

The Effect of Multivitamin supplementation on Reproductive Fitness in *Drosophila melanogaster*.

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Abstract

Nutritional supplementation plays a significant role in influencing reproductive behaviour and fertility in *Drosophila melanogaster*. The present study was carried out to evaluate the effect of multivitamin supplementation at different concentrations on the reproductive fitness of *D. melanogaster*. Experimental flies were cultured on standard wheat cream agar media (control) and media supplemented with multivitamin powder at concentrations of 6.75g (T1) and 13.5g (T2). Reproductive fitness parameters such as mating latency, copulation duration, and fertility were analysed.

The results revealed significant variation in reproductive traits among the experimental groups. Flies reared on T1 (6.75g) multivitamin-supplemented media exhibited the shortest mating latency, indicating faster initiation of mating compared to the control and T2 groups. In contrast, flies raised on T2 (13.5g) media showed the longest copulation duration, whereas the T1 group exhibited the shortest duration. Fertility analysis demonstrated that flies cultured on T1 media produced the highest number of offspring, while flies raised on T2 media showed the lowest fertility among the treatment groups. Statistical analysis using one-way ANOVA followed by Tukey's post hoc test confirmed significant differences among the dietary treatments.

The findings of the present study suggest that moderate multivitamin supplementation enhances reproductive fitness in *D. melanogaster*, whereas higher concentrations may adversely affect certain reproductive parameters. Thus, optimal levels of multivitamin supplementation may positively influence mating behaviour and reproductive success in *D. melanogaster*.

Keywords: *Drosophila melanogaster*, multivitamin supplementation, mating latency, copulation duration, fertility, reproductive fitness.

Introduction

Reproductive fitness is one of the most important biological parameters governing the survival and continuation of species. It is influenced by several physiological and behavioural traits such as mating latency, copulation duration, fertility, fecundity, viability, and lifespan. These reproductive parameters collectively determine the reproductive success of an organism and are therefore considered essential components of evolutionary fitness (Turner and Andersson, 1983). Numerous studies on *Drosophila melanogaster* have demonstrated that reproductive behaviour is highly sensitive to both genetic and environmental factors, particularly nutrition (Parsons, 1973; Banerjee and Singh, 1998).

Among various model organisms, *D. melanogaster* has been extensively used in studies related to reproductive biology, behavioural genetics, nutrition, aging, and evolutionary biology due to its short life cycle, easy maintenance, and well-characterized genome (Markow and O'Grady, 2006). Previous investigations have shown that mating behaviour in *Drosophila* is influenced by several factors including age, body size, environmental conditions, nutrient availability, and dietary composition (Guruprasad et al., 2008; Pathak et al., 2011; Prathiba et al., 2011). Variations in these factors significantly alter mating latency, copulation duration, and fertility, thereby affecting overall reproductive fitness.

Nutrition is considered one of the major environmental factors regulating reproductive success. Several studies have reported that dietary composition directly affects reproductive physiology and sexual behaviour in

Drosophila. Macronutrients such as proteins, carbohydrates, lipids, vitamins, and minerals are essential for maintaining normal reproductive functions and enhancing reproductive performance (Lee et al., 2008; Maklakov et al., 2008; Simpson and Raubenheimer, 2009). Research by Piper et al. (2011) and Solon-Biet et al. (2015) demonstrated that reproductive success is strongly associated with nutrient balance rather than total caloric intake alone. Nutritional quality also affects mate choice, courtship behaviour, sperm transfer, and offspring production in many insect species (Fanson and Taylor, 2011; Schultzhaus et al., 2017).

Vitamins are organic compounds required in small quantities for normal growth, metabolism, physiological maintenance, and reproductive health. Multivitamin supplements generally contain a combination of essential vitamins and minerals that play crucial roles in energy metabolism, cellular function, antioxidant defence, and reproductive processes. Vitamins such as vitamin A, vitamin B complex, vitamin C, vitamin D, and vitamin E are known to regulate gamete production, hormonal balance, cellular repair, and fertility in various organisms (Combs, 2012). Deficiency of these micronutrients often leads to impaired reproductive performance, reduced fertility, and physiological stress.

Recent studies have highlighted the beneficial effects of nutritional supplementation on reproductive fitness in *D. melanogaster*. Dietary supplements such as spirulina (Shreejani et al., 2023), whey protein (Purushotham et al., 2023), mass gainer (Jabbar et al., 2024), Ensure® nutritional supplement (Yashaswini et al., 2024), and chia seeds have shown significant effects on mating behaviour and fertility in *Drosophila*. These findings suggest that nutritional enrichment can modify reproductive parameters by influencing physiological condition and energy allocation.

Although several studies have investigated the effects of protein-rich and nutrient-rich supplements on reproductive fitness, limited information is available regarding the influence of multivitamin supplementation on mating behaviour and fertility in *D. melanogaster*. Since vitamins are essential regulators of metabolic and reproductive pathways, studying their effects on reproductive traits may provide important insights into the relationship between micronutrient availability and reproductive success.

Therefore, the present study was undertaken to investigate the effect of multivitamin supplementation at different concentrations on reproductive fitness in *D. melanogaster*. The study specifically focuses on important reproductive parameters such as mating latency, copulation duration, and fertility in flies reared on multivitamin-supplemented media under controlled laboratory conditions.

Materials and methods

Multivitamin tablets of the MuscleBlaze brand were purchased from Apollo Pharmacy (Mysore, Karnataka). The tablets were finely powdered using a clean mortar and pestle to obtain a uniform mixture. The powdered multivitamin was used for preparing experimental diets at different concentrations.

Establishment of stock

For the present investigation, the experimental Oregon K strain of *Drosophila melanogaster* was procured from the *Drosophila* Stock Centre, Department of Studies in Zoology, University of Mysore, Mysore.

The stock culture was maintained in culture bottles containing wheat cream agar medium. The medium was prepared by boiling 100 g of jaggery, 100 g of wheat cream rava, and 10 g of agar in 1000 ml of distilled water, followed by the addition of 7.5 ml of propionic acid. The flies were reared under controlled laboratory conditions at a temperature of $22 \pm 1^\circ\text{C}$, 70% relative humidity, and a 12 : 12 h light-dark cycle. The above cultured flies were used to establish the experimental flies with different treatment media.

Control media /Wheat cream agar media

This media was prepared by adding 100g of jaggery, 100g of wheat rava powder, 10g of Agar in 1000ml of boiling distilled water. To avoid fungal growth, 7.5ml of Propanoic acid was added.

Experimental Procedure

Virgin female and unmated male flies of *Drosophila melanogaster* were collected from control wheat cream agar media and from media supplemented with different concentrations of multivitamin powder [T1 (6.75g) and T2 (13.5g)] within three hours of eclosion. The collected flies were maintained separately and aged for five days under standardized laboratory conditions.

After aging, one virgin female and one unmated male from the same dietary treatment group were aspirated into a mating chamber without anesthesia and observed continuously for a period of one hour. Pairs that failed to mate within the observation period were discarded. For successfully mating pairs, two reproductive parameters were recorded: mating latency and copulation duration. Mating latency was defined as the time interval between the introduction of male and female flies into the mating chamber and the initiation of copulation. Copulation duration was recorded as the time interval from the initiation to the termination of copulation.

Following copulation, each successfully mated pair was transferred into separate culture vials containing their respective dietary media and maintained under controlled laboratory conditions. The flies were transferred to fresh media every seven days until death. The total number of offspring produced by each mating pair throughout their lifespan was counted and recorded as a measure of fertility.

A total of fifteen pairs were analysed separately for each experimental group, including control wheat cream agar media, T1 (6.75g multivitamin supplementation), and T2 (13.5g multivitamin supplementation).

Result

Figure 1: The effect of different concentrations of multivitamin supplementation on the mating latency of *D. melanogaster*.

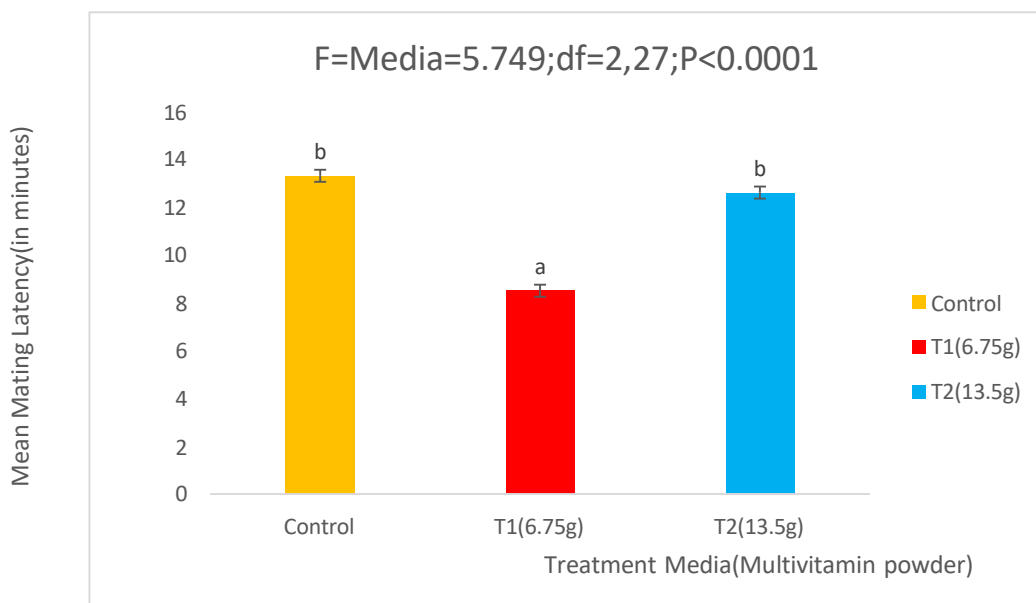


Fig.1: Different letters on the bar graph indicate significant variation between different dietary treatments by Tukey's post hoc test at 0.05 level.

Figure 1 illustrates the mean mating latency (in minutes) of *D. melanogaster* flies reared on diets supplemented with different concentrations of multivitamin powder [T1 (6.75g) and T2 (13.5g)] in comparison with the control diet. Flies raised on the control diet exhibited the highest mating latency. In contrast, flies fed with T1 (6.75g) multivitamin-supplemented diet showed the lowest mating latency. Flies raised on T2 (13.5g) multivitamin-supplemented diet exhibited intermediate mating latency.

A one-way ANOVA revealed a statistically significant difference in mating latency among the different dietary groups. Tukey’s post hoc test further indicated that the mating latency of flies reared on T1 diet was significantly lower than that of control and T2 groups. The control group showed significantly higher mating latency compared to both treatment groups. However, the T2 group exhibited intermediate latency values between control and T1 groups.

Figure 2: The effect of different concentrations of multivitamin supplementation on the copulation duration of *D. melanogaster*.

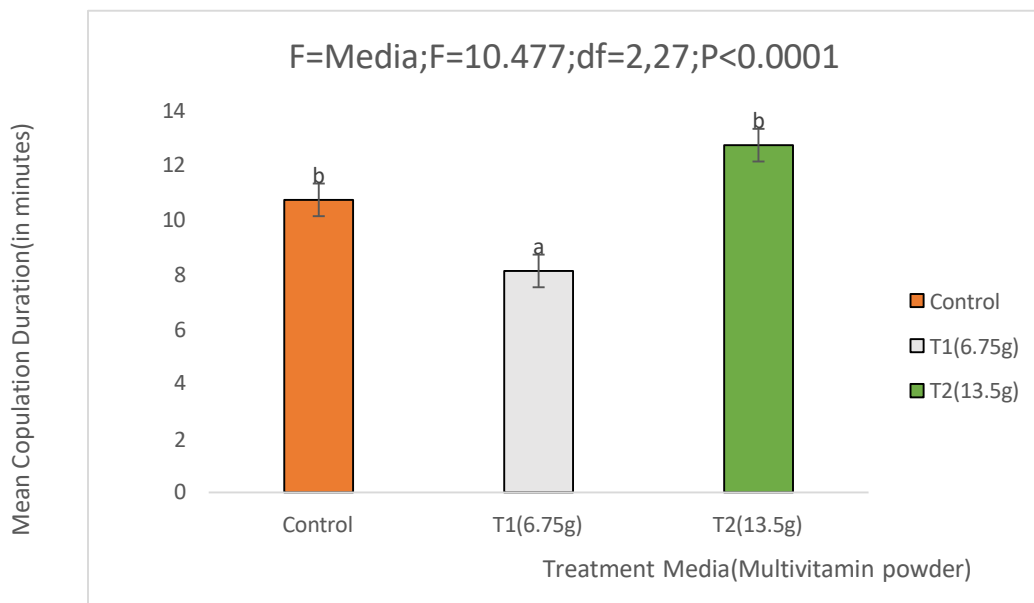


Fig.2: Different letters on the bar graph indicate significant variation between different dietary treatments by Tukey’s post hoc test at 0.05 level.

The mean copulation duration and standard error values of *D. melanogaster* reared on diets supplemented with different concentrations of multivitamin powder are presented in Figure 2. The results indicate that flies raised on T2 (13.5g) multivitamin-supplemented diet exhibited the highest copulation duration, whereas flies reared on T1 (6.75g) diet showed the shortest copulation duration. The control group displayed intermediate copulation duration.

A one-way ANOVA followed by Tukey’s post hoc test revealed statistically significant differences in copulation duration among the experimental groups. According to Tukey’s post hoc analysis, flies reared on T2 diet exhibited significantly longer copulation duration compared to T1 and control groups. The T1 group showed significantly shorter copulation duration than the control and T2 groups. The control group demonstrated intermediate copulation duration between the two treatment groups.

Figure 3: The effect of different concentrations of multivitamin supplementation on the fertility of *D. melanogaster*.

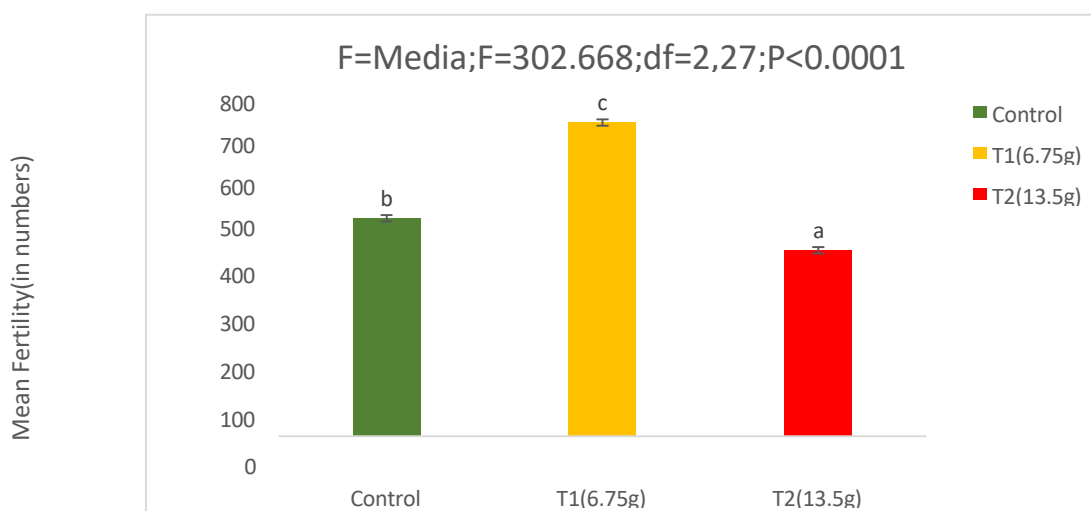


Fig.3: Different letters on the bar graph indicate significant variation between different dietary treatments by Tukey’s post hoc test at 0.05 level.

Figure 3 illustrates the mean fertility and standard error of *D. melanogaster* cultured on diets supplemented with different concentrations of multivitamin powder [control, T1 (6.75g), and T2 (13.5g)]. Based on the results, flies reared on T1 multivitamin-supplemented diet exhibited the highest fertility, whereas flies raised on T2 diet showed the lowest fertility among the treatment groups. The control group displayed intermediate fertility values.

A one-way ANOVA followed by Tukey's post hoc test revealed statistically significant differences in fertility among the different dietary treatments. Tukey's post hoc analysis suggested that flies reared on T1 diet showed significantly higher fertility compared to control and T2 groups. In contrast, flies reared on T2 diet exhibited significantly lower fertility than the T1 group. The control group showed intermediate fertility values between T1 and T2 groups.

Discussion

Reproductive fitness, physiological performance, and survival of organisms are greatly influenced by nutritional availability and dietary quality. In *Drosophila melanogaster*, nutritional composition plays a crucial role in regulating reproductive behaviour, mating success, fertility, and lifespan. Several earlier studies have demonstrated that dietary components significantly affect reproductive parameters such as mating latency, copulation duration, fecundity, and offspring production (Lee et al., 2008; Maklakov et al., 2008; Simpson and Raubenheimer, 2009). Nutrient availability influences energy allocation between maintenance, growth, and reproduction, thereby directly affecting reproductive success (Piper et al., 2011; Solon-Biet et al., 2015). In recent years, nutritional supplementation studies using different dietary components such as spirulina, whey protein, mass gainer, Ensure®, and chia seed have shown significant effects on reproductive fitness in *D. melanogaster* (Shreejani et al., 2023; Purushotham et al., 2023; Jabbar et al., 2024; Yashaswini et al., 2024). The present study investigated the influence of multivitamin supplementation at different concentrations on reproductive fitness parameters including mating latency, copulation duration, and fertility in

D. melanogaster.

In the present study, significant variation in reproductive parameters was observed among flies reared on different concentrations of multivitamin-supplemented diets. Flies cultured on T1 (6.75g) multivitamin diet exhibited the shortest mating latency and the highest fertility, indicating enhanced reproductive performance at moderate supplementation levels. In contrast, flies raised on T2 (13.5g) multivitamin diet showed increased copulation duration but comparatively reduced fertility. The control group generally exhibited intermediate or lower reproductive performance when compared to the treatment groups. These observations suggest that moderate multivitamin supplementation positively influences reproductive behaviour and reproductive success in *D. melanogaster*, whereas excessive supplementation may adversely affect certain reproductive parameters.

Mating latency is an important component of reproductive fitness and reflects male vigor, female receptivity, and mating efficiency. Shorter mating latency generally indicates greater sexual attraction and enhanced reproductive performance (Prakash, 1967; Pathak et al., 2011). Previous studies have demonstrated that nutritional status and dietary composition significantly influence mating latency in *Drosophila* species (Schultzhaus et al., 2017; Anitha and Krishna, 2020). In the present study (Fig. 1), flies reared on T1 (6.75g) multivitamin-supplemented diet exhibited the shortest mating latency, whereas control flies showed the highest latency. The T2 group displayed intermediate latency values. These findings suggest that moderate multivitamin supplementation improves mating efficiency and sexual responsiveness in *D. melanogaster*. Vitamins are known to play essential roles in metabolic activity, neuromuscular coordination, and physiological maintenance, which may contribute to improved courtship behaviour and faster mating initiation. Similar findings were reported by Lee et al. (2008) and Fanson and Taylor (2011), who observed that nutrient-rich diets positively affect reproductive behaviour and mating success in insects.

Copulation duration is another important reproductive parameter associated with sperm transfer efficiency and reproductive success. According to Spiess (1970), copulation behaviour represents an important

reproductive strategy in *Drosophila*, and its duration is influenced by genetic and environmental factors including nutrition. Several studies have shown that dietary quality, male body size, age, and physiological condition affect copulation duration in *Drosophila* species (Guruprasad et al., 2008; Hegde and Krishna, 1997). In the present study (Fig. 2), flies raised on T2 (13.5g) multivitamin-supplemented diet exhibited the longest copulation duration, while flies reared on T1 diet showed the shortest duration. The control group demonstrated intermediate values. Longer copulation duration observed in the T2 group may indicate prolonged sperm transfer or mate guarding behaviour by males, as previously suggested by Parker (1970) and Thornhill and Alcock (1983). However, prolonged copulation duration does not always guarantee enhanced fertility, since reproductive success also depends upon sperm quality, accessory gland proteins, and physiological compatibility between mating partners.

Fertility is considered one of the most important indicators of reproductive fitness and reproductive success. Nutritional quality directly affects gamete production, reproductive physiology, and offspring viability (Ramesh et al., 2014). Previous investigations have shown that balanced nutrient intake significantly enhances fertility in *Drosophila* (Maklakov et al., 2008; Solon-Biet et al., 2015). In the present study (Fig. 3), flies reared on T1 (6.75g)

multivitamin supplementation exhibited the highest fertility, whereas flies reared on T2 (13.5g) diet showed comparatively lower fertility. The control group displayed intermediate fertility levels. These results suggest that moderate multivitamin supplementation provides optimal micronutrient availability necessary for reproductive performance and offspring production. Vitamins are essential for cellular metabolism, enzymatic reactions, reproductive tissue maintenance, and antioxidant protection, all of which contribute to improved fertility. However, excessive supplementation may create nutritional imbalance or physiological stress, thereby reducing reproductive efficiency. Similar observations were reported by Piper et al. (2011), Schultzhaus et al. (2017), and Anitha and Krishna (2020), who demonstrated that optimal nutrient balance is essential for maximum reproductive performance in *Drosophila*.

The relationship between copulation duration and fertility observed in the present study suggests that these parameters may not always correlate directly. Although flies reared on T2 diet showed prolonged copulation duration, they did not exhibit the highest fertility. In contrast, flies raised on T1 diet displayed comparatively shorter copulation duration but produced the highest number of offspring. This indicates that reproductive success depends not only on mating duration but also on the efficiency of sperm transfer, sperm viability, and physiological condition of mating individuals. Similar findings were reported by Thornhill and Alcock (1983) and Parker (1970), who suggested that prolonged mating may function as mate guarding rather than solely increasing fertility. The present findings therefore indicate that moderate multivitamin supplementation enhances reproductive efficiency more effectively than higher concentrations.

Previous studies have demonstrated that reproductive fitness in *Drosophila* can also be influenced by several other factors including age, temperature, body size, environmental conditions, and nutrient composition (Prathiba et al., 2011; Santhosh et al., 2015; Somashekar et al., 2011). In the present investigation, flies of the same age were maintained under identical laboratory conditions, and the only variable factor was dietary supplementation. Therefore, the observed variations in mating latency, copulation duration, and fertility can be attributed primarily to the concentration and availability of multivitamin supplementation in the diet. Overall, the present study demonstrates that moderate multivitamin supplementation positively influences reproductive fitness in *D. melanogaster* and highlights the importance of micronutrient balance in regulating reproductive physiology and reproductive success.

Conclusion

Dietary supplementation with T1 (6.75g) multivitamin significantly enhanced reproductive fitness in *Drosophila melanogaster*, as evidenced by reduced mating latency and increased fertility. Although T2 (13.5g) supplementation increased copulation duration, it did not improve fertility when compared to T1 treatment. The results suggest that moderate multivitamin supplementation positively influences reproductive performance in *D. melanogaster*, whereas higher concentrations may not provide additional reproductive benefits.

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