



## **Effect of Prebiotics supplement on Pre-adult fitness in *Drosophila melanogaster***

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### **Abstract**

Prebiotics are a type of nutrient that can withstand the enzymatic and acidic digestion that takes place in the small intestine while still being broken down by the gut flora. For several health beverages with high fiber and vitamin content, prebiotic supplement products are manufactured. An organism uses the energy from food it consumes for growth, development, reproduction, and survival. Pre-adult development is one of the critical phases in an organism's survival. In the present study flies of *D. melanogaster* cultured in different concentration of prebiotic supplement (2.5%, 5%, 10%) and control media. Flies cultured in different concentrations of prebiotics supplement showed significant influence to adult development. From the results obtained in experiment, the rate of development from larva to pupa and pupa to adult found the flies grow faster in control media. Whereas, the rate of development from larva to pupa and pupa to adult showed slower in flies cultured from prebiotic treated media, mainly in 10% treated media. As the concentration of prebiotics supplement increases the growth of larva to pupa and pupa to adult decreased in flies. The percentage of hatchability was greater in control media flies compared to prebiotics treated flies in case of larva to pupa and pupa to adult hatchability. Further the rate of hatchability was less in 5% treated media and flies cultured in control media have showed a high rate of hatchability. Thus, these studies suggest that prebiotics supplement decreases pre adult fitness in *D. melanogaster*.

**Keywords:** prebiotics supplement, *D. melanogaster*, Nutrition, pre-adult fitness.

### **Introduction**

The most obvious way that environmental variation may affect fecundity and bodily condition is through nutritional impacts brought on by variations in the types of food that are readily available. Under natural circumstances, it can be difficult for many species to achieve their supplemental dietary needs for somatic and

reproductive growth (Raubenheimer and Simpson, 1999). Life-history features like illness susceptibility, fertility, reproduction, lifespan, and stress resistance are significantly influenced by the quantity and quality of nutrients consumed by organisms (Hoffmann and Parsons, 1991; Rion and Kawecki, 2007; Lee *et al.*, 2008). Studies that focus on the effects of nutrition frequently evaluate the morphological and physiological

responses of people exposed to various quality and amounts of nutrients. Animal survival and reproductive success depend on a healthy balance between energy intake and expenditure, (Pough, 1989; Sibly, 1991). This equilibrium depends on how food intake, digestion, and the distribution of newly obtained energy among various processes including maintenance, growth, and reproduction interact (Karasov, 1986). Food provides animals with energy and nutrition; hence diet can be regarded as a crucial factor that may have an impact on all aspects of their life histories (Sterner and Schulz, 1998; Tayloret *al.*, 2005) The investigation of how organisms alter their allocation of energy has been greatly aided by experimental changes to animal diets (Chown and Nicolson, 2004; Cruz-Neto and Bozinovic, 2004) Body tissues constantly require a particular amount and ratio of nutrients during development in order to achieve optimal growth and performance (Bauerfeind and Fischer, 2005). Characteristics like growth and reproduction can be affected by a lack or imbalance of fat, carbohydrates, or protein (Wang and Clark, 1995). In *D. melanogaster*, protein deficit lowers fertility and growth and in fruit-feeders, protein is frequently a limiting macronutrient (Mattson, 1980; Adams and Gerst, 1991; Hendrich *set al.*, 1991; Markow *et al.*, 1999; Markow *et al.*, 2001). Earlier research on *Drosophila* species (Geeth and Krishna, 2015) established that the organic fruits watermelon and chikku had a significant impact on pre-adult fitness. Although their study did not have positive effects on pre-adult development in *D. melanogaster*, the avocado and yogurt had demonstrated a positive effect on larva to pupa, pupal to adult viability, and rate of development in this species (Cleona Alexander and Krishna, 2018). Additionally (Alwyn's D Sowza and Krishna, 2015) discovered that consuming an alternate natural energy drink aided in preadult development when researching *D. melanogaster*. Prebiotics' impact on preadult fitness, however, has not been discovered. The current study has been conducted as a result.

Prebiotics are non-digestible meals that help the host by promoting the development of a particular strain of bacteria in the colon according to

(Gibson, 2004). This was reframed later, in 2004. In the small intestine, prebiotics are polysaccharides that can withstand acidic and enzymatic digestion and are utilised by probiotics that follow. The two types of saccharides are "oligosaccharides" and "galacto oligosaccharides." Many plants naturally contain prebiotics such as inulin, oligofructose, and lactulose oligolactose.

## **Materials and Methods**

### **Establishment of stock**

Experimental Oregon K strain of *D. melanogaster* used in the study was obtained from Drosophila stock center, Department of zoology, University of Mysore, Manasagangothri, Mysuru, used to establish experimental stock. The stock was cultured in bottles containing wheat cream agar media (100g of wheat powder, 100g of jaggery, 10g Agar the contents were boiled in 1000ml of distilled water and 7.5 ml propionic acid). Flies were maintained in laboratory conditions such as humidity of 70% and 12 hours dark: 12 hours light cycles and temperature of  $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The flies were used to conduct the experiments.

### **Establishment of experimental stocks**

Flies cultured in normal wheat cream agar media were used as control flies. And flies were obtained from above stock used to establish the experimental stock with different concentrations of dietary Prebiotic supplement (2.5%, 5%, 10%) per 100 ml of wheat cream agar media. Flies obtained from control and prebiotic treated flies were used in the present experiment.

### **Effect of prebiotic powder on rate of development in *D. melanogaster***

To examine, the effects of prebiotics on the rate of development, about twenty flies (virgin males and unmated males) of control and prebiotic treated flies were taken. The flies were introduced into vials and allowed them for about 24 hours. Later the flies were removed from the vial. The ten first instar larva were transferred by scooping

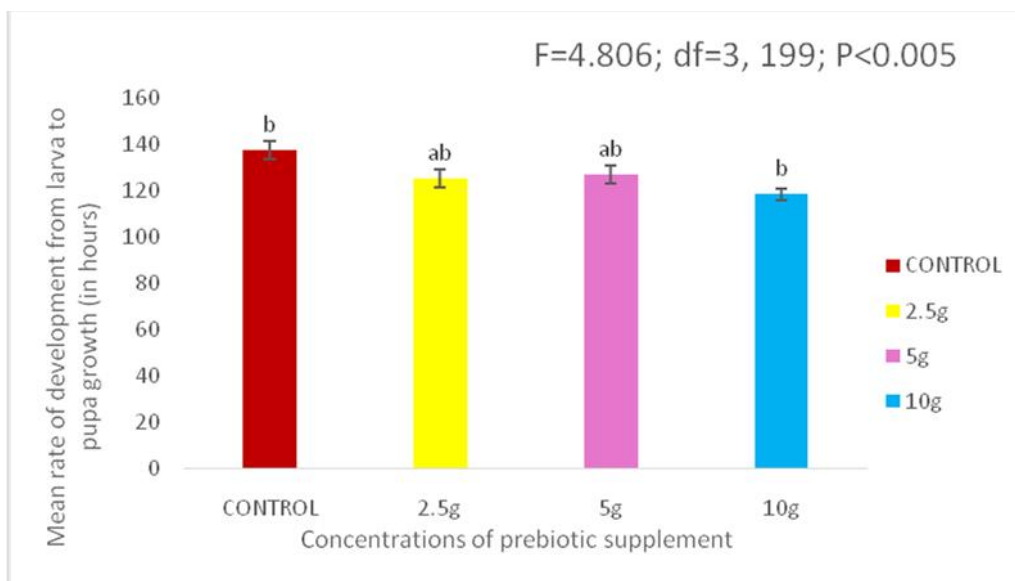
out the media from the vial the procedure follows for control and prebiotic treated flies. The first instar larva fed with different concentrations of prebiotic supplement (2.5%, 5%, 10%) and control media. To track the stages of larval development and percentage of hatchability, the

treated larvae in each vial were kept under control. Timings was recorded for the rate of development from larva to pupa and pupa to adult in hours. A total of 5 replicates were run for each of control and prebiotic treated flies to get the data for analyse the rate of development.

## Results and Discussion

### Effect of prebiotic supplement on rate of development in *D. melanogaster*

**Figure 1:** The effect of different concentration of prebiotic supplement on the rate of development from larva to pupa of *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



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

Diet is a significant environmental component that influences an organism's growth, development, reproduction, and survival. variable diets have variable nutritional availability in terms of both amount and quality. The purpose of the current study is to ascertain the benefits and drawbacks of prebiotic supplementation on the pre-adult development of *D. melanogaster*.

**Figure 1** shows mean  $\pm$  standard error of the rate of development from larva to pupa flies in control and prebiotic treated flies (2.5%, 5%, 10%). From the data obtained it was noticed the flies raised in control media had significantly faster rate of

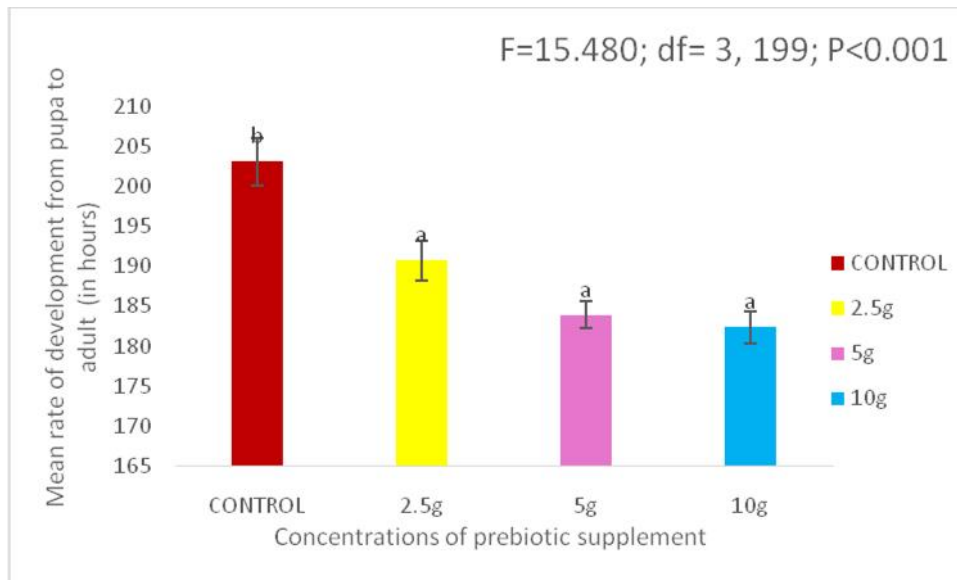
development compared to the flies fed with prebiotic treated media. The rate of development was found low in 10% prebiotic treated media. Further among prebiotic treated flies, the time taken for larva to pupa growth decreased with increasing as the concentration of prebiotic supplement.

The above data subjected to one-way ANOVA followed by Tukey's Post hoc test showed significance variation in rate of development from larva to pupa between the flies of different diet (**Figure 1**).

The Tukey's post hoc test showed that control media, 2.5% and 5% prebiotic treated media had significant highest rate of development from larva to pupa than 10% prebiotic treated flies. Further in

10% concentrated prebiotic treated flies shows nonsignificant rate of development from larva to pupa compared with control media flies.

**Figure 2:** The effect of different concentration of prebiotic supplement on the rate of development from pupa to adult of *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



**Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test**

**Figure 2** shows mean  $\pm$  standard error of the rate of development from pupa to adult flies in control and prebiotic treated flies (2.5%, 5%, 10%). From the data it was noticed the flies raised in control media had significantly faster rate of development from pupa to adult than flies fed with prebiotic treated media. The rate of development was found low in 10% prebiotic treated media. Further among prebiotic treated flies, the time taken for larva to pupa growth decreased with increasing as the concentration of prebiotic supplement.

The above data subjected to one-way ANOVA followed by Tukey's Post hoc test showed significance variation in rate of development from pupa to adult between the flies of different diet (**Figure 2**). Tukey's post hoc test showed that flies fed with control and prebiotic supplement different concentration media (2.5%, 5%, 10%)

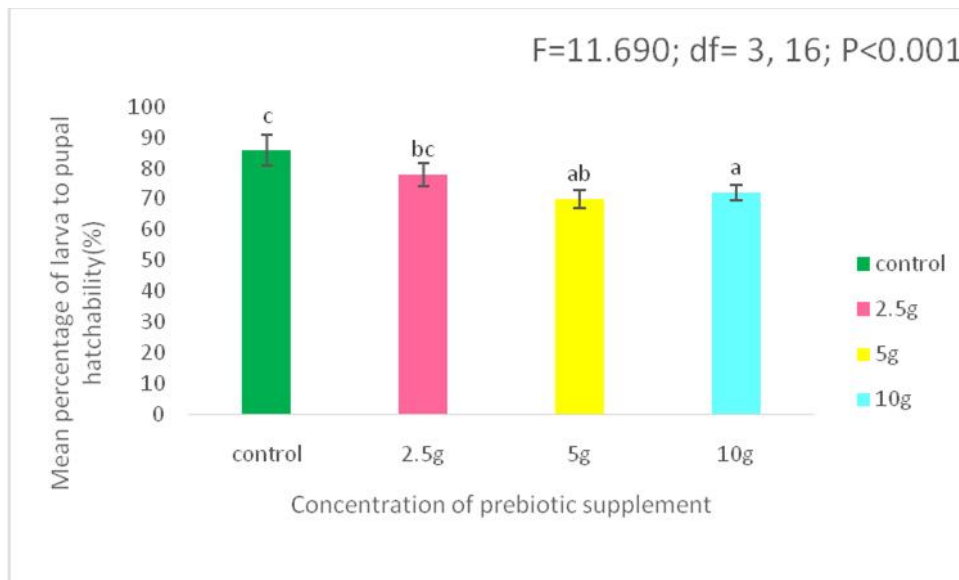
had significant rate of development from pupa to adult. Further in prebiotic treated flies the graph shows nonsignificant between different concentrations (2.5%, 5% and 10%). The nutrients present in wheat cream agar media and prebiotic supplement media were responsible for the observed variance in growth rates in control and prebiotics treated flies. According to the data obtained indicates larvae which raised on prebiotics supplement slower emergence. The mass of larvae fed on prebiotics-treated medium is lower than that of larvae fed on control media. Because prebiotics give less nutritional availability compared to control media preparation, larva take longer to convert the substrates into metabolized nutrients stores than when reared on varied amounts of prebiotic treated media.

According to earlier research (Vijendravarma *et al.*, 2010), people who consume diets deficient in nutrients may grow smaller. Poorer nutrition may be the cause of *Drosophila's* lower larval bulk and smaller adult size. It is also remarkable that prebiotic-treated flies consume food at a markedly higher rate as they mature. This increased food consumption could be a direct result of their better health or it could just be a result of their much smaller size. Our results indicates that protein rich prebiotic supplement media contains a nutrient balance that reduce size and reproductive rates but found to be adversely affecting lifespan. Further it decreases the pre adult fitness in *D. melanogaster*.

Recent research has also examined the pre-adult fitness of *D. melanogaster* using different diets. Organic fruits and vegetables have a positive impact on preadult fitness, according to (Chabra *et al.*, 2013). They discovered that flies fed organic fruits developed at a considerably faster rate. Similar to this, (Geetha and Krishna., 2015) discovered that organic fruits like chikku and watermelon have an impact on pre-adult development when researching *D. melanogaster*. In addition, (Alwyn's D Sowza and Krishna, 2015) discovered that alternative natural drinks were better for preadult development than both synthetic and natural juice. It was found from all of these research that the quantity and kind of nutrients consumed in the diet affected the pre-adult development of *D. melanogaster*.

**The effect of prebiotics supplement on percentage of pre-adult hatchability:**

**Figure 3:** The effect of different concentration of prebiotic supplement on larva to pupa hatchability of *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



**Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey’s post hoc test**

**Figure 3** shows mean ± standard error of the from larva to pupal hatchability in control and prebiotic treated flies (2.5%, 5%, 10%). From the data obtained it was noticed the flies raised in control

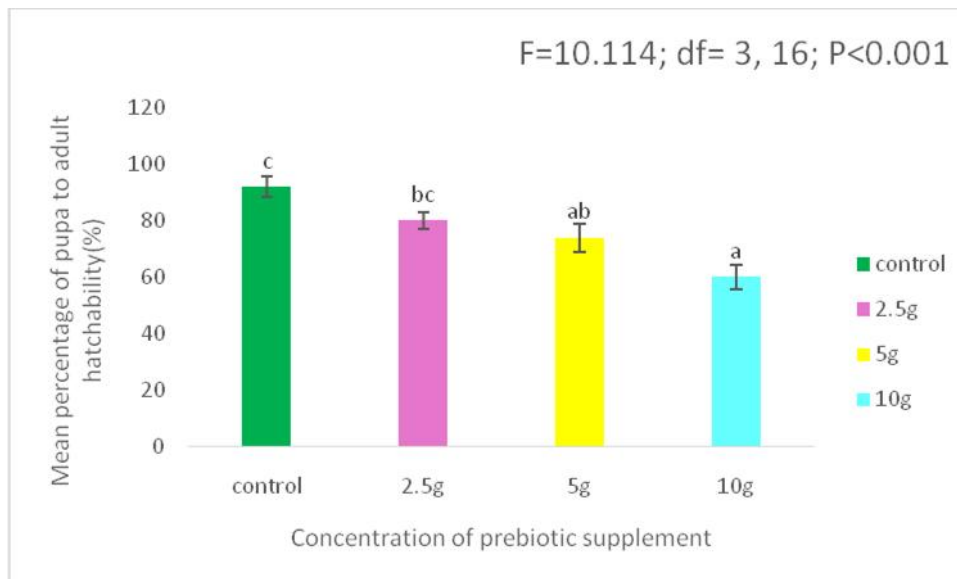
media had significantly faster rate of hatchability from larva to pupa compared to the flies fed with prebiotic treated media. The rate of hatchability was found low in 5% prebiotic treated media.

Further among prebiotic treated flies, the time taken for larva to pupal hatchability is same with increasing as the concentration of prebiotic supplement.

The above data subjected to one-way ANOVA followed by Tukey's Post hoc test showed significance variation in rate of hatchability from

larva to pupa between the flies of different diet (Figure 3). The Tukey's post hoc test showed that control media and 5% prebiotic treated media had significant rate of hatchability from larva to pupa. Further in 2.5% and 10% concentrated prebiotic treated flies shows significant rate of development from larva to pupa hatchability compared with control and 5% prebiotic treated media.

**Figure 4:** The effect of different concentration of prebiotic supplement on pupa to adult hatchability of *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



**Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test**

Figure 4 shows mean ± standard error of the from pupa to adult hatchability in control and prebiotic treated flies (2.5%, 5%, 10%). From the data obtained it was noticed the flies raised in control media had significantly faster rate of hatchability from pupa to adult compared to the flies fed with prebiotic treated media. The rate of hatchability was found low in 10% prebiotic treated media. Further among prebiotic treated flies, the time taken for pupa to adult hatchability decreased with increasing as the concentration of prebiotic powder.

The above data subjected to one-way ANOVA followed by Tukey's Post hoc test showed significance variation in rate of hatchability from

pupa to adult between the flies of different diet (Figure 4). The Tukey's post hoc test showed that control media and 10% prebiotic treated media had significant rate of hatchability from pupa to adult. Further in 2.5% and 5% concentrated prebiotic treated flies shows non significant rate of development from pupa to adult hatchability compared with control and 10% prebiotic treated media.

Viability is impacted by both chemical parameters such as pH and physical factors like as light, temperature, moisture, and so on. Egg to larval viability, larvae to pupae viability, pupae to adult viability are some of the phases that make up pre-adult viability.



It was found that from **Figure 2 and Figure 3**, shows there was significant variation in the pre adult rate of development in flies raised on prebiotics supplement had less larva to pupae and pupae to adult viability compared to flies fed on control media.

This indicates that control media operate better and support energy and necessary nutrients to promote pre adult fitness than prebiotic supplement which is needed for the pre adult development of larva to pupa and pupa to adult stage development. With temperature increases, the rate of *Drosophila* development tends to slow down. (Al-Saffar *et al.*, 1995; Gilbert and De Jong, 2001; Hartwell *et al.*, 2011). The viability will occur more quickly when the rate of development rises. This is so because both the control and prebiotics supplement flies were cultured in the similar lab environment; the only thing that varied was the nutrients in the diet. Thus, we conclude prebiotic supplement in the diet decreases the percentage of hatchability and rate of development in *D. melanogaster*.

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