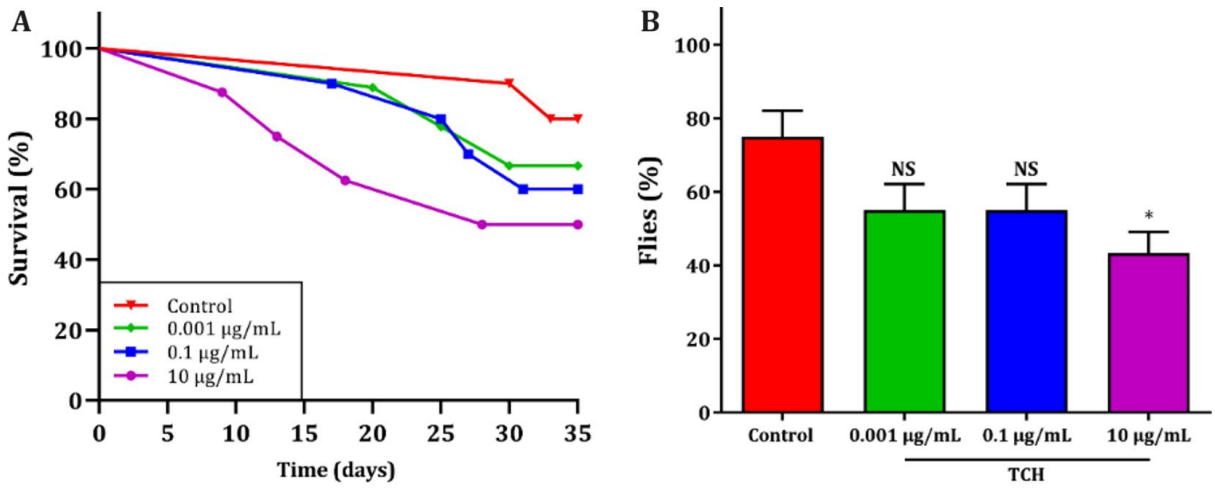

Figure 3. The administration of tetracycline hydrochloride (TCH) throughout the entire life cycle of *Drosophila melanogaster*, from larvae to adult flies, resulted in a notable decrease in the lifespan of the flies. Overall lifespan analysis (A) and selective analysis on day 35 (B) post TCH administration demonstrated reduced fly lifespan compared to the untreated control. NS: Not significant; *: $p<0.05$.

Flies exposed to higher concentrations of TCH exhibited a more pronounced decrease in lifespan than those exposed to lower concentrations or those not exposed to TCH. To statistically validate these observations, one-way ANOVA was conducted on day 35. The results indicated a significant reduction in the lifespan of flies exposed to a concentration of 10 µg/mL (Figure 3B). This concentration-dependent effect of TCH aligns with that reported in previous studies, emphasizing the critical role of antibiotic concentration in influencing the lifespan of *D. melanogaster* (Adembri *et al*., 2020; Osthoff *et al*., 2016). This pattern is consistent with the findings of previous studies on other antibiotics. Gökçe (2020) demonstrated that the effect of penicillin antibiotics on survival and development varied based on the administered concentration, causing a decrease in survival and significantly slowing down the developmental process at high concentrations (Üstündağ *et al*., 2020). Similarly, the administration of high concentrations of ciprofloxacin resulted in a decrease in fly survival and development (Liu *et al*., 2019). These cumulative findings underscore the concentration-dependent nature of TCH antibiotics and their consequential impact on the lifespan of *D. melanogaster*.

TCH affects cellular metabolic processes, such as oxidative phosphorylation and Krebs cycle activity, likely compensated by the increased activity of transaminase enzymes. This decrease in oxidative phosphorylation correlates with mitochondrial damage, which affects cellular function and health. Oxidative phosphorylation is the final stage of the cellular respiration pathway, which produces energy in the form of ATP within the mitochondria. Krebs cycle activity is involved in the breakdown of fatty acids, proteins, and carbohydrates into energy. In addition, TCH causes changes in the fly microbiome (Renault *et al*., 2018).

Disruption of gut microbiota growth in adult Drosophila resulting from extended exposure to tetracycline

To explore the possible relationship between reduced fly survival and changes in the gut microbiota, a Colony Forming Unit (CFU) assay was performed on adult flies from each experimental group on day 35. CFU analysis, a method for quantifying viable bacteria, was performed based on a previous study (Lee *et al*., 2019) to assess the effect of prolonged tetracycline exposure on the gut microbiota of adult Drosophila. The results revealed a significant reduction in the number of lactic acid bacteria at all tetracycline concentrations 0.001 µg/mL, 0.1 µg/mL, and 10 µg/mL) compared to the control group (Figure 4). This decrease in the abundance of lactic acid bacteria suggests a potential link between the observed decline in fly lifespan and disturbances in the gut microbiota. Similar findings have been reported in previous studies, associating reduced microbiota with decreased lifespan in Drosophila (Clark *et al*., 2015; Clark & Walker, 2018). However, the number of bacteria in all groups of flies exposed to TCH at concentrations of 0.001 µg/mL, 0.1 µg/mL, and 10 µg/mL showed similar results. This phenomenon arises because of the 100-fold difference in the concentration of TCH, which results in the same activity of reducing the number of bacteria. The effectiveness of TCH depends on its concentration; the higher the concentration, the greater the inhibition of bacterial growth (Ahmad *et al*., 2015).

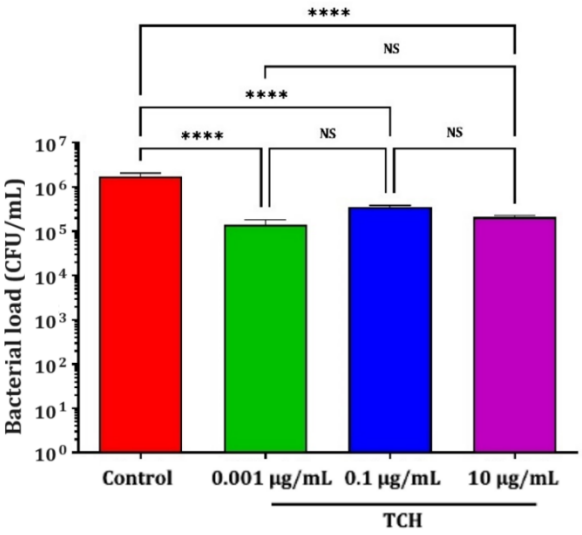

Figure 4. The Colony Forming Unit assay in flies using de Mann Rogosa Sharpe Agar medium. A substantial decrease in the abundance of lactic acid bacteria was evident across all TCH concentrations in comparison to the control group without TCH. However, there was no difference between all TCH concentration of 0.001 µg/mL, 0.1 µg/mL, and 10 µg/mL. NS: Not significant; ****: $p<0.0001$.

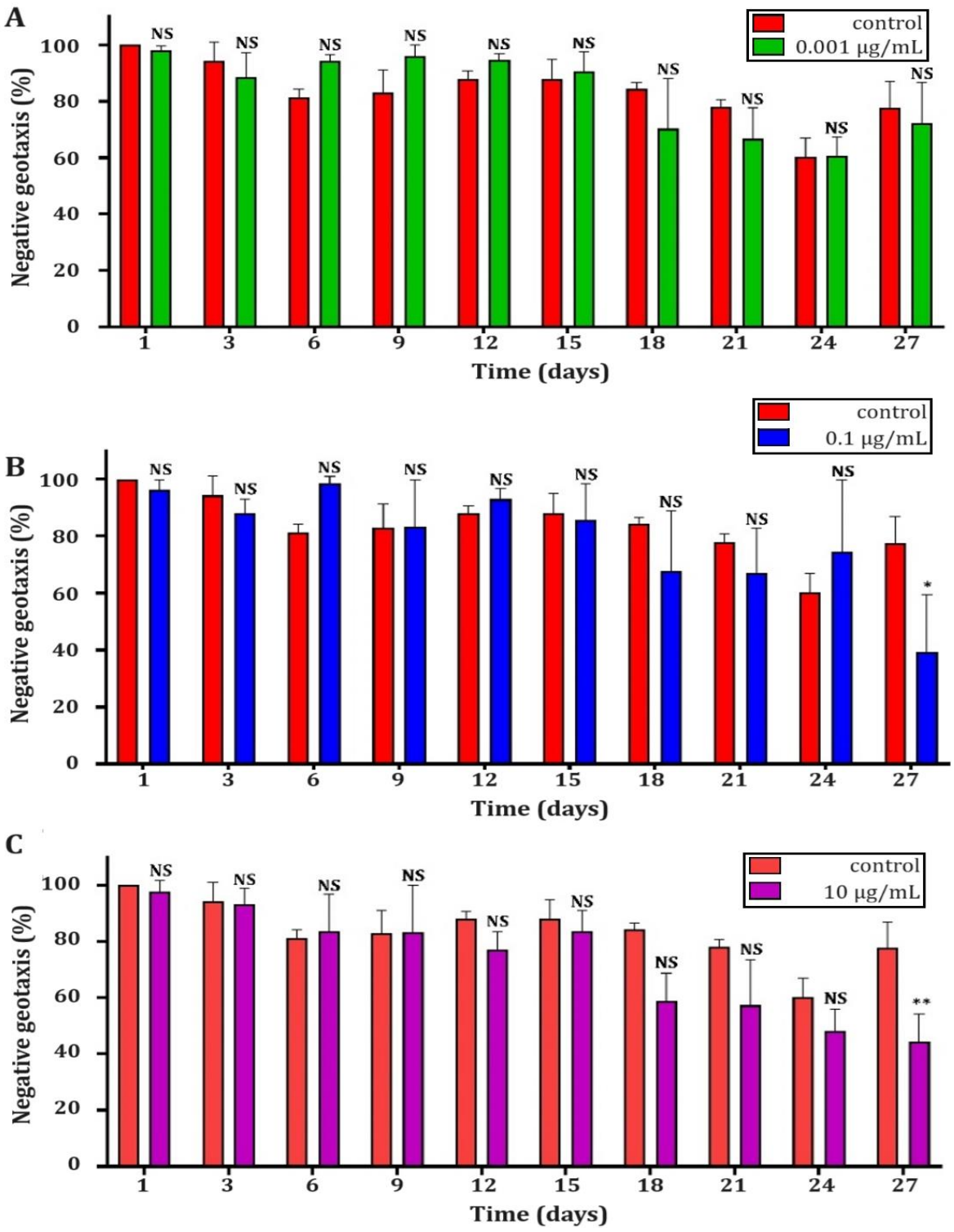

Figure 5. Impaired Drosophila locomotor upon tetracycline hydrochloride (TCH) treatment. The administration of TCH at a concentration of 0.001 µg/mL had no impact on the locomotion of adult flies (A), whereas the administration of TCH at concentrations of 0.1 µg/mL and 10 µg/mL disrupted the locomotion of adult flies (B, C). NS: Not significant; *: $p<0.05$; **: $p<0.01$.

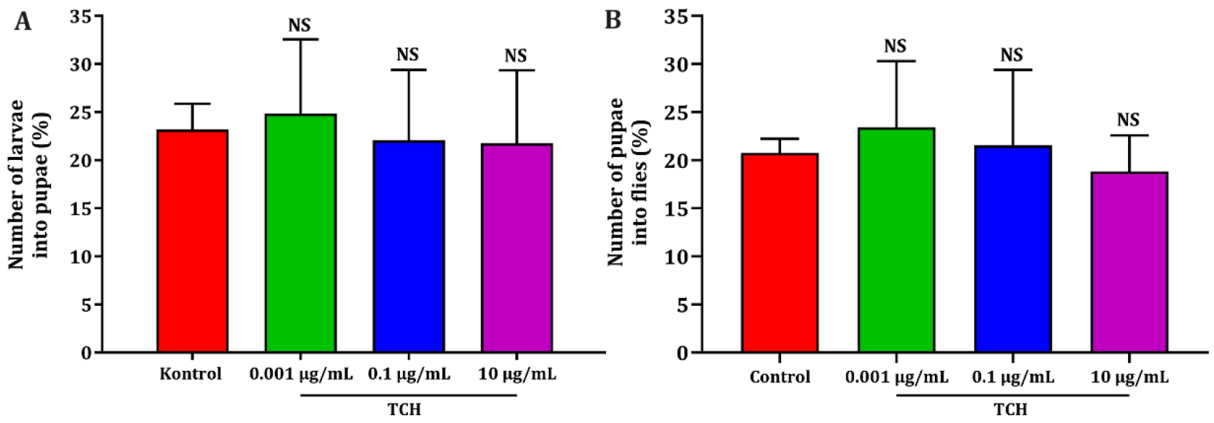

Figure 6. No significant difference in the number of pupae and flies between those exposed to tetracycline hydrochloride (TCH) and the control group not exposed to TCH, as shown by the number of larvae developing into pupae after five days of mating time (A) and the number of flies emerging from pupae (B). NS: No significant.

De Mann-Rogosa-Sharpe agar (MRSA), specifically designed for lactic acid bacteria, was used for this analysis. MRSA supports the growth of various lactic acid bacteria genera, including *Lactobacillus*, *Streptococcus*, *Pediococcus*, and *Leuconostoc*, which produce significant amounts of lactic acid (De Man, 1960; Nero *et al.*, 2006). This medium has been widely used to culture and quantify lactic acid bacteria in fly guts (Broderick *et al*., 2014; Pais *et al*., 2018).

Lactic acid bacteria are known for their ability to ferment carbohydrates, producing lactic acid. Their metabolic activities, including the breakdown of complex macromolecules and the generation of various products such as short-chain fatty acids, amines, bacteriocins, vitamins, and exopolysaccharides, have been well documented (Wang *et al*., 2021). The Drosophila gut microbiota includes species of the genus *Lactobacillus*, which are commonly found in mammals, including humans (Matos & Leulier, 2014).

Locomotor impairment of adult Drosophila due to prolonged exposure to TCH

We performed a negative geotaxis analysis to assess the impact of TCH exposure on the locomotor activity of adult Drosophila. Negative geotaxis analysis is a valuable tool for evaluating the behavioral responses of organisms and determining whether early life antibiotic exposure affects the activity level and mobility of flies (Ali *et al*., 2011). The results indicated that TCH administration at a concentration of 0.001 µg/mL did not significantly alter the locomotion of adult flies (Figure 5A). However, concentrations of 0.1 µg/mL and 10 µg/mL demonstrated a significant effect on day 27, with flies exhibiting impaired responses to gravity (Figures 5B and 5C). Notably, the influence of microbiota on the walking speed of Drosophila has been documented, with certain types of gut bacteria reported to reduce locomotor activity (Schretter *et al*., 2018).

Continuous exposure to TCH did not affect the reproductive activity of adult Drosophila.

Coordination of movement is essential not only for basic life activities but also for reproductive processes. Reproductive analyses were conducted to identify the conditions that might influence offspring count in flies (Boulétreau-Merle, 1982; Markow, 2015). Some studies have suggested that gut microbiota does not affect mating behavior in Drosophila (Jia *et al*., 2021; Leftwich *et al*., 2017; Selkrig *et al*., 2018). Consistent with previous research, the results of this study revealed no notable disparity in the number of pupae and adult flies between the groups exposed to TCH and the control group without TCH exposure. This analysis focused on a brief duration of TCH exposure limited to a five-day mating period.

Similar to the findings at the developmental stage, short exposure to TCH did not exhibit any reproductive effects. During its developmental stage, Drosophila have a restricted timeframe of approximately 10-12 days to transition into adult flies (Panchal & Tiwari, 2017). In contrast, the observed effects of decreased lifespan and

impaired locomotion manifested after a month of TCH exposure. Considering that Drosophila typically has a normal lifespan of approximately 90-100 days (Panchal & Tiwari, 2017), it is evident that the duration of TCH exposure plays a crucial role in influencing various aspects of Drosophila physiology.

Numerous studies have elucidated the intricate relationship between the gut microbiota and the health span in human populations (Claesson *et al*., 2012; Fan & Pedersen, 2021). However, this study uniquely delves into the ramifications of early life antibiotic administration on the gut microbiota and phenotypic profiles, including survival, locomotor function, and reproduction.

Tetracycline, a widely accessible antibiotic used for prophylaxis and infection management in humans and animals (di Cerbo *et al*., 2019), was the focus of this investigation. Despite well-established awareness of the potential side effects associated with tetracycline use, its prevalent administration in pediatric contexts persists (Enabulele *et al*., 2020). Hence, there is a compelling need for enhanced health education and widespread dissemination of information concerning tetracycline use, particularly when children are involved. The broad-spectrum activity of tetracycline extends beyond targeting Gram-positive and Gram-negative bacteria and encompasses a myriad of species, including those constituting the gut microbiota. Mechanistically, tetracyclines impede the attachment of aminoacyl-tRNAs to bacterial ribosomes, which is a pivotal step in cellular protein synthesis (Chopra & Roberts, 2001). Thus, tetracyclines can reduce the species diversity of gut microbiota, potentially promoting pathobiont overgrowth (Ramirez *et al*., 2020). Furthermore, tetracycline exposure can induce alterations in the gut bacterial metabolism, thereby influencing host immune responses and overall health (Keerthisinghe *et al*., 2019).

The use of *D. melanogaster* as a model organism in this study offers notable advantages, including a straightforward and cost-effective maintenance process and a genetic resemblance of approximately 75% to humans (Nainu *et al.*, 2019). Moreover, *D. melanogaster* possesses a gut microbiota similar to that of humans, notably featuring *Proteobacteria* and *Firmicutes* species (Cheng *et al*., 2018; Panchal & Tiwari, 2017; Tafesh-Edwards & Eleftherianos, 2023).

Given the outcomes of this research, our aim was to establish a foundation for exercising prudence in antibiotic use during early life and to stimulate further investigations that deepen our understanding of the intricate interactions among antibiotics, gut microbiota, and their consequential impact on health. These efforts are expected to mitigate the potential adverse effects of antibiotic administration.

In future research, it would be pertinent to explore whether the diminished survival observed when administering the antibiotic TCH exclusively to adult flies without larval-stage exposure yields comparable outcomes. This intriguing inquiry underscores the need for additional investigations into the potential influence of tetracycline administration during the larval stage on survival during the adult stage.

CONCLUSION

Exposure to tetracycline during early life results in impaired survival and movement in adult flies in a concentration-dependent manner. However, this exposure did not impair the fly development or reproduction at any concentration tested. There is a likelihood that the observed decrease in lactic acid bacteria in flies exposed to tetracycline may be associated with the impaired survival and locomotor functions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adembri, C., Novelli, A., & Nobili, S. (2020). Some Suggestions from PK/PD Principles to Contain Resistance in the Clinical Setting-Focus on ICU Patients and Gram-Negative Strains. *Antibiotics (Basel)*, *9*(10). https://doi.org/10.3390/antibiotics9100676

Ahmad, A., Zachariasen, C., Christiansen, L. E., Graesboll, K., Toft, N., Matthews, L., Damborg, P., Agerso, Y., Olsen, J. E., & Nielsen, S. S. (2015). Pharmacodynamic modelling of in vitro activity of tetracycline against a representative, naturally occurring population of porcine *Escherichia coli*. *Acta Vet Scand*, *57*, 79. https://doi.org/10.1186/s13028-015-0169-0

Ali, Y. O., Escala, W., Ruan, K., & Zhai, R. G. (2011). Assaying locomotor, learning, and memory deficits in Drosophila models of neurodegeneration. *J Vis Exp*(49). https://doi.org/10.3791/2504

Ashburner, M., & Roote, J. (2007). Maintenance of a Drosophila Laboratory: General Procedures. *Cold Spring Harbor Protocols*, *2007*(3), pdb.ip35. https://doi.org/10.1101/pdb.ip35

Boulétreau-Merle, J., Allemand, R., Cohet, Y., & David, J. R. (1982). Reproductive strategy in *Drosophila melanogaster*: Significance of a genetic divergence between temperate and tropical populations. *Oecologia 53*, *323–329*. https://doi.org/https://doi.org/10.1007/BF00389008

Broderick, N. A., Buchon, N., & Lemaitre, B. (2014). Microbiota-induced changes in *Drosophila melanogaster* host gene expression and gut morphology. *mBio*, *5*(3), e01117-01114. https://doi.org/10.1128/mBio.01117-14

Buchon, N., Broderick, N. A., Poidevin, M., Pradervand, S., & Lemaitre, B. (2009). Drosophila intestinal response to bacterial infection: activation of host defense and stem cell proliferation. *Cell Host Microbe*, *5*(2), 200-211. https://doi.org/10.1016/j.chom.2009.01.003

Chao, H., Sun, M., Ye, M., Zheng, X., & Hu, F. (2020). World within world: Intestinal bacteria combining physiological parameters to investigate the response of *Metaphire guillelmi* to tetracycline stress. *Environ Pollut*, *261*, 114174. https://doi.org/10.1016/j.envpol.2020.114174

Charroux, B., & Royet, J. (2012). Gut-microbiota interactions in non-mammals: what can we learn from Drosophila? *Semin Immunol*, *24*(1), 17-24. https://doi.org/10.1016/j.smim.2011.11.003

Cheng, L., Baonza, A., & Grifoni, D. (2018). Drosophila Models of Human Disease. *Biomed Res Int*, *2018*, 7214974. https://doi.org/10.1155/2018/7214974

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev*, *65*(2), 232-260 ; second page, table of contents. https://doi.org/10.1128/MMBR.65.2.232-260.2001

Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S., Harris, H. M., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G. F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace, M., Brennan, L., . . . O'Toole, P. W. (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature*, *488*(7410), 178-184. https://doi.org/10.1038/nature11319

Clark, R. I., Salazar, A., Yamada, R., Fitz-Gibbon, S., Morselli, M., Alcaraz, J., Rana, A., Rera, M., Pellegrini, M., Ja, W. W., & Walker, D. W. (2015). Distinct Shifts in Microbiota Composition during Drosophila Aging Impair Intestinal Function and Drive Mortality. *Cell Rep*, *12*(10), 1656-1667. https://doi.org/10.1016/j.celrep.2015.08.004

Clark, R. I., & Walker, D. W. (2018). Role of gut microbiota in aging-related health decline: insights from invertebrate models. *Cell Mol Life Sci*, *75*(1), 93-101. https://doi.org/10.1007/s00018-017-2671-1

de Man, J. C., Rogosa, M., & Sharpe, M. E. (1960). A Medium for the Cultivation of Lactobacilli. *Journal of Applied Bacteriology*, *23*, 130-135. https://doi.org/https://doi.org/10.1111/j.1365-2672.1960.tb00188.x

de Vries, L. E., Valles, Y., Agerso, Y., Vaishampayan, P. A., Garcia-Montaner, A., Kuehl, J. V., Christensen, H., Barlow, M., & Francino, M. P. (2011). The gut as reservoir of antibiotic resistance: microbial diversity of tetracycline resistance in mother and infant. *PLoS One*, *6*(6), e21644. https://doi.org/10.1371/journal.pone.0021644

di Cerbo, A., Pezzuto, F., Guidetti, G., Canello, S., & Corsi, L. (2019). Tetracyclines: Insights and

updates of their use in human and animal pathology and their potential toxicity. *The Open Biochemistry Journal*, *13*(1), 1-12. https://doi.org/10.2174/1874091x01913010001

Enabulele, J. E., Chukwumah, N. M., & Enabulele, O. (2020). Tetracycline use in children and knowledge of its oral implications among nursing mothers. *Pediatric Dental Journal*, *30*(3), 224-230. https://doi.org/10.1016/j.pdj.2020.08.003

Fan, Y., & Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol*, *19*(1), 55-71. https://doi.org/10.1038/s41579-020-0433-9

Gebrayel, P. N., C. Al Khodor, S. Bilinski, J. Caselli, E. Comelli, E. M. Egert, M. Giaroni, C. Karpinski, T. M. Loniewski, I. Mulak, A. Reygner, J. Samczuk, P. Serino, M. Sikora, M. Terranegra, A. Ufnal, M. Villeger, R. Pichon, C. Konturek, P. Edeas, M. (2022). Microbiota medicine: towards clinical revolution. *J Transl Med*, *20*(1), 111. https://doi.org/10.1186/s12967-022-03296-9

Gilbert, J. A., Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V., & Knight, R. (2018). Current understanding of the human microbiome. *Nat Med*, *24*(4), 392-400. https://doi.org/10.1038/nm.4517

Guo, X., Yu, Z., & Yin, D. (2023). Sex-dependent obesogenic effect of tetracycline on *Drosophila melanogaster* deteriorated by dysrhythmia. *J Environ Sci (China)*, *124*, 472-480. https://doi.org/10.1016/j.jes.2021.11.029

Jaya, A., Wahyudin, E., Djabir, Y. Y., Roska, T. P., Arfiansyah, R., Dirpan, A., & Nainu, F. (2021). Phenotypical Effect of Phosphodiesterase 5 (PDE5) Inhibitor on Behavioral Activities of Fruit Fly *Drosophila melanogaster*. *Biointerface Research in Applied Chemistry*, *12*(1), 222-229. https://doi.org/10.33263/briac121.222229

Jia, Y., Jin, S., Hu, K., Geng, L., Han, C., Kang, R., Pang, Y., Ling, E., Tan, E. K., Pan, Y., & Liu, W. (2021). Gut microbiome modulates Drosophila aggression through octopamine signaling. *Nat Commun*, *12*(1), 2698. https://doi.org/10.1038/s41467-021-23041-y

Keerthisinghe, T. P., Wang, M., Zhang, Y., Dong, W., & Fang, M. (2019). Low-dose tetracycline exposure alters gut bacterial metabolism and host-immune response: "Personalized" effect? *Environ Int*, *131*, 104989. https://doi.org/10.1016/j.envint.2019.104989

Kinross, J.M, Darzi, A.W., & Nicholson, J.K. (2011). Gut microbiome host interactions in health and disease. *Genome Medicine*. https://doi.org/https://doi.org/10.1186/gm228

Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S. A., Goossens, H., & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc Natl Acad Sci U S A*, *115*(15), E3463-E3470. https://doi.org/10.1073/pnas.1717295115

Kummerer, K. (2009). Antibiotics in the aquatic environment a review part I. *Chemosphere*, *75*(4), 417-434. https://doi.org/10.1016/j.chemosphere.2008.11.086

Landis, G. N., Doherty, D., & Tower, J. (2020). Analysis of *Drosophila melanogaster* Lifespan. In: Curran, S. (eds) Aging. *Methods in Molecular Biology*, *2144*. https://doi.org/https://doi.org/10.1007/978-1-0716-0592-9_4

Lee, H-Y., Lee, S-H., Lee, J-H., Lee, W-J., & Min, K-J. (2019). The role of commensal microbes in the lifespan of *Drosophila melanogaster*. *Aging*, *11(13)*. https://doi.org/10.18632/aging.102073

Leftwich, P. T., Clarke, N. V. E., Hutchings, M. I., & Chapman, T. (2017). Gut microbiomes and reproductive isolation in Drosophila. *Proc Natl Acad Sci U S A*, *114*(48), 12767-12772. https://doi.org/10.1073/pnas.1708345114

Liling, S., Puspita Roska, T., Arfiansyah, R., Maryam, F., & Nainu, F. (2021). Pharmacological Effect of *Muntingia calabura* Leaves on the Expression of *sod1* and *sod2* in Drosophila. *Biointerface Research in Applied Chemistry*, *11*(5), 12985-12992. https://doi.org/10.33263/briac115.12985 12992

Liu, J., Li, X., & Wang, X. (2019). Toxicological effects of ciprofloxacin exposure to *Drosophila melanogaster*. *Chemosphere*, *237*, 124542. https://doi.org/10.1016/j.chemosphere.2019.124542

Lu, X. M., & Lu, P. Z. (2019). Synergistic effects of key parameters on the fate of antibiotic resistance genes during swine manure composting. *Environ Pollut*, *252*(Pt B), 1277-1287. https://doi.org/10.1016/j.envpol.2019.06.073

Markow, T. A. (2015). Drosophila reproduction: Molecules meet morphology. *Proc Natl Acad Sci U S A*, *112*(27), 8168-8169. https://doi.org/10.1073/pnas.1510121112

Matos, R. C. & Leulier, F. (2014). *Lactobacilli* Host mutualism: learning on the fly. *Microb Cell Fact*. https://doi.org/https://doi.org/10.1186/1475-2859-13-S1-S6

Mills, S., Stanton, C., Lane, J. A., Smith, G. J., & Ross, R. P. (2019). Precision Nutrition and the Microbiome, Part I: Current State of the Science. *Nutrients*, *11*(4). https://doi.org/10.3390/nu11040923

Nainu, F., Nakanishi, Y., & Shiratsuchi, A. (2019). Fruit fly as a model organism in the study of human diseases and drug discovery. *J. Cent. Med. Educ. Sapporo Med. Univ*, *10*, 21-32. https://doi.org/10.15114/jcme.10.21

Nero, L. A., Beloti, V., DE Aguiar Ferreira Barros, M., Ortolani, M. B. T., Tamanini, R., & DE Melo Franco, B. D. G. (2006). Comparison of petrifilm aerobic count plates and de man rogosa sharpe agar for enumeration of lactic acid bacteria. *Journal of Rapid Methods & Automation in Microbiology*. https://doi.org/https://doi.org/10.1111/j.1745-4581.2006.00050.x

Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., & Pettersson, S. (2012). Host-gut microbiota metabolic interactions. *Science*, *336*(6086), 1262-1267. https://doi.org/10.1126/science.1223813

Nikolopoulos, N., Matos, R. C., Ravaud, S., Courtin, P., Akherraz, H., Palussiere, S., Gueguen-Chaignon, V., Salomon-Mallet, M., Guillot, A., Guerardel, Y., Chapot-Chartier, M. P., Grangeasse, C., & Leulier, F. (2023). Structure-function analysis of *Lactiplantibacillus plantarum* DltE reveals D-alanylated lipoteichoic acids as direct cues supporting Drosophila juvenile growth. *Elife*, *12*. https://doi.org/10.7554/eLife.84669

Osthoff, M., Siegemund, M., Balestra, G., Abdul-Aziz, M. H., & Roberts, J. A. (2016). Prolonged administration of beta lactam antibiotics - a comprehensive review and critical appraisal. *Swiss Med Wkly*, *146*, w14368. https://doi.org/10.4414/smw.2016.14368

Pais, I. S., Valente, R. S., Sporniak, M., & Teixeira, L. (2018). *Drosophila melanogaster* establishes a species-specific mutualistic interaction with stable gut colonizing bacteria. *PLoS Biol*, *16*(7), e2005710. https://doi.org/10.1371/journal.pbio.2005710

Panchal, K., & Tiwari, A. K. (2017). *Drosophila melanogaster* "a potential model organism" for identification of pharmacological properties of plants/plant-derived components. *Biomed Pharmacother*, *89*, 1331-1345. https://doi.org/10.1016/j.biopha.2017.03.001

Quigley, E. M. (2013). Gut Bacteria in Health and Disease. *Gastroenterology and Hepatology*.

Ramirez, J., Guarner, F., Bustos Fernandez, L., Maruy, A., Sdepanian, V. L., & Cohen, H. (2020). Antibiotics as Major Disruptors of Gut Microbiota. *Front Cell Infect Microbiol*, *10*, 572912. https://doi.org/10.3389/fcimb.2020.572912

Renault, D., Yousef, H., & Mohamed, A. A. (2018). The multilevel antibiotic-induced perturbations to biological systems: Early-life exposure induces long-lasting damages to muscle structure and mitochondrial metabolism in flies. *Environ Pollut*, *241*, 821-833. https://doi.org/10.1016/j.envpol.2018.06.011

Rothschild, D. W., O. Barkan, E. Kurilshikov, A. Korem, T. Zeevi, D. Costea, P. I. Godneva, A. Kalka, I. N. Bar, N. Shilo, S. Lador, D. Vila, A. V. Zmora, N. Pevsner-Fischer, M. Israeli, D. Kosower, N. Malka, G. Wolf, B. C. Avnit-Sagi, T. Lotan-Pompan, M. Weinberger, A. Halpern, Z. Carmi, S. Fu, J. Wijmenga, C. Zhernakova, A. Elinav, E. Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature*, *555*(7695), 210-215. https://doi.org/10.1038/nature25973

Rumata, N. R., Purwaningsih, D., Asbah, A., As'ad, M. F., Chadran, D., Emran, T. B., & Nainu, F. (2023). Phenotypical and molecular assessments on the pharmacological effects of curcumin in *Drosophila melanogaster*. *Narra J*, *3*(2). https://doi.org/10.52225/narra.v3i2.117

Sapkota, A., Sapkota, A. R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., & Lawrence, R. (2008). Aquaculture practices and potential human health risks: current knowledge and future priorities. *Environ Int*, *34*(8), 1215-1226. https://doi.org/10.1016/j.envint.2008.04.009

Schretter, C. E., Vielmetter, J., Bartos, I., Marka, Z., Marka, S., Argade, S., & Mazmanian, S. K. (2018). A gut microbial factor modulates locomotor behaviour in Drosophila. *Nature*, *563*(7731), 402-406. https://doi.org/10.1038/s41586-018-0634-9

Selkrig, J., Mohammad, F., Ng, S. H., Chua, J. Y., Tumkaya, T., Ho, J., Chiang, Y. N., Rieger, D., Pettersson, S., Helfrich-Forster, C., Yew, J. Y., & Claridge-Chang, A. (2018). The Drosophila microbiome has a limited influence on sleep, activity, and courtship behaviors. *Sci Rep*, *8*(1), 10646. https://doi.org/10.1038/s41598-018-28764-5

Siva-Jothy, J. A., Prakash, A., Vasanthakrishnan, R. B., Monteith, K. M., & Vale, P. F. (2018). Oral Bacterial Infection and Shedding in *Drosophila melanogaster*. *J Vis Exp*(135). https://doi.org/10.3791/57676

Sommer, F., & Backhed, F. (2013). The gut microbiota masters of host development and physiology. *Nat Rev Microbiol*, *11*(4), 227-238. https://doi.org/10.1038/nrmicro2974

Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., & Leulier, F. (2011). *Lactobacillus plantarum* promotes Drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. *Cell Metab*, *14*(3), 403-414. https://doi.org/10.1016/j.cmet.2011.07.012

Sun, M., Ye, M., Jiao, W., Feng, Y., Yu, P., Liu, M., Jiao, J., He, X., Liu, K., Zhao, Y., Wu, J., Jiang, X., & Hu, F. (2018). Changes in tetracycline partitioning and bacteria/phage-comediated ARGs in microplastic-contaminated greenhouse soil facilitated by sophorolipid. *J Hazard Mater*, *345*, 131-139. https://doi.org/10.1016/j.jhazmat.2017.11.036

Syamsidi, A., Rosa, R. A., Sulastri, E., Rahmah, N., Kamri, R. A., Rahma, N., Bin Emran T., Sharma R., Rabaan A. A., Nainu F., & Zubair M.S. (2023). Immunomodulatory Effect of *Begonia medicinalis* Ethanolic Extract in Drosophila. *Biointerface Research in Aplied Chemistry*, *13*(6). https://doi.org/10.33263/BRIAC136.558

Tafesh-Edwards, G., & Eleftherianos, I. (2023). The role of Drosophila microbiota in gut homeostasis and immunity. *Gut Microbes*, *15*(1), 2208503. https://doi.org/10.1080/19490976.2023.2208503

Üstündağ, G., Büyükgüzel, K., & Büyükgüzel, E. (2020). Penicillin Impact on Survivorship, Development, and Adult Longevity of *Drosophila melanogaster* (Diptera: Drosophilidae). *Journal of Entomological Science*, *55*(4). https://doi.org/https://doi.org/10.18474/0749-8004-55.4.560

Vajro, P., Paolella, G., & Fasano, A. (2013). Microbiota and gut-liver axis: their influences on obesity and obesity related liver disease. *J Pediatr Gastroenterol Nutr*, *56*(5), 461-468. https://doi.org/10.1097/MPG.0b013e318284abb5

Vaz, L. E., Kleinman, K. P., Raebel, M. A., Nordin, J. D., Lakoma, M. D., Dutta-Linn, M. M., & Finkelstein, J. A. (2014). Recent trends in outpatient antibiotic use in children. *Pediatrics*, *133*(3), 375-385. https://doi.org/10.1542/peds.2013-2903

Vuong, H. E., Yano, J. M., Fung, T. C., & Hsiao, E. Y. (2017). The microbiome and host behavior. *Annu Rev Neurosci*, *40*, 21-49. https://doi.org/10.1146/annurev-neuro-072116-031347

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Front Bioeng Biotechnol*, *9*, 612285. https://doi.org/10.3389/fbioe.2021.612285

Weiland, S. O., Detcharoen, M., Schlick-Steiner, B. C., & Steiner, F. M. (2022). Analyses of locomotion, wing morphology, and microbiome in *Drosophila nigrosparsa* after recovery from antibiotics. *Microbiology open*, *11*(3), e1291. https://doi.org/10.1002/mbo3.1291

World Health Organization. (2018). WHO report on surveillance of antibiotic consumption: 2016-2018 early implementation.

https://www.who.int/publications/i/item/9789241514880

Yu, Z., Shen, J., Li, Z., Yao, J., Li, W., Xue, L., Vandenberg, L. N., & Yin, D. (2020). Obesogenic effect of Sulfamethoxazole on *Drosophila melanogaster* with simultaneous disturbances on eclosion rhythm, glucolipid metabolism, and microbiota. *Environ Sci Technol*, *54*(9), 5667-5675. https://doi.org/10.1021/acs.est.9b07889

Zhang, K., Hornef, M. W., & Dupont, A. (2015). The intestinal epithelium as guardian of gut barrier integrity. *Cell Microbiol*, *17*(11), 1561-1569. https://doi.org/10.1111/cmi.12501