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Research Article

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The Ensure® nutritional supplement increases the Heat Resistance in *Drosophila melanogaster.*

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Abstract

The quantity and quality of nutrients taken by organisms have a significant impact on stress resistance, life-history features, and reproduction. The balance between energy uptake and expenditures is critical to animal survival and reproductive success. Extreme temperatures, dehydration, and food deprivation as well as the type of nutrition may have diverse effects on different age groups of males and females across species. In the present study, the flies of *D. melanogaster* flies were cultured in wheat cream agar media, mixed diet, and Ensure® treated media to understand the effect of Ensure® powder on the heat resistance. Our result revealed that the flies which fed Ensure® treated media had greater heat resistance than mixed diet followed by control media (Treat>mixed>control). Ensure® enhances heat resistance in *Drosophila melanogaster*.

Keywords: Heat resistance, *Drosophila melanogaster*, virgins, mated, Diets.

Introduction

A crucial component of habitat quality is the likelihood that an organism may experience excessive heat stress (Huey, 1991), resistance to thermal extremes may be closely correlated with changing temperatures. The simplest way that environmental change can impact on stress resistance, fecundity and physical condition is though nutritional impacts brought on by changes in the availability of different food types. In general, diet can be categorized as either quantitative (i.e., food availability) or qualitative (i.e., food consumption). Out of these two, the quantitative effects are evident since animals obtain their energy and other nutritional needs from food; consequently, under a wide range of natural conditions, there is a positive relation between food availability and fitness of an organism. On the other hand, qualitative effects are frequently divided into two categories, namely, non-nutritive effects and nutritive effects

two broad categories that can be used to categorize diet effects are food availability and food composition (Pough, 1989;Sibly,1991).

The model organism *Drosophila melanogaster* has been extensively employed in the scientific sciences, *Drosophila* is a popular choice for life span studies because of its 60-80-day lifespan. Additionally, 60% of the fruit fly genes have mammalian orthologs. As a result, metabolic and signaling pathways are much conserved (Staats *et al.*,2018). *Drosophila* maintenance and reproduction are relatively inexpensive and don't call for expensive equipment. Comparatively to studies using laboratory rodents like rats and mice, experimental *Drosophila* research fewer ethical concerns. The use of *Drosophila* as a model organism in food and nutrition research is growing. Feed intake, body composition, locomotor activity, intestinal barrier function, microbiota, cognition, fertility, aging, and life span can be systematically determined in *Drosophila* in response to dietary factors (Staats *et al*., 2018). Furthermore, diet induced pathophysiological mechanisms including inflammation and stress responses may be evaluated in the fly under defined experimental conditions. Both intrinsic and extrinsic factors known to affect all biochemical, physiological, and developmental changes that take place in an organism have an impact on the overall growth, development, and reproduction of an organism (Sterner and Schulz, 1998; Taylor *et al.*, 2005).

Environmental stress is defined as the lack of acceptable or sufficient food supplies, which deprives a population of normal nutrients (White, 1993). Many species individuals must endure times of famine or exposure to unsatisfactory nutrition. In areas where food is likely to be less plentiful or momentarily less consistent, positive selection for resistance to famine stress is anticipated.

As is frequently observed when insects are restricted to food low in protein relative to carbohydrate (Raubenheimer 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 decreases fitness (Simpson et al., 2004, Warbrick-Smith *et al.*, 2006). Several variables can affect an organism's ability to withstand stress. In response to climate shifts, organisms may go into coma, undergo physiological stiffening, or produce metabolites that enable them to withstand temperature extremes (Sorensen *et al*., 2003; Sorensen *et al.*, 2005; Lalouette *et al*., 2007). Additionally, an organism may adjust its growth cycle or change how much energy it devotes to growth to make up for nutritional stress and lower body size, delaying the reproductive phase (Reichling and German, 2000; Lobe *et al.*, 2006).

Ensure® powder is commonly used for the diagnosis or treatment of muscular hypertrophy, immune function, weight loss and residue diet. Ensure® is a balanced diet, food which support nutritional health, weight gain, and general health and it also negatively correlated with diarrhea, nausea, abdominal bloating, complete exhaustion and increased pulse, but the severity of these condition is very less, but there is no evidence documented about how the Ensure® powder effects on heat resistance in *Drosophila melanogaster*.

People may often feel weak and exhausted as they age due to a decrease in bone density and muscle mass. In order to enhance bone and muscular development, immunological function, and overall health. Ensure® is a comprehensive, well balanced diet that includes HMB, macronutrients (high quality protein, fat and carbohydrates) and micronutrients (vitamins and minerals). It is the world's most popular nutritional supplement drink, Ensure® provides thirty-two nutrients, including high quality protein, calcium, zinc, vitamin C, vitamin D and iron. It also contains a unique substance called HMB. HMB, or β-Hydroxy-β-Methyl butyrate, is an amino acid that promotes and preserves muscle growth. Although it includes eleven immune boosting nutrients-vitamins A, C, E, B6, iron, D, Folate, Zinc, and copper-Ensure® is a high protein nutrient, the peoples of all ages take this drink (Roland and Roy Curtiss, 2015).

There is no published data about the impact of Ensure® nutrition supplements on an organism's ability to withstand environmental stressors like starvation resistance. Therefore, present study has been undertaken in *Drosophila* to study the effect of Ensure® on the heat resistance.

Materials and Methods

The Ensure® nutrition supplement powder was purchased from Medplus pharmacy shop, Srirampura, Mysuru, Karnataka, India. This Ensure®nutrition supplement powder was used to prepare the experimental media.

Establishment of stock: -

Experiment Oregon K strain of *Drosophila melanogaster* used in the study was collected from *Drosophila* stock center. 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 what cream rava, 10g of Agar was boiled in 1000ml distilled water and 7.5ml of propionic acid was added). Flies were maintained in laboratory conditions such as humidity of 70% and 12 hours dark 12 hours light cycles and temperature 22º ±10°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 50g of jaggery, 50g of wheat cream rava powder, 5g of agar boiled in 500ml distilled water and 3.5ml of propionic acid added to it.;

Ensure® nutritional supplement (referred to as Ensure®) media: Ensure® media was prepared from 50g of jaggery, 50g of Ensure® nutritional supplement powder, 5g of agar boiled in 500ml of distilled water, and 3.5ml of propionic acid added to it;

Mixed(Wheat cream + Ensure®)media: Mixed media is prepared from 50g of jaggery, 25g of wheat cream powder and 25g of Ensure® nutritional supplement powder, 5g of agar boiled in 500ml of distilled water and 3.5ml of propionic acid added to it]. The flies emerged from the wheat cream agar media and other experimental treated media were maintained above. These flies were used to study the heat resistance experiment in *D. melanogaster*.

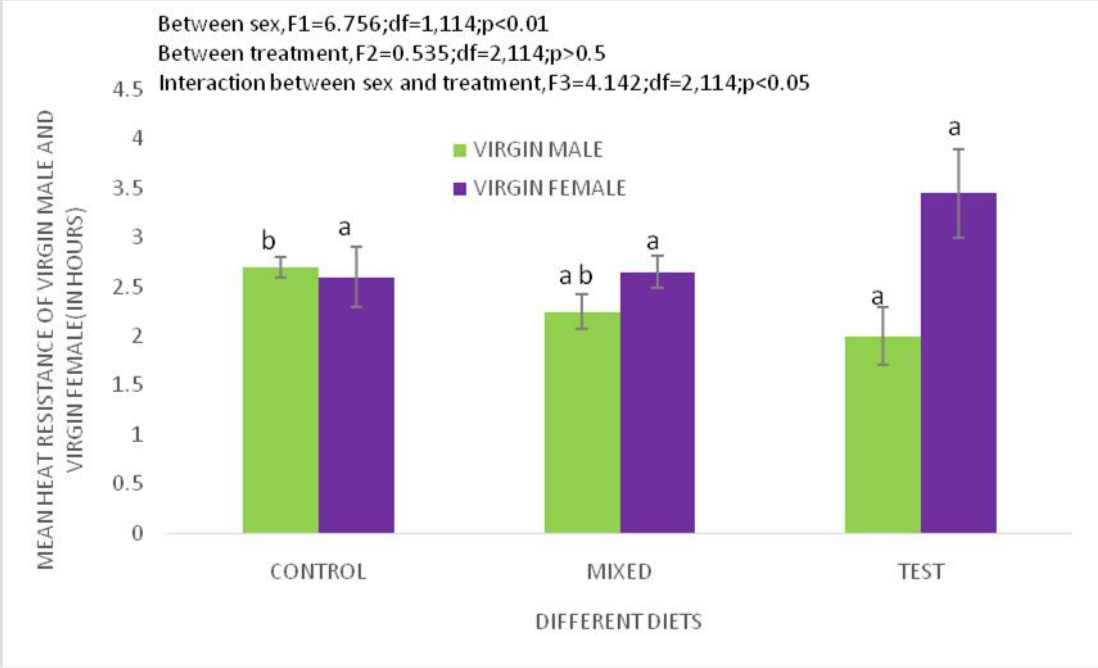
Experimental procedure: -

Heat resistance: For heat resistance experiment, control, mixed and test treated mated and unmated flies were used. Twenty flies (control, mixed, test flies) in a vial were kept in the incubator for 37°C.

The flies were observed for every 5 minute of interval until the death of each fly. The heat resistance was observed in minutes, and a total of twenty flies observed in each of the three media. A separate experiment was run for both males and females.

Results

Figure 1: Effect of the Ensure® nutrition supplement on the heat resistance in the virgin male and virgin female of *Drosophila melanogaster.*

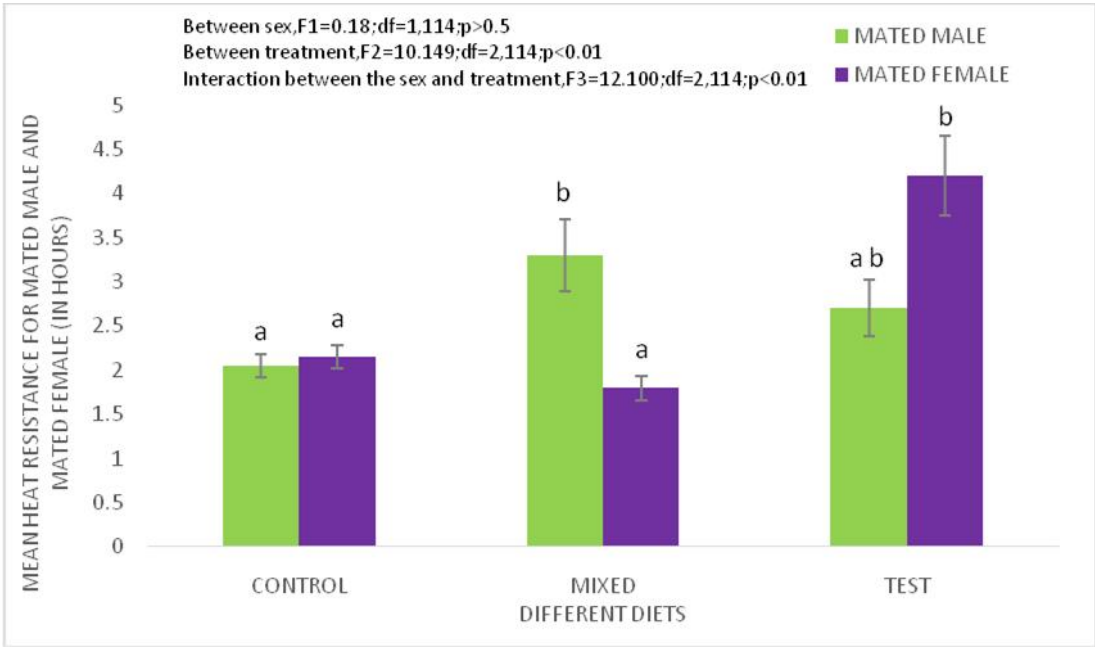


The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

The mean and standard error values of the heat resistance of virgin male and virgin female flies raised with Ensure®, mixed, and wheat cream agar media are provided in figure-1. According to diet it was noticed that heat resistance was greater in the test media that is Ensure® compared to the control media.

The result was found that the virgin female had the greater heat resistance than the virgin males in different diets. The above data were subjected to the Two-way ANOVA followed by the Tukey's post hoc test showed the significant variation between sexes. Non-significant variation observed between the diets.

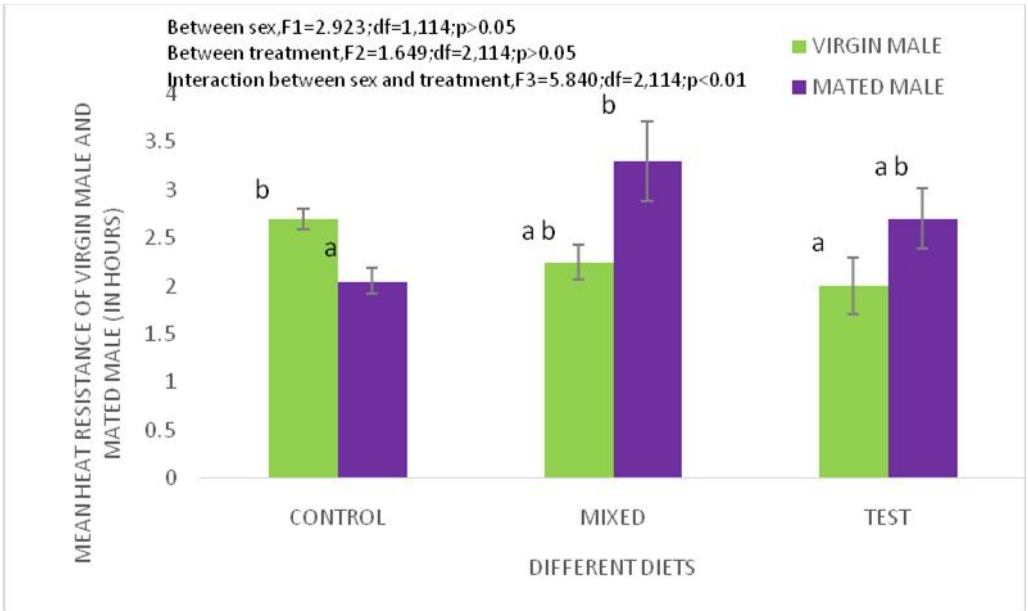
Figure 2: Effect of Ensure® nutrition supplement in the mated male and mated female of *D. melanogaster*.



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

In fig-2 shows the mean result of heat resistance of mated males and mated females raised in different diets, these data were subjected to Two-way ANOVA followed by Tukey's post hoc test. According to the observation, significant variation was observed between the diets and insignificant variation between the sexes. The female flies of test media have more heat resistance capacity compared to other media.

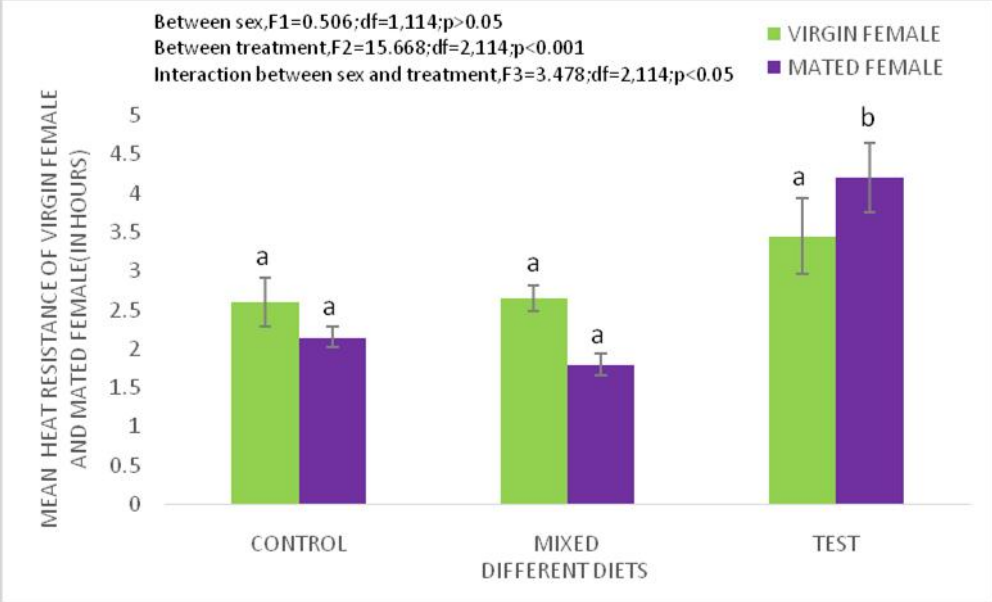
Figure 3: The effect of Ensure® nutrition supplement in virgin male and mated male of *D. melanogaster*.



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.

The mean of the heat resistance of virgin male and mated male flies of all three diets are showed in figure-3. According to the data, noticed that heat resistance was greater in the mated males of mixed and test media. The above data were subjected to Two-way ANOVA followed by Tukey's post hoc test showed non-significant variation between the treatments and sexes.

Figure 4: Effect of Ensure® nutrition supplement on the heat resistance on the virgin female and mated female of *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.

Figure 4 shows the mean and standard error value of the heat resistance of virgin female and mated female flies raised in different diets. According to the data obtained the heat resistance was greater in mated females of Ensure® media compared to mixed and wheat cream agar media. These data were subjected to Two-way ANOVA followed by Turkey's post hoc test showed significant variation between the diets and non-significant variation between the sexes.

Discussion

The availability of a nutritional diet is known to influence on various environmental stress such as thermal, starvation etc. In the present study, flies developed in Ensure® treated media was significantly greater heat resistance compared to flies raised in mixed followed by control media figure 2. This is because the Ensure® contain significantly greater protein content compared to mixed and control media. These differences in the heat resistance of *D. melanogaster* were caused by difference in the quality and amount of dietary nutrients contained in the wheat cream agar media and Ensure® treated media. The ability of insect to adapt and reduce the effects of changing climatic conditions is based on stress-related traits. A well-studied phenomenon, especially in insects is the impact of physiological or morphological changes brought on by hardening or acclimation on temperature tolerance (Maness and Hutchison, 1980).

Our study also supported by Sisodia and Singh (2012) who also found the flies raised on protein-enriched medium are more heat resistant than those raised on carbohydrate enriched medium. Fly development on a diet high in protein can withstand heat shock more quickly than fly developed on a diet high in carbohydrate. Our Ensure® product also rich in carbohydrate than protein but according to our study compared to mixed and wheat cream agar media, our Ensure® treated media is rich in protein.

Though the overall impact of them is uncertain in a number of factors, including sex, physical condition, and diet, help the organism to resist the stress (Harshmann and Schmid, 1996). Mated males were more resistance to heat than compared to unmated males this may be due to release of pheromones during copulation may responsible for resistance to withstand the stress, figure-3.

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.

While studies on *D. melanogaster* has shown that mated females are more stress resistance than virgins (Ballard, 2008; Hoffmann, 2005; Rion and Kawecki, 2007). This may be due to accessory gland protein that males transfer to female during copulation, together with seminal gland proteins and relax a complex protein mixture known as seminal fluid, which is delivered to the female along sperm (Chen, 1984; Monsma and Wolfner, 1988; Wolfner, 2002).

Also, in the present study, the female (mated and unmated) raised in Ensure® treated media had greater heat resistance compared to control and mixed diet. In males (mated and unmated) fed with mixed diet had a greater heat resistance compared to test and control.

From this experimental study we can conclude that flies which developed in Ensure® treated media had greater heat resistance and withstand environmental stress than compared to flies which fed with mixed and wheat cream agar media.

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