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Research Article

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The Effect of Mass Gainer on The Heat resistance in *Drosophila melanogaster*

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Abstract

Quality and quantity of nutrients affects stress tolerance, life-history features, and reproduction of an organism. Animal survival and the success of their reproductive processes depends on maintaining a balance between energy intake and use. *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 heat resistance. The results showed that flies that were given 20g of mass gainer media had greater heat resistance compared to flies that were given mass gainer of 10g which had an average heat resistance whereas flies that were reared on wheat cream agar media showed the least heat resistance. The present study also reveals that female flies were more heat resistant than males on these three diets. In addition, mated female flies and unmated female flies, mated female flies had greater heat resistance on these three types of diets. However, in male we found variation in heat resistance between mated and unmated males. Thus, mass gainer having high protein increases heat resistance in *D. melanogaster*.

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

Introduction

A common model organism in the study of physiological and evolutionary reactions to different types of stress is *D. melanogaster* (Hoffmann *et al*., 2003, Sinclair *et al*., 2007, Kristensen *et al*., 2008). But little is known about how nutrition affects life history features and performance under heat stress and studies frequently undervalue the significance of diet in their design (Prasad *etal*., 2003).

Consequently, there is an increasing need to look at the influence of food behind variation in traits that are crucial to fitness. Fly lifespan has been demonstrated to be influenced by a wide range of variables, including nutrition, temperature, cage density, and social environment. Temperature and gender characteristics have a significant impact on stress resistance features, which include tolerance to heat, cold, hunger, and desiccation. Temperature is perhaps the most important extrinsic factor in relation to ectotherms because it influences almost all rate-dependent processes ranging from biochemical kinetics to life history traits (Cossins and Bowler, 1987;Hochachkaand Somero, 2002). The widely studied and cosmopolitan species *D. melanogaster* can be found at temperatures ranging between 11°C and 32 °C, increasing temperature is one of the most ubiquitous impacts of climate change and has widespread impacts on life history traits (Rincon *et al*., 2014).

Food and other nutritional sources are essential to the growth, survival, and maintenance of normal health of all living things. The body undergoes metabolic changes in response to an abundance of food in order to store resources for times of starvation or other activities requiring energy consumption (Krittika and Yadav, 2024). Insect resilience to environmental stress is mostly determined by nutrition (Andersen *et al*., 2010; Djawdan *et al*., 1997; Sisodia and Singh, 2012).

The effect of nutrition on thermal tolerance depends on several interacting factors including sex, marital status, and age. Although the physiological and biochemical basis of the thermal response is becoming clearer through metabolic and physiological studies (Overgaard *et al*., 2007, Doucet *et al*., 2009, Colinet *et al*., 2012, Kostál *et al*., 2012, Storey.,2012, Teets and Denlinger, 2013). Age, marital status, and sex are some of the interacting elements that determine how nutrition affects temperature tolerance. While metabolic and physiological studies are providing more clarity on the physiological and biochemical underpinnings of the temperature response.

Compared to adults raised on a diet high in carbohydrates, *D. melanogaster* bred on a diet high in protein exhibits greater heat and desiccation tolerance, but less recovery from cold coma (Andersen *et al*., 2010). In the presence of the amino acids tyrosine and phenylalanine, which are building blocks for the manufacture of melanin and which together give insects their capacity to withstand dehydration and harden their cuticles, may provide an answer. By modulating the deposition of cuticular wax and lowering air loss from evaporation at high temperatures, lipids also indirectly increase heat resistance (Yosef *et al*., 2022).

Protein powders are a well-liked and widely used supplement among athletes that help with skeletal muscle growth and repair, general health enhancement, and post-exercise recovery. Athletes are also counselled to increase their muscle mass and refrain from oxidising proteins during prolonged exercise (Memet *et al*., 2014). While some scientists think that taking too many protein supplements could be detrimental to one's health, others do not. A related study found that taking supplements and eating a lot of protein may be detrimental to kidney function (Baskan and Sezen, 2023).

A mass gainer, sometimes referred to as a "weight gainer," is a powder designed to take the place of meals in order to increase muscle mass. Most mass gainers are heavy in fat, protein, and carbohydrates to promote an energy excess and the synthesis of muscle protein. Mass gainers may also contain extra muscle-building ingredients like beta-hydroxymethylbutyrate (HMB) and creatine monohydrate to expedite the healing process. A powdered supplement called a "mass gainer" mixes protein and carbs and is typically used to enhance body mass (Campbell *et al*., 2008).

These days, mass gainers are used by a lot of people, particularly body builders and athletes, because of their advantageous effects on nutrition and health. Despite numerous studies showing that mass gainer consumption can increase protein synthesis, increases in muscle protein net balance, and increases body weight, among other benefits, in various model organisms, there is no published data on how mass gainer impacts an organism's ability to withstand heat or other environmental stresses (Campbell *et al*., 2008). Therefore, this study has been undertaken to determine the influence of mass gainer on heat 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° C± 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 heat resistance experiment in *D. melanogaster*.

Experimental procedure

Heat resistance: To study heat resistance five days old unmated (virgins) and mated flies obtained from wheat cream agar, 10g mass gainer and 20g mass gainer were used. Ten 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 37° C (sublethal temperature) in water bath and resistance to heat of each fly was observed in 10 minutes interval until its death by taking out the vial each time from the water bath. A total of ten flies were observed for each of the wheat cream agar, 10g mass gainer media and 20g mass gainer media. Separate experiment was carried out for mated and unmated flies.

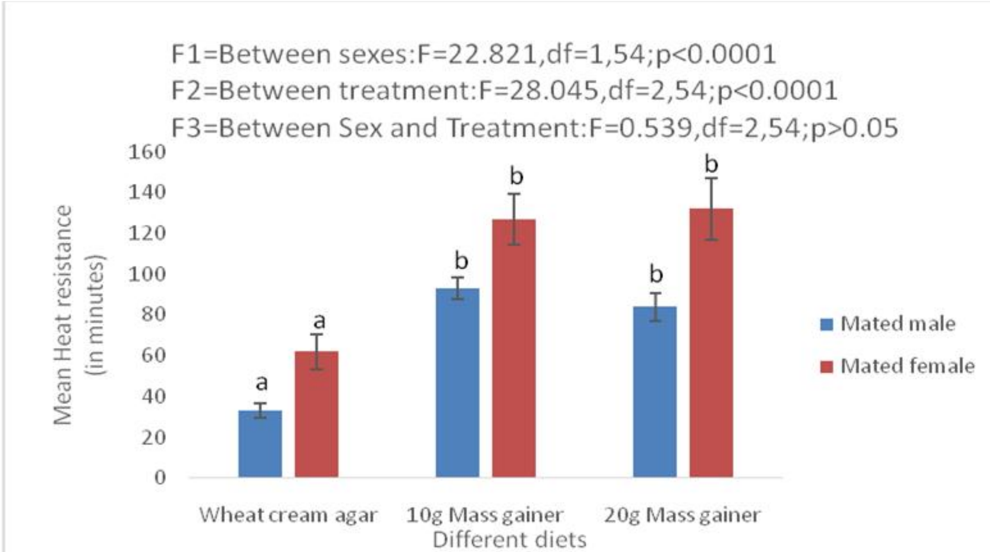
Results

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

The mean and standard error value of the heat 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 heat 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 heat resistance in 20g of mass gainer.

The above data was subjected to the Two-way ANOVA followed by the Tukey’s post hoc test showed the significant variation in heat resistance between the treatment and between sexes. However, non-significant variation was observed between the interaction between sex and treatment.

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



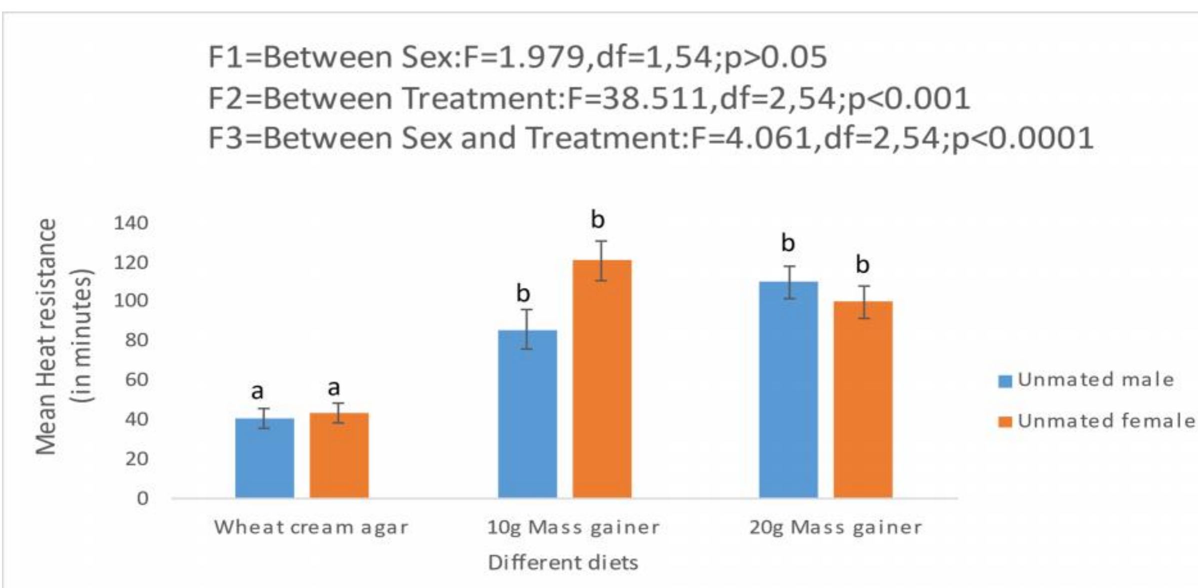
The different letters on the bar graph indicate the significant variation between the different diet byTukey's post hoc test at 0.05 level.

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

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

The data was analysed using a Two-way ANOVA followed by Tukey's post hoc test, revealing significant variation in heat resistance was found between the treatments and between the interaction between sexes and treatment. However in heat resistance non-significant variation was observed between sexes.

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



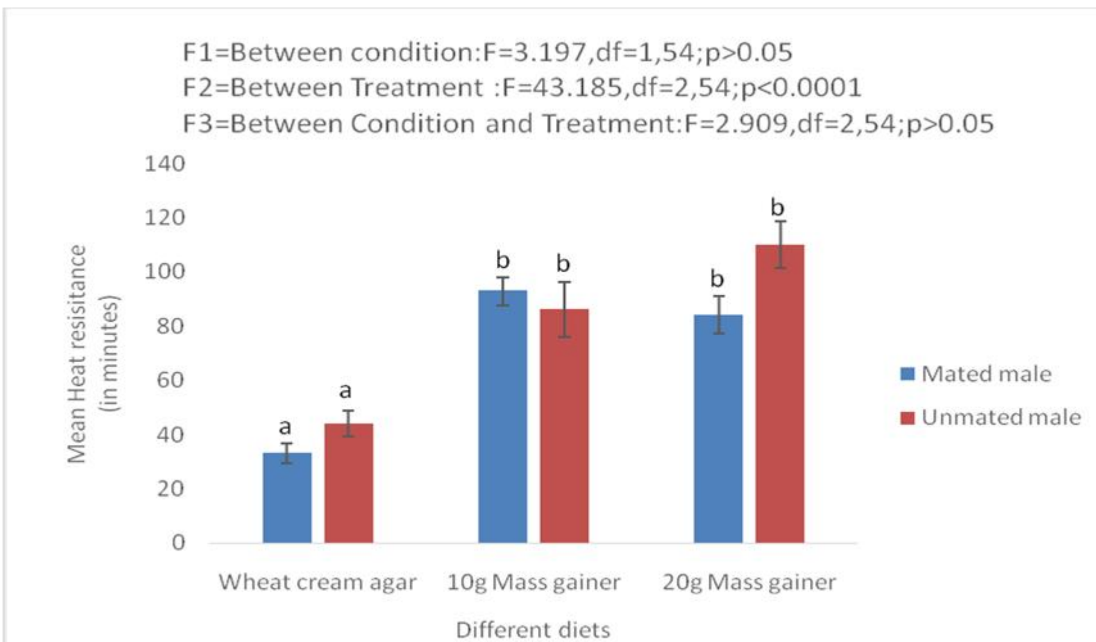
The different letters on the bar graph indicate the significant variation between the different diet byTukey's post hoc test at 0.05 level.

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

Figure 3 provides the mean and standard error values for the heat 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 heat 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 unmated males exhibited greater heat resistance than mated males in different diets.

By using Two-way ANOVA followed by Tukey's post hoc test, the data was analysed, which revealed significant variations in heat resistance was found between treatment and non-significant variation was observed between interaction between condition and treatment.

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



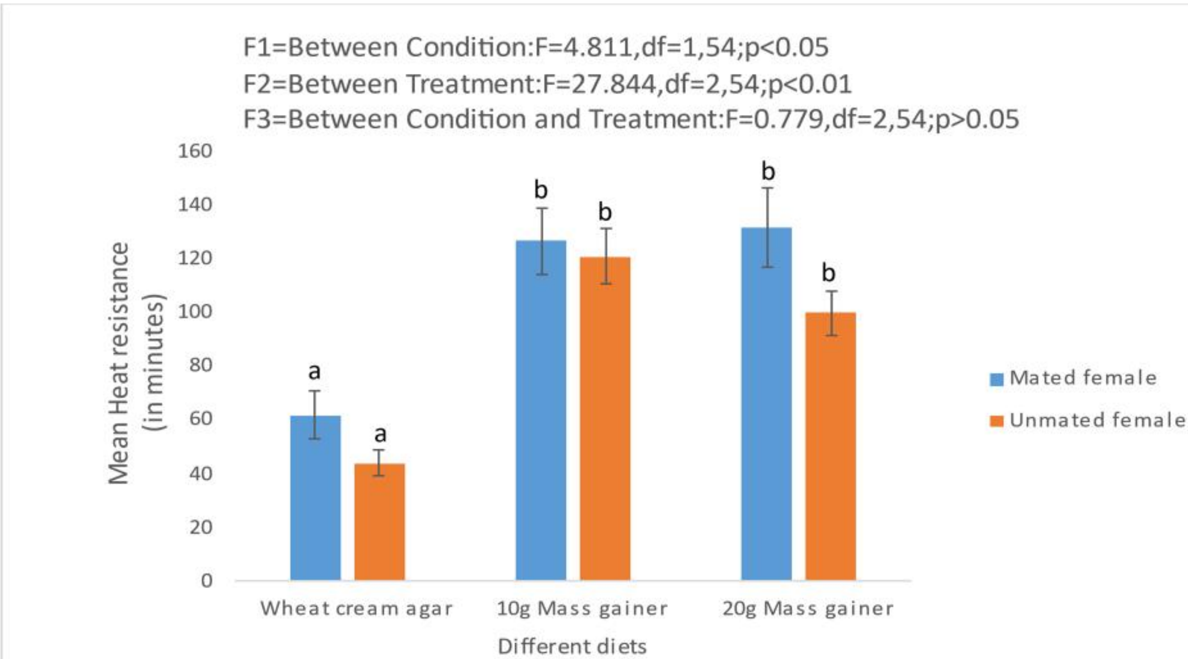
The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Effect of the mass gainer on the heat resistance of the mated female and unmated female in *D. melanogaster*

The heat 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 heat 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, mated females demonstrated a higher level of heat resistance than unmated females.

The Two-way ANOVA and Tukey's post hoc test applied to the above-mentioned data revealed significant variation in heat resistance between the treatments. There was a non-significant variation in heat resistance between the interaction between condition and treatment.

Figure 4: Effect of the mass gainer on the heat 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 by Tukey's post hoc test at 0.05 level.

Discussion

Animal survival and the success of their reproductive processes depend on maintaining a balance between energy intake and use. One distinctive aspect of life is the capacity of organisms, known as phenotypic plasticity, to modify their physiology, behaviour, or development in response to external stimuli. In response to alterations in embryonic nutrition, stress tolerance and reproduction provide one of the most well-known and significant instances of phenotypic plasticity (Sisodia and Singh, 2012).

The consequences of dietary protein deficit for other holometabolous insects have been extensively studied, and protein is a crucial macronutrient for growing larvae. In comparison to flies produced from a diet richer in carbohydrates, *D. melanogaster* adults with excess protein in their diet were more resilient to heat and desiccation stress (Yosep *et al*., 2022).

The impact of mass gainers on *D. melanogaster* capacity to tolerate heat is the present focus of research. According to study findings, flies treated with mass gainer had much greater success rates than control flies. This indicates that the inclusion of mass gainers in the diet may improve *D. melanogaster* capacity to withstand heat (Figure 1-4) demonstrates that the capacity to tolerate heat is significantly influenced by the quantity and quality of nutrients ingested. This validates previous research on how the protein and carbohydrate content of larval diet influences adult *D. melanogaster* resistance to heat stress and desiccation. Heat knockdown resistance was discovered to be significantly influenced by nutrition and sex (Anderson *et al*., 2020). In comparison to flies developed on the carbohydrate-enriched medium, male and female flies developed on the protein-enriched medium survived, on average, 18% and 19% longer.

In both the control and treated flies, the current investigation also discovered that females had greater heat tolerance than males (Fig. 1-2). Female appear to have an advantage over male in that they are generally heavier than male, which can improve fertility and increase tolerance to heat stress, drought, and cold coma. The results of the experiment indicate that while males survive better on larval medium rich in carbohydrates, females develop more successfully on media rich in proteins, indicating that the needs of the two sexes differ during the stages of development and growth. Previous investigations on *D. melanogaster* have shown its peculiarities, including sex-specific responses in life history (Matzkin *et al*., 2007; Sørensen *et al*., 2007; Yadav and Singh, 2007; Kristensen *et al*., 2008).
Further, in this study we also studied the variation in the heat resistance in the mated and unmated male and females were studied (Figure 4) revealed that heat resistance was greater in mated females compared to unmated females according to, study revealed that mating can alter heat resistance in *D. melanogaster* females. The most likely reason for this is that mated females consumed more food and built up more lipids than male (Carvalho *et al*., 2006; Lee *et al*., 2013). Mating in female *D. melanogaster* is also known to increase food intake, suppress sexual receptivity, stimulate egg production, and transfer male seminal fluid peptides to females (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 fulfil their increased energy requirements for egg laying(Service, 1989; Goenaga *et al*., 2012).

In the present study we found unmated male had more heat resistance than mated male (figure 3), Thus, males might show greater longevity and heat stress resistance because they are able to respond faster to the stress by inducing Hsps (Kristensen *et al*., 2003) the heat shock proteins (Hsps) have been shown to regulate both stress resistance and life span (Tower, 2011)

Thus, from our experiment, we conclude that20g of mass gainer provide more energy and helps for longer food storage to withstand the heat resistance than the 10g of mass gainer and wheat cream agar. Further heat resistance increased with increasing concentration of mass gainer.

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