International Journal of Zoology Studies
www.zoologyjournals.com
ISSN: 2455-7269
Received: 11-06-2024, Accepted: 02-07-2024, Published: 25-07-2024
Volume 9, Issue 3, 2024, Page No. 7-11

The effect of ensure® nutritional supplement on the longevity in *Drosophila melanogaster.*

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Abstract
Nutrition has long been recognized as an important factor in influencing the health and lifespan of animals, including flies. In the present study, the flies of *Drosophila melanogaster* are cultured in control, mixed, and the test media. To understand the effect of Ensure® nutritional supplement on longevity, the results revealed that, the flies fed with the Ensure® media had significantly greater lifespan, compared to mixed and control media. Further in the present study among the males and females, the females showed a longer life span than the males in all three diets. Hence these studies suggest that the consumption of the Ensure® Salutary affects the longevity of *Drosophila melanogaster*. Thus, the longevity sequences of flies in our experiments are as follows Test> Mixed> control media. Suggests that consumption of Ensure® increases the life span of *Drosophila melanogaster.*

Keywords: Longevity, survival curve, lifespan, nutrition, *Drosophila melanogaster*

Introduction

Organism longevity is a quantitative trait determined by both environmental and genetic components. *Drosophila melanogaster* proved one of the most useful model systems for exploring genetic determination of life span. A vital extrinsic component that can impact an organism's growth, development, and survival is its nutrition. Diet is a significant extrinsic factor that can influence an organism's growth, development, and survival (Sisodia and Singh 2012) [19]. It was studied that dietary restriction by diluting the food medium can extend the life span of *Drosophila. Drosophila* results in a state characterized by reduced fecundity, increased starvation resistance, and higher total lipid levels. Food had a major impact on life span and reproductive output. Piper *et al*. (2011) [16]. The diet that an organism consumes and its impact on life history traits can be divided into two categories: quantity, which depends on food availability, and quality, which depends on the nutritional makeup of food (Sisodia and Singh, 2012) [19].

Numerous factors, including extrinsic factors like predation, malnutrition, and other environmental factors, as well as intrinsic elements like aging rates, have an impact on the lifetime of wild animals. Recent work with flies shows that longevity is controlled by the ratio of consumed protein relative to carbohydrates. Given that reduced insulin and/or insulin-like growth factor (IGF) and target rapamycin (TOR) signaling increase *Drosophila* lifespan. Nutrients sensing protein and carbohydrate may occur in the fat body through signals to hypothalamic –neurons in the fly brain and, thus, control the secretion of insulin-like peptides that regulate longevity, (Marc Tatar, Stephanie Post, *et al*. 2014). 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 supports nutritional health, weight gain, and general health it also negatively correlated with Diarrhea, Nausea, Abdominal bloating, complete exhaustion, and Increased pulse, but the severity of these conditions is much less, but there is no evidence documented about how the Ensure® powder effects on the longevity of the organism. Therefore, the study is undertaken to address the effect of Ensure protein supplement on the longevity of *Drosophila melanogaster.*

Lifespan is a quantitative trait that is impacted by a number of variables, including sex, age, genetic makeup(epigenetics), and environment (Paaby and Schmid, 2009) [15]. Extended lifespan in *Drosophila* is frequently linked to a variety of characteristics, including lipid contents, development time, body size, biochemical defenses, and resistance to environmental stressors (starvation, desiccation, and cold), which can correlate positively or negatively (Vermeulen and Loescheke, 2007; wit *et al*. 2013: Deepashree *et al*. 2017) [7, 23, 24].

According to Finch (1990) [8], and Charlesworth (1994), aging is the steady functional decrease of an organism that results in death. However, several hypotheses have been put up to explain the evolutionary pressures involving fitness and reproduction that affect species longevity (Hughes and Reynolds, 2005) [9]. In many species, there are disparities between the average lifespan of the sexes, and females often outlive males (Lints *et al*. 1983; Austad and Fischer, 2016) [4, 12]. The reported variations in life span between the sexes are frequently inferred from wild populations and attributed to risk-taking behavior, feeding habits, and sexual competition, which may have little to do with sex differences in intrinsic aging rates (Austad and Fischer, 2016) [4]. The sexual selection theory (Trivers, 1972) [21] postulates that lifelong investment and reproductive effort trade-off, resulting in sex differences in ROS generation and antioxidant defenses. The studies are not done using Ensure® nutritional supplement yet we are proceding to observe the effect on its lifespan.

Materials and methodology

The Ensure® powder was purchased from the Med Plus pharmacy shop, in Sriramapura, Mysuru, Karnataka, India. This Ensure® protein Powder is used to prepare the experimental media.

Establishment of stock

The experiment stock was established using the Oregon K stain of Drosophila melanogaster, which was sourced from the Drosophila Stock Center, Department of Studies in

Zoology, University of Mysore, Manasagangotri, Mysuru. The flies were raised using wheat cream agar media (100g of jaggery, 100g of wheat powder, 10g of agar was boiled in 1000 ml of distilled water, and 7.5 ml of propionic acid was added to avoid fungal growth). The flies were kept in a controlled environment with 70% humidity, a 12:12 photoperiod (dark and light cycles), and a temperature of 22°_+1° degrees Celsius. We utilized these flies to carry out our experiment.

Experimental procedure

From the wheat cream agar, Ensure® nutritional supplement, mixed media treated *Drosophila melanogaster* culture virgin females, and unmated male flies were isolated within 3 hours of eclosion from the control media. These flies were aged for 5 days. Virgin females and unmated males are individually aspirated into the mating chamber. If mating occurs, these mated pairs were transferred to a vial containing control media, once in seven days until their death. And note down the number of flies that die every week. A total of ten pairs were made separately for each of their respective media and separate experiments were carried out for the virgin female, mated female, unmated male, and mated male flies in *Drosophila melanogaster*.

Results and discussion

Analysis of survival curve

The survival curve was calculated for the longevity of unmated males, virgin females, and mated males, and mated females. Two functions that are dependent on time are of particular interest: The survival function and the Hazard function. The survival function S(t) is defined as the probability of dying at the time having survived until that time. The graph of S(t) against t is called the survival curve. The Kaplan-Meier method was used to estimate this curve from observed survival times without assuming an underlying probability distribution. Two survival curves were compared using a statistical hypothesis test called the log-rank test, which is used to test the null hypothesis that there is no difference between survival curves, i.e, the probability of an event occurring at a point in time is for each media.

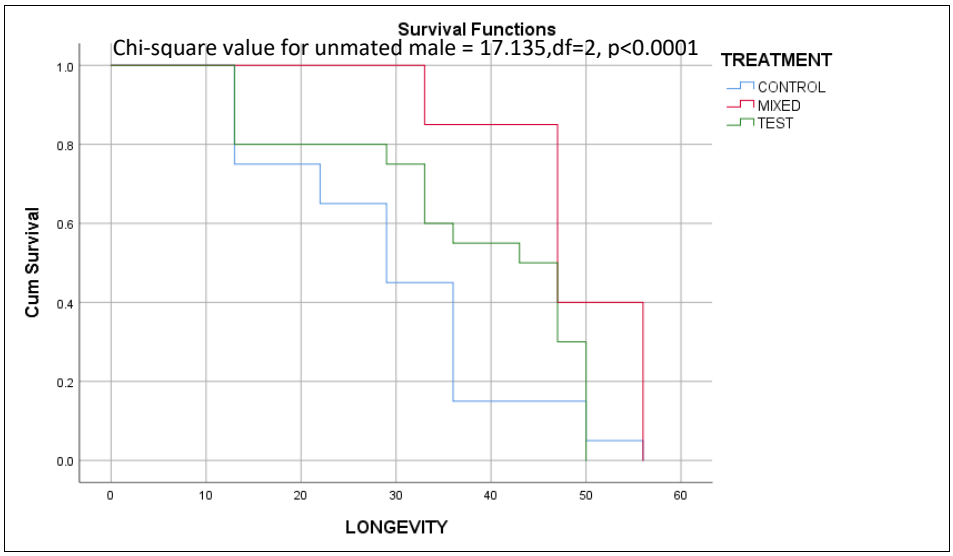

Fig 1: Unmated male

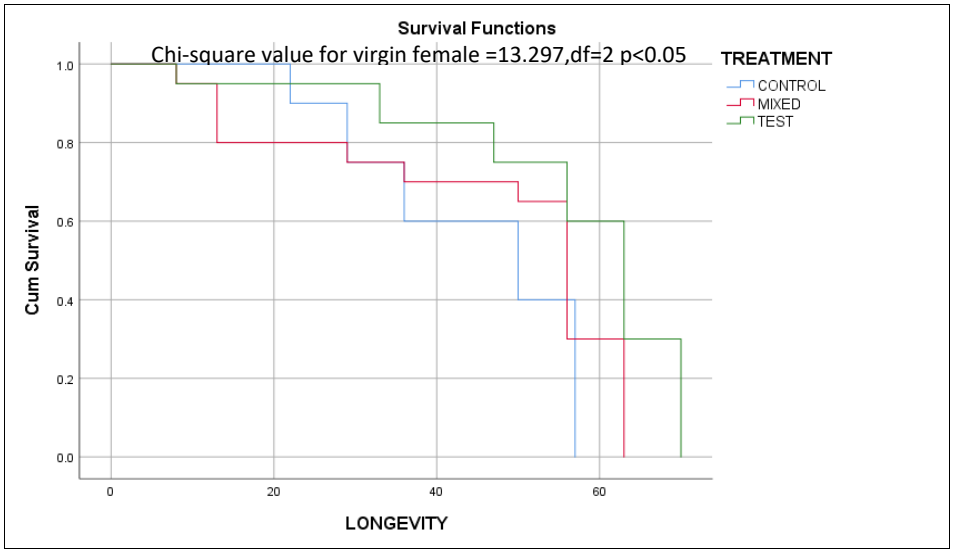

Fig 2: Virgin female

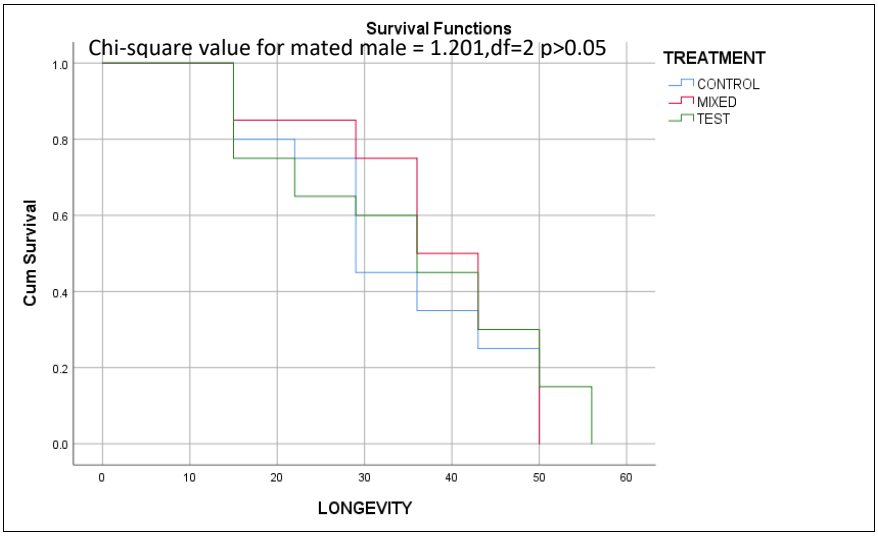

Fig 3: Mated male

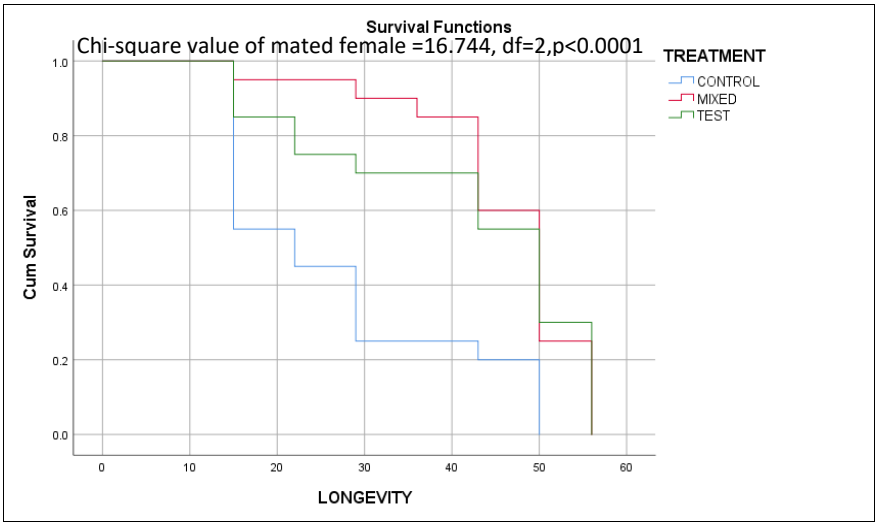

Fig 4: Mated female

Sex difference in survivability is determined by the Kalphan-Meier analysis of *Drosophila melanogaster* fig 1, fig 2, fig 3, and fig4 respectively. The graph presents the survivorship of unmated male, female, and mated males, and females in a control media bottle. The log-rank test revealed a statistically significant variation (Chi-square value for unmated male = 17.135, $p<0.0001$ and the virgin female =13.297, $p<0.05$), and insignificant variation (Chi-square value for mated male = 1.201, $p>0.5$) while significant variation in the mated female (Chi-square value =16.744, $p<0.0001$).

Aging is defined as a study of functional deterioration in an organism that results in death (Finch,1990; Charlesworth,1994) [8], several theories have been proposed to explain the evolutionary selection relating fitness and reproduction that influence a species' longevity. (Hugher and Reynolds,2005). A slow loss of biological function, and physiological process linked to longevity or of main interest for medical and biological study are the hallmarks of aging, a complicated biological process. Lifespan is a quantifiable characteristic feature that depends on several variables such as diet, environment, sex, age, and genetic background (epigenetics). (Schmidt and Paddy, 2009). One of the major external elements influencing an organism's ability to reproduce, develop, and survive is its nutritious food. Furthermore, the most crucial factors affecting an organism's ability to survive and lengthen its life are the type and quantity of nutrients it consumes. Male and female lifespan differences are common in laboratory species such as *C.elegans, D. melanogaster*, and *Mus musculus.* (Tower and Arbeitma 2009; Austard and Fisher 2016) [20].

The Ensure® nutritional supplement contains various nutrients, carbohydrates and protein are the two nutrients that constitute the majority. The present study is undertaken to know the effect of Ensure® on the longevity of *Drosophila melanogaster*. The flies fed with the Ensure® nutritional supplement lived significantly greater than those of flies fed with wheat cream agar media (control) media and the mixed media. This suggests the amount of quantity and quality of nutrients present in the wheat cream agar media, mixed and Ensure® media is responsible for the variation in the longevity of *Drosophila melanogaster. The* Ensure® nutritional supplement contains high carbohydrates and proteins along with some other vitamins and minerals which may have influenced the physiology of the flies therefore enhancement in longevity is observed.

By comparing the mated females and males in all the 3 diets, females had lived longer than males, because males often engage in more aggressive and risky behaviors to complete mating, which leads to higher stress and injury

rates, the hormonal shifts can also lead to increased metabolic rate and oxidative stress, which can reduce the lifespan. the virgin flies have more lifespans than mated flies because of mating significantly requires more energy and in this case the flies are virgin therefore the energy is stored and utilized only for the basic required work by the flies. Figures 1 and 2 reveal the significant variation in virgin males and females and Figures 3 and 4 reveal the significant variation between mated males and females.

Nutrient intake has a profound influence on the lifespan across a wide range of species, with both very low and very high levels of dietary protein (Piper *et al*. 2011) [16]. Our study does not support Brook *et al.* (2021) who while studying *Drosophila* also found the high protein and carbohydrate ratios reduced the life span in flies. Theodore *et al.* (1999), suggest that nutrition should be neutral for selecting lifespan in *Drosophila* and also proved that the lifespan is decreased under conditions of high nutrition as nutrition increases, lifespan decreases. In most insects, dietary restriction, and in particular a reduced protein intake, increases lifespan (Simpson *et al*. 2012). All the above studies suggest that the nutritional variation in the dietary components is responsible for the increases or decreases in the life span of *Drosophila melanogaster*. The Ensure® supplement is a nutritional food that may result in an increased life span in flies.

In the present study, The survival curve and log-rank analysis between the males and females evidently revealed the longevity between the sexes that is females are significantly lived longer than the males in all three different diets. Several studies suggest that females have larger guts than males helps to consume more amount of nutritional food in turn extends their lifespan.

There are several studies which shown that variations in environmental conditions such as temperature, light, age, and stress also influence the survival of the flies and these factors also influence the lifespan of the flies in a sex-specific manner. However, in our study, we maintained the flies in the laboratory condition, the same aged flies which fed with the different diets used to study longevity the hence observed variation in the experimental results is due to the variation in the quantity, and quality of the nutrients in the diet.

Hence from our study in *Drosophila melanogaster*, we can conclude that nutrition is one of the key factors that influences the longevity of the organism. The flies raised on the Ensure® supplement showed the highest longevity than the wheat cream agar and mixed diet. Thus, the longevity of sequences of our experiments is as follows Ensure® >Mixed>Wheat cream agar media. Suggests that the consumption of The Ensure® supplement increases the life span of the *Drosophila melanogaster*.

Acknowledgement

The authors would like to thank the Chairman Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru, and also extend the gratitude to *Drosophila* Stock Center, National Facility, University of Mysore for providing the facilities to carry out the major project work.

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