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The Effect of Mass Gainer on the Starvation Resistance in *Drosophila melanogaster*

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Abstract

Drosophila melanogaster is a model organism, researchers often assess traits such as starvation resistance. Dietary factors are one of the significant external environmental elements that impact starvation resistance. *D. melanogaster* was cultured on wheat cream agar, 10g mass gainer, and 20g mass gainer. This experiment was carried out to determine the effect of mass gainer on starvation resistance. The results showed that flies that were given 20g of mass gainer media had greater starvation resistance compared to flies that were given mass gainer of 10g which had an average starvation resistance whereas flies that were reared on wheat cream agar media showed the least starvation resistance. The present study also reveals that female flies were more starvation resistant than males on these three diets. In addition, mated female flies and unmated female flies had variation in starvation resistance on these three types of diets. However, in male we found variation in starvation resistance between mated and unmated males. Thus, mass gainer having high protein increases starvation resistance in *D. melanogaster*.

Keywords: Diet, Starvation resistance, *D. melanogaster*, Mated, Virgins.

Introduction

Starvation is the ability of an animal to withstand a lack of food. Usually, starvation can occur briefly or over a long period of time. If long-term starvation continues, for example due to seasonal cycles and the absence of food sources, this can cause animal death (McCue, 2010). Many animal species struggle to obtain food and reproductive fitness is directly correlated with an animal's capacity to forage (Chippindale *et al*., 1996; Wayne *et al*., 2006).

The most common environmental problem animals face is food scarcity, and an animal's diet and nutritional status impact its ability to survive prolonged periods of starvation. Although the ecology and evolution of starvation resistance have been studied extensively using fruit flies of the genus *Drosophila*, the nutritional basis of starvation resistance has not been thoroughly investigated (Lee and Jang, 2014).

A decrease in the amount of food sources available is typical in natural populations. For the wider study of evolution and its possible effects for human health, the physiology of starving adaption is of interest. Hoffmann and Harshman (1999) conducted a comparative analysis of patterns of variation withinand between populations of *D. melanogaster* species that were subjected to drought and starvation.

The nutritional value of a feed is a determining factor that influences an animals starvation resistance, because an animal physiological ability to survive starvation depends on its nutritional status and feeding history. As a heterotroph, *D. melanogaster* must consume, digest, and assimilate available nutrients. The food consumed is used for growth, reproduction or storage. The amount of nutrients an animal receives during this process depends on the rate and frequency of food consumption, as well as the efficiency of digestion and assimilation of the nutrients consumed. In *Drosophila*, the level of lipid stores in the body is considered the most important determinant of resistance to starvation, but little is known about how the relative proportions of body nutrients (proteins, carbohydrates, and lipids) are formed (Lee and Jang, 2014).

The quantity and quality of an organism's food intake greatly influences life history traits such as disease susceptibility, fecundity, reproduction, longevity, and stress tolerance (Kiran and Krishna, 2023). In general, impacts on diet can be classified as quantitative (i.e. food availability) or qualitative (i.e. food composition). The quantitative impact is clear because animals obtain energy and other nutrients from food. How much food an animal receives through this process depends on the rate and frequency of food consumption, as well as the efficiency of digestion and absorption of the nutrients consumed (Singh and Sisodia, 2012). This feeding response is known to be tightly controlled in some animals through regulatory feedback loops that can be modulated by the quantity and quality of nutrients in the diet (Simpson, *et al*2012). Energy has long been thought to be the sole focus of aging animals, but it has now become clear that animals balance their intake of different nutrients to maximize their fitness.

There is strong evidence that higher fat content in adults is the cause of increased starvation tolerance. According to some evidence, almost all variation in starvation resistance is due to this characteristic. For example, in a series of strains selected for starvation or modification of life history features, Chippindale *et al.*,(1996) evaluated lipid levels and starvation. When all this was taken into account, they found there was an almost perfect relationship between lipid levels and hunger. In a comparison of allozyme genotypes and the way lipid and starvation levels change with age, lipid relationships have also been reported from a number of starvation selection strains resulting from various base populations (Offmann and Harshman, 1999).

A popular and extensively used supplement among athletes, protein powders assist build and repair of skeletal muscle as well as improve overall health and aid in post-exercise recovery. Athletes are also advised to build more muscle mass and avoid breaking down proteins while engaging in extended activity (Memet *et al*, 2014). Although protein supplements seem to have benefits, other scientists believe that taking too much of them could be harmful to one's health. According to a related study, consuming a lot of protein and using supplements could harm renal function (Baskan and Sezen, 2023).

Mass gainer, often known as a "weight gainer," is a powder that is intended to replace meals with the goal of gaining muscle mass. In order to encourage an energy surplus and the synthesis of

muscle protein, the majority of mass gainers are high in fat, protein, and carbohydrates. In order to speed up recuperation, mass gainers may also include additional muscle-building components like beta-hydroxymethylbutyrate (HMB) and creatine monohydrate. Mass gainer is a powdered supplement that combines carbohydrates and protein which is usually used to increase body mass(Campbell *et al*, 2008).

These days, a lot of people especially athletes and body builders consume mass gainers due to their beneficial effects on nutrition and health. There is no published data on how mass gainer impacts an organism's ability to withstand starvation or other environmental stresses, despite numerous studies demonstrating that its consumption can increase protein synthesis, increases in muscle protein net balance, and increase body weight, among other benefits, in various model organisms(Campbell *et al*, 2008). Therefore, this study has been undertaken to determine the influence of mass gainer on starvation resistance in *D. melanogaster*.

Materials and Methods

The mass gainer was purchased through Flipkart App from A207, Lane No. 9, No. 4, Mahipalpur, Delhi, 110037, India. This mass gainer was used to prepare the experimental media.

Establishment of stock

Experimental Oregon K strain of *D. melanogaster* used in the study was collected from *Drosophila* stock center. Department of studies in Zoology, University of Mysore, Mysore 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 1000 ml distilled water and 7.5 ml of propionic acid was added]. Flies were maintained in laboratory conditions such as humidity of 70% and 12 hours dark and 12 hours light cycles and temperature 22± 1° C.

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 100g of jaggery, 100g of wheat cream rava , 10g of agar boiled in 1000ml distilled water and 7.5 ml of propionic acid added to it.

20g of Mass gainer media: is prepared from100g of jaggery, 80 g of wheat cream rava, 20g of mass gainer powder, 10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it.

10g of mass gainer media: is prepared from 100g of jaggery, 90g of wheat cream rava and 10g of mass gainer powder,10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it]. The flies emerged from the wheat cream agar media and other experimental treated media under the same laboratory conditions as mentioned above 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 wheat cream agar, 10g mass gainer and 20g mass gainer were used. Fifteen flies (unmated male / unmated female, mated male / mated female) were observed by transferring them to empty vials with each vial containing 5 flies.

These vials were kept at 22± 1° C under constant light condition and resistance to starvation of each fly was observed in 1 hour interval until its death. A total of 3 replicates (each with 5 flies) were carried out for each of the wheat cream agar, 10g mass gainer and 20g mass gainer media. Separate experiment was carried out for mated and unmated flies.

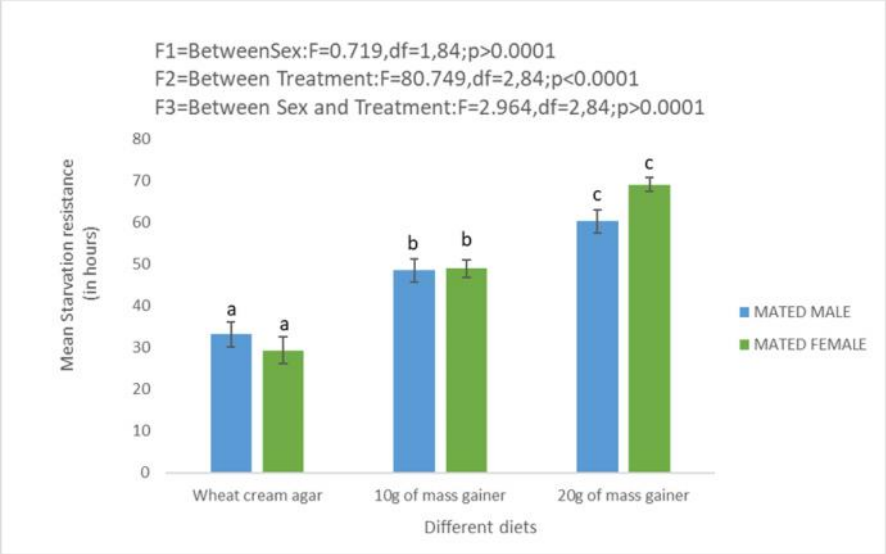
Results

Effect of the mass gainer on the starvation resistance in the mated male and female of *D. melanogaster*

The mean and standard error value of the starvation resistance of mated male and female flies raised with wheat cream agar, 10g of mass gainer and 20g of mass gainer media are provided in the figure 1. According to data it was noticed that starvation resistance was greater in the 20g of mass gainer compared to the wheat cream agar and 10g of mass gainer diet. The result was found that the mated female had the greater starvation resistance than mated males in different diet.

The above data was subjected to the Two-way ANOVA followed by the Tukey's post hoc test showed the significant variation in starvation resistance was found between the diets, between sexes and interaction between the diet and sex. However, significant variation observed between the male and female of wheat cream agar media and 20g of mass gainer. Further in starvation resistance non-significant variation was observed between male and female in the 10g of mass gainer.

Figure 1: Effect of the mass gainer on the starvation resistance in the mated male and female of *D. melanogaster*.



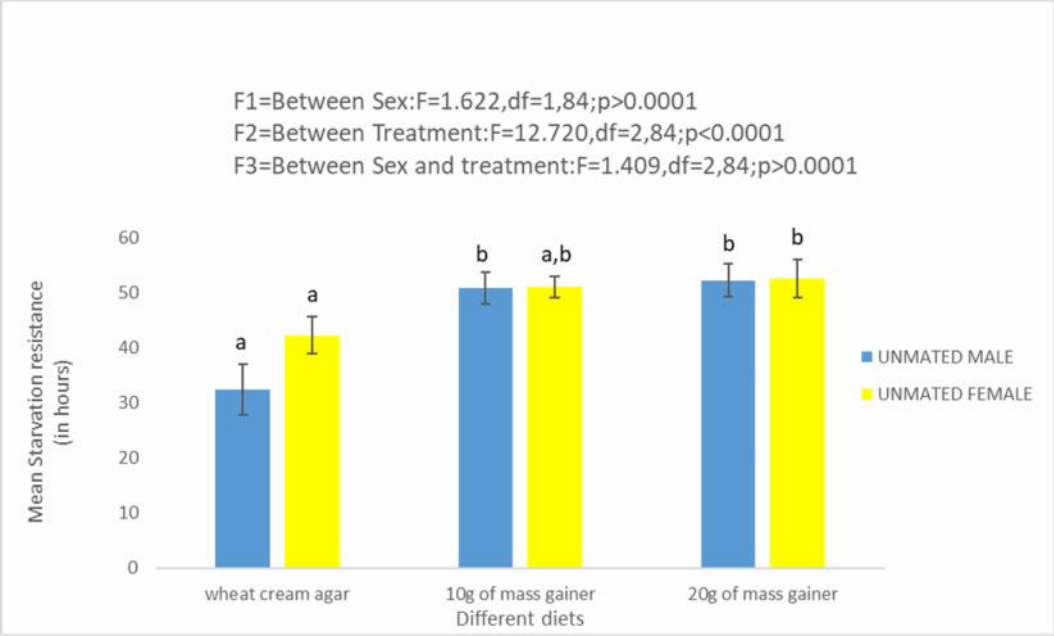
The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Effect of the mass gainer on the starvation resistance on the unmated male and female of *D. melanogaster*

Data in Figure 2 shows that the mean and standard error values for starvation resistance were higher in unmated male and female flies raised on a diet with 20g of mass gainer compared to those raised on wheat cream agar or 10g of mass gainer. The results indicated that unmated females exhibited greater starvation resistance than mated males across different diets.

The data was analyzed using a Two-way ANOVA followed by Tukey's post hoc test, revealing significant variation in starvation resistance was found between the diets, between sexes, and the interaction between diet and sex. However in starvation resistance non-significant variation was observed between males and females in the 10g and 20g mass gainer media, while starvation resistance had significant difference between males and females on the wheat cream agar media.

Figure 2: Effect of the mass gainer on the starvation resistance on the unmated male and female of *D. melanogaster*



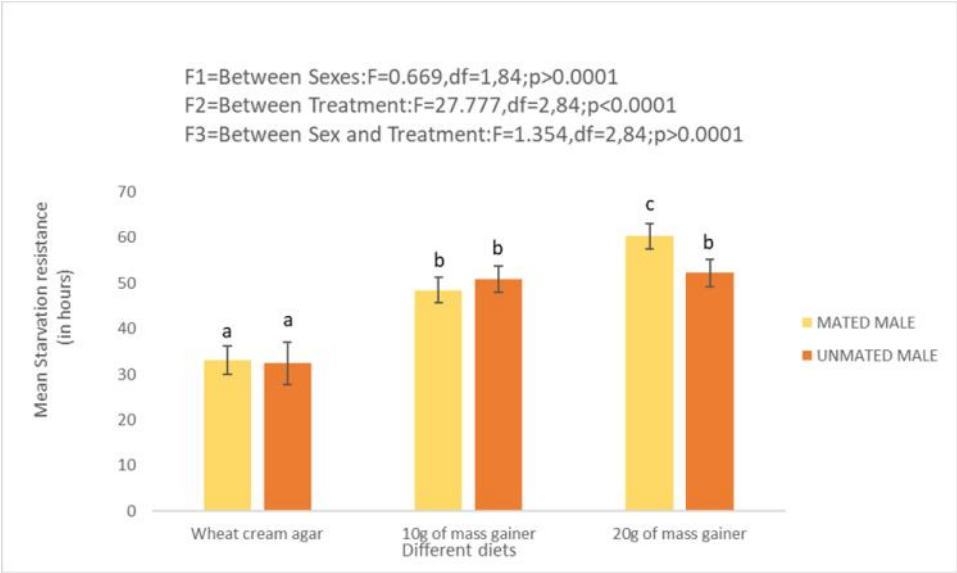
The different letters on the bar graph indicate the significant variation between the different diet byTukey's post hoc test at 0.05 level.

Effect of the mass gainer on the starvation resistance on the mated male and unmated male of *D. melanogaster*

Figure 3 provides the mean and standard error values for the starvation resistance of mated male and unmated male flies raised in wheat cream agar, 10g of mass gainer, and 20g of mass gainer media. The data indicates that starvation resistance was highest in flies raised in the 20g mass gainer diet compared to those in wheat cream agar and the 10g mass gainer media. Additionally, it was found that mated males exhibited greater starvation resistance than unmated males in different diets.

By using Two-way ANOVA followed by Tukey's post hoc test, the data was analyzed, which revealed significant variations in starvation resistance was found between sexes, between diets, and the interaction between diet and sex. However in starvation resistance significant variation was observed between mated and unmated males on the 10g and 20g mass gainer diets, while non-significant starvation resistance differences were found between mated and unmated males on the wheat cream agar diet.

Figure 3: Effect of the mass gainer on the starvation resistance of the mated and unmated male in *D. melanogaster*



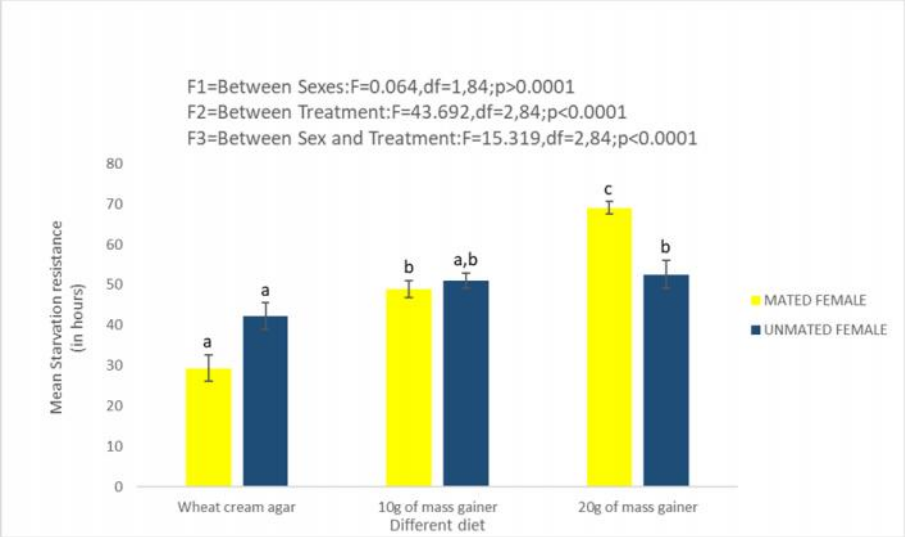
The different letters on the bar graph indicate the significant variation between the different diet byTukey's post hoc test at 0.05 level.

Effect of the mass gainer on the starvation resistance of the mated female and unmated female in *D. Melanogaster*

The starvation resistance of mated female and unmated female flies reared on wheat cream agar, 10g of mass gainer, and 20g of mass gainer media is shown in Figure 4 along with the mean and standard error values. According to the results, flies raised on the 20g mass gainer media showed the highest level of starvation resistance when compared to those raised on the 10g mass gainer media and wheat cream agar media. Furthermore, it was noted that, irrespective of media, unmated females demonstrated a higher level of starvation resistance than mated females.

The Two-way ANOVA and Tukey's post hoc test applied to the above-mentioned data revealed significant variation in starvation resistance was found between the sexes, between diets, as well as an interaction between the diet and sex. There was a significant difference in starvation resistance was noticed between the mated and unmated females of the 20g mass gainer diet and wheat cream agar. When 10g of mass gainer diet was given to both mated and unmated females, there was non-significant difference in starvation resistance was noticed.

Figure 4: Effect of the mass gainer on the starvation resistance of the mated and unmated female in *D. Melanogaster*



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

Discussion

Diet affects many aspects of fly development, including sex, health, reproduction, and even the organism's ability to survive and starvation resistance. In this present study, in the *D. melanogaster* results (figure 1-4) revealed that starvation resistance was greater in the 20g of mass gainer compared to 10g mass gainer media and wheat cream media.

Our Study also confirms the study of Sisodia and Singh (2012), that is dietary effect of the nutrients present in food affecting the Starvation resistance of *D. ananassae*, they demonstrated that, in comparison to flies raised on protein-enriched medium, those raised on carbohydrate-enriched medium exhibited more starvation resistance. Physiological alterations necessary for greater starvation resistance will probably come at the expense of other fitness-related characteristics.

Shreeraksha *et al*., (2023) who while studying Spirulina Supplements effect on starvation resistance in*D. Melanogaster* have also showed that Spirulina treated flies showed significantly greater starvation resistance in *D. melanogaster*. This is because Spirulina has a high protein concentration compared to the control media, thereby *Drosophila* flies mobilize their stored energy reserves built up during times of high nutrition availability in times of extreme food scarcity. Similar studies have also been carried out by Kiran and Krishna (2023),they studied the effect of the Jeeni millet traditional mix on the starvation resistance in *D. melanogaster*. The results showed that flies fed with mixed media (wheat cream+ jeeni millet) had greater resistance to starvation. Millet is a well-known nutritious cereal very nutrient-dense and high in protein as well as carbohydrate. Known amount, quantity, and quality of nutrition in food is responsible for variation in resistance to starvation. Thus these studies in *Drosophila* suggests that quality of nutrients affects the starvation resistance.

In the present study we have cultured the flies in same temperature and humidity and other conditions, further we have used same aged flies therefore observed variation in the starvation resistance was not resulted due to the age, and environmental conditions in the flies.

In the present study, we also studied the starvation resistance of the male and female flies in 20g of mass gainer, 10g of mass gainer and wheat cream agar media. The results (Figure1-2) revealed that female flies were significantly had greater starvation resistance than those of male flies in the three different diets. We can explain the results by some possible mechanisms i.e.1) Females have a relatively higher fat and or protein content, 2) a higher portion of this content is available for energy metabolism (i.e., females with starvation have a lower residual fat and/or protein content than males), and 3) females use energy more effectively (Rion and Kawecki, 2007). Insects provide ample evidence of sexual dimorphism in characteristics involved in starvation resistance. Compared to males, mated females consumed more food and therefore accumulated a higher amount of lipids (Lee *et al*, 2013).

Aggarwal (2014), who investigated the physiological implications of sex and population specific variations in *D. leontia* starvation resistance. Under conditions of starvation stress, females stored higher amounts of body lipids and glycogen and used both of these energy sources, whereas starved males only used digested body lipids as a source of energy. Improved energy storage or a slower rate of depletion of these reserves are related to increased starvation resistance (Hoffmann and Harshman,1999). In *D. melanogaster* sexual dimorphism with reference to starvation resistance was also shown by Kiran and Krishna (2023), Shreeraksha *et al*., (2023), Mambaand Krishna (2023), they also found that females had significantly greater starvation resistance than males.

Further, in this study we also studied the variation in the starvation resistance in the mated and unmated male and females were studied. (Figure 4) revealed that starvation resistance was greater in mated females compared to unmated females according to (Jang and Lee, 2015), study revealed that mating can alter starvation resistance in *D. melanogaster* females.

Mating has been demonstrated to have significant effects on starvation resistance especially in *D. melanogaster*. Mating in female *D. melanogaster* is known to increase food intake, suppress sexual receptivity, stimulate egg production, and transfer male seminal fluid peptides to females, the primary cause of the rise in starvation resistance in female *D. melanogaster* is the post-mating increase in food consumption and the ensuring rise in lipid storage (Rush *et al*., 2007). More recently, it has also been shown that mating can cause a female *D. melanogaster* midgut to significantly expand, thus increasing their post-ingestive nutrition usage, allowing mated females to fulfill their increased energy requirements for egg laying(Service, 1989; Goenaga *et al*., 2012).

In the present study we found mated male had more starvation resistance than unmated male (figure 3), our study supports the work of Krishna and Uchenna (2013), decrease in the reproductive stress faced by mated male flies can be attributed to the increase in their starvation resistance, hence there is a reduced reproductive stress, the antioxidants further reduce these stress levels in the treated flies in turn increasing their starvation resistance.

Thus from our experiment, we conclude that the dietary protein: carbohydrate (P: C) ratio, rather than the diet's caloric content or the carbohydrate alone, had a significant impact on *Drosophila* starvation resistance.

20g of mass gainer provide more energy and helps for longer food storage to withstand the starvation resistance than the 10g of mass gainer and wheat cream agar. The consumption of the food with 20g of mass gainer enhances the starvation resistance.

Thus in *D. melanogaster* mass gainer increases starvation resistance, in all the diet studied female had greater starvation resistance than male flies. Furthermore the mated males and females had greater resistance to the starvation compared to unmated male and female.

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