



## **Whey protein increases starvation resistance in *Drosophila melanogaster***

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### **Abstract**

Stress is any change in environment that reduces the fitness of an organism. Environmental stress can be influenced by external environmental factors like diet. Food is crucial for the organisms for their growth, development, health, reproduction and survival. The quality and quantity of nutrients in different diets influence the variation in the starvation resistance. The current study demonstrates the effect of the Whey protein on the starvation resistance in *Drosophila melanogaster* which is cultured in Wheat cream agar media, Mixed media and Whey protein media. The results suggest that, flies fed with Whey protein media had greater starvation resistance than Wheat cream agar and mixed media. This shows that Whey protein provides sufficient nutrition to withstand starvation. This study also shows that females (virgin and mated) have more starvation resistance than that of males (virgin and mated) in all the diets. Variation in the starvation resistance was seen in both mated and unmated male and female flies. Thus, the starvation resistance was greater in flies cultured in Whey protein media.

**Keywords:** Diet, Starvation resistance, *Drosophila melanogaster*, Whey protein, mated, unmated.

### **Introduction**

Starvation resistance is a crucial as well as a common stressor with ecological, organismal, and evolutionary importance (McCue, 2010). An animal is only considered starvation resistant if it can withstand extended intervals of food deprivation. It is a phenotypic trait determined by the duration an animal can survive in food

deprived condition (Hoffmann and Harshman, 1999). It is a quantitative trait which exhibits Significant genetic diversity in both natural populations (van Herrewege and David, 1997; Karan and Parkash, 1998) as well as response to artificial selection (Harshman and Schmid, 1998; Harshman *et al.*, 1999). It is unknown if the selection takes place in developmentally specified phases or is maintained through development,

however starvation resistance is likely influenced by developmental processes that contribute to an organism's size, metabolic characteristics, and brain function (Brown *et al.*, 2019).

The physiological capacity of an animal to withstand food deprivation depends on their nutritional status and dietary background; the primary environmental factor influencing starvation resistance is the nutritional composition of food (Jensen *et al.*, 2010; Laparie *et al.*, 2012). Individuals of many species have to suffer from starvation or inadequate nutrition. Positive selection favoring resistance to starvation stress is expected in environments where food supplies are likely to be scarcer or more erratic in the short term. When insects are fed a diet that is nutritionally unbalanced, compensatory feeding for the limiting component results in an excess of other nutrients being consumed, as is often the case when they are restricted to meals low in protein compared to carbohydrates (P:C) (Raubenheimer and Simpson, 1999). This could lead to an increase in fat accumulation and a loss in fitness (Simpson *et al.*, 2004; Warbrick-Smith *et al.*, 2006).

A potent organism used to examine starvation resistance is *Drosophila melanogaster*, a fruit fly (Rion and Kawecki, 2007; Gibbs and Reynolds, 2012). Due to significant genetic heterogeneity in natural populations of *D. melanogaster*, laboratory selection tests have demonstrated a quick response for starvation resistance (Chippindale *et al.*, 1996; Baldal *et al.*, 2006). Temperature has significant impact on starvation resistance (Gibbs and Reynolds, 2012). According to Sisodia and Singh (2010), stress resistance qualities in *Drosophila* frequently differ across latitudinal clines, suggesting that selection either directly or indirectly influences resistance traits. Due to its short generation period and ease of care, *D. melanogaster* has made it possible to conduct experimental evolution research on starvation resistance.

Whey protein use a high-quality protein powder from cow's milk. Milk has two proteins: casein (approximately 80%) and whey protein

(approximately 20%). Whey contains less than 1% proteins comprising mainly  $\alpha$ -lactoglobulin ( $\alpha$ -LG),  $\beta$ -lactalbumin ( $\beta$ -LA), bovine serum albumin (BSA), immunoglobulins and protease peptone, as well as several minor proteins including lactoferrin, lactollin, glycoproteins, lactoperoxidases and transferrin. Whey proteins remain soluble at pH 4.6 and 20<sup>0</sup>C after removal of casein from milk. Whey is highly bioavailable and boasts having the highest biological value (BV) of any protein source. Therefore, it is an excellent choice for building body mass. It is used in various products like infant formulas, food supplements, sport bars and beverages to meet variety of health goals for people of all ages.

Now a days people are using Whey protein to boost muscle protein synthesis and to build lean muscle mass. Muscle Asylum Premium Whey protein contain following nutritional value per 40g: 24g protein, 5.2g BCAAs, low carbohydrate with no sugar. It also contains digestive enzymes and flavor (banana). It provides essential amino acids like isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, EAAs, SEAAs, and glutamic acid. All studies show how it is helpful in health but there is no evidence documented about how it affect starvation resistance. Therefore, the study under taken to address the effects of The Whey protein on the starvation resistance in *D. melanogaster*.

## **Materials and Methods**

Muscle Asylum Premium Whey protein, Banana flavor was purchased from online platform supplied from H2H Innovations Pvt.Ltd. distributors.

### **Establishment of stocks**

*D. melanogaster* of Oregano K strain were collected from the Drosophila Stock Centre, Department of studies in Zoology, University of Mysore, Manasagangotri, Mysuru, Karnataka, India. The collected flies were cultured in wheat cream agar media (100g of jaggery, 100g of wheat rava powder, 10g of Agar in 1000ml of boiling distilled water. To avoid fungal growth,

7.5ml of Propionic acid was added). These flies were maintained in laboratory condition like temperature around  $22\pm 1^{\circ}\text{C}$ , humidity around 70% RH and with 12:12 dark and light cycle (Photoperiodism). The obtained flies were used in establishment of experimental stock with different 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 Propionic acid was added.

### **Whey protein media/ treated media**

It was prepared by adding 100g of jaggery, 100g of Muscle Asylum Premium Whey protein (Banana flavor) powder, 10g of Agar in 1000ml of boiling distilled water and 7.5ml of Propionic acid.

### **Mixed media**

It was prepared by adding 100g of jaggery, 50g of Wheat rava powder, 50g of Muscle Asylum Premium Whey protein (Banana flavor) powder, 10g of Agar in 1000ml of boiling distilled water and 7.5ml of Propionic acid.

Flies were transferred and maintained in same laboratory condition as mentioned above.

### **Starvation Resistance**

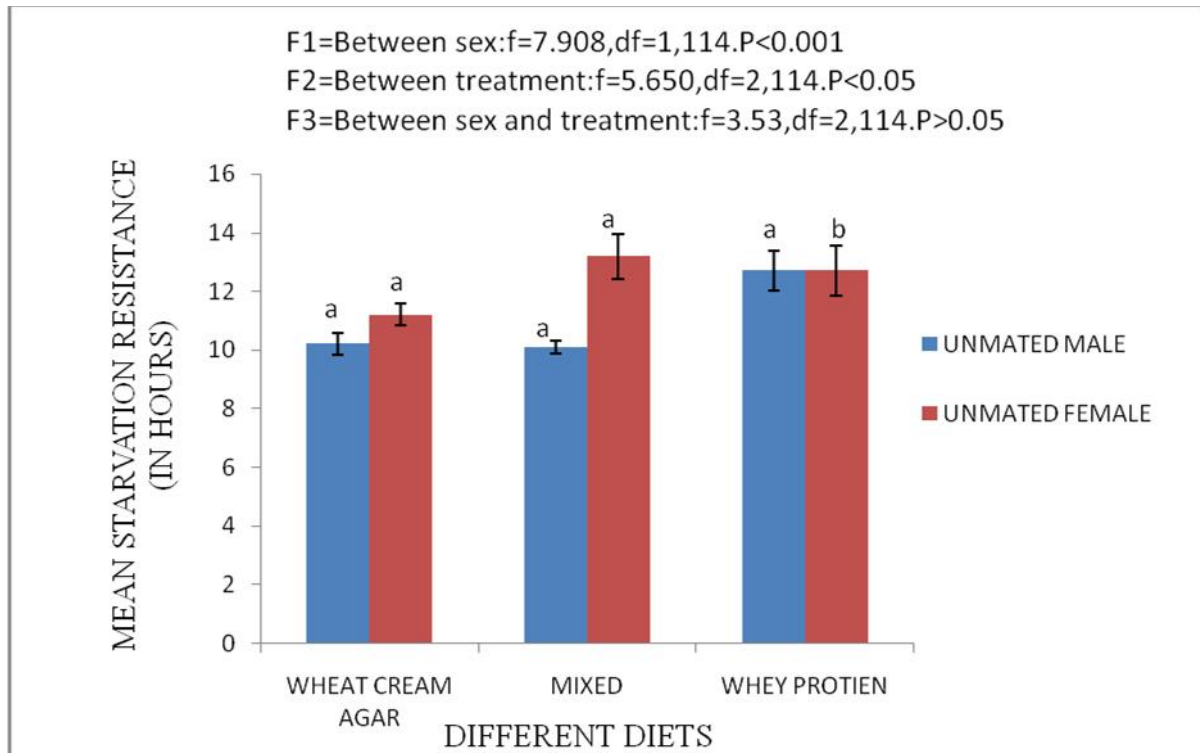
For studying starvation resistance, both virgin and mated 5 days old flies were taken from control, mixed and treated media. Twenty flies of each sex (male and female) from each of control, mixed and treated media were transferred to empty vials (each vial with 5 flies) which are plugged with cotton. These vials were kept in laboratory condition as mentioned above and the resistance towards the starvation of each fly was observed in the time interval of 1 hour until its death. Separate experiments were carried for mated and unmated flies.

## **Results**

### **Unmated flies**

**Fig.1** showed the mean and standard error value of starvation resistance in unmated male and unmated female flies cultured in Wheat cream agar media, Mixed media and Whey protein media. This data show that unmated female flies had more starvation resistance than unmated male flies in all provided diets. Among unmated females, flies cultured in Mixed media showed highest and flies cultured in Wheat cream agar diet showed the least starvation resistance. Among unmated males, flies cultured in Whey protein showed highest and more or less same starvation resistance in other two diets. Both unmated male and female flies cultured in Whey protein showed equal amount of starvation resistance. The above starvation resistance data subjected to two-way ANOVA followed by Tukey's Post hoc test showed significant variation in time taken by unmated male and female flies cultured in control, mixed and treated diet to survive in food deprived condition. However, insignificant variation in starvation resistance was noticed interaction between treatment and sex. Tukey's post hoc test showed significant difference in Starvation resistance between control, mixed and Whey protein media.

**Figure 1: Effect of Whey protein on starvation resistance in Unmated male and female of *Drosophila melanogaster***



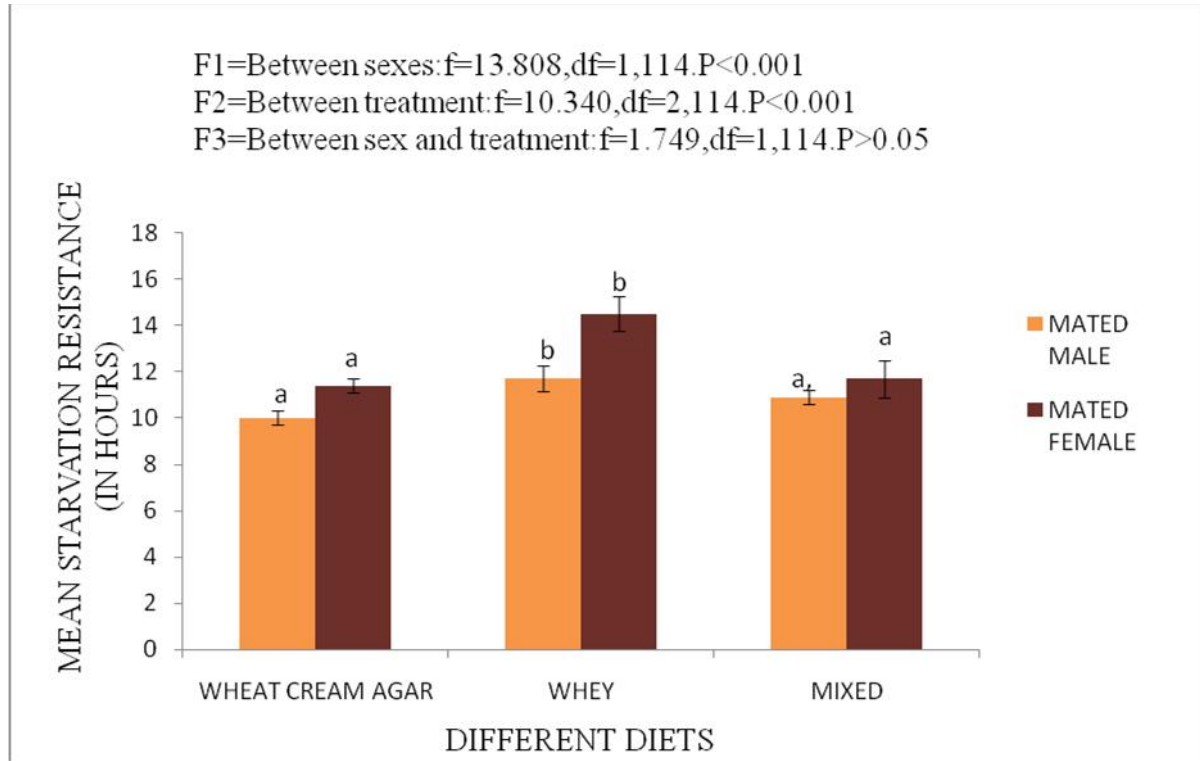
The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey’s Post Hoc test.

**Mated flies**

The mean and standard error value of starvation resistance in mated male and mated female flies cultured in Wheat cream agar media, Mixed media and Whey protein media are shown in **Fig.2**. This data showed that mated female had shown greater starvation resistance than mated males in all the diet provided. Among mated females, flies raised in Whey protein media showed greater starvation resistance compared to more or less same in Mixed and Wheat cream agar media. Among mated males, flies cultured in

Whey showed highest starvation resistance than others. The above starvation resistance data subjected to two-way ANOVA followed by Tukey’s Post hoc test showed significant variation in time taken by mated male and female flies cultured in control, mixed and treated diet to survive in food deprived condition. However, insignificant variation in starvation resistance was noticed interaction between treatment and sex. Tukey’s post hoc test showed significant difference in Starvation resistance between control, mixed and Whey protein media.

**Figure 2: Effect of Whey protein on starvation resistance in Mated male and female of *Drosophila melanogaster***



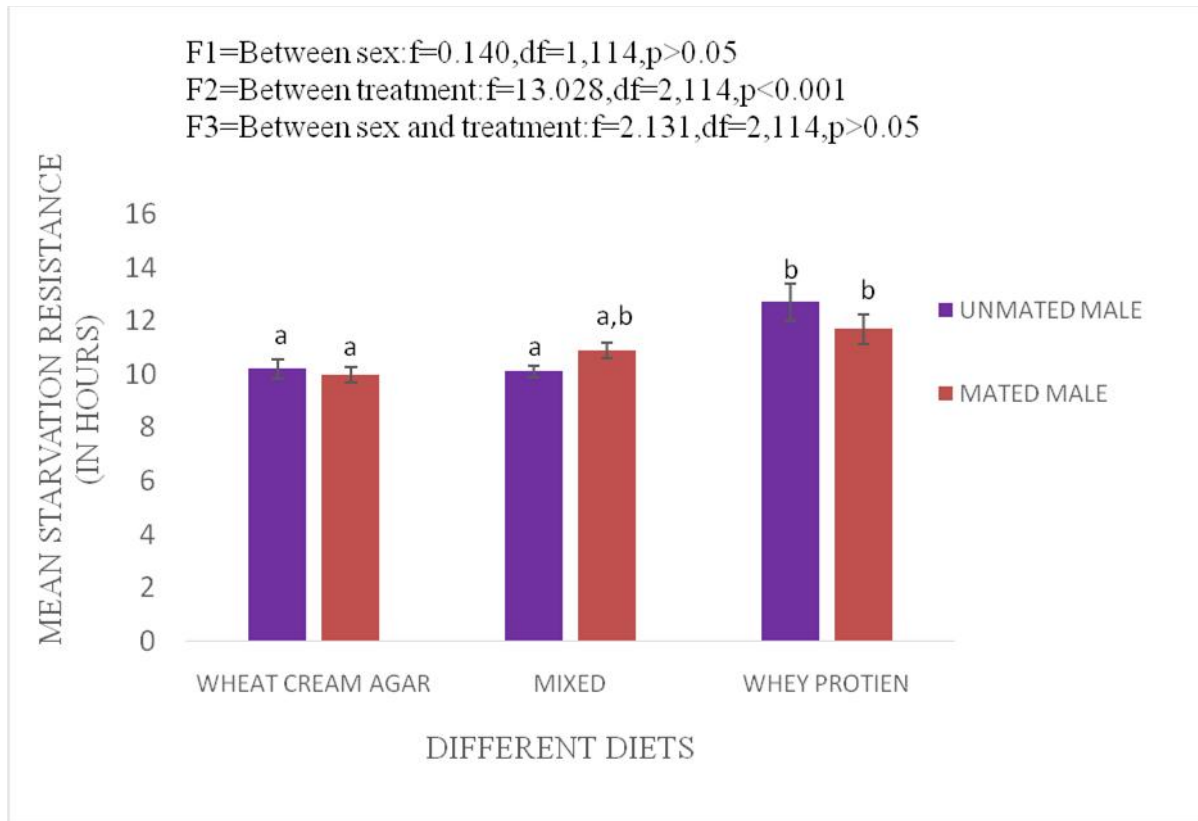
The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey’s Post Hoc test.

**Unmated male and mated male flies**

In **Fig. 3** the mean and standard error value of starvation resistance in unmated male and mated male flies cultured in Wheat cream agar media, Mixed media and Whey protein media is represented. This data showed that starvation resistance was greater in both unmated and mated male flies raised in Whey protein media compared to Mixed and Wheat cream agar media. Among mated males, flies raised in Wheat cream agar media showed less starvation and unmated male flies raised in both Wheat cream agar and Mixed

media showed more or less equal starvation resistance. The above starvation resistance data subjected to two-way ANOVA followed by Tukey’s Post hoc test showed significant variation in time taken by mated male and female flies cultured in control, mixed and treated diet to survive in food deprived condition. However, insignificant variation in starvation resistance was noticed interaction between treatment and sex. Tukey’s post hoc test showed significant difference in Starvation resistance between control, mixed and Whey protein media.

**Figure 3: Effect of Whey protein on starvation resistance in Mated male and female of *Drosophila melanogaster***



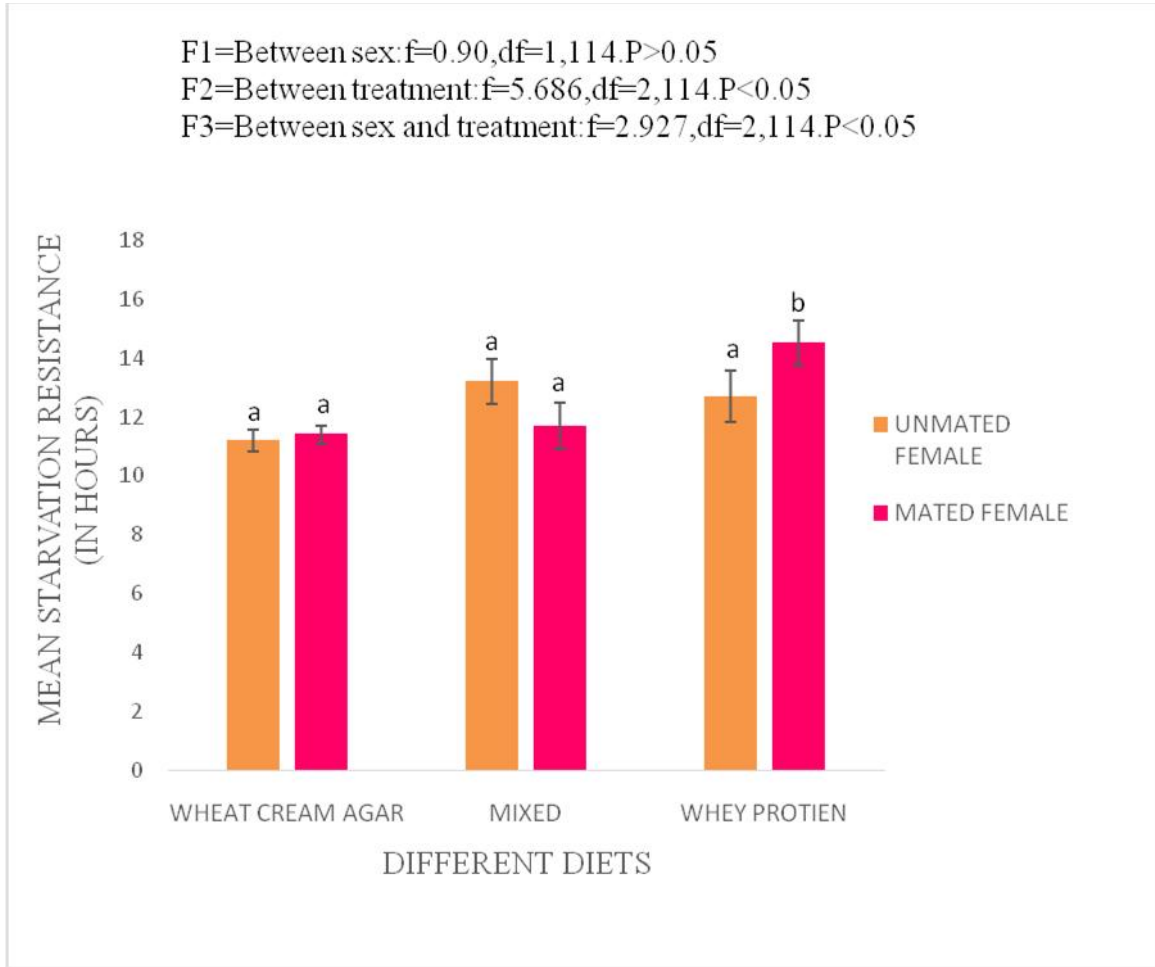
The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test.

### Unmated female and Mated female flies

The average starvation resistance in unmated female and mated female flies cultured in Wheat cream agar media, Mixed media and Whey protein media is represented in **Fig. 4**. This data showed that the Unmated female flies raised in Mixed media showed greater and flies raised in Wheat cream agar showed less starvation resistance. Among the mated females, flies cultured in Whey had shown greater and flies cultured in other two diets shown more or less

equal resistance towards starvation. The above starvation resistance data subjected to two-way ANOVA followed by Tukey's Post hoc test showed significant variation in time taken by mated male and female flies cultured in control, mixed and treated diet to survive in food deprived condition. However, insignificant variation in starvation resistance was noticed interaction between treatment and sex. Tukey's post hoc test showed significant difference in Starvation resistance between control, mixed and Whey protein media.

**Figure 4: Effect of Whey protein on starvation resistance in Mated male and female of *Drosophila melanogaster***



The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey’s Post Hoc test.

**Discussion**

Food is crucial for the organisms for their growth, development, health, reproduction and survival. The food availability and nutrient value in available food is responsible for the resistance of starvation.

In the present study, the results showed that starvation resistance in flies cultured in Whey protein had greater than Mixed diet which had an average and wheat cream agar media had least (**Fig.1-4**). This suggest that variation in starvation resistance depends on the quality and nutrition of the diet. Insects have to depend on previously

acquired and stored energy reserves since they are unable to gain additional energy during starvation. To increase Starvation resistance, *Drosophila* uses three mechanisms. It stores more energy in the form of lipids, carbohydrates and proteins, slowly consumes it according to activity levels and metabolic rates, or tolerate the loss of larger portion of their initial energy supply. Some studies have shown that starvation resistance is due to increased lipid accumulation in terms of TAGs in *D. melanogaster* (Chippindale *et al.*, 1996; Harshman *et al.*, 1999).Jang and Lee, (2018) tested effect of temperature on starvation resistance in *D. melanogaster* by varying temperature from 18 to 23<sup>0</sup>C and found that

exposure to high temperature during starvation resulted in reduced starvation resistance. This is due to increased metabolic rate and faster decreased levels in lipid. Prakash *et al.*, (2014) demonstrated that low humidity (35% RH) reduces starvation resistance as well as body lipid levels and higher humidity (85%RH) increases starvation resistance in *D. melanogaster*. In this study as the flies were maintained in uniform temperature ( $22\pm 1^{\circ}\text{C}$ ) and humidity (around 70%) suggesting no significant effect of humidity and temperature on Starvation resistance. In the study by Kiran and Krishna (2023), found that flies cultured in Mixed media showed greater starvation resistance than treated media (Jeeni millet) but our study confirms the study by Shreeraksha *et al.*, (2023) that protein rich diet (spirulina) increases starvation resistance.

According to present study, the results (**Fig.1 and Fig.2**) showed that female flies had greater starvation resistance than that of male flies in all three diets (Wheat cream agar, Mixed and Whey protein). This can be due to variations in energy metabolism during starvation. In many organisms, males and females need different nutrition. This causes variations in starvation resistance in sex-specific manner (Hoyenga and Hoyenga, 1982). In *D. melanogaster*, the pattern of sexual dimorphism in starvation resistance depends on strain, mating status, age and assay condition (Service, 1989; Huey *et al.*, 2004; Vermeulen *et al.*, 2006; Matzkin *et al.*, 2009). During starvation females use body lipids and glycogen which are stored in high amount as energy source, while males digest body lipids as energy source. Improved starvation resistance is correlated with increased energy storage or slower breakdown of these reserves (Hoffmann and Harshman, 1999; Rion and Kaweck, 2007; Gibbs and Reynolds, 2012). In addition to variations in the composition of a particular tissue/compound that facilitate energy acquisition, sex-specific starvation resistance may also arise from differences in how that different sexes are capable of using specific tissue/compounds for energy acquisition (Aggarwal, 2014).

In this study, the results (**Fig. 3**), the unmated male flies of Wheat cream agar media and Whey protein media showed greater starvation resistance than mated males. In *D. melanogaster*, starvation resistance also depends on mating (Service, 1989; Rush *et al.*, 2007; Goenaga *et al.*, 2012). The transfer of the sperms and accessory gland proteins Acp's and also loss of energy sexual intercourse which is not in the case of unmated males. This may have increased starvation resistance in unmated males. But in case of mated male flies in Mixed media, may had both carbohydrate and protein source which may enough to compensate the protein loss during mating.

In this study, the results (**Fig.4**), shows that, mated females have greater starvation resistance in Wheat cream agar and Whey protein media. Compared to mated male, mated females accumulate more amount of lipids due to intake of large amount of food (Carvalho *et al.*, 2006). This is because in females, mating increases food intake, decreases sexual receptibility, and stimulates egg production. These impacts are the resultants of sex-peptides from male seminal fluid that are transmitted (Chen *et al.*, 1988; Herndon and Wolfner, 1995).

In our result (**Fig. 4**), the starvation resistance was found to be greater in unmated female in Mixed media. This may be due to less availability of protein in the diet. And also mating and the increase in egg production are costly in terms of survival (reviewed in Harshman and Zera, 2006; Flatt, 2011). Virgin female might have reabsorbed nutrients from eggs to cope up with starvation. Some recent studies reveal that oosorption i.e., autophagic reabsorption of oocytes increases starvation resistance in virgin females.

Numerous internal and external factors such as nutrition, social interactions, age, genetic diversity, and temperature also have an impact on the physiological relationships associated with starvation (Vermeulen *et al.*, 2006; Pijpe *et al.*, 2007; Rush *et al.*, 2007; Lee and Jang, 2014).



From this study, we noticed that the quality and quantity of nutrients in different diets were responsible for the variation in the Starvation resistance.

With this study, we can conclude that the Whey protein increases starvation resistance in *D. melanogaster*. Further, female had significantly greater starvation resistance than those of male flies in all the diets studied. Furthermore, variations in starvation resistance was noticed in both mated and unmated males and females.

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