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The Effect of Chia seed on Longevity in *Drosophila melanogaster*

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ABSTRACT:

Nutrition plays a vital role in influencing both the health and lifespan of various organisms, including *Drosophila melanogaste*r. In the present study, *D. melanogaster* flies were cultured on wheat cream agar medium and diets supplemented with different concentrations of chia seeds (5g, 10g, and 15g) to evaluate the effect of chia seed supplementation on longevity. The results demonstrated that flies fed with chia seed supplemented diets showed, a increased lifespan compared to those fed with the control diet (wheat cream agar). Furthermore, when comparing the survival of different groups by sex and mating status, females consistently lived longer than males across all diets. Specifically, unmated females displayed the highest longevity, followed by mated females, unmated males, and mated males. These findings suggest that chia seed supplementation, particularly at higher concentrations, positively affects the lifespan of *D. melanogaster*. The overall order of longevity observed in this study is: 15g chia seed > 10g chia seed > 5g chia seed > control. This study concludes that increasing the concentration of chia seed in the diet extends the lifespan of *D. melanogaster*.

Keywords: Nutrition, *Drosophila melanogaster*, longevity, chia seed

Introduction

Diet plays a crucial external factor in shaping an organism's growth, development, and survival (Sisodia and Singh, 2012) [20]. Research had shown that dietary restrictions, even in the absence of starvation, can influence both lifespan and reproductive success across various species, including mammals and nematodes. Piper *et al*., (2011) [17] emphasized that nutrition significantly affects life expectancy and reproductive performance. The effects of diet on life history traits can be categorized into two main aspects: quantity, which relates to food availability, and quality, which concerns the nutritional composition of the food (Sisodia and Singh, 2012) [20]. Dietary changes have been found to extend the lifespan in numerous animal species, including fruit flies.

Multiple elements impact the longevity of wild animals. These include extrinsic factors such as predation, poor nutrition, and environmental conditions, as well as intrinsic ones like the natural aging process. Genetic factors that influence traits related to fitness and nutritional signaling pathways also contribute to longevity, which is considered a quantitative trait. Other influences on lifespan include sex, age, genetic background (including epigenetic factors), and the environment (Paaby and Schmidt, 2009) [16]. In *Drosophila*, increased lifespan is often associated with various traits such as lipid reserves, developmental timing, body size, biochemical defenses, and resistance to environmental stressors like starvation, dehydration, and cold. These traits may correlate positively or negatively with longevity (Vermeulen and Loeschcke, 2007; Wit *et al*., 2013; Deepashree *et al*., 2017) [24, 25, 7].

According to Finch (1990) [8] and Charlesworth (1994) [5], aging is defined as a gradual functional decline that eventually leads to death. Several theories have been proposed to explain the evolutionary forces related to fitness and reproduction that influence species longevity (Hughes and Reynolds, 2005) [9].

In many species, males and females differ in average lifespan, with females typically living longer (Lints *et al*., 1983; Austad and Fischer, 2016) [13, 4]. These sex-based differences are often observed in wild populations and are commonly attributed to risk-taking behavior, feeding strategies, and sexual competition. However, such factors may not fully explain differences in intrinsic aging rates between sexes (Austad and Fischer, 2016) [4]. The sexual selection theory proposed by Trivers (1972) [22] suggests that the trade-off between lifelong reproductive effort and investment leads to sex differences in the production of reactive oxygen species (ROS) and in antioxidant defenses.

Numerous experiments have investigated how various nutritional supplements and natural products affect longevity. Research involving Jeeni millet (Kiran and Krishna, 2023), Spirulina (Shreejani and Shreeraksha, 2023), Whey protein by (Darshan *etal*., 2024), Mass gainer (Sultana e*tal*., 2024) and Ensure nutritional supplement (Kishore *etal*., 2024) has shown that both the quality and concentration of these dietary components significantly influence the longevity of *D. melanogaster*.

However, the potential effects of chia seeds on longevity remain unexplored. Chia seeds are widely recognized for their health benefits. They are rich in omega-3 fatty acids, dietary fiber, protein, and antioxidants, and are known to support cardiovascular health, aid digestion, and assist in weight regulation (Cristiane Freitas Rodrigues et al., 2018) [12]. Therefore, this study is designed to assess how chia seed consumption influences the reproductive fitness of *D. melanogaster*.

Nutritionally, chia seeds typically consist of 18–30% fiber, 4–5% ash, 15–25% protein, 30–33% fats, and 26–41% carbohydrates. They also contain high levels of natural antioxidants, including myricetin, quercetin, kaempferol, and caffeic acid, which help maintain their rich polyunsaturated fatty acid (PUFA) content. These antioxidants may also counteract the harmful effects of reactive oxygen and nitrogen species, which are often linked to obesity-related conditions (Cristiane Freitas Rodrigues *et al*., 2018) [12].

Materials Method

In this study, chia seeds were procured from Loyal World, a grocery store in Mysore, Karnataka. Sold under the brand name True Elements, the seeds were finely ground into a powder to create the experimental medium. Before being used in the treatment process, the powdered seeds were stored under suitable conditions for later use.

Establishment of stock

The Oregon K strain of *Drosophila melanogaster* was sourced from the *Drosophila* Stock Centre at the Department of Zoology, University of Mysore. This strain was used to create a stock culture maintained in bottles containing wheat cream agar medium. The standard medium was prepared by boiling 100 grams each of jaggery and wheat flour, along with 10 grams of agar in 1000 ml of distilled water, followed by the addition of 7.5 ml of propionic acid. The flies were kept under controlled laboratory conditions, including a 12-hour light/dark cycle, 70% relative humidity, and a temperature of 22 ± 1°C.

These stock flies were then used to set up experimental groups, each reared on a different diet: Control Diet (Wheat Cream Agar): This standard medium was made using 100 g of jaggery, 100 g of rava powder, and 10 g of agar dissolved in 1000 ml of boiling distilled water. Once mixed, 7.5 ml of propionic acid was added. Wheat Cream Agar with 5 g Chia Powder: Prepared using the same base ingredients (100 g jaggery, 100 g rava powder, 10 g agar in 1000 ml boiling water), followed by the addition of 7.5 ml of propionic acid. After the mixture cooled, 5 g of chia powder was added and blended thoroughly. Wheat Cream Agar with 10 g Chia Powder: Following the same base preparation and addition of propionic acid, 10 g of chia powder was incorporated once the mixture had cooled and was mixed evenly. Wheat Cream Agar with 15 g Chia Powder: Using the same procedure, 15 g of chia powder was added to the cooled mixture and mixed thoroughly after incorporating 7.5 ml of propionic acid.
Flies raised on each of these diets, under identical lab conditions, were then evaluated for their longevity.

Experimental procedure

Longevity for mated flies

Virgin female and unmated male flies were collected within three hours of emerging (eclosion) from their respective media: wheat cream agar and wheat cream agar with 5g,10g and 15g chia seed powdered media. These flies were aged for five days. Afterward, individual virgin females and unmated males were introduced into mating chambers. Once mating occurred, each pair was transferred to a vial containing their original medium. These transfers were done once every seven days and continued until the flies died. The number of deaths was recorded daily. A total of thirty flies were monitored separately for each media type: wheat cream agar and wheat cream agar with 5g,10g and 15g chia seed powdered media.

Longevity for unmated flies

Five days old unmated male and female *D. melanogaster* flies were collected from four types of media: wheat cream agar and wheat cream agar with 5g,10g and 15g chia seed powdered media. For the longevity experiments, fifteen unmated males and fifteen unmated females were selected from each media type, total thirty flies per media condition. These flies were placed in separate vials according to their respective media. The number of living and dead flies was recorded daily. Longevity experiments were also conducted separately for virgin females, mated females, mated males, and unmated males. On the seventh day, all flies were transferred to fresh vials containing the same type of media and continued to be monitored until death.

Results

Analysis of survival curve Survival curve was calculated for longevity of males and females. Two functions that are dependent on time are of particular interest: The survival function and Hazard function. The survival function S(t) is defined as the probability of dying at time t having survived until that time. The graph of S(t) against t is called the survival curve. The Kaplan- Meir method was used to estimate this curve from observed survival times without assuming an underlying probability distribution. Two survival curves were compared using a statistical hypothesis test called the log-rank test, which is used to test null hypothesis that there is no difference between survival curves, i.e., the probability of an event occurring at any point of time is for each media 15 trials were made for each of the Wheat cream agar, and Wheat cream agar with 5g, 10g and 15g chia seed powder. Separate experiment was carried out for virgin female, mated female, mated male and unmated male in *D. melanogaster*. The results revealed that the females fly lived longer than the male flies in all the four diets. According to the experimental analysis as the chia seed concentration increases life span increases in *D. melanogaster*.

Table 1: Test of equality of survival distribution for Control (Wheat cream agar) and Wheat cram agar with 5g, 10g and 15g Chia seed supplementation treated mated male and female flies.

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	145.307	3	0
Breslow (Generalized Wilcoxon)	128.373	3	0
Tarone-Ware	136.814	3	0
Test of equality of survival distributions for the different levels of Treatment.			

Table 2: Test of equality of survival distribution for Control (Wheat cream agar) and Wheat cram agar with 5g, 10g and 15g Chia seed supplementation treated unmated male and female flies.

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	175.053	3	0
Breslow (Generalized Wilcoxon)	155.688	3	0
Tarone-Ware	165.157	3	0
Test of equality of survival distributions for the different levels of Treatment.			

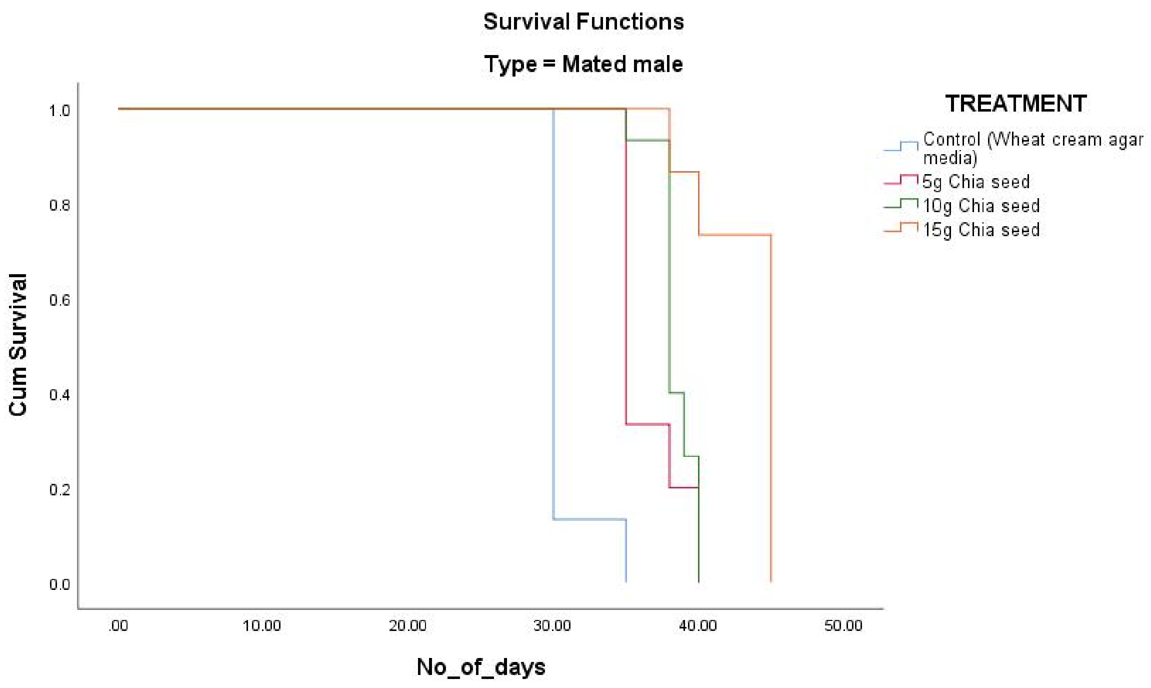

Fig 1: The effect of Chia seed supplementation on longevity of mated male in *D. melanogaster*.

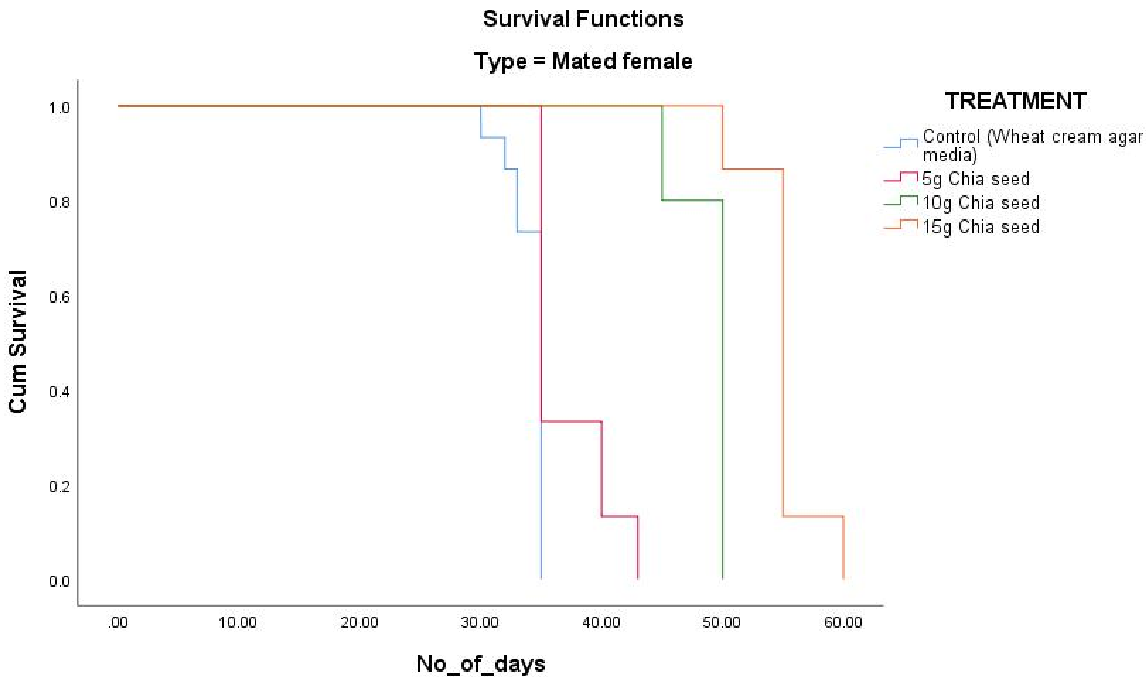

Fig 2: The effect of Chia seed supplementation on longevity of mated female in *D. melanogaster*.

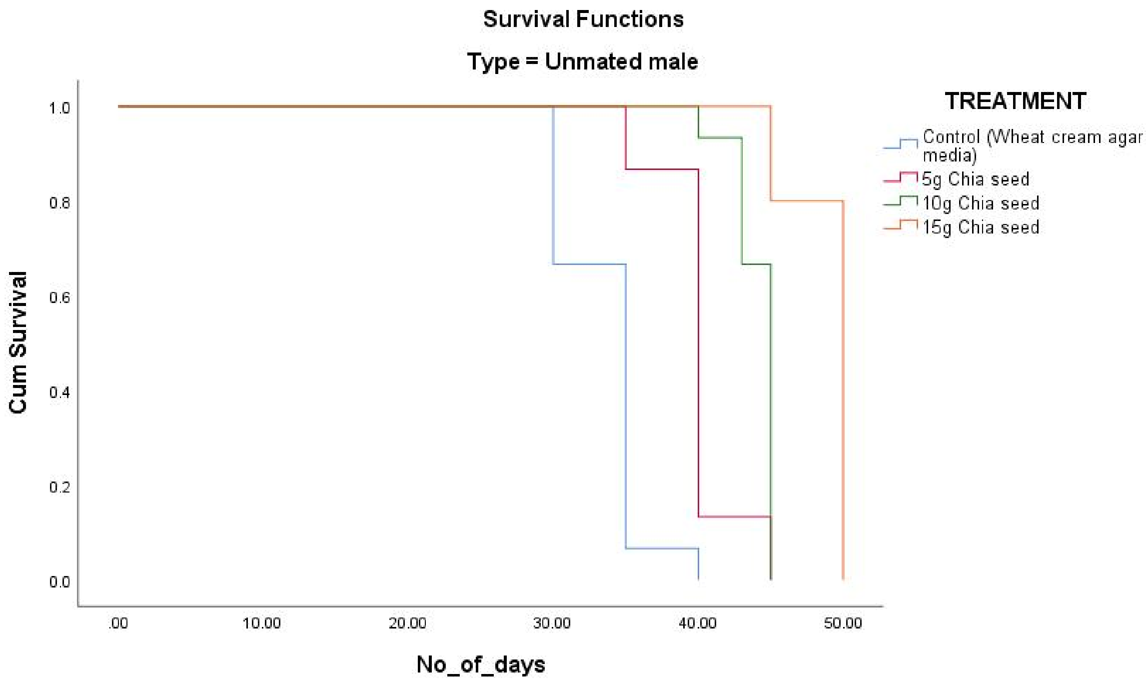

Fig 3: The effect of Chia seed supplementation on longevity of unmated male in *D. melanogaster*.

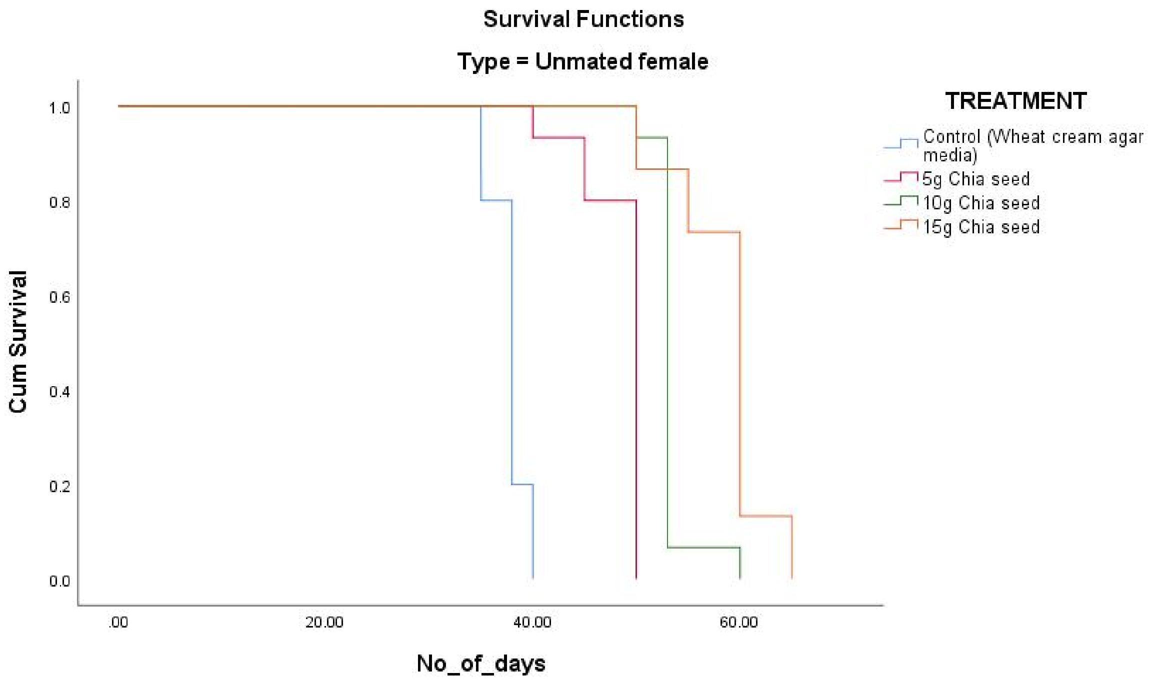

Fig 4: The effect of Chia seed supplementation on longevity of unmated female in *D. melanogaster*.

Sex difference in the survivability of *D. melanogaster* as determined by the Kaplan-Meier survival analysis (Fig 1 : Mated male, Fig 2: Mated female, Fig 3: Unmated male and Fig 4: Unmated female). The graphs represent the survivorship of mated male, mated female, unmated male and unmated female flies housed separately in vials of control media and different concentration of chia seed treated media (5g, 10g and 15g). The log-rank test revealed a statistically significant difference in the survivorship between mated male, mated female, unmated male and unmated female flies (Chi square value for Mated male and female=145.307, Unmated male and female=175.053 and P<0.001).

Discussion

Aging is a complex biological phenomenon characterized by the progressive decline of physiological and cellular functions over time. Understanding the molecular mechanisms, metabolic pathways, and physiological processes associated with aging and longevity is a major focus of medical and biological research. Lifespan is a polygenic and multifactorial trait, influenced by several factors such as sex, age, genetic background (including epigenetic modifications), and environmental factors diet being one of the most critical among them (Paaby & Schmidt, 2009) [16]. Diet plays a key role in modulating reproduction, stress tolerance, growth, development, and ultimately the survivability of organisms. Both the quality and quantity of nutrients are crucial in determining the lifespan of an organism. In the present study, we investigated the effects of varying concentrations of Chia seed supplementation (5g, 10g, and 15g) on the lifespan of *D. melanogaster* using wheat cream agar media as the control. The nutritional composition of Chia seeds includes a rich profile of essential fatty acids, proteins, dietary fiber, antioxidants, and minerals, making them a highly nutritious food source. Kaplan-Meier survival analysis (Figures 1-4) and Chi square test (Table 1 and 2) showed significant differences in longevity across different treatment groups. The control group (wheat cream agar media) consistently showed a reduced lifespan across all fly types (unmated males, unmated females, mated males, and mated females), while flies fed with Chia seed-supplemented diets exhibited increased lifespan as the concentration increased. The results suggest that while Chia seeds are nutritionally dense, the high protein and carbohydrate content, along with possible antioxidant overload, may have contributed to increased longevity in *D. melanogaster*. Supporting this, Dsouza and Krishna (2015) [1] found that flies fed on natural energy drinks lived significantly longer than those consuming synthetic alternatives. Additionally, other studies (Le Bourg, 2001; Magwere *et al*., 2004) [12,14] suggest that high concentrations of dietary antioxidants may also reduce lifespan, possibly due to oxidative stress imbalances or metabolic shifts.

Various studies suggest that nutritional differences in dietary components can influence the lifespan of *D. melanogaster*. Chia seeds, which are rich in protein, carbohydrates, and antioxidants, are considered a "high-nutrition" food. Unlike some other nutrient-dense diets that may shorten lifespan, flies fed with chia seeds showed an increase in lifespan, indicating a potential positive effect of chia seed supplementation on longevity.

In laboratory species such as *C. elegans*, *D. melanogaster*, and *Mus musculus*, notable differences in lifespan between males and females have been frequently observed (Tower and Arbeitman, 2009; Austad and Fischer, 2016) [21, 4]. In many species, females often outlive males (Lints *et al*., 1983; Austad and Fischer, 2016) [13, 4]. These lifespan differences are commonly inferred from observations in wild populations and are frequently attributed to factors such as risk taking behavior, feeding habits, and sexual competition. However, these factors may not necessarily reflect sex-specific differences in intrinssic aging rates (Austad and Fischer, 2016) [4]. To better understand sex-specific aging, examining lifespan variations within a single species is valuable. In *Drosophila*, sex-based differences in lifespan have been documented, and these differences are thought to result from fundamentally different energy requirements between males and females (Hunt *et al*., 2011; Magwere *et al*., 2004; Velasco and Medina, 2014) [14, 23]. In the present study, survival curves and log-rank test analysis clearly demonstrated that females lived significantly longer than males across all four dietary conditions. Several studies suggest that females possess larger guts than males, allowing them to consume greater amounts of nutritional food, which may contribute to their extended lifespan. Additionally, lipid metabolism appears to play a role in enhancing longevity.

According to Vermeulen *et al*. (2005) [24], resistance to oxidative stress, such as that induced by paraquat, can impact *Drosophila* lifespan. Archer *et al*. (2013) [2] also reported sex-based differences in oxidative stress and lifespan in the cricket *Gryllodes sigillatus*. Similarly, Chaudhuri *et al*. (2007) [6] observed sex-specific differences in oxidative stress induced mortality in *Drosophila* treated with paraquat. In particular, older females showed significantly higher catalase activity compared to males of the same age. Furthermore, longer-lived females maintained stronger superoxide dismutase (SOD) activity even after ethanol exposure.

In our current study, we also found that female flies outlived males across all four dietary conditions. However, we did not measure oxidative stress levels in the flies. Numerous studies have shown that environmental factors such as temperature, light, age, and stress can influence fly survival and affect lifespan in a sex-specific manner. Nonetheless, in our experiment, flies were maintained under controlled laboratory conditions. All flies were of the same age and fed different diets. Therefore, the observed lifespan variations are likely attributable to differences in the quantity and quality of nutrients in the diets.

Hence from our study in *D. melanogaster*, we can conclude that nutrition plays a significant role in influencing the organism's longevity. Flies raised on media containing chia seeds showed increased longevity compared to those on other diets. Among the different concentrations tested, the sequence of longevity observed was as follows: 15g chia seed > 10g chia seed > 5g chia seed > control. This suggests that the consumption of chia seeds increases the lifespan of *D. melanogaster*.

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