



## The Effect of Cerelac on The Heat Resistance in *Drosophila melanogaster*

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### Abstract

The quality and quantity of nutrients play a crucial role in influencing stress tolerance, life-history traits, and reproduction in organisms. The survival and reproductive success of animals depend on maintaining a balance between energy intake and expenditure. In this study, *Drosophila melanogaster* was raised on wheat cream agar and cerelac-based diets containing 10%, 20%, and 30% concentrations. Findings revealed that flies fed with 20% and 30% cerelac exhibited higher heat resistance than those on the 10% cerelac diet, which showed moderate resistance. Flies reared on wheat cream agar alone demonstrated the lowest resistance to heat stress. Additionally, female flies consistently showed higher heat resistance than males across all diet types. Among males, mated individuals had better heat resistance than unmated ones, while this difference was not evident in females. These results suggest that cerelac with its high protein contents increases heat resistance in *D. melanogaster*.

**Keywords:** Nutrition, *Drosophila melanogaster*, heat resistance, mated, unmated, cerelac.

### Introduction

An organism's capacity to withstand temperature fluctuations is influenced by multiple interconnected variables, such as gender, reproductive condition, and developmental stage. (Storey., 2012, Teets and Denlinger, 2013) <sup>[37, 38]</sup>. Research has revealed that the biological and chemical foundations of temperature resistance are becoming increasingly understood through studies examining metabolic and physiological adaptations (Overgaard *et al.*, 2007, Doucet *et al.*, 2009, Colinet *et al.*, 2012, kostal *et al.*, 2012,) <sup>[24]</sup>.

Various factors including reproductive condition and gender represent key variables that influence how dietary intake affects an organism's ability to tolerate temperature changes. (Sisodia ana singh, 2010) <sup>[32]</sup> Although metabolic and physiological research provides valuable insights into the biological mechanisms underlying temperature tolerance, there remains a need for more comprehensive understanding of these complex interactions.

*Drosophila melanogaster* serves as a widely utilized model species for investigating physiological and evolutionary responses to various stress conditions (Hoffmann *et al.*, 2003; Sinclair *et al.*, 2007; Kristensen *et al.*, 2008) <sup>[31]</sup>. However, research gaps remain regarding the impact of nutritional factors on life history characteristics and performance during thermal stress, with many studies failing to adequately consider dietary influences in their experimental frameworks (Prasad *et al.*, 2003) <sup>[25]</sup>.

Therefore, there is a growing necessity to examine how dietary variation affects traits that are fundamental to organismal fitness. Multiple factors have been shown to influence fly longevity, encompassing nutritional

components, environmental temperature, population density, and social surroundings (Sisodia ana singh, 2010) <sup>[33]</sup>. Both temperature conditions and sex-related traits significantly affect an organism's capacity to withstand stress, including resistance to thermal extremes, cold exposure, starvation, and dehydration. Among environmental variables, temperature stands out as particularly crucial for ectothermic organisms since it affects nearly all metabolic processes, from biochemical reaction rates to life history characteristics (Cossins and Bowler, 1987; Hochachka and Somero, 2002). The extensively researched and globally distributed *D. melanogaster* thrives across a temperature spectrum from 11°C to 32°C, with increasing temperature.

In comparison to adult *D. melanogaster* raised on carbohydrate-rich diets, those reared on protein-rich diets demonstrate enhanced tolerance to heat and desiccation, although they show a decreased ability to recover from cold-induced comas (Andersen *et al.*, 2010). The amino acids tyrosine and phenylalanine, which serve as precursors for melanin synthesis, play a crucial role in boosting insects' resistance to dehydration and in strengthening their cuticles. These effects are possibly linked to changes in cuticular wax deposition and reduced moisture loss at elevated temperatures. Additionally, lipids contribute to thermal resistance (Yosef *et al.*, 2022).

Cerelac, a popular brand of instant cereal, is widely used as a complementary food for infants and young children. Produced by Nestlé, Cerelac has long been a staple in households, providing essential nutrients to support infants as they move from breastfeeding or formula feeding to consuming solid foods (Shally Awasthi Narayan U. Reddy *et al.* 2020.)

Cerelac, a popular infant cereal produced by Nestlé, is known for its nutrient-rich composition and ease of digestion, making it ideal for infants transitioning to solid foods. Typically made from grains such as rice, wheat, oats, or barley and fortified with essential vitamins and minerals, Cerelac is tailored to meet the dietary needs of young children (Djawdan, *et al*, 1998).

Cerelac is product with different nutrients no researchers was used this product earlier, so we have undertaken this product to check out cold resistance in *D. melanogaster*.

### Method and Material

"The Cerelac (nestle, ragi nachni apple flavor) was purchased from a Shree medicals store #14, shop no.1, 6<sup>th</sup> main, Vinayakanagara, MYSURU-570012. used as a dietary component in the experimental setup

### Establishment of Stock

The *D. melanogaster* flies used in this study were sourced from the *Drosophila* stock center, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysuru. The collected flies were cultured in wheat cream agar media composed of 100 g jaggery, 100 g rava powder, and 10 g agar dissolved in 1000 ml boiling distilled water, with 7.5 ml propionic acid added to inhibit fungal growth. The flies were maintained under laboratory conditions at a temperature of  $22 \pm 1^\circ\text{C}$ , approximately 70% relative humidity, and a 12:12 hour light-dark cycle.

### Establishment of Experimental Stock

**Wheat cream agar media (Control media):** This was made using 100g of jaggery, 100g of rava powder, and 10g of agar in 1000ml of boiling distilled water, along with 7.5ml of propionic acid.

### Treated Cerelac Media:

10% of cerelac media was prepared using 100g of jaggery, 90g of rava powder, 10g of cerelac powder, 10g of agar powder in 1000ml of boiling distilled water, along with 7.5ml of propionic acid.

20% of cerelac media was prepared using 100g of jaggery, 80g of rava powder, 20g of cerelac powder, 10g of agar

powder in 1000ml of boiling distilled water, along with 7.5ml of propionic acid.

30% of cerelac media was prepared using 100g of jaggery, 60g of rava powder, 30g of cerelac powder, 10g of agar powder in 1000ml of boiling distilled water, along with 7.5ml of propionic acid. The flies obtained from above 3 media were used to conduct heat resistance experiment in *D. melanogaster*.

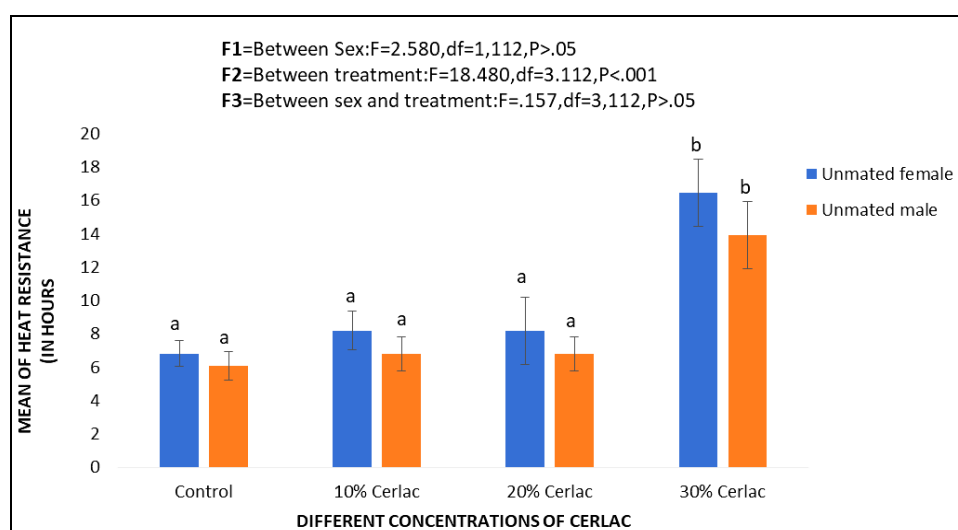
### Experimental Procedure

To study the heat resistance, 5 days old virgin, mated male and female *Drosophila* flies from the wheat cream agar, 10%, 20%, and 30% cerelac media were used. Five flies from each media were transferred to separate empty vials (plugged with cotton) and kept in water bath at  $37^\circ\text{C}$ . For every 5 minutes interval, the vials were observed until the death of each fly. A total of three replicates were observed for each of wheat cream agar media, 10%, 20% and 30% cerelac media each replicate contains 5 flies. Separate experiments were carried out for unmated (male and female flies) and mated flies (male and female).

### Result

Figure 1: showed the mean and standard error value of heat resistance in unmated female and unmated male flies cultured in Wheat cream agar media, 10%, 20% and 30% cerelac media. According to this data, heat resistance was greater in 30% of cerelac media, more or less similar in wheat cream agar media, 10% and 20% cerelac media. Further, this data also showed that unmated female flies had more heat resistance than unmated male flies in 30% of cerelac diets.

The above heat resistance data subjected to two-way ANOVA followed by Tukey's Post hoc test showed significant variation in time taken by unmated female and male flies cultured wheat cream agar media, 10%, 20% and 30% cerelac media in to survive in heat temperature and interaction between treatment and sex. However, insignificant variation in heat resistance was noticed in sex Tukey's post hoc test showed significant variation in heat resistance in unmated female flies of all diets of cerelac media.



**Fig 1:** Effect of cerelac on heat resistance in unmated female and unmated male of *Drosophila melanogaster*

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

Figure 2: showed the mean and standard error value of heat resistance in mated female and mated male flies cultured in Wheat cream agar media, 10%, 20% and 30% cerelac media.

According to this data, heat resistance was greater in 20% and 30% cerelac media, more or less similar in wheat cream agar media and 10% cerelac media. Further, this data also showed that mated male flies had more heat resistance than mated female flies in 20% of cerelac diets and mated female flies had more heat resistance than mated male in 30% of cerelac. The above heat resistance data subjected to two-way ANOVA followed by Tukey's Post hoc test showed significant

variation in time taken by mated female and male flies cultured wheat cream agar media, 10%, 20% and 30% cerelac media in to survive in heat temperature. However, insignificant variation in heat resistance was noticed in sex and interaction between treatment and sex. Tukey's post hoc test showed significant variation in heat resistance in mated female flies of 30% cerelac media.

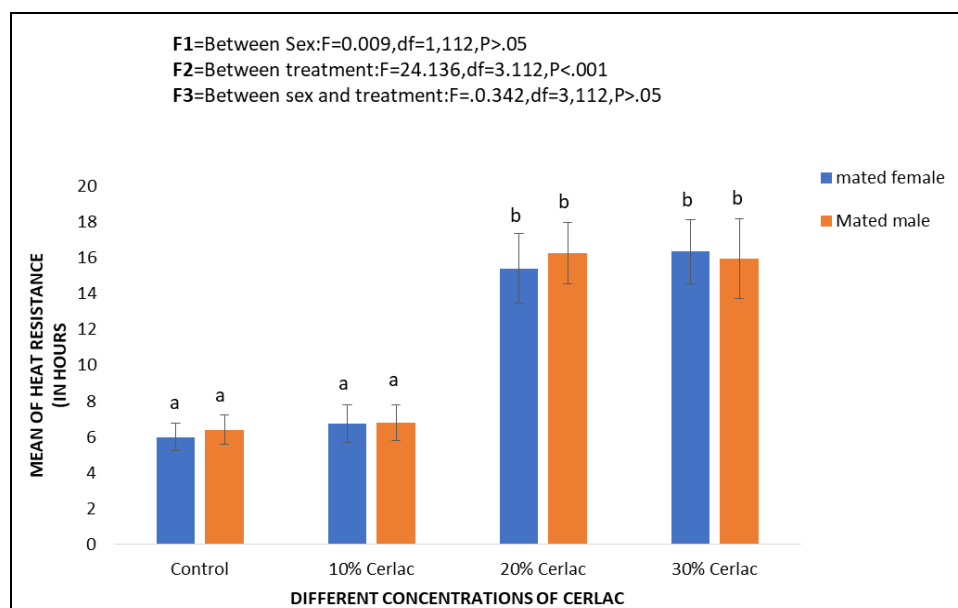


Fig 2: Effect of cerelac on heat resistance in mated female and mated male of *Drosophila melanogaster*

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

Figure 3: showed the mean and standard error value of heat resistance in unmated female and mated female flies cultured in Wheat cream agar media, 10%, 20% and 30% cerelac media. According to this data, heat resistance was greater in 20% and 30% cerelac media, more or less similar in wheat cream agar media and 10% cerelac media. Further, this data also showed that unmated female flies had more heat resistance than mated male flies in all diets.

The above heat resistance data subjected to two-way ANOVA followed by Tukey's Post hoc test showed significant variation in time taken by unmated female and mated male flies cultured wheat cream agar media, 10%, 20% and 30% cerelac media in to survive in heat temperature. However, significant variation in heat resistance was noticed between treatment and insignificant variation was noticed interaction between sex and treatment and between sexes. Tukey's post hoc test showed significant variation in heat resistance in unmated female flies of wheat cream agar media, 10%, 20% and 30% cerelac media.

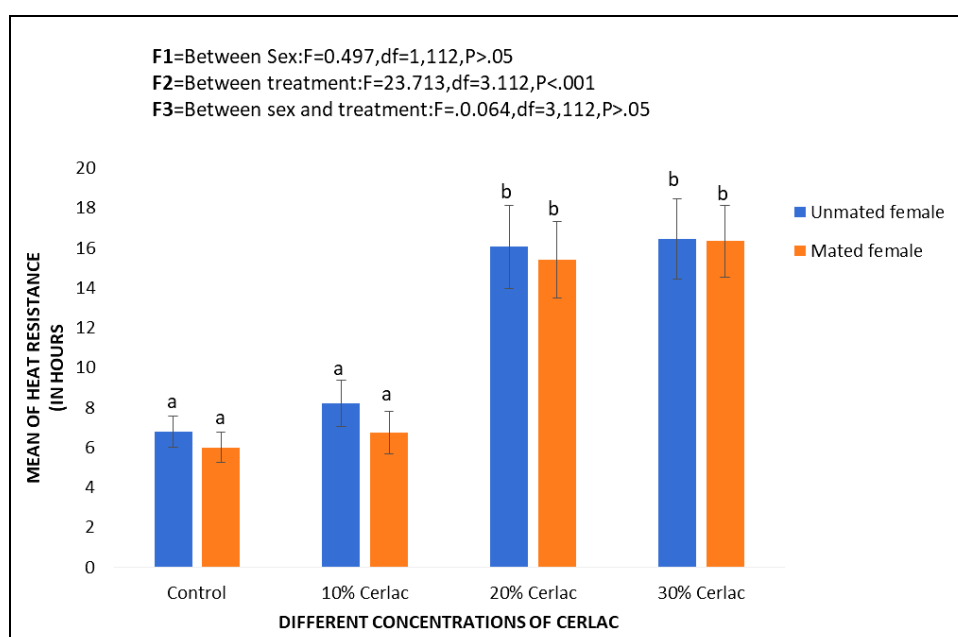


Fig 3: Effect of cerelac on heat resistance in unmated female and mated female of *Drosophila melanogaster*

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

Figure 4: The mean and standard error value of heat resistance in unmated male and mated male flies cultured in Wheat cream agar media, 10%, 20% and 30% cerelac media. This data showed highest heat resistance in flies fed with 20% and 30% cerelac media compared to other two diets. The result also showed that mated male flies had greater heat resistance than unmated male flies in 20% and 30% cerelac media and

more or less equal in wheat cream agar media and 10% of cerelac media.

The above data was subjected to two-way ANOVA followed by Tuckey's Post hoc test showed significant variation in heat resistance between treatment. However, insignificant variation in heat resistance was noticed in interaction between diet and sex between sexes. Tukey's post hoc test showed significant variation in heat resistance in unmated male and mated males flies of wheat cream agar and 10% of cerelac media and mated flies of in other two diets.

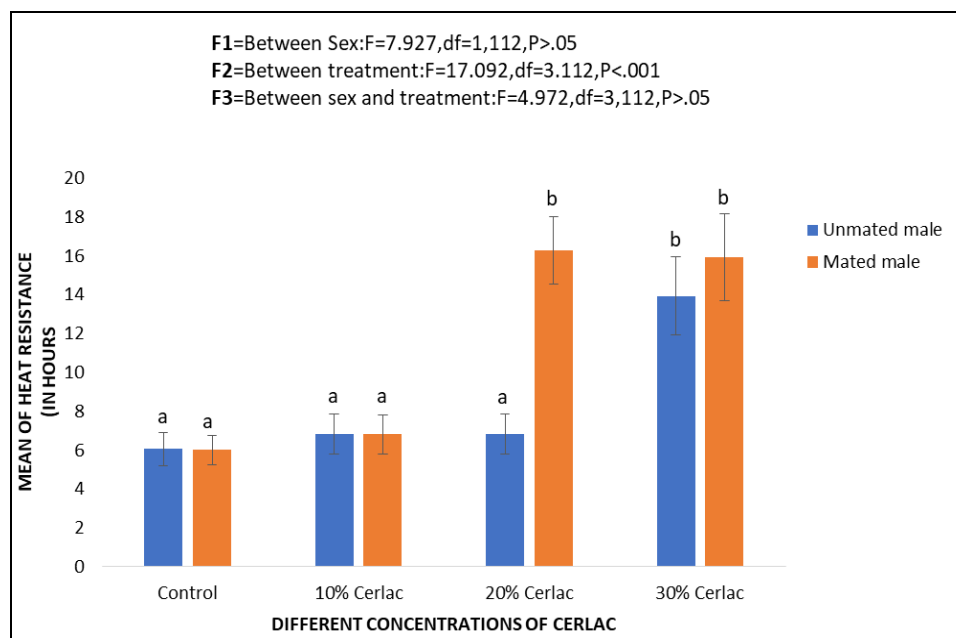


Fig 4: Effect of cerelac on heat resistance in unmated male and mated male of *Drosophila melanogaster*

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

## Discussion

Physical health, sex, and nutritional status are among the factors that help organisms endure stress, although their overall effects remain somewhat ambiguous (Harshmann and Schmid, 1998). Diet, in particular, serves as a critical environmental factor influencing stress, including thermal and starvation stress. Due to its significance in helping insects adjust to fluctuating environmental conditions, numerous studies have explored stress-related traits across various insect species.

The current findings (Fig. 1-4) shows that flies consuming a 30% cerelac-enriched diet exhibited higher resistance to heat stress compared to those on a 10% and 20% cerelac media, which displayed moderate and lowest heat resistance, and those fed with wheat cream agar, which showed the lowest resistance. This indicates that both the availability and nutritional quality of food influence heat tolerance. Although the mechanism behind improved heat knockdown resistance in protein-fed flies is not fully understood, it may involve the upregulation of heat shock proteins, which are central to stress responses (Srensen *et al.*, 2005; Sinclair *et al.*, 2007) [31]. Species that have historically been exposed to high temperatures are likely to activate the heat shock response and develop genetic diversity in heat resistance. This diversity may include variations in the expression levels of heat shock proteins, amino acid sequences, or the thermal resilience of structural and enzymatic proteins (Krishna and Koushik, 2014). Though the current study did not directly measure HSP

expression, the results imply that the high protein and carbohydrate content in the cerelac diet could have enhanced the flies' capacity to tolerate heat stress. The different concentrations of cerelac diets offered moderate energy, while the wheat cream agar diet provided the least, correlating with the observed levels of thermal resistance. These findings align with those of Sisodia and Singh (2012) [33], who concluded that flies fed with protein-rich diets had significantly better heat resistance than those on carbohydrate-dominant diets.

Our findings also highlight sexual dimorphism in heat resistance. Specifically, female *Drosophila melanogaster* exhibited greater resistance to heat stress compared to males (see Fig. 1 & 2). One possible explanation is that females may consume more food than males, leading to greater lipid accumulation. Supporting this, females have been reported to possess higher fat and protein content relative to males, which are crucial for energy metabolism (Carvalho *et al.*, 2006; Lee *et al.*, 2013). This implies that females primarily derive energy from stored fats and glycogen. On the other hand, heat-tolerant males rely on their lipid reserves for energy, which depletes their energy stores and reduces their capacity to withstand heat.

Further analysis (Fig. 3) revealed that unmated females were more heat resistant than mated females. This could be due to a relatively lower protein intake in unmated females. Moreover, the physiological demands of mating and increased egg production can negatively affect survival, as discussed in Flatt (2011) and Harshman and Zera (2006). It is also plausible that virgin females reabsorb nutrients from undeveloped eggs to endure thermal stress. Recent studies suggest that oosorption,



or the autophagic reabsorption of oocytes, enhances heat tolerance in virgin females.

Additionally, heat resistance appears to be influenced by mating status. As shown in our results (Fig. 4), unmated males demonstrated greater thermal tolerance compared to their mated counterparts. This difference could be attributed to the energetic costs associated with mating, such as the transfer of sperm and accessory gland proteins (ACPs), which unmated males do not experience, thereby preserving their energy and enhancing heat resistance. (Ravi Ram and Wolfner 2017).

While numerous internal and external factors such as nutrition, age, environmental temperature, and social interactions can affect an organism's stress response in our study same aged flies and were cultured in same temperature. Therefore the observed differences in heat tolerance were therefore likely driven by the variation in nutrient type and intake, which significantly influenced the thermal resilience of *D. melanogaster*. Different experiments were conducted by using different products on drosophila in heat resistance experiment under same environmental conditions but differences in concentrations of cerelac diets. Because of different nutrients present in cerelac our result shows increased heat tolerance in *D. melanogaster*.

## Conclusion

Hence our experimental study we can conclude that, the flies developed with cerelac supplemented diet had the greater resistance to the heat than the flies fed with wheat cream agar media further heat resistance increased with increasing the concentration of cerelac. Thus these studies suggesting that quality and quantity of nutrients present in cerelac affects heat resistance in *D. melanogaster*.

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