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The Effect of Chia Seed on Reproductive Fitness in *Drosophila melanogaster*

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Abstract

Nutritional components play a vital role in influencing the growth, development, reproductive success, and physiological traits of *Drosophila melanogaster*. In this study, we investigated the impact of chia seed supplementation at varying concentrations (5g, 10g, and 15g) on reproductive fitness parameters, including mating latency, copulation duration, and fertility.

Experimental flies were cultured on standard wheat cream agar (control) and chia seed-enriched media at different concentrations. The results revealed a statistically significant variation in mating behavior and reproductive outcomes among the groups. Flies fed with 5g of chia seed exhibited the shortest mating latency, indicating faster initiation of mating compared to control flies, which showed the longest delay. Copulation duration was significantly higher in the 5g group, followed by the 10g and 15g groups, while the control group had the shortest duration, suggesting enhanced sperm transfer potential in chia-fed flies.

Fertility analysis showed that the 5g chia seed group had the highest progeny count, whereas the control group produced significantly fewer offspring. Intermediate fertility levels were recorded in the 10g and 15g groups.

Thus these studies suggests that nutrient present in chia seed increases reproductive fitness in *D. melanogaster*.

Keywords: *Drosophila melanogaster*, chia seed, mating latency, copulation duration, fertility, nutrition.

Introduction

Reproductive fitness is largely governed by biological processes closely tied to reproduction. These processes include factors such as viability, lifespan, offspring production, fecundity, latency in viability, and the time interval between matings. Turner and Andersson (1983) [45] emphasized that these factors collectively define the fertility aspect of fitness, offering a broad interpretation of fertility. Numerous studies involving different *Drosophila* species have explored sexual behaviors, with a particular focus on key courtship patterns, genetic regulation, sensory cues, and the roles played by both sexes in mating behavior and frequency (Parsons, 1973; Banerjee and Singh, 1998) [27, 3]. Research consistently demonstrates that differences in fertility significantly contribute to evolutionary selection, as shown in various studies using *Drosophila* as a model (Turner and Andersson, 1983) [45].

Traits such as male body size and age are known to influence reproductive success in *Drosophila*, while nutrition is another critical factor affecting reproductive outcomes. Nutrient availability has been shown to impact sexual selection and mate preferences (Janicke *et al*., 2015; Kunz and Uhl, 2015) [13, 16]. Studies examining diet have consistently found that optimal nutritional intake enhances lifetime fecundity (Lee *et al*., 2008; Maklakov *et al*., 2009; Pirk *et al*., 2010; Solon-Biet *et al*., 2015) [17, 19, 31, 41]. However, the ideal macronutrient balance comprising fats, proteins, and carbohydrates varies between sexes and species. *D. melanogaster* is widely used as a model organism to investigate how nutrition influences reproductive fitness.

As species evolve, they develop the ability to recognize traits in potential mates that signal high reproductive potential (Andersson, 1994; Maynard-Smith and Harper, 2003) [1, 21]. Recent research indicates that reproductive success is more closely tied to nutrient composition—especially the protein to carbohydrate ratio rather than total caloric intake (Piper *et al*., 2011; Fanson and Taylor, 2011; Simpson and Raubenheimer, 2009) [30, 6, 39]. This is expected, as physiological and sex-specific requirements often demand distinct nutrient profiles. Various studies (Fricke *et al*., 2008; Maklakov *et al*., 2008; Vargas *et al*., 2010; Gosden and Chenoweth, 2011) [7, 19, 46, 9] suggest that dietary composition influences how organisms allocate resources between survival and reproduction. Natural selection likely favours mechanisms that enable organisms to rapidly adjust resource allocation strategies in response to dietary changes and for potential mates to recognize these physiological changes.

Several experiments on reproductive fitness have been carried out using different nutritional supplements and natural products. Studies involving Jeeni millet (Kiran and Krishna, 2023) [12] and Spirulina supplement (Shreejani and Shreeraksha, 2023) [29] have demonstrated that the quality and concentration of these dietary elements significantly impact the reproductive fitness of *D. melanogaster*.

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Despite extensive research, no study to date has explored the effects of chia seeds on reproductive fitness. Chia seeds are well known for their numerous health benefits. They are rich in omega-3 fatty acids, dietary fiber, protein, and antioxidants, and are recognized for promoting heart health, improving digestion, and supporting weight management (Cristiane Freitas Rodrigues *et al*., 2018) [12]. Consequently, this study aims to evaluate the impact of chia seed consumption on the reproductive fitness of *D. melanogaster*.
Chia seeds typically contain 18–30% dietary fiber, 4–5% ash, 15–25% protein, 30–33% fats, and 26–41% carbohydrates. They are also abundant in natural antioxidants such as myricetin, quercetin, kaempferol, and caffeic acid, which help preserve their high polyunsaturated fatty acid (PUFA) content. These antioxidants may help mitigate the damaging effects of reactive oxygen and nitrogen species, which are commonly associated with obesity-related disorders (Cristiane Freitas Rodrigues *et al*., 2018) [12].

Materials and Methods

For this study, chia seeds were sourced from Loyal World, a grocery store located in Mysore, Karnataka. Marketed under the brand name True Elements, the seeds were ground into a fine powder to prepare the experimental medium. Prior to their use in the treatment procedure, the powdered seeds were stored under appropriate conditions for future application.

Establishment of Stock

The Oregon K strain of *Drosophila melanogaster* was obtained from the *Drosophila* Stock Centre, Department of Zoology, University of Mysore, Mysore. These flies were used to develop a stock culture grown in bottles containing a wheat cream agar medium. The medium consisted of 100 grams each of jaggery and wheat flour, along with 10 grams of agar, all boiled in 1000ml of distilled water, with 7.5ml of propionic acid added. The flies were maintained under laboratory conditions with a 12-hour light and 12-hour dark cycle, 70% relative humidity, and a temperature of 22 ± 1°C.
Cultured flies were used to establish experimental groups, each raised on a specific dietary medium. [Control Medium (Wheat Cream Agar): The standard diet, known as wheat cream agar, was made by dissolving 100g of jaggery, 100g of rava powder, and 10g of agar in 1000ml of boiling distilled water. Once fully mixed, 7.5ml of propionic acid was added. Wheat Cream Agar with 5g Chia Powder: This variant was prepared by dissolving 100g of jaggery, 100g of rava powder, and 10g of agar in 1000ml of boiling distilled water. After thorough mixing, 7.5ml of propionic acid was added while still hot. Once the mixture had cooled, 5g of chia powder was added and blended evenly. Wheat Cream Agar with 10g Chia Powder: Following the same base preparation, 100g each of jaggery and rava powder, and 10g of agar were mixed into 1000ml of boiling distilled water. After adding 7.5ml of propionic acid to the hot solution, the mixture was allowed to cool, then 10g of chia powder was added and mixed thoroughly. Wheat Cream Agar with 15g Chia Powder: This version followed the same preparation steps: 100g of jaggery, 100g of rava powder, and 10g of agar were combined with 1000ml of boiling distilled water. After mixing and adding 7.5ml of propionic acid to the hot solution, the medium was cooled before incorporating 15g of chia powder, ensuring even distribution.
Flies grown on each of these media under standardized lab conditions were assessed for reproductive fitness.

Experimental Procedure

Virgin female and unmated male flies were collected from wheat cream agar and from media supplemented with 5g, 10g, and 15g of chia seed powder within three hours of eclosion. These flies were then aged for five days. Each virgin female and unmated male was aspirated into a mating chamber and observed for one hour. If mating did not occur within this period, the flies were discarded. For pairs that mated, two parameters were recorded: mating latency (the time elapsed between the introduction of the male and female into the mating chamber and the initiation of copulation) and copulation duration (the time elapsed from the initiation to the termination of copulation for each pair).Successfully mated pairs were then transferred to vials containing their respective media and maintained there, with transfers occurring every seven days until the flies died. The total number of offspring produced by each pair was recorded as a measure of fertility. Fifteen pairs were tested separately for each of the media types: wheat cream agar, and media supplemented with 5g, 10g, and 15g of chia seed powder.

Results

Effect of Chia Seeds Nutritional Supplement on Mating Latency in the *Drosophila melanogaster*.

Figure 1 illustrates the mean mating latency (in minutes) of *D. melanogaster* flies reared on diets supplemented with varying concentrations of chia seed (5g, 10g, and 15g), in comparison to a control diet. The flies raised on the control diet exhibited the highest mating latency. In contrast, flies fed with the 5g chia seed diet demonstrated the lowest latency. Flies fed with 10g and 15g chia seed diets showed intermediate mating latency.
A one-way ANOVA revealed a statistically significant difference in mating latency among the different diet groups. Tukey's post hoc test further indicated that the mating latency in control flies was significantly higher than in all chia seed diet groups. The latency in flies fed 5g chia seed was significantly lower than in those fed 10g and 15g. However, there was no significant difference between the 10g and 15g groups by Tukey's post hoc test.

Figure 1: The effect of different concentration of chia seed supplementation on the Mating latency of *D. melanogaster*. [Control diet- wheat cream agar media; chia seed diet (5g, 10g, 15g concentration)]

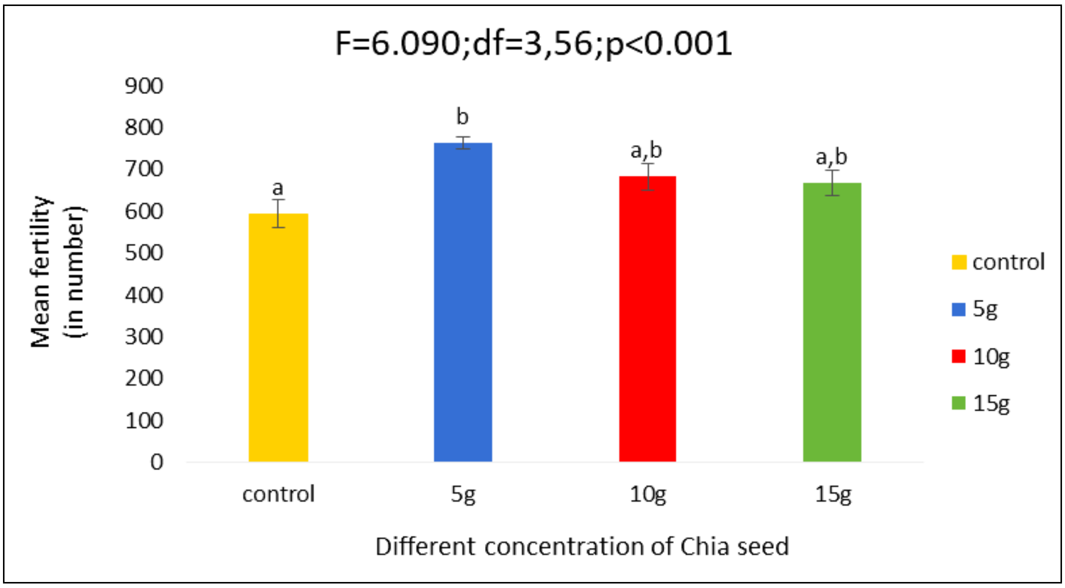

Fig 1: The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Effect of Chia Seeds Nutritional Supplement on Copulation Duration in the *Drosophila melanogaster*.

The mean copulation durations and standard error values for *D. melanogaster* raised on diets with varying concentrations of chia seeds are presented in Figure 2. The results show that flies raised on a diet supplemented with 5g of chia seed exhibited the longest copulation duration, while those on the control diet had the shortest.

A one-way ANOVA followed by Tukey's post hoc test revealed a statistically significant difference in copulation durations among the groups. According to Tukey's post hoc analysis, flies fed a 5g chia seed diet showed significantly longer copulation durations compared to all other groups. In contrast, flies fed with 10g and 15g of chia seed did not differ significantly from each other but had longer durations than the control group. The control group had significantly shorter copulation durations than the 5g group but was not significantly different from the 10g and 15g groups.

Figure 2: The effect of different concentration of chia seed supplementation on the Copulation duration of *D. melanogaster*. [Control diet- wheat cream agar media; chia seed diet (5g, 10g, 15g concentration)]

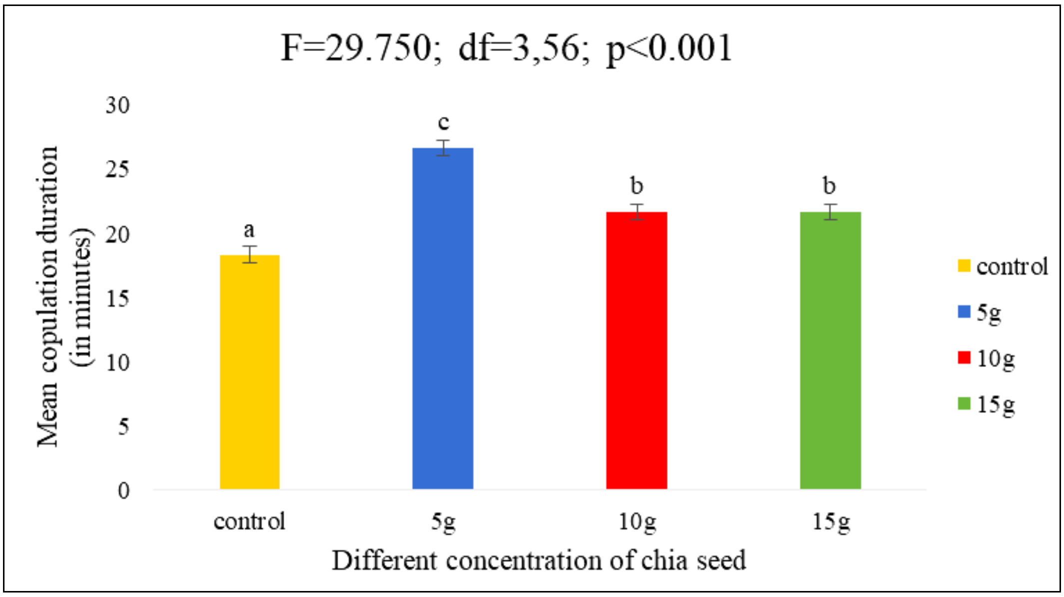

Fig 2: The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Effect of Chia Seeds Nutritional Supplement on Fertility in the *Drosophila melanogaster*.

Figure 3 illustrates the mean fertility and standard error of *D. melanogaster* when cultured on diets supplemented with varying concentrations of chia seed (control, 5g, 10g, and 15g). Based on the results, flies fed with 5g of chia seed exhibited the highest fertility rate, significantly greater than the control group. Fertility rates for flies fed 10g and 15g chia seed were intermediate, showing no significant difference compared to the control or the 5g group.

A one-way ANOVA followed by Tukey's post hoc test revealed a statistically significant difference in fertility among the different dietary treatments. The Tukey test results suggest that the 5g chia seed diet led to significantly higher fertility than the control, while the 10g and 15g treatments showed moderate fertility values, not significantly different from either the control or the 5g group.

Figure 3: The effect of different concentration of chia seed supplementation on the Fertility of *D. melanogaster*. [Control diet- wheat cream agar media; chia seed diet (5g, 10g, 15g concentration)]

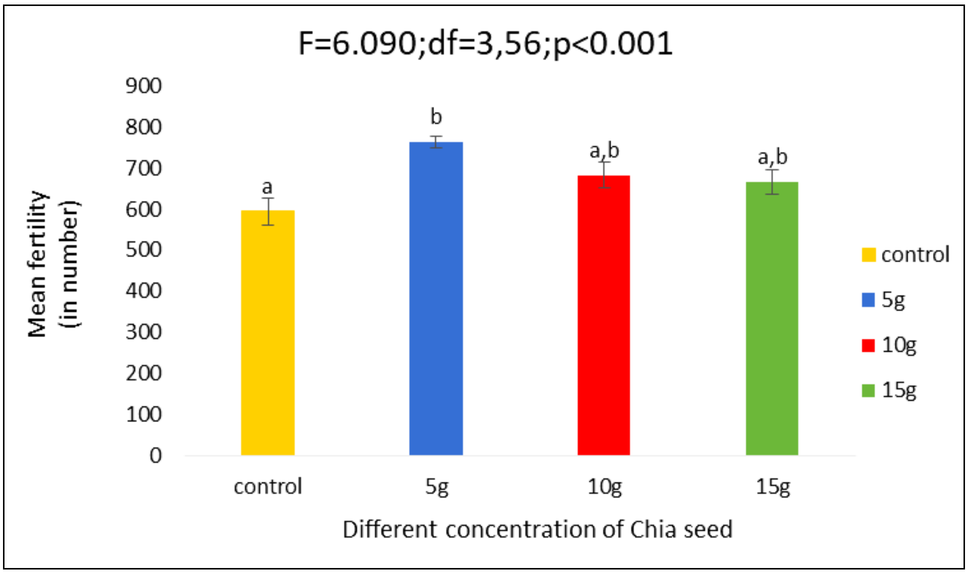

Fig 3: The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Discussion

Reproductive success, energy balance, and health maintenance in animals are closely linked to their nutritional intake. In *D. melanogaster*, the availability and quality of food significantly influence reproductive parameters. The current study explores how different concentrations of chia seed affect reproductive fitness in *D. melanogaster*, focusing specifically on mating latency a key component of mating behavior.

Mating latency refers to the amount of time taken by a male and female *D. melanogaster* from initial interaction to the initiation of copulation. It is an important indicator of mating behavior and reproductive fitness. Shorter mating latency suggests higher male vigor, greater female receptivity, and stronger sexual attraction between mates.

Mating latency is recognized as an important indicator of fitness. Previous studies, such as Prakash (1967) [23], have demonstrated a positive correlation between mating speed and fertility in *D. robusta*. Mating latency also reflects male vigor, female receptivity, and mutual sexual attraction. A shorter latency period generally indicates higher mating vigor (Pathak *et al*., 2011) [20].

In the present study the significant variation in mating latency was noticed between different diet as illustrated in (Figure 1). Flies fed with a 5g chia seed diet exhibited the shortest mating latency, indicating the highest mating speed. This group showed significantly faster mating behavior compared to the control which displayed the longest latency and thus the slowest mating activity. The 10g and 15g chia seed diets resulted in intermediate mating latencies, both significantly lower than the control but higher than the 5g group. These findings suggest that the nutritional profile provided by chia seed supplementation positively influences reproductive behavior, particularly mating speed. Diets rich in chia seed especially at a 5g concentration may enhance mating efficiency in *D. melanogaster*. This is in line with earlier reports by Schultzhaus *et al*. (2017) [28] and Anitha & Krishna (2020) [2], who noted the impact of protein to carbohydrate ratios on mating latency in *Drosophila* species. Overall, the quality of nutrients, such as those in chia seeds, appears to be a crucial factor in determining reproductive fitness in fruit flies.

Copulation duration is another important parameter of reproductive fitness in *D. melanogaster*, reflecting both male reproductive strategy and the potential for successful sperm transfer. Variations in this trait can be influenced by both genetic factors and environmental conditions, including diet.

According to Spiess (1970) [43], copulation represents the culmination of male or female courtship behavior in many animals. The duration of copulation is a critical factor for reproductive success, as it directly influences the amount of sperm transferred to the female's reproductive tract. This ensures greater chances of fertilization and offspring production. Copulatory behavior, including its duration, is known to be affected by several factors such as genotype, environmental conditions, the size and age of both sexes, and strain differences (Guruprasad *et al*., 2008) [10]. In *Drosophila*, as in many other insects, the male typically dictates the duration of copulation (Parsons, 1973) [27]. In the current study (Fig. 2), flies raised on a diet supplemented with different concentrations of chia seeds exhibited significant variation in copulation duration. The highest mean copulation duration was observed in the group fed with 5g of chia seed, followed by the 10g and 15g groups, which showed statistically similar durations. The control group showed the shortest copulation time. These results indicate that the 5g chia seed supplementation significantly enhanced copulation duration compared to other groups. This suggests that chia seed, particularly at 5g concentration, may contain optimal levels of nutrients such as proteins and essential fatty acids that enhance reproductive behaviors in *Drosophila*. This finding aligns with the observations of Anitha and Krishna (2020) [2], who reported that dietary variations in protein and carbohydrate content influence copulation traits. Similarly, previous studies have shown that copulation duration in *Drosophila* is influenced by male body size, age, and diet (Guruprasad *et al*., 2008) [10]; larger males typically exhibit longer mating times (Hegde and Krishna, 1997) [12].

Fertility is a key parameter influencing reproductive fitness in organisms. It is measured as the number of viable offspring produced, is a direct indicator of reproductive success. Nutritional quality plays a crucial role in determining fertility rates, as both male and female reproductive physiology are dependent on adequate nutrient intake.

In the present study (Fig. 3), we examined the effect of different concentrations of chia seed on fertility to evaluate its impact on reproductive potential. Among the different groups, the 5g chia seed supplementation resulted in the highest fertility, significantly greater than the control group. The 10g and 15g concentrations also showed elevated fertility levels compared to the control, although the differences were not statistically distinct from either the control or the 5g group. Overall, fertility followed the pattern: 5g > 10g ≈ 15g > control. These results suggest that dietary chia seed positively influences fertility, particularly at moderate concentrations. This is consistent with previous findings in *D. melanogaster*, where variations in macronutrient composition, such as protein and carbohydrate ratios, influenced reproductive outcomes. High protein content has been associated with increased fertility, while diets high in sucrose tend to reduce reproductive success (Anitha and Krishna, 2020; Ramesh *et al*., 2014) [2, 26].

From the results shown in the copulation duration (Fig. 2) and fertility (Fig. 3) graphs, it can be inferred that the diet containing different concentrations of chia seed had a significant impact on both reproductive parameters. Flies in the control group exhibited the shortest mean copulation duration and correspondingly produced the least number of progeny. This could suggest that shorter copulation times limit the transfer of adequate sperm or seminal components, ultimately resulting in lower fertility. Interestingly, flies fed with 5g of chia seed demonstrated the longest copulation duration which may reflect an attempt to enhance sperm transfer. However, despite this prolonged mating period, the fertility rate was not the highest, but only moderately improved. This indicates that longer copulation alone may not guarantee higher fertility if sufficient accessory gland proteins or sperm are not efficiently transferred. In contrast, flies fed with 10g and 15g chia seed concentrations showed moderate copulation durations but had relatively high fertility outputs. This suggests that even with slightly shorter mating durations compared to the 5g group, these flies might have efficiently transferred greater quantities of sperm and seminal fluids, thereby enhancing reproductive success. These patterns suggest that copulation duration and fertility do not always correlate linearly. While longer copulation can provide more opportunity for sperm transfer, the quality and efficiency of the transfer process potentially influenced by dietary content play a critical role. However, it is important to note that our study did not quantify the actual amounts of sperm or accessory gland proteins transferred during mating. Previous research on *Drosophila* had similarly shown that prolonged copulation is often associated with increased sperm transfer in many insect species (Thornhill and Alcock, 1983) [34]. Furthermore, extended copulation may also serve as a form of mate guarding by males (Parker, 1970; McLain, 1989) [18, 17].

Studies on *Drosophila* species have shown that reproductive fitness can be influenced by factors such as male age (Prathiba *et al*., 2011) [18], female age (Somashekar *et al*., 2011) [42], and environmental conditions like temperature (Santhosh *et al*., 2015) [36] and light also affect reproductive fitness of flies. In the present study, however, flies of the same age were used and kept under identical laboratory conditions, with the only variable being the type of diet they were reared on. Therefore, the observed differences in mating traits such as mating latency, copulation duration and fertility in *D. melanogaster* can be attributed to the availability, quantity, and quality of the nutritional intake.

Conclusion

Dietary supplementation with 5g of chia seed significantly enhanced reproductive fitness in *D. melanogaster*, as evidenced by reduced mating latency, increased copulation duration, and improved fertility. Increasing the concentration beyond 5g did not yield further significant reproductive benefits, suggesting that 5g is the most effective dosage among those tested.

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