



The effect of muesli on heat resistance in *Drosophila melanogaster*

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Abstract

The study examines how nutrient-rich muesli influences stress tolerance and reproductive traits in *Drosophila melanogaster*. Flies were cultured on wheat cream agar supplemented with 10 g, 20 g, and 30 g of muesli to evaluate their resistance to heat stress. Results indicated that flies fed with 20 g and 30 g muesli showed higher heat resistance compared to those given 10 g muesli, while control flies had the lowest tolerance. Female flies generally exhibited greater heat resistance than males across all diet conditions. Among females, mated individuals performed better than unmated ones, whereas mated males showed slightly improved resistance compared to unmated males. Overall, the findings suggest that increasing muesli concentration enhances heat resistance in *Drosophila melanogaster*.

Keywords: Diet, heat resistance, *D. melanogaster*, mated, virgins

Introduction

Drosophila melanogaster is widely used as a model organism for studying physiological and evolutionary responses to environmental stress due to its well-characterized genetics and high adaptability (Greenspan, 2004; Hoffmann & Harshman, 1999). While numerous studies have examined stress resistance traits in this species, the influence of diet on physiological responses remains relatively underexplored. In particular, the role of nutritional composition in shaping life-history traits and performance under heat stress is not yet fully understood.

There is growing evidence that diet plays a fundamental role in determining fitness-related traits. Both environmental and biological factors—including temperature, nutrition, population density, and social interactions—collectively influence lifespan and stress tolerance (Partridge *et al.*, 2005; Simpson & Raubenheimer, 2012) [7]. Among these, temperature is one of the most influential abiotic factors affecting ectothermic organisms, as it directly regulates metabolic rate, enzyme kinetics, and overall physiological performance (Angilletta, 2009). As a result, tolerance to thermal stress, particularly heat resistance, is closely associated with both environmental exposure and the organism's internal physiological condition.

Drosophila melanogaster exhibits a relatively broad thermal tolerance range; however, increasing global temperatures present significant challenges that can impact survival, development, and reproductive success (Hoffmann *et al.*, 2003; Sørensen *et al.*, 2013) [8]. Nutritional status plays a crucial role in determining the organism's ability to cope with such stress, as it influences energy reserves, metabolic flexibility, and physiological resilience (Lee *et al.*, 2008) [9]. Adequate nutrition supports the maintenance of homeostasis during stress by fueling energy-demanding protective mechanisms.

Recent studies have shown that dietary composition significantly affects thermal tolerance. Protein-rich diets have been associated with improved resistance to heat and desiccation stress, likely due to their role in maintaining structural integrity and supporting enzymatic processes, whereas carbohydrates provide essential energy required during stress responses (Andersen *et al.*, 2010) [10]. Lipids also contribute significantly to heat tolerance by stabilizing

cellular membranes and minimizing water loss, thereby preserving cellular integrity under elevated temperatures (Yosef *et al.*, 2022; Arrese & Soulages, 2010) [11].

Emerging research further emphasizes the importance of diet quality in regulating metabolic and oxidative stress pathways. Nutrient-rich diets have been shown to enhance antioxidant defenses and reduce cellular damage caused by reactive oxygen species, which are typically elevated under heat stress conditions (Liu, 2013; Oczkowski *et al.*, 2024) [3, 14]. In addition, dietary fibers such as β -glucan have been reported to improve metabolic efficiency and maintain physiological stability, thereby enhancing stress tolerance (Wood, 2007; Tosh & Chu, 2015; Muroya *et al.*, 2025) [12, 13, 15].

In this context, muesli represents a nutritionally diverse food composed of whole grains, nuts, seeds, and dried fruits. It provides a balanced combination of carbohydrates, proteins, and lipids, along with dietary fiber, essential micronutrients, and bioactive compounds (McKevith, 2004; Slavin, 2013) [1, 2]. Oat-derived β -glucan, a key component of muesli, has been shown to enhance metabolic regulation and reduce physiological stress responses (Wood, 2007; Oczkowski *et al.*, 2024) [12, 14]. Furthermore, the presence of antioxidants such as polyphenols contributes to the reduction of oxidative damage, which is a major consequence of heat stress (Fardet, 2010; Liu, 2013) [3, 4].

Collectively, these nutritional properties suggest that muesli may enhance heat resistance by improving energy availability, stabilizing cellular structures, and strengthening antioxidant defense systems. Despite increasing evidence on the role of diet in stress physiology, the effects of complex, mixed diets such as muesli on heat tolerance remain largely unexplored in model organisms.

Therefore, the present study aims to evaluate the effect of muesli on heat resistance in *Drosophila melanogaster*, with the objective of understanding how a nutritionally balanced diet influences physiological adaptation to elevated temperature stress.

Establishment of stock Experimental

Oregon K strain of *D. melanogaster* used in the study was collected from Drosophila stock Centre, 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 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 $22 \pm 1^\circ \text{C}$.

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 in1000ml distilled water and 7.5 ml of propionic acid added to it].

30g of Muesli powder media: is prepared from 100g of jaggery, 80 g of wheat cream rava, 30g of Muesli powder, 10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it.

20g of Muesli media: is prepared from 100g of jaggery, 90g of wheat cream rava and 20g of Muesli powder, 10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it.

10g of Muesli powder: 10g of Muesli powder media: is prepared from 100g of jaggery, 80 g of wheat cream rava,10g of Muesli 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*

Materials and Methods

The Muesli was purchased from Loyal World Supermarket,1011, Udayaravi road, KuvempuNagara, Mysuru, Karnataka 570023, India. This Muesli was used to prepare the experimental media.

Experimental procedure

Heat resistance: To study heat resistance five days old unmated (unmated) and mated flies obtained from wheat cream agar, 10g Muesli powder, 20g Muesli powder and 30g Muesli powder 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 37°C under constant light condition and resistance to heat resistance 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 Muesli, 20g Muesli and 30g Muesli media. Separate experiment was carried out for mated and unmated flies.

Results

Effect of the Muesli 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 Muesli, 20g of Muesli and 30g of Muesli media are provided in the figure 1. According to data it was noticed that heat resistance was greater in the 20g of Muesli compared to the wheat cream agar and 10g of Muesli diet. The result was found that the mated female had the greater heat resistance in 20g of Muesli. 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 diet and between sexes. However, non-significant variation was observed between the interaction between sex and diet.

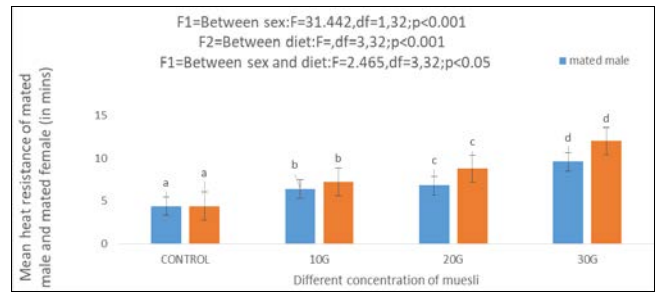


Fig 1: The Effect of Muesli on the heat resistance of the mated male and mated female in *D. melanogaster*

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

Effect of the Muesli 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 Muesli compared to those raised on wheat cream agar or 20g of Muesli. 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 media and between the interaction between sexes and media. However in heat resistance non-significant variation was observed between sexes and media.

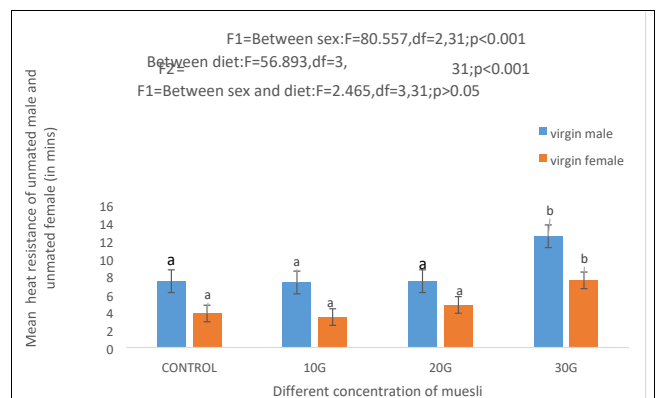


Fig 2: The Effect of Muesli on the heat resistance of the unmated male and unmated female in *D. melanogaster*

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

Effect of the Muesli 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 Muesli, 20g of Muesli and 30g of Muesli media. The data indicates that heat resistance was highest in flies raised in the 20g muesli diet compared to those in wheat cream agar and the 10g Muesli 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 media

between the sexes and between interaction between sexes and media.

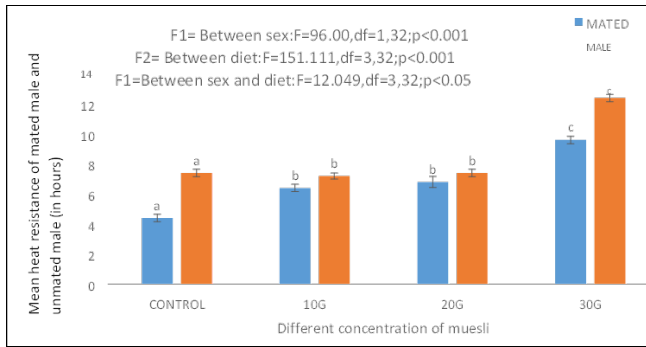


Fig 3: The Effect of Muesli on the heat resistance of the mated male and unmated male in *D. melanogaster*

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

Effect of the Muesli 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 Muesli, 20g of Muesli and 30G of Muesli media is shown in Figure 4 along with the mean and standard error values. According to the results, flies raised on the 20g muesli media showed the highest level of heat resistance when compared to those raised on the 10gMuesli 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 between the sexes between media and with the interaction between sex and media

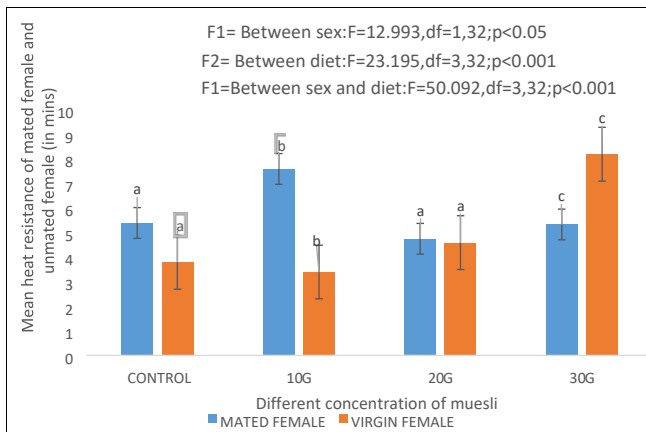


Fig 4: The Effect of Muesli on the heat resistance of the mated female and unmated female in *D. melanogaster*

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

Discussion

The survival of animals and the success of their reproductive processes depend on maintaining a proper balance between energy intake and energy expenditure. One of the defining features of living organisms is phenotypic plasticity, which refers to their ability to alter physiology,

behaviour, and development in response to environmental changes. In insects, stress tolerance and reproductive performance are among the most important examples of such plasticity, especially when influenced by variations in nutrition during development (Sisodia and Singh, 2012).

Protein is an essential macronutrient for the growth and development of holometabolous insect larvae, and the effects of protein deficiency have been widely studied. Previous research has shown that adult *Drosophila melanogaster* reared on protein-rich diets exhibit greater tolerance to heat and desiccation stress than flies raised on carbohydrate-rich diets (Yosep *et al.*, 2022). In the present study, the focus was to evaluate how muesli supplementation affects heat resistance in *D. melanogaster*. The findings revealed that flies reared on muesli-supplemented media showed significantly greater heat tolerance than those reared on control media. Results presented in Figures 1–4 indicate that both the quantity and quality of nutrients consumed strongly influence heat resistance.

These observations support earlier studies demonstrating that the balance of protein and carbohydrates in larval diets affects adult resistance to heat stress and desiccation. Anderson *et al.* (2020) reported that both diet and sex significantly influence heat knockdown resistance. In their study, male and female flies raised on protein-enriched media survived approximately 18% and 19% longer, respectively, than flies reared on carbohydrate-enriched diets. Similarly, in the current study, females consistently displayed greater heat resistance than males in both control and treated groups (Figures 1–2).

The higher heat tolerance observed in females may be due to their generally larger body size and greater energy reserves compared to males. Increased body mass can improve fertility and enhance resistance not only to heat stress but also to drought and cold shock.

Experimental evidence suggests that males survive better on carbohydrate-rich larval diets, whereas females perform better on protein-rich diets, indicating that the nutritional requirements of the two sexes differ during growth and development. Such sex-specific differences in life-history traits have also been reported in earlier studies on *D. melanogaster* (Matzkin *et al.*, 2007; Sorensen *et al.*, 2007; Yadav and Singh, 2007; Kristensen *et al.*, 2008).

The present study also examined differences in heat resistance between mated and unmated male and female flies. Results (Figure 4) showed that mated females had greater heat resistance than unmated females. This supports earlier findings that mating alters stress resistance in female *D. melanogaster* (Carvalho *et al.*, 2006; Lee *et al.*, 2013). One likely explanation is that mated females consume more food and accumulate more lipid reserves than unmated females. In addition, mating in female flies increases food intake, stimulates egg production, suppresses sexual receptivity, and involves the transfer of male seminal peptides, all of which contribute to physiological changes that improve stress tolerance (Rush *et al.*, 2007). Mating has also been shown to enlarge the female midgut, thereby enhancing nutrient absorption and enabling females to meet the higher energy demands of reproduction (Service, 1989; Goenaga *et al.*, 2012).

Interestingly, the study found that unmated males showed greater heat resistance than mated males (Figure 3). This may be because unmated males can respond more rapidly to heat stress by inducing heat shock proteins (Hsps), which

are known to regulate both stress resistance and lifespan (Kristensen *et al.*, 2003; Tower, 2011). These proteins help protect cellular structures under stressful conditions, thereby improving survival.

Overall, the results of this experiment indicate that a 20 g muesli diet provides more effective energy reserves and supports longer survival under heat stress compared to 10 g muesli and wheat cream agar media. Heat resistance increased progressively with increasing concentrations of muesli, suggesting that muesli supplementation significantly enhances the ability of *D. melanogaster* to tolerate high-temperature stress.

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