



Effect of fruit body extract of *Pleurotus ostreatus* mushroom on longevity in *D. melanogaster*

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Abstract

Diet is important in preserving long-term health. Lowering the dietary protein: carbohydrate ratio, in particular, can promote longevity. Nutritional programming refers to the long-term effects of nutrition on adult physiology, disease, and lifespan that occur throughout the formative phases. The effect of varied concentrations of *Pleurotus ostreatus* (oyster) mushroom fruit body extract were supplemented along with control wheat cream agar media using *D. melanogaster* were utilized to study longevity between male and female flies in the current study. It was discovered that oyster mushroom treated flies live longer than flies grown in control diet further grown in control diet. Further flies grown in various concentrations of oyster mushroom lived longer with increasing concentrations of oyster mushroom. It was reasonable to believe that the abundant nutrients contribute to a longer life span. In addition, in both control and mushroom treated females lived longer than males. Thus, our finding show that rearing flies in varying concentrations of oyster mushroom enhances longevity in *D. melanogaster*.

Keywords: Diet, dietary protein, *D. melanogaster*

Introduction

Nutrition is a fundamental predictor of life span, the rate of aging, and reproductive capacity (Weindruch and Walford, 1982; Chippindale *et al.*, 1993; Good and Tatar, 2001; Walker *et al.*, 2005; Fontana *et al.*, 2010) [9, 15, 33, 34]. The majority of this research has concentrated on the effects of adult dietary quantity and quality, providing significant contributions to the study of aging. For instance, a burgeoning field devoted to describing the mechanism and particular nutrient dependencies of the effect has been created as a result of the discovery that life span extension upon dietary restriction occurs across a wide range of animal species (Weindruch and Walford, 1982; Austad 1989; Chippindale *et al.*, 1993; Grandison *et al.*, 2009) [3, 9, 16, 34]. According to a growing body of research, however (Gluckman and Hanson, 2004; Boggs and Freeman, 2005; Brakefield *et al.*, 2005; Cleal *et al.*, 2007; Gluckman *et al.*, 2008; Barrett *et al.*, 2009; Joy *et al.*, 2010; Dmitriew and Rowe, 2011) [5, 6, 7, 10, 12, 14, 19], developmental nutrition may also have profound effects on adult traits, such as life span and fecundity. Many species have longevity disparities between the sexes, and females frequently outlive men (Lints *et al.*, 1983; Austad and Fischer, 2016) [4, 22]. According to (Austad and Fischer, 2016) [4] reported variations in longevity between the sexes are frequently inferred from wild populations and attributed to risk-taking behavior, feeding habits, and sexual competition differences that may have little to do with inherent sex differences in aging rates. A trade-off between investment in reproductive effort and lifespan results in sex differences in ROS generation and antioxidant defenses, according to the sexual selection theory (Trivers, 1972) [31]. However, further research is needed to fully understand how oxidative stress affects sexual selection. To understand sex-specific aging, it can be helpful to compare the lifespans of the sexes within a species (Austad and Fischer, 2016) [4]. According to recent research on the cricket *Gryllodes sigillatus*, oxidative stress may have contributed to the evolution of lifespan disparities and aging (Archer *et al.*, 2013). However effect of quality and quantity of nutrient present in the diet vary from food to food therefore present study has been undertaken to study

the effect of *P. ostreatus* (oyster) mushroom on longevity in *D. melanogaster*.

P. ostreatus may be cholesterol-free and low in calories, carbs, fat, and sodium, they are now highly prized and regarded as functional dietary ingredients. They provide essential nutrients like riboflavin, selenium, potassium, niacin, proteins, and fiber when they are placed next to one another (Akyuz *et al.*, 2010; Sahoo *et al.*, 2022) [1, 28]. According to several studies (Zhang *et al.*, 2012) [37], the consumption of mushrooms is rising quickly all over the world because they are a rich source of bioactive substances like laccase, functional protein glucans, ubiquinone-9, nebroleolysin, and glycoprotein, proteoglycans, pleuran (-1, 3-glucan with galactose, and mannose), pleurostrin (peptide), minerals (Fe, Ca, K, P, and Na), dietary. Therefore the present study was taken to study the effect of oyster mushroom on longevity in *D. melanogaster*.

Materials Method

Collection of fruit body extract of *Pleurotus ostreatus* (oyster) mushroom

The fruit body extract of oyster mushroom powder was purchased from the ROOTED (Active naturals). Delivered by Amazon app by online.

Establishment of Stock

Oregon K strain of *D. melanogaster* provided by *Drosophila* stock center, Department of Zoology, University of Mysore, Mysuru. was used in the investigation. This stock was used in the present study, was cultured in bottles containing wheat cream agar medium (100g jaggery, 100g wheat powder, 10g agar, 1000ml distilled water, and 7.5 ml propionic acid were added). Flies were kept in a lab environment with 70% humidity, 12-hour cycles of darkness and light, and a temperature of 22°C ±1°C.

Experimental media preparations

To get experimental media, the different concentrations (2.5g, 5g, 10g) of *P. ostreatus* (oyster) mushroom powder was weighed and mixed thoroughly with 100ml of wheat cream agar media. To obtain 2.5g, 5g and 10g experimental

media. Flies cultured in wheat cream agar media were considered as control. These experimental flies were also maintained in same laboratory conditions as described above. Flies obtained from control and experimental flies were used in the present experiment.

Effect of fruit body extract of *P. ostreatus* mushroom diet on longevity in *D. melanogaster*

Five to six days old virgin female and unmated males obtained from control and *P. ostreatus* (oyster) mushroom

treated flies were used. Pair wise mating experiment was made. These mated pairs were transferred into labelled vials containing control wheat cream agar media and different concentration of oyster mushroom diet. Once in a seven days until the death of each fly. A total of twenty mated pairs were made separately for each of control and oyster mushroom treated flies.

Result and Discussion

Table 1A: Effect of different concentration of *Pleurotus ostreatus* (oyster) mushroom diet on mean and median survival time in male flies (*D. melanogaster*). [Control diet-wheat cream agar media; *P. ostreatus* (oyster) mushroom diet (2.5g, 5g, 10g)].

| GROUPS | Mean ^a | | | | Median | | | |
|---------|-------------------|------------|-------------------------|-------------|----------|------------|-------------------------|-------------|
| | Estimate | Std. Error | 95% Confidence Interval | | Estimate | Std. Error | 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound | | | Lower Bound | Upper Bound |
| CONTROL | 46.700 | 1.833 | 43.108 | 50.292 | 41.000 | 3.354 | 34.426 | 47.574 |
| T1 | 41.600 | 2.320 | 37.053 | 46.147 | 41.000 | 4.930 | 31.338 | 50.662 |
| T2 | 43.500 | 2.459 | 38.681 | 48.319 | 41.000 | 5.870 | 29.495 | 52.505 |
| T3 | 45.800 | 2.069 | 41.744 | 49.856 | 41.000 | 3.913 | 33.330 | 48.670 |
| Overall | 44.400 | 1.094 | 42.256 | 46.544 | 41.000 | 2.039 | 37.003 | 44.997 |

a. Estimation is limited to the largest survival time if it is censored.

Table 1B: Test for equality of survival distribution for the different levels of groups of control and *P. ostreatus* (oyster) mushroom diet treated male flies:

| Overall Comparisons | | | |
|--------------------------------|------------|----|------|
| | Chi-Square | df | Sig. |
| Log Rank (Mantel-Cox) | 6.490 | 3 | .090 |
| Breslow (Generalized Wilcoxon) | 5.004 | 3 | .171 |
| Tarone-Ware | 5.367 | 3 | .147 |

Test of equality of survival distributions for the different levels of GROUPS.

Table 2A: Effect of different concentration of *Pleurotus ostreatus* (oyster) mushroom diet on mean and median survival time in female flies (*D. melanogaster*). [Control diet-wheat cream agar media; *P. ostreatus* (oyster) mushroom diet (2.5g, 5g, 10g)].

| GRUOP | Mean ^a | | | | Median | | | |
|---------|-------------------|------------|-------------------------|-------------|----------|------------|-------------------------|-------------|
| | Estimate | Std. Error | 95% Confidence Interval | | Estimate | Std. Error | 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound | | | Lower Bound | Upper Bound |
| CONTROL | 45.900 | 1.721 | 42.526 | 49.274 | 45.000 | 4.025 | 37.111 | 52.889 |
| T1 | 42.900 | 2.539 | 37.923 | 47.877 | 44.000 | 2.236 | 39.617 | 48.383 |
| T2 | 45.600 | 1.358 | 42.938 | 48.262 | 47.000 | 1.095 | 44.853 | 49.147 |
| T3 | 46.800 | 2.037 | 42.807 | 50.793 | 45.000 | 4.450 | 36.279 | 53.721 |
| Overall | 45.300 | .976 | 43.387 | 47.213 | 45.000 | 1.032 | 42.978 | 47.022 |

a. Estimation is limited to the largest survival time if it is censored.

Table 2B: Test for equality of survival distribution for the different levels of groups of control and *P. ostreatus* (oyster) mushroom diet treated female flies

| Overall Comparisons | | | |
|--------------------------------|------------|----|------|
| | Chi-Square | df | Sig. |
| Log Rank (Mantel-Cox) | 6.254 | 3 | .100 |
| Breslow (Generalized Wilcoxon) | 1.463 | 3 | .691 |
| Tarone-Ware | 2.660 | 3 | .447 |

Test of equality of survival distributions for the different levels of GRUOP.

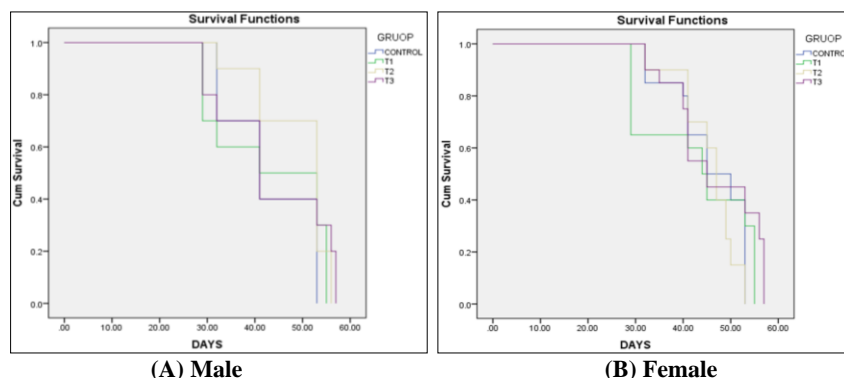


Fig 1 A&B: The effect of *P. ostreatus* (oyster) mushroom on the longevity of the male and female in *Drosophila*

Figure 1: Sex difference in the survivability of as determined by the Kaplan-Meier survival analysis of *D. melanogaster* [A] Male, [B] Female. The graphs represent the survivorship of male and female flies housed separately in vials (n=80; 20 vials with 10 flies each per group per sex). The log-rank test revealed a statistically significant. Difference in the survivorship between males and females (Chi square value for male=6.490; P<0.05, Female=6.254; P<0.05).

Note: In the Figure 1[A and B] T1 represented as 2.5g, T2 represented as 5g, T3 represented as 10g concentration of *Pleurotus ostreatus* (oyster) mushroom diet.

Statistical analysis

Survival curve was calculated for longevity of males and females. Two functions that are dependent on time are of particular interest: The survival function and Hazard function. The survival function S(t) is defined as the probability of dying at time t 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 null hypothesis that there is no difference between survival curves, i.e., the probability of an event occurring at any point of time is for each media 20 trials were made for each of the control wheat cream agar media and *Pleurotus ostreatus* (oyster) mushroom media.

Discussion

In *Drosophila*, longevity is connected to stress tolerance (Rose 1984; Arking 1987; Rose *et al.*, 1992; Hercus *et al.*, 2003; Bublly and Loeschcke, 2005; Niveditha *et al.*, 2017) [2, 8, 18, 24, 26, 27]. The current study (Figure 1 A and B), (Table 1A and 2A) of by the chi square test (Table 1B and 2B) shows significant on life span of flies in control and oyster mushroom diet. Furthermore the flies fed with *P. ostreatus* (oyster) mushroom had increase the life span with increasing concentration of oyster mushroom diet in both male and female flies. This suggest that the quantity of nutrients present in the oyster mushroom diet gave positive impact on longevity in *D. melanogaster*. Lifespan is a quantitative attribute that is impacted by a variety of factors such as gender, age, genetic background (epigenetics), and environment (Paaby and Schmidt, 2009) [25]. Extended lifespan in *Drosophila* is frequently associated with a variety of characteristics, including resistance to environmental stressors (starvation, desiccation, and cold), lipid content, development time, body size, biochemical defenses, and so on, which correlate either positively or negatively (Vermeulen and Loeschcke, 2007; Wit *et al.*, 2013; Deepashree *et al.*, 2017) [11, 32, 35]. Resistance to oxidative stress is vital in extending life (Rose, 1984; Service, 1987; Hoffman and Parsons, 1989; Niveditha *et al.*, 2017) [24, 27]. Despite various studies on transgenic flies (long-lived mutants) to investigate the association between oxidative stress resistance and longevity, there are few investigations on artificially selected extended lifespan phenotypes (ELPs) (Arking *et al.*, 1991; Mockett *et al.*, 2001) [23]. The current study (Figure 1 and 2) also demonstrated that females of *D. melanogaster* live longer than males. Male-female lifespan differences are widespread

in laboratory species such as *C. elegans*, *D. melanogaster*, and *Mus musculus* (Tower and Arbeitman, 2009; Austad and Fischer 2016) [4, 30]. The *D. melanogaster* (Oregon K) model employed in this work is a useful one for studying gender differences in aging since females live longer than males, as evidenced by the survival curves. Previous findings on *Drosophila* survival suggest that mortality rates display gender differences with age, which could be attributable to differential aging in the sexes. Endogenous ROS were positively connected with age-related mortality, which was consistent with previous research showing an adverse association between oxidative stress and lifespan (Ku *et al.*, 1993; Sohal *et al.*, 1995). (Archer *et al.*, 2013) [21, 29] found evidence for sex variations in oxidative stress in connection to lifespan in the cricket, *Gryllodes sigillatus*. A recent study found that overall SOD and catalase activities drop with aging in both sexes. Where an earlier study found that whether or not male fruit flies were kept in mixed-sex groups affected the magnitude of life span changes in response to diet (Zajitschek *et al.*, 2013) [36]. Because increased reproduction frequently reduces life span (Harshman and Zera, 2007; Kenyon 2010; Dmitriew and Rowe, 2011) [13, 17, 20], it is critical to understand how nutritional manipulations affect longevity in environments with varying reproductive potentials (i.e., the extent to which females can reach their full reproductive potential). There are several studies was shown that life span influenced by various factors such as environmental factors i.e., stress, temperature, light etc., in our study we used same age, laboratory conditions. But the flies fed with different diets with different concentrations used to study the longevity on *D. melanogaster*.

Thus these study suggest that the life span also influenced by quality and quantity of nutrients in the diet. The *P. ostreatus* (oyster) mushroom enhances the life span with increasing concentration of mushroom in *D. melanogaster*.

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