**Impact Factor (SJIF): 6.092** **E-ISSN: 2583-1615**

International Journal of Research in Academic World

**Received**: 21/May/2024 **IJRAW:** 2024; 3(6):96-99 **Accepted:** 25/June/2024

# The Effect of Whey Protein on the Cold Resistance in *Drosophila Melanogaster*

**[1]Harshitha S, [2]Manaswini D Kashyap, [3]Darshini M, [4]Naveenashree N, [5]Purushotham MR, [6]Darshan BK and [*7]Krishna MS**

[1, 2, 3, 4, 5, 6, *7] Department of Zoology, Drosophila Stock Centre, University of Mysore, Manasagangotri, Mysuru, Karnataka, India.

**Abstract**

Stress resistance in an organism may be influenced by various factors including morphological, physiological and behavioural changes. Resistance to stress is influenced by quality and quantity of nutrition present in diet. The current study demonstrates the effect of the Whey protein on the cold 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 cold resistance than Wheat cream agar and mixed media. This shows that Whey protein provides sufficient nutrition to withstand cold. This study also shows that females (virgin and mated) have more starvation resistance than that of males (virgin and mated) in all the diets. Further, both mated male and female flies had greater cold resistance than those of unmated flies. Thus, Whey protein increases cold resistance in *D. melanogaster*.

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

## Introduction

Fitness related characteristics in insects, including growth, immunological response, stress tolerance, fertility, and longevity are influenced by the composition of their diet (Burger *et al*., 2007) [1]. Insect distribution and abundance can be impacted by frequent shifts in their surrounding environmental conditions. Stress resistance in an organism may be influenced by various factors including morphological, physiological and behavioural changes. At high latitudes, it is expected that the environmental stress will cause species to shift the usage of their resources, giving survival a greater priority than reproduction. This is due to the fact that in order for an organism to survive the winter, it must produce a wide range of expensive metabolites and proteins in addition to beginning to store resources (Lee, 1991).

Due to its wide inter and intraspecific variation in the species' thermal tolerance, high degree of heat tolerance, and status as a model organism, *Drosophila* is a valuable model for relating the effects of temperature on biochemistry and physiology to ecological patterns and processes. Cold resistance has a major impact on the distribution and abundance of insects, and temperature acclimation has a major impact on the phenotypes of organismal stress resistance, particularly in small ectotherms like *Drosophila.* Because the nature and degree of resistance vary so greatly between and within species (Stanely *et al*., 1980; Kimura, 1988) [16 9].

Life history methods systematically divide resources between reproduction and survival, and the relative importance of each is impacted by a range of internal and external factors (Stearns, 1992, 2000; Roff, 2002) [17, 18, 12]. Given that a number of these factors-like age, density, and reproductive status-change during the course of an organism's existence. Different allocations will be preferred via selection at different times or under different situations. Consequently, organisms typically adapt by plastically changing the relative distribution of resources in response to environmental cues, particularly in situations where environmental changes are predictable (Scheiner, 1993) [14].

Now a days people are using Whey protein to build lean muscle mass and boost muscle protein synthesis. Whey protein is a high quality cow's milk protein powder. Casein makes up around 80% of the protein in milk, whereas whey protein makes up about 20%. Less than 1% of proteins are found in whey, primarily in the forms of immunoglobulins, β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), lactoperoxidase, lactoferrin, lactollin, glycoproteins and transferrin. The Muscle Asylum Premium Whey protein have the following nutritional value per 40g (one serving): 24g of protein, 5.2g of BCAAs, no sugar, and minimal carbs. It also has flavour (banana) and digestive enzymes. It contains glutamic acid and important amino acids such as leucine, valine, lysine, isoleucine, methionine, phenylalanine, threonine, tryptophan, and histidine. There are various studies shows the health benefits of Whey protein but there is no evidence documented on the effect on cold resistance. Therefore, the study was undertaken to address the effect of the Whey protein on the cold resistance in *D. melanogaster*.

***Corresponding Author:** Krishna MS

## Materials and Methods

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

## Establishment of Stock

*D. melanogaster* (Oregon K strain) flies were obtained from *Drosophila* stock centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru. The obtained flies were cultured in bottles with wheat cream agar media (prepared by adding 100g jaggery, 100g rava powder, 10g agar in 1000ml boiling distilled water and 7.5ml propionic acid to prevent fungal growth). These flies were maintained in laboratory conditions with temperature of 22±1$^0$C, 12:12 light and dark cycle and humidity level of approximately 70% RH.

## Establishment of Experimental Stock

Flies from the above stocks were used to culture experimental stocks in different media.

**Wheat Cream Agar Media (Control Media):** Was prepared by 100g of jaggery, 100g of rava powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

**Whey Protein Media (Treated Media):** Was by 100g of Muscle Asylum Premium Whey protein powder, 100g of jaggery and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

**Mixed Media** was prepared by 50g of rava powder, 50g of Muscle asylum Premium Whey protein powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid]. These flies were maintained under the laboratory condition mentioned above and used to study cold resistance in *D. melanogaster*.

## Experimental Procedure

To study the cold resistance, five days old virgin and mated male and female *Drosophila* flies from the wheat cream agar, whey protein, and mixed media were used. Five flies from each media were transferred to separate empty vials (plugged with cotton) and kept in refrigerator at 5$^0$ C. For every one-hour interval, the vials were observed until the death of each fly. A total of sixty flies were observed for each of wheat cream agar media, mixed media and whey protein media. Separate experiments were carried out for unmated and mated flies.

## Results

### Effect of Whey Protein on Cold Resistance in Unmated Male and Unmated Female on *Drosophila Melanogaster*

Fig.1 showed the mean and standard error value of cold resistance in unmated male and unmated female flies cultured in Wheat cream agar media, Mixed media and Whey protein media. According to this data, cold resistance was greater in whey protein media, average in wheat cream agar media and least in mixed media. Further, this data also showed that unmated female flies had more cold resistance than unmated male flies in all provided diets. The above heat resistance data subjected to two-way ANOVA followed by Tuckey'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 cold temperature. However, insignificant variation in cold resistance was noticed in interaction between treatment and sex. Tukey's post hoc test showed significant variation in cold resistance in unmated male and female flies of wheat cream agar and mixed media and unmated male flies of whey protein media.

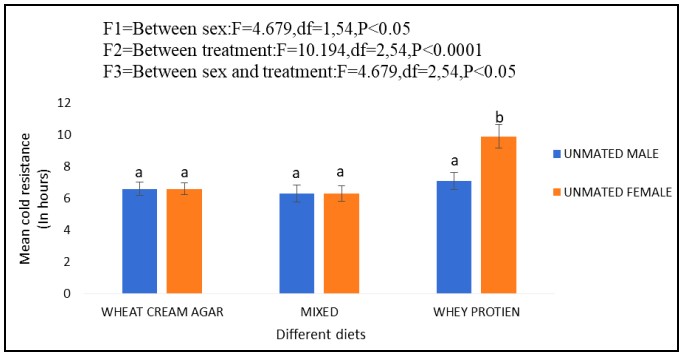


**Fig 1:** Effect of Whey protein on cold resistance in unmated male and unmated female of *Drosophila melanogaster*

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

### Effect of Whey Protein on Cold Resistance in Mated Male and Mated Female on *Drosophila Melanogaster*

The mean and standard error value of cold resistance in mated male and mated female flies cultured in Wheat cream agar media, Mixed media and Whey protein media were represented in Fig. 2. The flies cultured in whey protein showed greatest cold resistance when compared to, average in mixed media and least in wheat cream agar media. Further, this data also showed that mated female flies had more cold resistance than mated male flies in all provided diets. The above heat resistance data subjected to two-way ANOVA followed by Tuckey's Post hoc test showed significant variation in cold resistance by mated male and female flies cultured in control, mixed and treated diet. However, insignificant variation in cold resistance was noticed in interaction between treatment and sex. Tukey's post hoc test showed significant variation in heat resistance in mated male and female flies of wheat cream agar and mixed media.

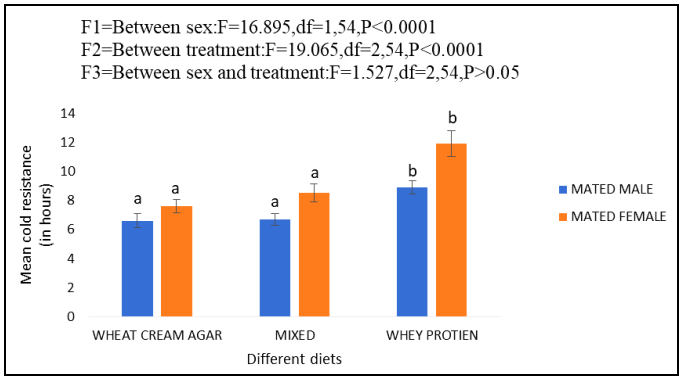


**Fig 2:** Effect of Whey protein on cold resistance in mated male and mated female of *Drosophila melanogaster*

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

### Effect of Whey Protein on Cold Resistance in Virgin (Unmated) Male and Mated Male on *Drosophila Melanogaster*

The mean and standard error value of cold resistance in unmated male and mated male flies cultured in Wheat cream agar media, Mixed media and Whey protein media are provided in Fig.3. This data showed highest cold resistance in flies fed with whey protein media compared to other two diets. The result showed that mated male flies had greater cold resistance than unmated male flies in mixed and whey

protein media and more or less equal in wheat cream agar media. The above data was subjected to two-way ANOVA followed by Tuckey's Post hoc test showed significant variation in cold resistance in between diets and in between sex. However, insignificant variation in cold resistance was noticed in interaction between diet and sex. Tukey's post hoc test showed significant variation in cold resistance in unmated and mated males flies of wheat cream agar and mixed media and unmated flies of whey protein media.

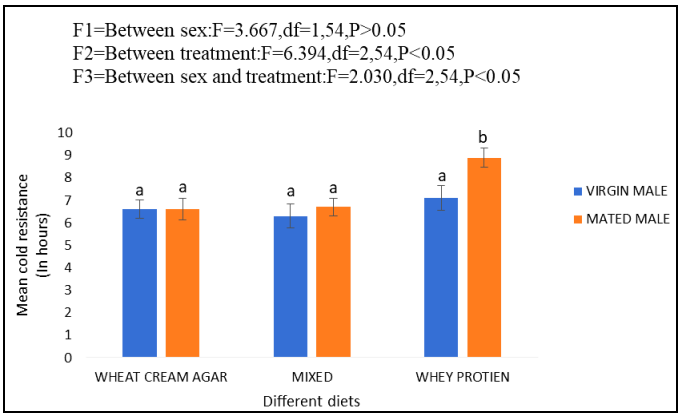


**Fig 3:** Effect of Whey protein on cold resistance in unmated male and mated male of *Drosophila melanogaster*

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

**Effect of Whey Protein on Cold Resistance in Unmated Female and Mated Female on *Drosophila Melanogaster***

The mean and standard error value of cold resistance in unmated female and mated female flies cultured in Wheat cream agar media, Mixed media and Whey protein media are represented in Fig.4. This data showed greater cold resistance in flies cultured in whey protein media compared to other two media. The result showed that mated female flies had greater cold resistance than those of unmated female flies in all three diets. The above data was subjected to two-way ANOVA followed by Tuckey's Post hoc test showed significant variation in cold resistance in between diets and in between sex. However, insignificant variation in cold resistance was noticed in interaction between diet and sex. Tukey's post hoc test showed significant variation in heat resistance in unmated and mated females flies of wheat cream agar and mixed media.

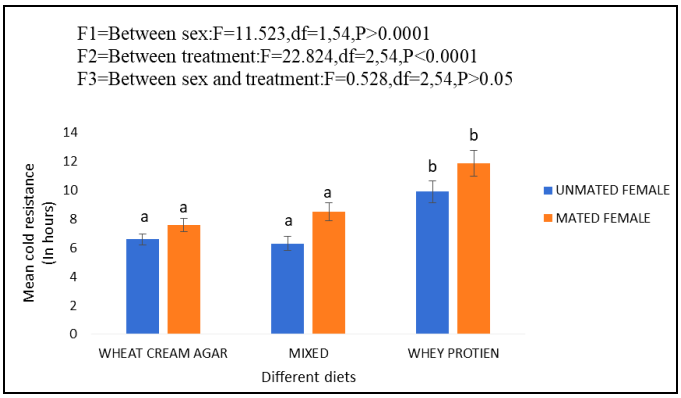


**Fig 4:** Effect of Whey protein on cold resistance in unmated female and mated female of *Drosophila melanogaster*

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

**Discussion**

Stress related characters differ in insects and other organisms has been studied widely due to the ability of insects to adopt and ability to cope up the changes in climate. Tolerance of an organism to thermal stress can be resultant of the variations in the nutrition, sex, and genetic composition. In insects, availability of diet and dietary composition have an influence on cold resistance.

In the present study, the results (Fig. 1-4) reveals that flies fed with whey protein have greater tolerance towards cold and variations in cold resistance can be seen in flies fed with wheat cream agar media and mixed media. This suggests that, the quality, and amount of nutrition available in diet have an influence on variation of cold resistance. Whey protein is rich with amino acids which provide energy to withstand the cold resistance in *D. melanogaster*. This suggests that variations in the flies' tolerance to cold were impacted by the food's quality and availability of nutrients. The high protein content in Whey protein have helped *D. melanogaster* to resist cold.

There is evidence that selecting for cold tolerance raises the lipid level (Chen and Walker, 1994) [3]. Furthermore, Kimura (1982) [8] discovered that the acquisition of cold tolerance in *Drosophila* has been linked to the accumulation of haemolymph sugars. Furthermore, the flies' ability to withstand cold temperatures was found to be negatively affected by a larger concentration of sugars in their diet.

Our data also supports sexual dimorphism with reference to cold resistance. In results (Fig. 1 and 2), shows that female flies had more cold resistance than those of male flies in all the diets provided. This suggests that, males and females responded to cold differently in terms of changes in gene expression, which suggests that they mostly use the same mechanisms to modify their physiology. It has also been suggested by a number of studies that the nutritional makeup influences cold resistance in both sexes. Age, condition, life cycle, and other characteristics are likely to have an impact on how much is traded off against cold stress. However, remarkably little research has been done on one potentially significant factor: sex. It is found in this study that in all three diets, females were more resistant to cold against men. It may vary between genders as a result of variations in regulatory architecture or proportional disparities in the costs of life or reproduction (Grath and Parsch, 2016; Mank, 2017) [7, 11].

Additionally, we observed that the flies' cold tolerance differs in mated and unmated situations, and that sexual variations also affect the flies' thermal stress. As previous researches have suggested, mating may not be harmful to male flies and may even benefit them in terms of cold resistance. This is supported by the results of the current study (Fig. 3), which indicate that mated males exhibit greater cold resistance than virgin males. In *D. melanogaster*, females may benefit from increased mating activity and rate. Thus, it makes sense that, similar to female flies, post-mating emergence could lead to increased cold tolerance compared to virgin flies. To improve the *D. melanogaster's* thermal resistance, more energy is used in our study because we did not assess the amount of food consumed. A further explanation that could be offered is that the pheromones that female flies produce during copulation may constantly expose mated male flies to different stimuli, changing their physiology and potentially giving them a higher tolerance to cold than virgin males. Several studies have demonstrated, in contrast to our work, that mated male

flies lose their energy during mating and transfer sperm and accessory gland proteins (Acps), making them more susceptible to the cold than virgin flies.

In comparison to the unmated (virgins) females, the mated females have a higher degree of resistance against cold, as depicted in Figure 4. The findings of our study support the findings of Goenga *et al*. (2012), who, in their studies of *D. melanogaster*, also showed that mated females show a greater tolerance to stress than virgins. Researchers Ravi Ram and Wolfner (2007) [12] and Carvalho *et al*., (2006) have demonstrated that female *Drosophila* increase in food intake following mating, which may provide a physiological explanation for the variations in stress resistance between mated and virgin females. Moreover, the nuptial gifts that males provide to females during mating may also be the reason. Specifically, males transfer accessory gland proteins during mating, while seminal gland proteins transferred to female during copulation. The complex protein mixture that makes up the seminal fluid, which is secreted by the accessory gland and carried to the female after copulation, is created by the gland (Wolfner, 2002) [19]. Among mated females, accessory gland proteins (Acps) induce physiological and behavioral alterations (Gillot, 2003) [5]. This could be the reason married women are more resistant to the cold than virgins. With the use of our findings, we are able to explain why the mating condition benefits the physiological behaviour of *D. melanogaster*, helping both sexes adapt to and endure the cold.

While there are a lot of internal and external variables that influence stress resistance in an organism, such as nutrition, social interactions, age, and environmental temperature, genetic variation can also have an impact. To investigate *D. melanogaster's* cold tolerance, however, we kept the same age and temperature of the flies in our study while giving them varied meals. The difference in *D. melanogaster's* cold resistance was thus caused by variations in the quantity and quality of nutrients included in the diet.

## Conclusion

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

## Acknowledgement

The Authors extend their gratitude to the Chairman, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru, and also would like to thank the *Drosophila* stock center, University of Mysore for providing the facilities to carry out the above major project work.

## References

1. Burger JM, Hwangbo DS, Corby-Harris V, Promislow DE. The functional costs and benefits of dietary restriction in Drosophila. Aging Cell. 2007; 6:63-71.
2. Carvalho GB, Kapahi P, Anderson DJ, Benzer S. Allocrine modulation of feeding behavior by the sex peptide of Drosophila. Curr. Biol. 2006; 16:692-696.
3. Chen CP, Walker VK. Cold shock and chilling tolerance in *Drosophila*. Journal of insect Physiology. 1994; 40(08):661-669
4. Chen CP, Denlinger DL, Lee RE. Jr Responses of mom diapausing flesh flies to low rearing temperatures: developmental rate, cold tolerance, and glycerol concentrations. Annals of the entomological society of America. 1987; 80:790-796.
5. Gillott C. Male accessory gland secretions: Modulators of Female Reproductive Physiology and Behavior. Annu Rev Entomol. 2003; 48:163-184.
6. Goenaga J, Mensch J, Fanara JJ, Hasson E. The effect of mating on starvation resistance in natural populations of *Drosophila melanogaster*. Evol Ecol. 2011; 26:813-823
7. Grath S, Parsch J. Sex based gene expression. Annual review of genetics, 2016, 50(1).
8. Kimura MT. Inheritance of cold hardiness and sugar contents in two closely related species, Drosophila takahashii and Drosophila lutescens. Jpn. J. Genet. 1982; 57:575-580
9. Kimura MT. Adaptations to temperate climates and evolution of overwintering strategies in the *Drosophila melanogaster* species group. Evolution. 1988; 42(6):1988-1297
10. Lee Bourg E. Oxidative stress, aging and longevity in Drosophila melanogaster. FEBS Lett. 2001; 498:183-6
11. Mank JE. The transcriptional architecture of phenotypic dimorphism. Nat. Ecol. Evol. 1, 0006, 2017.
12. Ravi Ram K, Wolfner MF. Seminal influences: Drosophila ACPs and the molecular interplay between males and females during reproduction. Integr Comp Biol. 2007; 47(3):427-45.
13. Roff DA. Life history evolution. Sinauer associates; Sunderland, MA, 2002.
14. Scheiner S. Genetics and evolution of phenotypic plasticity. Annu. Rve. Ecol. Syst. 1993; 24:35-68.
15. Shankar JR, Bansal GK. A study on health benefits of Whey proteins. *International journal of Advance biotechnology and research*. 2013; 4:15-19.
16. Stanley SM, Parsons PA, Spence GE, Weber L. Resistance of species of Drosophils melanogaster subgroup to environmental extremes. *Aust J Zool*. 1980; 28(3):413-421.
17. Stearns SC. The evolution of life histories. Oxford University press, Oxford, 1992.
18. Stearns SC. Life history evolution: succession, limitations, and prospects. Naturwiss. 2000; 87:467-486.
19. Wolfner MF. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. Heredity. 2002; 88:85-93.