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The effect of the Ensure® nutrition supplement on the starvation resistance in *Drosophila melanogaster.*

Aishwarya K C, Ashwini M, Shilpashree B M, Suma S, Tanmayi Kishore, Yashaswini S P and*M S Krishna
Department of studies in Zoology, University of Mysore, Manasagangotri,
Mysuru-560006, Karnataka, India.
*Corresponding author email – *drosokrish@gmail.com / bmshilpashree2003@gmail.com*

Abstract

The nutrient quality and quantity have a significant impact on the stress resistance, life cycle characteristics, and reproduction of organisms. Animal life and reproduction depend on maintaining a balance between energy acquisition and expenditure. Adaptability is the ability of an organism to alter its development, physiology, or behavior in response to external factors. The diet plays a crucial role as an external component that influences environmental stress. In the study conducted, *Drosophila melanogaster* flies were cultured in wheat cream agar media, test media (Ensure®), and mixed media to understand the effect of Ensure® nutritional supplement powder on starvation resistance. The results showed that the flies fed with the mixed media had greater starvation resistance than those fed with test media (Ensure®), which exhibited average resistance to starvation, and those raised on wheat cream agar media, which showed the least resistance to starvation. This suggests that the mixed diet provided more energy and nutrients, enabling the flies to withstand starvation for longer periods. Additionally, the study revealed that females were more resistant to starvation than males across all three diets.

In the study conducted, it was found that among mated and virgin *Drosophila melanogaster* flies, virgin males and females were more resistant to starvation than mated males and females across all three diets. The study suggested that the supplementation of Ensure® along with wheat cream agar in the diet in equal proportions provided sufficient energy and food storage to withstand starvation for longer periods. Therefore, the sequence of starvation resistance observed in the study was as follows: mixed media > test media (Ensure ®) > wheat cream agar media.

Keywords: Diet, Starvation resistance, *Drosophila melanogaster*, Mated virgins.

Introduction

Starvation resistance refers to an animal's capacity to endure extended periods of not eating. which, considering that starvation is the most common environmental stress experienced by animals living in habitats where food availability fluctuates and is unpredictable, is a phenotypic characteristic of enormous biological, ecological, and evolutionary significance (McCue, 2010).

Stress resistance, life span, fertility, and life history features are all strongly influenced by the quantity and quality of nutrients that an organism consumes. The body goes into starvation mode when there are extended periods of low-calorie intake (Theo Brenner-Roach, 2018). Following extended fasts, the body starts burning muscle and lean tissue for fuel once it has used up all of its body fat. The majority of animal's experience periods of its body fat. The majority of animal's experience periods of this kind of food scarcity, thus it is predicted that they would develop adaptations to increase their resilience to starvation (Rion and Kaweeki, 2007). Slowing down metabolism and/or building up energy-storing compounds are two ways to become resistant to starvation (Harshman and *et al.*, 1998). The period of death following the start of food deprivation is known as starvation resistance and it is calculated as the final known midline crossing for a particular organism (Bharucha and *et al.*, 2008). Adult flies that have been completely devoid of food can be used to investigate starvation resistance (Kwang and Jang, 2014). Additional supplementation of energy rich nutrients triggers to utilize during starvation (Gomez pinilla, 2008). As a result, research on starvation resistance is crucial for understanding related problems with human health, such as links between dietary intake and the onset and progression of metabolic disorders (Schwasinger Schmidt, 2010). Due to their high degree of evolutionary conservation with vertebrates, *Drosophila* is the finest animal model for studying energy utilization under starvation because it is difficult to examine in humans (Christopher Hardy, *et al*., 2017).

According to Baker and Thummel research, an organism's ability to withstand starvation is always dependent on its physiological reactions, which lead to a more effective use of energy during times of starvation. Triglycerides and glycogen are the two main energy metabolizing molecules that are lipids and carbohydrates. The capacity to withstand starvation is linked to dietary energy storing substances including lipids, proteins and carbohydrates. Storing more energy, particularly in the form of fats and carbohydrates, is the fundamental process that improves survival under conditions of starvation stress. Increased storage of energy utilization and an overall reduction in the minimal resources necessary for survival (Ballard *et al*., 2008).

The primary research organism utilized to examine the nearest and ultimate reasons of starvation resistance has been the fruit fly *D. melanogaster* (Gibbs and Reynolds, 2012; Hoffmann and Harshman, 1999; Rion and Kaweeki, 2007). An examination of starvation resistance has been conducted in many selected lines of *D. melanogaster*. The majority of research on *Drosophila's* starvation resistance has examined fruit flies genetic or evolutionary responses to sudden food shortages at the species, population and intrapopulation levels (Hoffman and parsons, 1991; Hoffman and Harshman, 1999; Matzkin *et al.*, 2009). The dipteran *Drosophila* serves as an effective model organism for investigations on stress responses in adaptive evolution. Between humans and Drosophila, the metabolic pathways and molecular roles of certain components in food absorption, transport and storage are conserved (Baker and Thummel, 2007; Ruden *et al.*, 2005). *Drosophila* artificial selection experiments have shown patterns of variation both within and across populations (Hoffmann and Harshman, 1999; Zera and Harshman, 2021). Twenty-three distinct species of *Drosophila*, including those in the group's *melanogaster, obscura, willistoni, saltens, nanoptera* and *replete,* have been the subject of starvation resistance studies (Matzkin *et al.*, 2009). According to the results, *D. pseudoobscura* and *D. simulans* had poor starvation resistance capacity. Male *D. melanogaster* flies with increased hunger resistance were seen in a research on starvation resistance in flies raised on a semolina-jaggery diet (Chattopadhyay *et al.*, 2015).

People may often feel weak and exhausted as they age due to a decrease in bone density and muscle mass. In order to enhance bone and muscular development, immunological function, and overall health. Ensure® is a comprehensive, well balanced diet that includes HMB, macronutrients (high quality protein, fat and carbohydrates) and micronutrients (vitamins and minerals). It is the world's most popular nutritional supplement drink. Ensure® provides thirty-two nutrients, including high quality protein, calcium, zinc, vitamin C, vitamin D and iron. It also contains a unique substance called HMB. HMB, or β-Hydroxy-β-Methyl butyrate, is an amino acid that promotes and preserves muscle growth. Although it includes eleven immune boosting nutrients-vitamins A, C, E, B6, iron, D, Folate, Zinc, and copper-Ensure® is a high protein nutrient, the peoples of all ages take this drink (Roland and Roy Curtiss, 2015).

There is no published data about the impact of Ensure® nutritional supplements on an organism's ability to withstand environmental stressors like starvation resistance. Therefore, present study has been undertaken in *Drosophila* to study the effect of Ensure® on the starvation.

Materials and methods

The Ensure® nutritional supplement powder was purchased from Medplus pharmacy shop, Sriramapura, Mysuru, Karnataka, India. This Ensure® nutritional supplement powder was used to prepare the experimental media.

Establishment of stock

Experimental Oregon K strain of *Drosophila melanogaster* used in the study was collected from *Drosophila* stock center. Department of studies in Zoology, University of Mysore, Mysuru and this stock was cultured in bottles containing wheat cream agar media (100g of jaggery, 100g of wheat cream rava, 10g of Agar was boiled in 1000ml distilled water and 7.5ml of propionic acid was added). Flies were maintained in laboratory conditions such as humidity of 70% and 12 hours dark 12 hours light cycles and temperature 220± 10ºc.

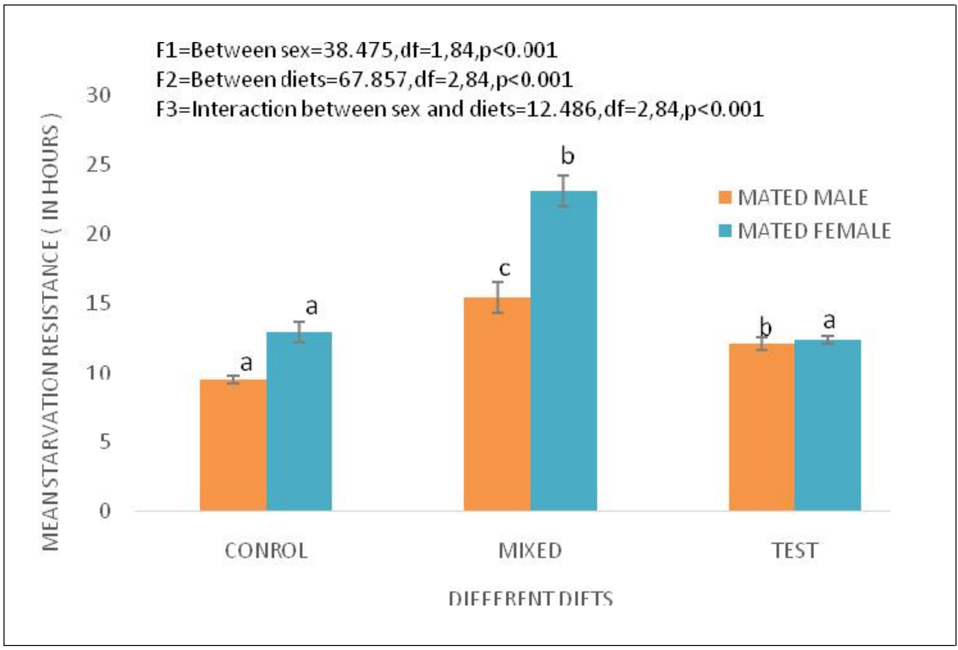
The flies obtained as above were used to establish the experimental stock with different diet media [**Wheat cream agar media:** Wheat cream agar media was prepared from 50g of jaggery, 50g of wheat cream rava powder, 5g of agar boiled in 500ml distilled water and 3.5ml of propionic acid added to it.; **Ensure® nutritional supplement (referred as Ensure®) media:** Ensure® media was prepared from 50g of jaggery, 50g of Ensure® nutritional supplement powder, 5g of agar boiled in 500ml of distilled water and 3.5ml of propionic acid added to it; **Mixed(Wheat cream+ Ensure®) media:** Mixed media is prepared from 50g of jaggery, 25g of wheat cream powder and 25g of Ensure® nutritional supplement powder, 5g of agar boiled in 500ml of distilled water and 3.5ml of propionic acid added to it.]. The flies emerged from the wheat cream agar media and other experimental treated media were maintained under the same laboratory conditions as mentioned above. These flies were used to study the starvation resistance experiment in *D. melanogaster*.

Experimental procedure

Starvation resistance: To study starvation resistance five days old unmated(virgins) and mated flies obtained from Ensure®. Wheat cream agar, and mixed diet were used to. Fifteen flies (male and female) obtained from test media (Ensure®), mixed media, control media (wheat cream agar media) were transferred to a new empty vial and plugged with a cotton. These vials were kept at 25ºc under constant light condition and resistance to starvation of each fly was observed in 2 hours of intervals until its death. A total of three replicates were carried out of each of the wheat cream agar, Ensure® media and mixed diet.

Results

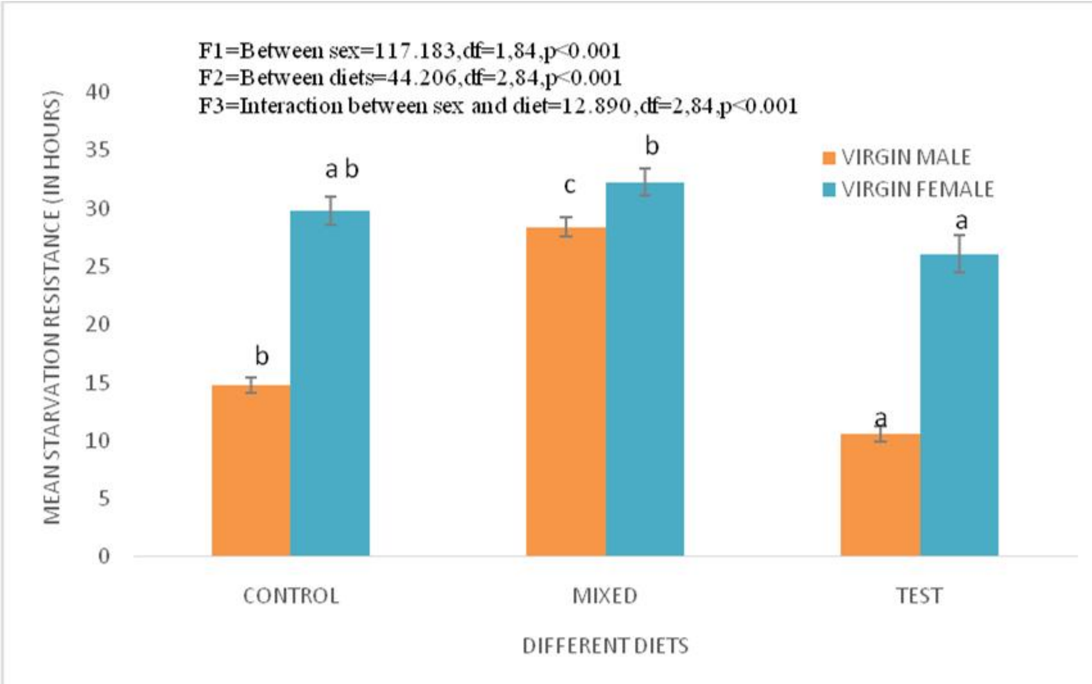
Figure 1: Effect of the Ensure® nutritional supplement on the starvation resistance in the mated male and female of *D. melanogaster.*



The different letters on the bar graph indicates the significant variation between the different diet by Tukey's post Hoc test at 0.05 level.

The mean and standard error value of the starvation resistance of mated male and female flies raised with Ensure®, mixed and wheat cream agar media are provided in the figure 1. According to diet it was noticed that starvation resistance was greater in the mixed media compared to the wheat cream agar and Ensure® diet. The result was found that the mated female had the greater starvation resistance than mated males in different diet. The above data were subjected to the two-way ANOVA followed by the Tukey's post hoc test showed the significant variation between the diets and sexes. However, non-significant variation observed between the male and female of wheat cream agar media compared to the mixed diet which had the significant variation between the male and female as well as the Ensure® diet flies had the non-significant with wheat cream agar and mixed diet.

Figure 2: Effect of Ensure® nutritional supplement in the virgin male and female of *D. melanogaster*.

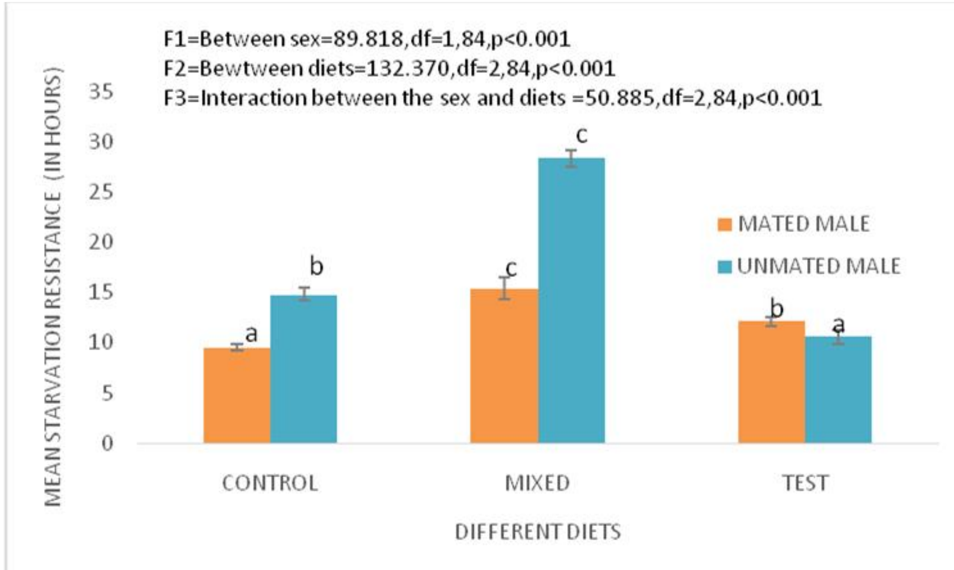


The different letters on bar graph indicates the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

The mean and standard error value of the starvation resistance of unmated male and female flies raised with Ensure®, mixed and wheat cream agar media are provided in the figure 2. According to data it was noticed that starvation resistance was greater in the mixed media compared to the wheat cream agar and Ensure® diet. The result was found that the unmated female had the greater starvation resistance than unmated males in different diet.

The above data were subjected to the two-way ANOVA followed by the Tukey's post hoctest showed the significant variation between the diets, sexes and also interaction between the sexes and diets. Non-significant variation observed between the females of control and mixed media and males had the significant variation between the different diets.

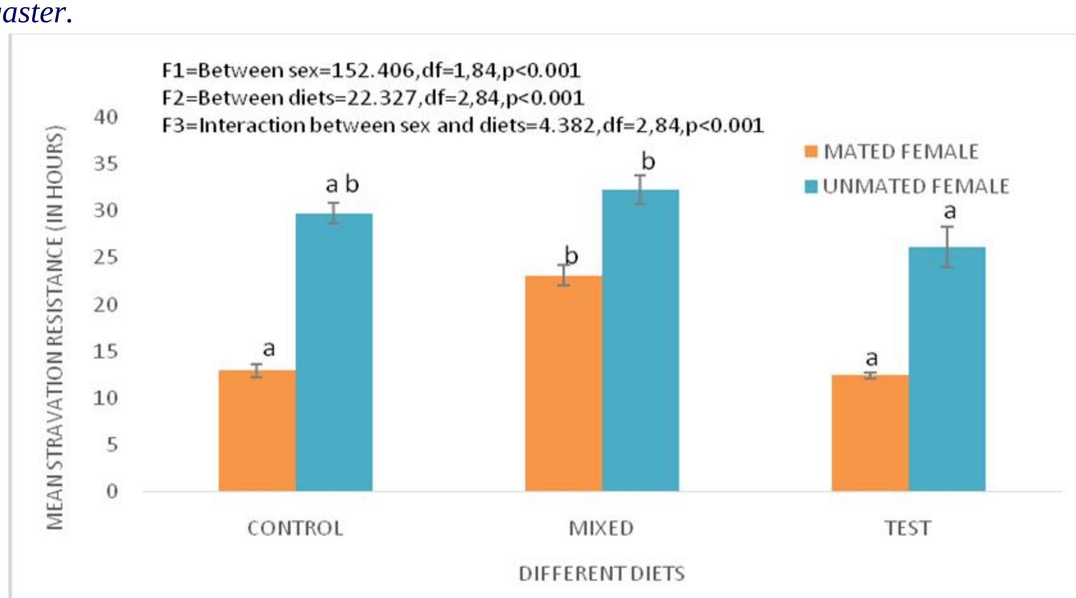
Figure 3: Effect of the Ensure® nutritional supplement on the starvation resistance on the mated male and unmated male of *D. melanogaster.*



The different letters on bar graph indicates the significant variation between the different diet by tukey's post hoc test are 0.05 level.

The mean and standard error value of the starvation resistance of mated male and unmated male flies raised with Ensure®, mixed and wheat cream agar media are provided in the figure 3. According to data it was noticed that starvation resistance was greater in the mixed diet compared to the wheat cream agar and Ensure® diet. The result was found that the unmated male had the greater starvation resistance than mated males in different diet. The above data were subjected to the two ANOVA followed by the Tukey's post hoctest showed that significant variation between the diet and sexes. Significant variation observed between the mated males of the mixed and wheat cream agar diet whereas Ensure® diet had the nonsignificant variation with mixed and wheat cream agar diet. The unmated males had the non-significant variation in the different diets.

Figure 4: Effect of Ensure® nutritional supplement on starvation resistance in unmated and mated female of *D. melanogaster*.



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

The mean and standard error value of the starvation resistance of mated male and unmated female flies raised with Ensure®, mixed and wheat cream agar media are provided in the figure 4. According to the data obtained the starvation resistance was greater in the mixed media compared to the control (wheat cream agar) and test media (Ensure® nutritional supplement). The result was found that the unmated females had the greater starvation resistance than the mated females in different diets.

The data were subjected to two-way ANOVA followed by Tukey's post Hoc test showed the non- significant variation between the diets, sex and interaction between the diets and sexes. However, non-significant variation observed between the mated female and unmated female of (wheat cream agar) control media, mixed media diet as well as the Ensure® nutritional supplement had the non-significant variation between the sexes and diets.

Discussion

Starvation resistance is caused by a number of variables, including sex, food, physiology, development and environmental circumstances (Sisodia and Singh, 2012; Sebastian and Krishna, 2023; Kiran and Krishna, 2023). A frequent abiotic stress on organisms is change in food supply and quality, which is caused by variations in food availability and its nutrients across various diets (Rion and Kaweeki, 2007). Furthermore, nutritional quality and quantity in the diet are critical for preventing environmental stressors like starvation. The Ensure® nutrition supplement powder contains the various nutrients (per 100g) such as 62.38g of carbohydrates, 15.10g of protein, 422 Kcal of energy, 11.50g of fat, 421mg of calcium, 8.00mg of iron and 35g of total sugars. Therefore, present study was undertaken to understand the effect of Ensure® nutritional supplement on the starvation resistance using *D. melanogaster* as a model organism.

The results of our experiment (Fig 1-4) demonstrate that the flies fed with mixed media exhibited higher resistance to starvation compared to control and test media (Ensure®). In the mixed media, in the ratio of carbohydrate and protein, carbohydrate content was higher compared to control and test media there by supporting flies to resist starvation. Our research also supports the findings of Sisodia and Singh (2012), which examined how food nutrients affect *D. ananassae* ability to withstand starvation. North Indian population of *D. ananassae*, which fed on protein enriched fruits had lowest starvation resistance compared to South Indian flies which fed with carbohydrates. The findings indicated that flies raised on a diet high in carbohydrate exhibited greater starvation resistance than those raised on a high protein diet. Physiological changes necessary for increased starvation resistance will probably come at the expense of other fitness-related features. Further Lee and Jang (2014), discovered that *Drosophila* were better able to resist starvation by sequestering more lipid reserves when fed on diets containing higher proportions of carbohydrate. Thus, the observed difference in starvation resistance across control, mixed and test media was caused by variations in the quantity and quality of nutrients present in each of these media. In *D. melanogaster* studies have also been carried out using various products such as Jeeni Millet traditional mix (Kiran and Krishna, 2023), and spirulina supplement on starvation resistance. These studies have also pointed out that quantity and quality of nutrients in the diet had significant influence on the starvation resistance. Further these studies have also mentioned that flies fed with high protein diet had significantly greater starvation resistance compared with other diets.

Figure 1-2, revealed that both unmated and mated female flies were significantly had greater starvation resistance than those of both mated and unmated male flies in the three different diets i.e., test diet, wheat cream agar and mixed diet. Our research supports the studies of Kiran and Krishna, (2023); Sebastian and Krishna, (2023); Shreeraksha *et al.*, (2023). In their research also, the female flies showed greater resistance to starvation compared to males. The result can explain in the following way, female flies are larger than the male flies due to the accumulation of more lipids and glycogen. During starvation, these stored lipids and glycogen metabolized (breakdown) to supply energy. The female utilizes energy more effectively (Kiran and Krishna, 2023). Hoffman and Harshman (1999) explained in relation to the body weight, according to them body weight is an indicator, that measure the total amount of energy stored chemicals that an organism is carrying. Lee and Jang, (2014) confirmed that when the lipid reserves in the body of flies were completely exhausted, death due to starvation occurred. According to Shreeraksha *et al.,* (2023), the fact that females use the lipid and glycogen contents as an energy source may explain why they exhibit greater tolerance to starvation. However, the starved male exhibit reduced resistance to starvation as a result of breaking down body lipids as a source of stored energy. Earlier studies in *Drosophila* (Matzkin *et al.*, 2009) revealed sex-specific patterns of starvation resistance in fifteen distinct species of *Drosophila* genus. The pattern of sexual dimorphism in starvation resistance has been shown to vary greatly even within *D. melanogaster,* depending on factors like as age, strain, mating status, and test circumstance (Service, 1989; Huey *et al.*, 2004; Vermeulen *et al.,* 2006; Matzkin, 2009). Many organisms exhibit sex-specific expressions of starvation resistance as a result of the nutritional needs that differ between males and females. In the present study we have used same strain, age and media including environmental factors, only differences were sex. Therefore, observed result in starvation resistance was due to the sex of the individual.

In the present study we also conducted effect of starvation resistance on the mated males and females, in figure-2, our study revealed that mated females flies have significantly greater resistance to starvation. The post mating increase in food consumption and corresponding increase in fat storage are the primary factors contributing to the rise in starvation resistance in female flies (Rush et.al., 2007; Sebastian and Krishna, 2023; Harshitha and Krishna, 2023). The transfer of male seminal fluid peptides to female *Drosophila* at the time of copulation also increases the resistance to starvation (Kiran and Krishna, 2023). More recent studies have also demonstrated that mating cause the female *Drosophila* midgut to expand considerably, which enables mated females to satisfy their higher energy requirements for egg production by optimizing their post- ingestive nutrition use (Reiff *et.al.,* 2015).

Additionally, we investigated the differences is starvation resistance between mated and unmated (virgin) flies in this study. The results showed that the unmated males were more resistant to starvation than the mated males (figure-3), this is due to the fact that mated males lose energy during mating and transfer sperm and accessory gland proteins (ACPs); in contrast, unmated males do not lose energy or sperm, which may lead to increased starvation resistance in unmated males. Male starvation tolerance was impacted by mating status differently (Kiran and Krishna, 2023).

Figure-4, showed that the starvation resistance of mated and unmated females differed from one another. In other words, in the three distinct diets-test media (Ensure®), mixed and wheat cream agar media-unmated females showed more substantial resistance to starvation than mated females. Because mating is an energy expensive process, the mated female might have lost most of the energy during copulation, hence unmated females showed greater resistance to starvation. Our result supports the studies of Kiran and Krishna, (2023). They reasoned in a following way, the cost of mating in females is attributed to seminal fluid products from the main cells of the male accessory gland and increasing exposure to these products increase the death rate of females and may cause them to die of starvation earlier than unmated females. This could be one explanation for the observed results, as reported by Chapman *et al.,* (1996). Although a recent research found that oosorption- the autophagic reabsorption of oocyte- may indicate that virgin female flies are more resistant to starvation than mated females, it is still possible that virgin females employ the nutrients reabsorbed from eggs to tolerate fasting (Kiran and Krishna, 2023). The survival of mated female *D. melanogaster* flies during starvation may be lower than that of virgin female flies due to the fact that was demonstrated by Fowler and Partridge, (1989) that exposure to male flies, mating and remating results in a reduced life span in the female. Himuro and Fujisaki, (2010) research on the seed bug *Togo hemipterus*, which revealed that virgins longer than non-virgins under starving conditions, further corroborated our finding. Contrary to our findings, a number of other researches (Sebastian and Krishna, 2023; Shreeraksha *et al.,* 2023) revealed that mated females had a higher tolerance for starvation than virgins. It has been demonstrated that food intake increases in *Drosophila* females after mating (Carvalho *et al.,* 2006), among other female post-mating responses (Ravi Ram and Wolfner, 2007). In fact, these findings may offer a physiological explanation for the variations in starvation resistance between mated and unmated females.

In our research, we have considered individuals of the same age, strain as well as environmental factors. The variation in our results can be attributed to the quality and quantity of the nutrients present in the different diets. There by, starvation resistance is influenced by the amount of nutrients present in the diet.

Thus, from our study it was noticed that the test media (Ensure® nutritional supplement) decreases starvation resistance in *D. melanogaster*. Further sexual dimorphism in starvation resistance was observed in that female flies showed greater resistant to starvation compared to male flies. Furthermore, we found variation in starvation resistance between unmated and mated, males and females.

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