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Impact of High-Sugar Diet-Induced Hyperglycemia on Gut Microbiota and Reproductive Fitness in *Drosophila melanogaster*

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Abstract:

This research aims to determine how hyperglycemia caused by high-sugar diet affects gut microbiota composition and reproductive fitness of *Drosophila melanogaster*. The experimental groups were kept in controlled conditions and evaluated through various age periods. It was found that there was a clear decrease in microbial diversity with the loss of beneficial taxa such as *Lactobacillus* and enrichment of opportunistic bacteria such as *Enterobacter* and *Serratia* under high-sugar conditions. There was an increase in bacterial load and strong dysbiosis, especially as one gets older. Physiological examination revealed a decrease in the thorax length, the body size index, and the change in metabolic pattern, pointing to the inadequacy of nutrient utilization. This had a significant impact on reproductive performance with reduced fecundity, ovariole number, and egg-to-adult viability in hyperglycemic groups. Statistical analyses revealed that diet and age, and their interaction, have a significant effect on microbial and physiological parameters. The findings highlight the importance of diet in terms of host-microbe interactions, and in accelerating physiological and reproductive decline.

Keywords: Hyperglycemia, Gut microbiota, *Drosophila melanogaster*, High-sugar diet, Dysbiosis, Reproductive fitness

1. Introduction

The interactions between diet, metabolism, and gut microbiota have come to be the focus of current biological and biomedical studies. Excessive sugar consumption has received special consideration, among dietary factors, because excess sugar consumption is closely related to metabolic disorders, including obesity, insulin resistance, and type 2 diabetes. Hyperglycemia, which is a condition that is typified by an increase in the level of blood glucose, does not only interfere with host metabolic pathways, but also its effects on the composition and functionality of the gut microbiota are profound. The gut microbiome, a dynamic and complex community of microorganisms, is important in the regulation of host physiology including the metabolism of nutrients, immune functions, and reproductive processes. Thus, it is crucial to determine the ways in which high-sugar diets alter the composition of microbial communities and the fitness



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of hosts to understand the mechanisms underlying the diet-induced metabolic dysfunction. (Wong et al., 2014; Baenas & Wagner, 2022).

Drosophila melanogaster has become a widely accepted model organism to study metabolism and microbiome-related processes due to its short life cycle, genetic tractability and conserved metabolic pathways that closely resemble those of higher organisms, including humans. It is an excellent system to study host-microbe interactions in response to dietary changes, due to its simplicity of its gut microbiota, which is mainly composed of a limited number of bacterial taxa, such as *Lactobacillus* and *Acetobacter*. Past research has established that dietary composition plays a significant role in shaping the microbial structure of *Drosophila*, and thus, affect host development, metabolism and lifespan (Broderick and Lemaitre, 2012; Ridley et al., 2012). In particular, high-sugar diets have been demonstrated to induce obesity-like phenotypes and insulin resistance in *Drosophila*, which can serve as a valuable model to study hyperglycemic conditions. (Palanker Musselman et al., 2011).

The gut microbiota can be considered an ecological system of the host, where the taxa of microbes can be linked to cooperative and competitive relationships, which contribute to nutrient cycling and metabolic homeostasis. The beneficial microorganisms that are known to improve host growth and metabolic efficiency are *Lactobacillus plantarum* and *Acetobacter pomorum* (Erkosar et al., 2013; Consuegra et al., 2020). But dietary perturbations, especially a high sugar intake, can distort this balance, causing microbial dysbiosis with loss of diversity and increased growth of opportunistic or pathogenic bacteria. These dysbiotic states are linked to the inability to maintain a proper metabolic rate, oxidative stress, and dysfunction of physiological functions (Clark et al., 2015; Arias-Rojas and Iatsenko, 2022).

Besides metabolic effects, hyperglycemia has been cited to affect reproductive fitness which is a key ingredient of organismal survival and evolutionary success. Reproductive measures of fecundity, ovariole number and viability of offspring are sensitive predictors of physiological status, and are closely associated with nutritional condition. It has been demonstrated that dietary imbalance, such as excessive intake of carbohydrates, can have an adverse effect on reproductive output by changing the allocation of energy, hormonal balance, and cellular processes during oogenesis (Lushchak et al., 2014; Henry et al., 2020). Under metabolic stress conditions, reproductive decline in *Drosophila* is frequently accelerated and this suggests that hyperglycemia might also play a pivotal role in modulating reproductive aging.

There is a mounting body of evidence, that demonstrates that the gut microbiota can be a central mediator between diet and host physiology, and between metabolic and reproductive outcomes. Microbial metabolites, such as short-chain fatty acids, vitamins, and amino acids, play a role in host energy balance and reproductive processes by interacting with signaling pathways, including insulin and TOR pathways (Ankrah et al., 2021; Wong et al., 2014). High-sugar conditions may disrupt microbial composition, which can then disrupt these interactions and result in a reduced efficiency of nutrient utilization and reduced reproductive performance.



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Furthermore, a rise in opportunistic bacteria in the conditions of hyperglycemia can cause inflammatory reactions and loss of gut integrity, which further contributes to physiological deterioration. (Zhang et al., 2017; Whon et al., 2017).

Another problematic aspect that affects the gut microbiota composition and the host physiology is aging. Natural changes in microbial diversity and community structure occur with aging of organisms, usually resulting in a loss of stability and increased vulnerability to dysbiosis. Age-related changes in microbiota have been linked to impaired intestinal functioning and reduced lifespan in *Drosophila* (Clark et al., 2015). Such age-related alterations can be enhanced when combined with dietary stress, such as the consumption of high-sugar diets. The role of diet and aging in the interaction between host and microbe is therefore critical in understanding the role of hyperglycemia in the host-microbe interactions.

Although there have been immense improvements in the discipline, the need to employ integrated studies that can simultaneously determine the impacts of hyperglycemia on intestinal microbiota, metabolic characteristics, and reproductive fitness of different life stages is still there. However, most past studies have looked at microbial composition or physiological consequences in isolation without any comprehensive study of how these two are related to each other. This study fills this gap by taking a holistic approach, integrating microbial diversity analysis, morphometric analysis, and reproductive analysis to give a comprehensive understanding of diet-induced changes in *Drosophila melanogaster*. The study will help clarify the mechanisms by which hyperglycemia affects host physiology and microbial ecology, through the examination of independent and interactive impacts of diet and age.

Literature Review

Diet, intestinal microbiota and host physiology interaction have become a significant subject of biological research, especially with regard to metabolic disorders such as hyperglycemia. Microbiota of the gut is fundamental in ensuring metabolic balance, immune, and overall physiological homeostasis of the host. *Drosophila melanogaster* has been taken up in recent years as a potent model organism to study these interactions due to its relatively simple gut microbiome and conserved metabolic pathways (Wong et al., 2014). The composition of the gut microbiota in *Drosophila* is highly diet, environment and age dependent, making it an ideal system to study diet induced microbial changes.

Diet has been mentioned to be one of the most important factors that determine the gut microbial composition. The feeding behavior has a direct impact on the acquisition and maintenance of microbial communities, since microbes are constantly ingested by the environment and established in the gut (Microbiome in *Drosophila* gut). Research has revealed that dietary composition modifications especially high-sugar diets can have significant effects on the microbial diversity and abundance. As an example, the study has proven that high-sugar diets cause a decrease in microbial diversity and alteration in bacterial composition, which is often accompanied by the loss of beneficial taxa and an enrichment of opportunistic species



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(Voulgari-Kokota et al., 2024). This microbial imbalance has been related to compromised host physiology and poor developmental outcome.

It has been known to cause metabolic dysfunction in *Drosophila melanogaster*, including obesity-like phenotypes, insulin resistance, and disrupted lipid metabolism caused by high-sugar diets. It has been experimentally confirmed that a high sugar intake impairs the normal metabolic pathways and causes physiological stress (Salami, 2020). Moreover, the gut microbiota is a mediating factor in such metabolic responses. According to Wong et al. (2014), gut microbes help *Drosophila* to accommodate nutrient-poor or imbalanced diets by increasing nutrient use and metabolism. But in high-sugar environments this positive interaction is disturbed resulting in a decreased metabolism efficiency and an increased vulnerability to physiological deterioration.

Figure 1 provides published comparative evidence for diet-related glucose changes in *Drosophila melanogaster* (Baenas & Wagner, 2022).

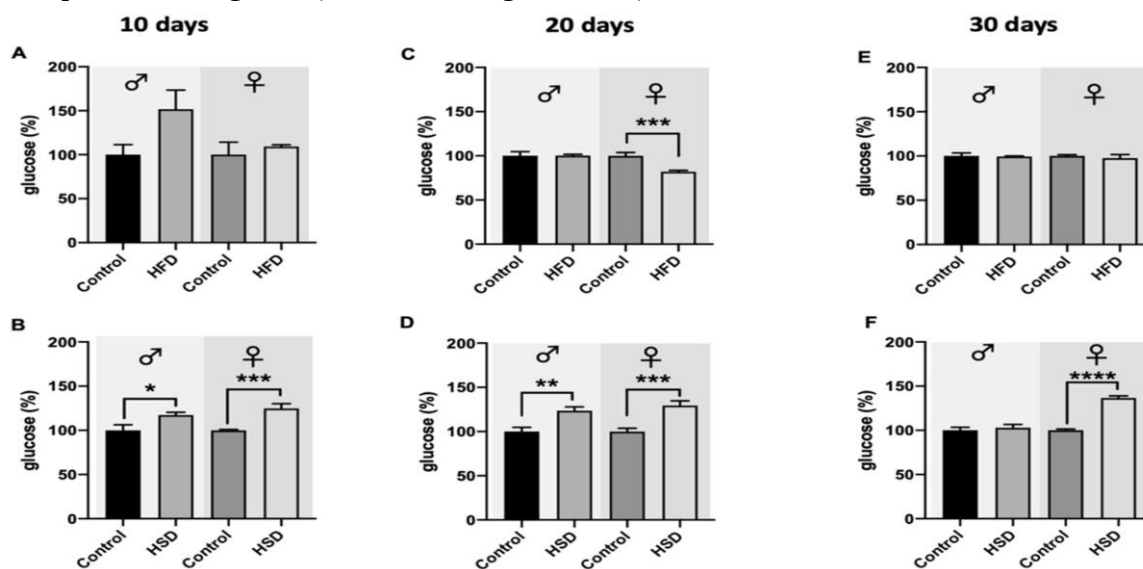


Figure 1. Whole-fly glucose levels under high-fat and high-sugar diets

Baenas and Wagner (2022)

The idea of microbial dysbiosis in the presence of hyperglycemia has been a popular topic of the recent researches. High sugar diets are shown to selectively favor some bacterial taxa that are capable of efficiently utilizing excess sugar, thus outcompeting beneficial microbes. As an example, it has been shown that the excess of sucrose causes the loss of the beneficial lactic acid bacteria and encourages the predominance of the taxa that metabolize sugars, thus, reducing the microbial diversity and undermining the health of the host organism. Equally, dietary changes (long-term) have been identified to cause persistent alterations in microbial composition, indicating that diet-induced microbial shifts can have long-term effects on host physiology (Bombin et al., 2021).

Besides the metabolic effects, gut microbiota have been demonstrated to be critical in controlling reproductive fitness in the *Drosophila melanogaster*. The reproductive characteristics, including fecundity, ovariole development, and viability of offspring are closely associated with the nutritional status and composition of microbes. Research has shown that helpful bacteria enhance reproduction by promoting nutrient uptake and controlling hormonal signals that mediate oogenesis. But these processes can be adversely affected by dietary imbalance, especially high sugar consumption. As an example, it has been demonstrated that sugar diets can change ovary physiology and decrease ovary output, and that reproductive systems are sensitive to alterations in diets and microbes.

Another critical determinant that affects gut microbiota and host physiology is aging. Changes in microbial composition with age have been extensively reported with less microbial diversity and more prone to dysbiosis. Aging in *Drosophila* has been linked to changes in microbial communities that harm intestinal functioning and are involved in physiological aging. These age-related changes are usually compounded when combined with dietary stressors such as high-sugar diets and the resultant deterioration of both microbial and host systems accelerated. Recent research has demonstrated that diet and age synergistically interact to influence age-related microbial changes and physiological aging. (Jelena et al., 2025).

The review model in Figure 2 illustrates how age-associated changes in microbiota and immune regulation can compromise intestinal homeostasis (Arias-Rojas & Iatsenko, 2022).

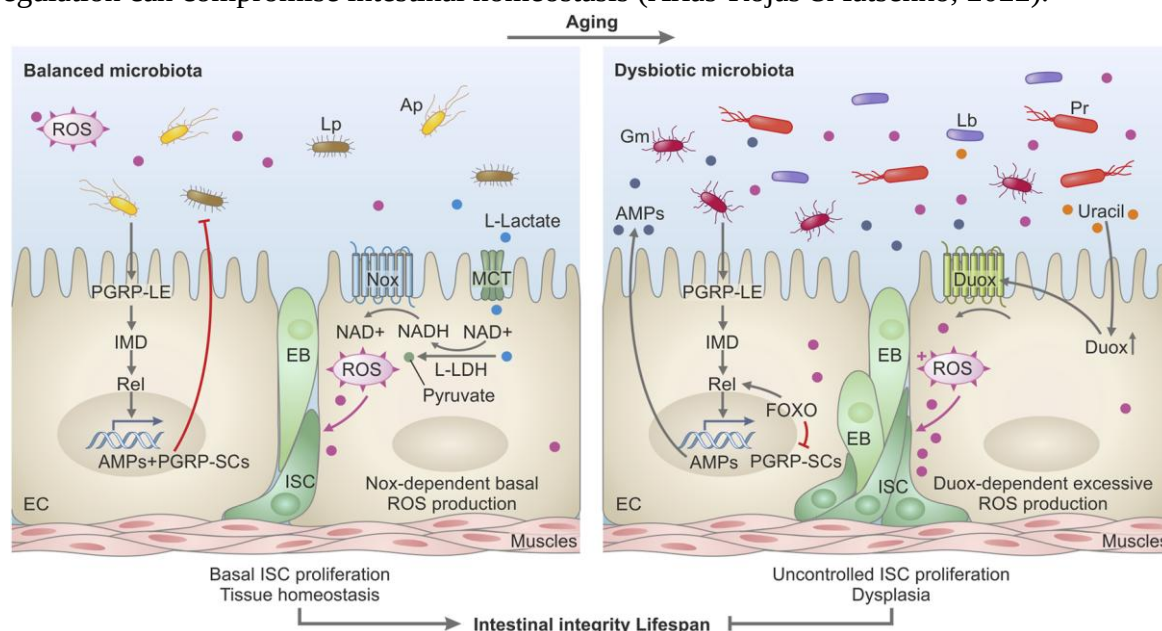


Figure 2. Microbiota and intestinal homeostasis during *Drosophila* aging

The mechanisms of the host-microbe interactions of gut microbiota are not limited to metabolism and reproduction but also to immune functioning and regulation of the lifespan. The dietary interventions and especially the ones that involve high sugar content have been found to cause disruption in the immune homeostasis and predispose to infections. As an



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example, it has been shown that diets rich in sugar can change the gut environment, resulting in increased oxidative stress and activation of immune pathways, which ultimately shorten lifespan and healthspan in *Drosophila* (Qush et al., 2023). These results underscore the intricate interaction between diet, microbiota and host well-being. The other significant point of the research of gut microbial life is the idea of the cooperation of microbes and their intermetabolic interactions. Under normal gut conditions, there is a cooperative relationship between the microbial taxa, which in turn aid in the growth and metabolic stability of the host. Nonetheless, in the high-sugar conditions, these interactions are frequently disturbed because of the overload of substrates, which cause competitive relations and low efficiency of nutrient utilization. This change in cooperative to competitive microbial networks is a characteristic of dysbiotic ecosystems and is associated with the loss of host physiological performance (Hung et al., 2025).

Although the relationship between diet, microbiota, and host physiology has made significant progress in the existing literature, there are still several gaps in the literature. Most of the studies have only concentrated on each of these elements separately, e.g. microbial composition or their metabolic consequences, not as a whole unit. It is necessary to conduct studies that will simultaneously examine the microbial diversity, physiological characteristics and reproductive fitness to give the comprehensive understanding of the diet-induced changes. Also, the diet/aging interaction has not been studied extensively, especially with respect to hyperglycemia and its chronic impact on host-microbe interactions.

In sum, the literature has been categorical that high-sugar diets have far reaching repercussions in the composition of gut microbiota, their metabolic activity and their reproductive fitness in *Drosophila melanogaster*. The loss of microbial balance by hyperglycemic conditions results in loss of diversity, altered metabolic pathways and impaired physiological performances. Moreover, diet-aging interaction is a key factor that contributes to these effects, which illustrates the need to consider various factors when conducting microbiome studies. The current research is based on these findings by offering a combined analysis of microbial, physiological, and reproductive parameters and thus contribute to the further understanding of the ecological and functional implications of diet-induced hyperglycemia.

3. Research Methodology

The research is a combination of experimental, microbiological, and statistical methods to investigate the effects of dietary sugar on microbial diversity and physiological characteristics and reproductive performance at various age stages. An experimental design was used in a controlled laboratory based experiment design, which ensured reliability and reproducibility of the results.

3.1 Research Design

The experimental research design with comparative- longitudinal approach forms the basis of the current research. Two dietary groups control and high-sugar diet (HSD) were designed and



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observations were made at different time points (5, 20 and 40 days) to examine the effects according to age. Both independent (diet and age) and interaction effects of the independent factors on the microbial and physiological parameters can be tested using it.

3.2 Study Organism

The model organism that will be used in this study is the *Drosophila melanogaster* which is widely known to be the best to study the metabolic and microbiome systems due to the fact that it has a short life cycle, well characterized genetics and preserved metabolic pathways. The use of female flies was primarily in reproductive and morphological studies due to their applicability in the fecundity and ovary researches.

3.3 Experimental Groups and Diet Composition

Two experimental groups were established:

- **Control Group:** Fed on standard cornmeal–yeast–agar medium containing approximately 5.4% w/v sugar.
- **High-Sugar Diet Group (HSD):** Fed on a modified semi-defined diet containing 30.6% w/v (1.7 M) sugar to induce hyperglycemic conditions.

The dietary treatments were designed to simulate normal metabolic conditions and chronic hyperglycemic stress, respectively.

3.4 Controlled Experimental Conditions

All experiments were conducted under standardized laboratory conditions to minimize environmental variability:

- **Temperature:** 25°C
- **Light Cycle:** 12-hour light: 12-hour dark cycle
- **Humidity:** 60%

These conditions ensured optimal growth, metabolic stability, and consistent circadian rhythms across all experimental groups.

3.5 Sample Size and Replication

Adequate sample sizes were maintained to ensure statistical validity:

- **Total Flies per Group:** 100–120 (3 replicates)
- **Fecundity Assay:** 30 females per replicate
- **Morphological Analysis:** 27–30 females per replicate
- **Microbiota Analysis:** 3–5 biological samples per replicate

All experiments were performed in triplicate to ensure reproducibility and reduce experimental error.

4: Results and Data Analysis

This study is the presentation of the experimental results of the research. The findings are systematically presented in tables and respective paragraph-wise discussions that cover the diversity of microbes, physiological peculiarities and the reproductive performance.



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4.1 Microbial Diversity Analysis

Table 1: Alpha Diversity Indices Across Diet and Age

Age (Days)	Diet	Shannon Index (H')	Simpson Index (D)	Chao1 Richness	Observed OTUs $\pm$ SD
5	Control	2.47 $\pm$ 0.08	0.82 $\pm$ 0.02	38 $\pm$ 3	42 $\pm$ 4
5	HSD	1.98 $\pm$ 0.06	0.74 $\pm$ 0.03	29 $\pm$ 2	30 $\pm$ 3
20	Control	2.31 $\pm$ 0.05	0.80 $\pm$ 0.03	36 $\pm$ 2	39 $\pm$ 3
20	HSD	1.67 $\pm$ 0.07	0.65 $\pm$ 0.04	25 $\pm$ 3	28 $\pm$ 2
40	Control	2.05 $\pm$ 0.09	0.76 $\pm$ 0.03	33 $\pm$ 2	35 $\pm$ 3
40	HSD	1.32 $\pm$ 0.08	0.52 $\pm$ 0.05	21 $\pm$ 3	24 $\pm$ 2

The alpha diversity indices show clearly that there is a great loss in microbial diversity under high-sugar diet (HSD) conditions as compared to the control group. The values of the Shannon Index and Simpson Index are always lower in HSD-fed flies indicating reduced microbial richness and evenness. The decrease is further enhanced with age, especially at the age of 40 days, where HSD flies have the lowest values of diversity. It means that hyperglycemia has high selective pressure on the gut microbiota, and results in the loss of beneficial taxa and dominance of a few opportunistic species. The decreasing Chao1 richness and the OTUs that are observed also support the simplification of the microbial community. On the whole, the evidence indicates that diet and aging interactively to lead to microbial dysbiosis.

4.2 Microbial Community Variation

Table 2: PERMANOVA Results for Gut Microbial Communities

Source of Variation	df	Sum of Squares	Mean Square	F-value	p-value
Diet (Control vs HSD)	1	2.154	2.154	5.83	0.001
Age (5, 20, 40 days)	2	2.982	1.491	4.04	0.002
Diet $\times$ Age	2	1.473	0.736	3.02	0.030
Residual	12	4.428	0.369	—	—

The results of the PERMANOVA test confirm that diet and age have significant effects on the composition of gut microbial communities. The p-value of diet ($p = 0.001$) is highly significant which means that dietary sugar is the key factor that contributes to microbial variation. Age is also significantly effective, showing natural changes in the microbial composition with age. Notably, diet and age have a significant interaction, indicating that the effect of hyperglycemia increases with age. This interplay underscores the joint effect of intrinsic and extrinsic factors in the structure of gut microbiota.



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4.3 Taxonomic Composition

Table 3: Relative Abundance of Major Bacterial Phyla (%)

Phylum	Control (5d)	Control (20d)	Control (40d)	HSD (5d)	HSD (20d)	HSD (40d)
Firmicutes	41.2	39.6	37.8	35.1	27.3	19.4
Proteobacteria	33.4	35.9	37.5	41.8	47.2	53.7
Bacteroidetes	17.8	16.5	15.2	14.1	12.0	9.5
Actinobacteria	7.6	8.0	9.5	9.0	10.6	11.8

The analysis at the phylum level shows that there is an apparent change in microbial composition when the conditions are hyperglycemic. Firmicutes, including the helpful bacteria, *Lactobacillus* decrease dramatically in flies fed HSD, especially with age. Proteobacteria, in turn, demonstrate a significant growth, which proves the development of opportunistic and potentially pathogenic taxa. This change portrays a change of a balanced microbial environment to a dysbiotic environment. The progressive changes in control groups are natural aging, whereas drastic changes in HSD groups are associated with the disruptive nature of high-sugar diets.

4.4 Bacterial Load Analysis

Table 4: Bacterial Load Comparison (CFU/ml)

Group	Mean CFU/ml ($\pm$ SD)
Control	$2.5 \times 10^5 \pm 0.3$
High-Sugar Diet	$4.8 \times 10^5 \pm 0.5$

The bacterial load analysis indicates that there is significant increase in CFU/ml in flies that have been fed using HSD compared to controls. This demonstrates that conditions characterized by high sugar content are conducive to the growth of microbial species, especially the rapid growth of opportunistic microbial species. Nevertheless, this rise is not due to better microbial health but an indication of dysbiosis and imbalance. The increased variability in the HSD group itself is another indication of instability in the control of microbes. The findings suggest the conclusion that hyperglycemia increases the abundance of microbes and disrupted the microbial balance.

4.5 Reproductive Performance

Table 5: Fecundity (Average Eggs per Female)

Age (Days)	Control	HSD
5	65 ± 5	52 ± 4
10	58 ± 4	41 ± 3
20	42 ± 3	25 ± 2

The fecundity of both groups decreases progressively with age, with the decrease in the case of the flies fed on HSD. Reduced egg production in the HSD groups demonstrates that the ovarian functionality and the capacity to reproduce is impaired. This deterioration is probably



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because of metabolic stress, hormonal imbalance, and microbial dysbiosis. The findings clearly indicate that hyperglycemia has adverse effects on reproductive fitness and it increases reproductive aging.

4.6 Egg-to-Adult Viability

Table 6: Egg-to-Adult Viability (%)

Group	Viability (%)
Control	88% $\pm$ 3
High-Sugar Diet	70% $\pm$ 4

The viability results indicate that there is a strong decrease in survival between the egg and adult stages in HSD conditions. It indicates that hyperglycemia has a detrimental influence on the developmental processes, which may be caused by the imbalance in the metabolic processes and disruption of the microbes. This reduced viability implies decreased fitness of offspring and the larger effect of dietary sugar on life-history traits.

4.7 Morphometric Analysis

Table 7: Mean Thorax Length (mm)

Age	Control	HSD
5	1.12 $\pm$ 0.05	0.98 $\pm$ 0.06
20	1.10 $\pm$ 0.07	0.95 $\pm$ 0.05
40	1.00 $\pm$ 0.08	0.88 $\pm$ 0.07

Although the length of the thorax decreases with age in both groups, the decrease is always greater in HSD-fed flies. This means that there is an impaired somatic growth in hyperglycemic conditions. The findings indicate that a high amount of sugar interferes with the absorption of nutrients and anabolic functions, resulting in a smaller size of the body and its structural integrity.

4.8 Body Weight and BSI

Table 8: Body Weight and BSI

Age	Diet	Body Weight (mg)	BSI (mm ³)
5	Control	1.25	1.40
5	HSD	1.32	0.94
40	Control	1.10	1.00
40	HSD	0.85	0.68

The results indicate that HSD-fed flies have a biphasic response whereby there is an increase in weight and then a decrease. Although the body weight at an early age is high, BSI is low, which is a sign of fat accumulation as opposed to actual growth. As age increases weight and BSI decrease rapidly, a metabolic exhaustion. These results indicate the adverse effect of hyperglycemia on energy usage and structural formation.



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4.9 Ovariole Number

Table 9: Mean Ovariole Number per Female

Age	Control	HSD
5	18.0 ± 1.2	15.2 ± 1.5
20	16.3 ± 1.3	13.0 ± 1.2
40	14.5 ± 1.4	10.8 ± 1.6

During the HSD conditions, the number of ovarian cells decreases considerably, which is evidence of impaired ovarian development. The decrease is further advanced with age, indicating rapid reproductive aging. This decrease can probably be attributed to impaired hormonal signalling and metabolic imbalance.

4.10 Summary of Results

The findings indicate that the hyperglycemia induced by high-sugar diets in *Drosophila melanogaster* significantly changes the composition of gut microbiota, reduces the microbial diversity, alters the physiological characteristics, and decreases the reproductive fitness of the insect. Diet-induced dysbiosis, metabolic imbalance, and physiological decline are the combined effects of diet and aging, which explains the critical role of diet in determining host-microbiota interactions.

5. Conclusion

The current study clearly shows that a hyperglycemic state induced by a high-sugar diet significantly alters the gut microbiota composition, and negatively affects physiological and reproductive fitness in *Drosophila melanogaster*. The results show that microbial diversity decreases significantly, and the process is associated with a transition to the opportunistic state in the microbiome, and an overall increase in a microbial load. These microbial changes are closely linked with impaired metabolic control, reduced somatic growth and a strong reduction in reproductive performance, including reduced fecundity, ovariole number and offspring viability. Additionally, the paper emphasizes the synergistic effect between diet and aging, in which hyperglycemia makes physiological degradation and microbial instability with age. The high correlations found between microbial diversity and host fitness parameters highlight the importance of gut microbiota on sustaining metabolic and reproductive fitness. In sum, the research yields extensive evidence that dietary sugar is a significant environmental factor that affects host microbe interactions, and the overall fitness of organisms.

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