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The Ensure® Nutritional supplement increases the Cold resistance in *Drosophila melanogaster.*

Tanmayi Kishore, Yashaswini . S.P., Shilpashree. B. M., Suma .S.,

Ashwini .M., Aishwarya. K.C.

Corresponding author :- M.S. Krishna*

Department of Studies in Zoology, Drosophila Stock Centre, University of Mysore, Manasagangotri, Mysuru-560006. Karnataka, India.

ABSTRACT

Nutrition consumed by an organism provides energy for the survival and other vital activities to perform properly in the body of the organisms. Environmental stress is one of the important factor that the organism should face during its life time. A healthy balanced diet should be maintained by the organism to lead a better life and resist to the surrounding stress. In the present study the flies of *Drosophila melanogaster* were cultured in different media (wheat cream agar media, Ensure® nutrition supplement media and mixture to these two media) to check the cold resistance of the flies. Our result showed that the flies which fed on Ensure® nutrition supplement media showed greater resistance than control and mixed media. We also noticed that mated females were more resistant to cold than the mated male flies. Hence this experiment suggest that Ensure® treated media increased the cold resistance in *Drosophila melanogaster.*

Keywords: Cold resistance, virgin, mated, control, mixed, and test, *Drosophila melanogaster.*

INTRODUCTION:

An organism's capacity to tolerate stress can be influenced by a wide range of factors, which can lead to physiological and behavioral changes. Additionally, it was shown that an organism's experience with climate change could lead to physiological changes like a coma, the hardening process, the production of metabolites, and the ability to withstand extreme temperatures (Srensen; Nielson *et al.*, 2005; Lalouette; Kostal *et al.*, 2007). Life history features like fecundity, fertility, longevity, and stress resistance are subsequently impacted by these changes. More food restriction or light fasting can also result in increased tolerance to stress, such as cold stress.(Wenzel ,2006; Smith, Hoi *et al.*, 2007). Recent studies (Prasad et al., 2003; Jenki *et al.*, 1997; Bijlsm et

al., 1996) have shown that characteristics of organisms such as longevity, fertility, susceptibility to disease, and stress tolerance are highly impacted by the amount and caliber of nutrients they eat.

According to evolutionary scientists, tolerance to difficult environments is a major concern (Hoffman and Parsons, 1991; Bijisma and Loeschcke, 1997). Severe conditions increase physiological strain, which influences the direction of stress tolerance selection. For ectotherm animals, especially insects, temperature is an important abiotic factor that can cause heat or cold stress. There has been much less research on cold tolerance, with the majority of studies concentrating on the consequences of very low temperatures, such as freezing tolerance and cryoprotection (Leather *et al.*, 1993). Studying sublethal reactions in cold-stressed insects can help us learn more about the physiological mechanisms that both encounter and prevent cold-related damage as well as fitness consequences. However, these responses are often disregarded and overlooked in the literature.

The importance of the fruit fly *Drosophila melanogaster* as a model organism in nutrition research is becoming more widely acknowledged. The makeup of the experimental diets needs to be carefully considered while performing nutritional studies on fruit flies, in addition to complicated diets that frequently consist of wheat, yeast, sugar, and agar. Moreover, chemically designed diets can be fed to drosophila. Although the precise macro and micronutrient content of these so-called holistic meals is defined, the quantitative nutrient requirements of flies are still unknown and require more research. (Gerald Rimbach, Thomar Roder, and Kai Luersen in 2019).

It is also used as a model organism in evolutionary biology and ecophysiology, where it is studied along with related physiological mechanisms. Various diets, many of which are nutrient-rich and differ from foods found in the natural environment, are used in different facilities to feed *Drosophila* cultures, along with development time, dry body mass, and other characteristics. The nutritional contents of the standard were analyzed and addressed in relation to the phenotypic data.

One important extrinsic factor that can affect an organism's survival, growth, and development is its diet (Sisodia and Singh, 2012). It has been shown that food restrictions that do not result in famine impact the longevity and procreative capacity of a wide range of species, including nematode worms and mammals. Studies demonstrated that diet significantly affected both longevity and fertility (Piper and others, 2011). There are two categories for how an organism's diet affects its life history traits: quantity, which is determined by the availability of food, and quality, which is determined by the nutritional content of food (Sisodia and Singh, 2012). It's been shown that Ensure® is a complete, balanced nutrition with all essential nutrients.

Ensure® provides good nutrition to help keep strong and healthy and also helps malnourished adults improve their nutritional status and body weight. 32nutruents to help people stay strong and active and provide high-quality protein. Ensure has a balanced macronutrient ratio supporting optimum protein utilization. Two servings, Ensure® meets 80% of the RDA for calcium to support strong bones. By nature, it is free of gluten and it can be consumed after reconstituting either along with water or with milk. Changing an animal's food can lengthen its lifespan including that of flies. Ensure powder is primarily indicated for immunological function, weight loss, muscle

hypertrophy, and low-residue diets. Make sure the powder is primarily indicated for immunological function, weight loss, muscle hypertrophy, and low-residue diets.

MATERIALS AND METHODOLOGY:

Establishment of stock:

The experimental stock of *Drosophila melanogaster* was established using the Oregon K strain, which was obtained from the Drosophila Stock Center, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysuru. To cultivate the flies, wheat cream agar media (100g jaggery, 100g wheat powder, 10g agar boiled in 1000 ml distilled water, and 7.5ml propionic acid added to prevent fungal growth) was used. The flies were kept in a laboratory setting with 70% humidity, a 12:12 photoperiod (dark and light cycles), and a temperature of 22°_+ 1° degrees Celsius. We carried out our experiment with these flies.

Establishment of experimental stock:

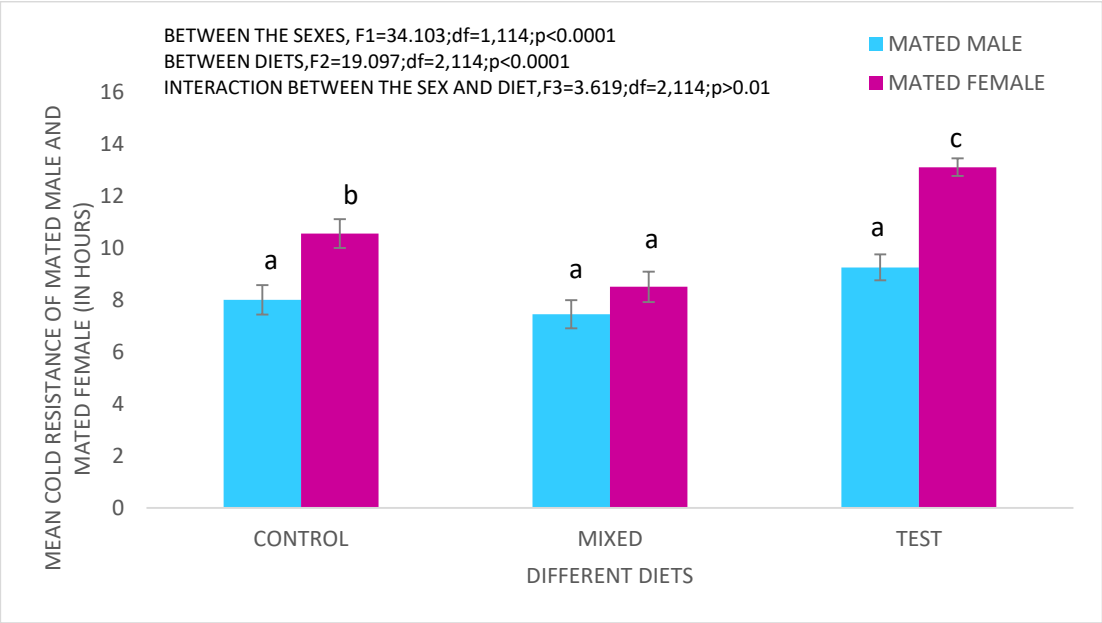
The flies obtained as above were used to establish the experimental stock with different diet media [Wheat cream agar media: Wheat cream agar media was prepared from 100g of jaggery, 100g of semolina (wheat rava powder) 10g of agar boiled in 1000ml of distilled water and 7.5ml of propionic acid added to it; Ensure® powder media or test media was prepared from 100g of jaggery, 100g of ensure powder, 10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it; Mixed (wheat cream + Ensure® powder (1:1) media): Mixed media is prepared from 100g od jaggery, 50g of semolina (wheat cream powder, 50g of Ensure® powder,10g of agar boiled in 1000ml of distilled water and 7.5ml of propionic acid added to it.] The flies that emerged from Wheat cream agar media and other experimental treated media were maintained using the above conditions and were used to study the cold resistance experiment in *Drosophila melanogaster.*

Experimental procedure:

To study Cold resistance, 5-day-old mated (male and female) and unmated(male and female) flies of *Drosophila melanogaster* from control and treated (mixed and test) media were used. Twenty flies from each media were transferred into vials. These flies were kept at -5°c in the refrigerator and observed for every two-hour interval until the death of each fly. A separate experiment was run for both males and females. The data obtained was subjected to Two-way ANOVA followed by Tukey's post hoc test using the SPSS package 26 version.

RESULTS:

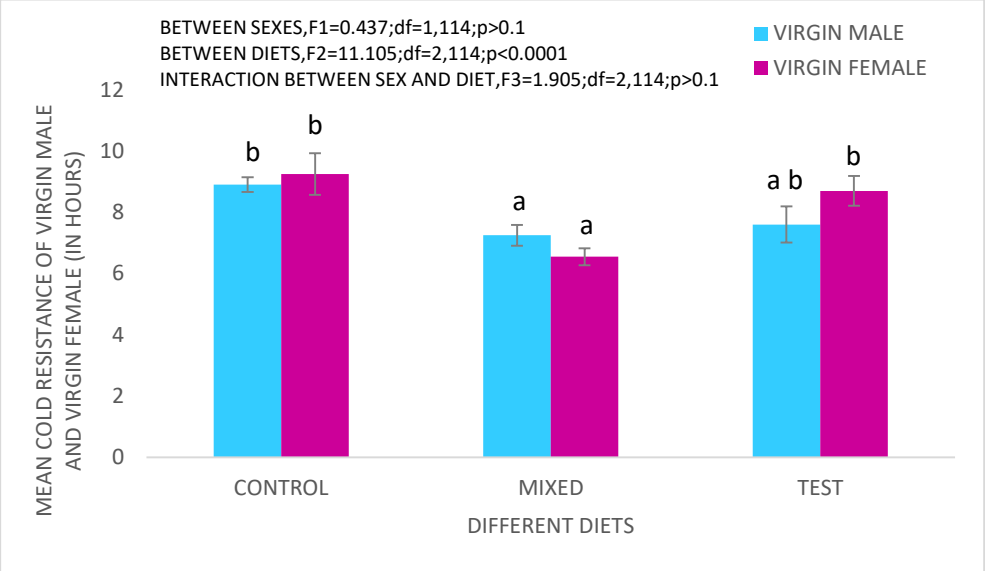
Figure 1: the effect of Ensure® nutritional supplement in mated male and mated female of *Drosophila melanogaster.*



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.

The control mated males and female flies had lesser cold resistance compared to the other 2 diets as shown in Figure 1. The data was subjected to Two way ANOVA followed by Tukey's post hoc test which showed a significant variation in cold resistance of mated male and female flies raised in test media and control media, between sexes. The mated males were less cold-resistant than mated females in all different media. Among the mixture and test media-treated flies, the test flies were more significantly cold-resistant by Tukey's post hoc test.

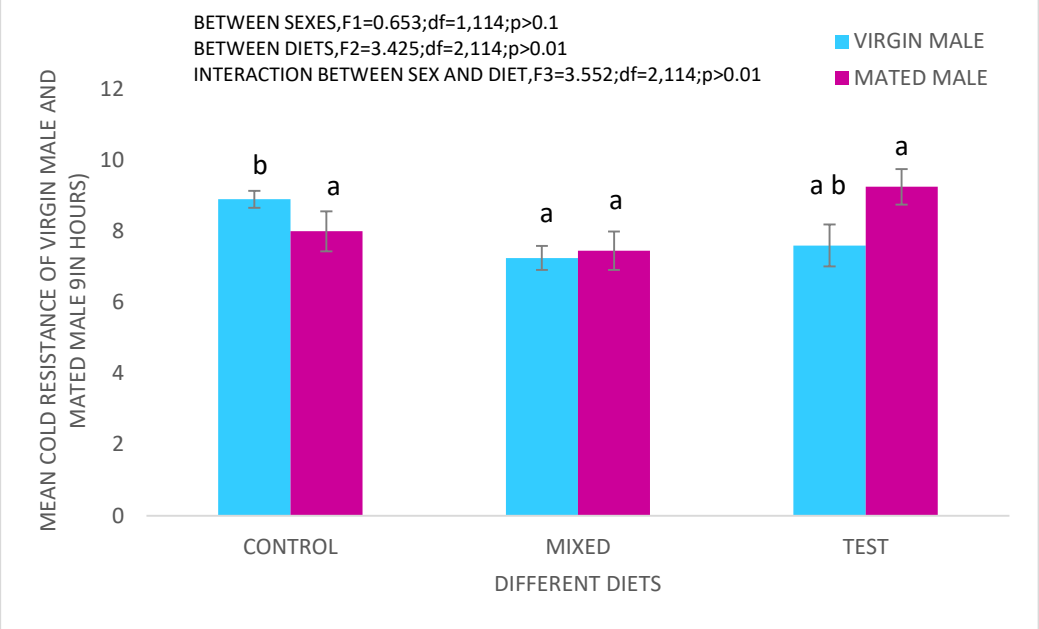
Figure 2: The effect of Ensure® nutritional supplement in unmated male and virgin females *Drosophila melanogaster.*



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.

In Figure 2 the graph shows the mean values of cold resistance in virgin males and virgin females where a significant variation was observed between the treatments. The virgin female flies of control and test media were more resistant to cold compared to the virgin males. The data was subjected to Two-way ANOVA followed by Tukey's post hoc test and the virgin flies of control media were found to be more significantly resistant to cold compared to other media.

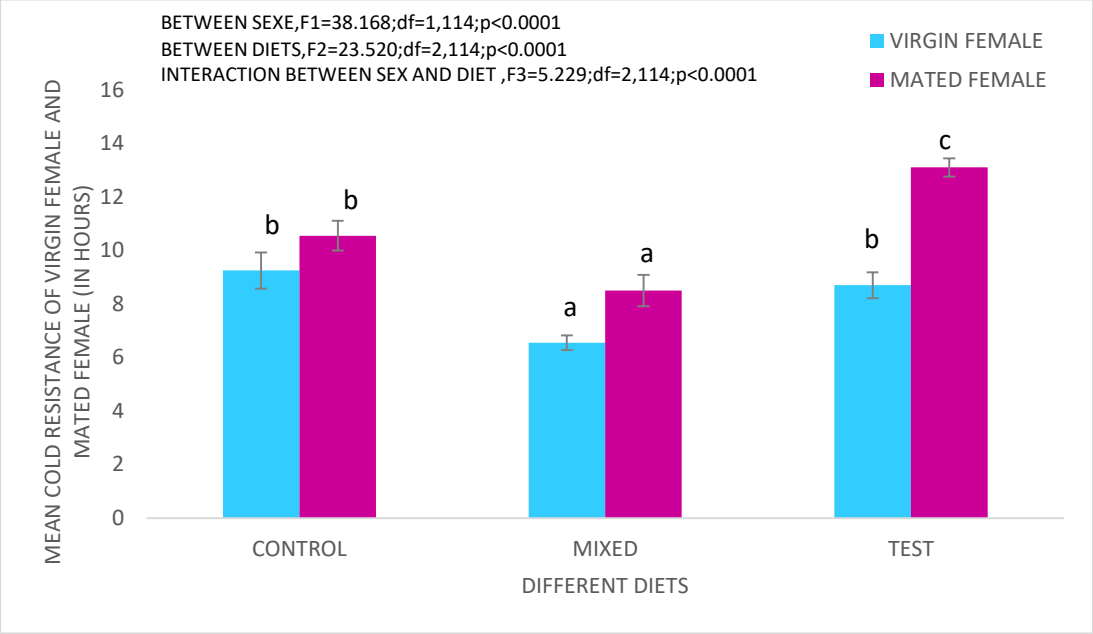
Figure 3: Effect of Ensure® nutritional supplement on the mated male and unmated males of *Drosophila melanogaster.*



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.

The mean and standard error of the control and treated flies graph showed a non-significant variation in between the sexes and diets of virgin males and mated males in Figure 3. The data was subjected to Two-way ANOVA followed by Tukey's post hoc test. The comparision between results of the virgin male flies and mated male flies, the mixture and test treated mated flies were more resistant to cold compared to virgin males. Further we can observe that among ensure treated flies the resistance increased in increasing the concentration of the supplement. The control treated flies were significantly lesser resistant to cold.

Figure 04: Effect of Ensure® nutritional supplement on the mated females and virgin females of *Drosophila 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.

Cold resistance data of control, mixed and test flies are provided in the Figure 04. From the data obtained it was noticed that in both mated and virgin female flies, the lowest cold resistance was observed in the virgin flies of mixed media, and highest cold resistance in the mated females of test media. The comparision between the virgin and mated females showed that more resistant was found in the mated female flies in all the different medias. The data was subjected to Two-way ANOVA followed by Tukey's post hoc test showed the significant variation between the sexes and diets and also interaction between the sex and diet.

DISCUSSION:

For growth, development, physiology, starvation resistance, and survival, food is a crucial component that all organisms need. The quantity and quality of nutrients that an organism consumes have a significant impact on life history processes like reproduction and stress tolerance (Sisodia and Singh, 1988). Ensure® influence on *D. melanogaster's* ability to withstand cold was therefore the subject of the current investigation. The cold resistant of Ensure® treated flies was found to be substantially higher than that of control flies. This indicates that adding Ensure® to meals may improve *D. melanogaster's* ability to withstand cold (Fig 1-4). Physiological changes are required for increased cold tolerance, which are likely to impair other fitness-related traits. By reducing the amount of food, notably protein (yeast), available to adult flies, caloric restriction increases their resilience to cold

, with up to a twofold difference between females previously fed ad labium yeast and those given no yeast. (Chown and Nicolson, 2004; McCue, 2010; Laparie et al., 2012; Ribeiro et al., 2010; Burger et al., 2017).

The Ensure® treated media contain more protein compared to mixed and wheat cream agar media. When insects are restricted to food low in protein relative to carbohydrate (Raubenheier and Simpson, 1999)compensatory feeding for the limiting nutrients results in the over ingestion of other nutrients. This may lead to increased lipid storage and decreased fitness (Simpson et al., 2004, Warbrick-Smith et al., 2006). Several variables can affect an organism's ability to withstand stress.

In the present study also found that females were stronger cold resistance than males in both control and treated flies (Fig 1-4). This is because females in species of *Drosophila* are larger and heavier than male flies in contrast to them. In many organisms, males and females were likely to respond to resistance in different ways, mostly because their nutritional requirements and methods of usage differ (Hoyenga et al., 1982). *D. melanogaster*, considerably affect the pattern of sexual dimorphism in cold resistance.

When exposed to ecologically significant temperature variations, adults of *D. melanogaster*, like many other insects, can quickly increase their thermal tolerance. lower the thermal threshold for survival by 1-2°c, and the improved cold resistance may also be crucial for other fitness-related features including sustained activity and reproductive behavior in colder climates (Chen et al., 1987). In the present study, the cold resistance of mated and unmated males and females of *D. melanogaster* was also examined. It was found that unmated males considerably outperformed mated males in both the control and Ensure® treated flies in terms of cold resistance. The most likely reason for this is that mated females consumed more food and built up more lipids than males (Carvalho et a., 2006; Lee et al., 2013). It has also been demonstrated in more recent research that female *D. melanogaster* can dramatically increase their midgut size during mating. Mated females were able to meet their heightened energy requirements for egg production by increasing their post-ingestive nutrient consumption.

Thus, these studies suggests that Ensure® supplement in the diet enhances cold resistance in *D. melanogaster*. Further cold resistance increased with increasing concentration of Ensure® supplement.

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