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The Effect of the Ensure[®] Nutrition Supplement on the Rate of Development and Percentage of Hatchability in *Drosophila melanogaster*

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Abstract

An organism needs energy from its nutrition-rich food for growth, development, reproduction, and survival. In the present study, *Drosophila melanogaster* flies were cultured on wheat cream agar, Ensure[®], and mixed medium to determine the impact of Ensure[®] on the *Drosophila melanogaster* % of hatchability and rate of development. The results revealed that the rate of development of larva to pupa and pupa to adult was significantly slower in Ensure[®] media and larva to pupa and pupa to adult rate of development was significantly faster in the control media compared to test media and mixed media which showed more or less similar rate of development of control media. Overall the development time from larva to adult was faster in the mixed and control media compared to the test media which had the least preadult rate of development. Further the % hatchability of larva to pupa and pupa to adult was significantly greater in the mixed media compared to wheat cream agar media and test media. Control diet showed the least percentage of hatchability compared to mixed and test diet. This suggest that mixed diet provides the required nutrition to increases the rate of development and % of hatchability. This study suggests that the consumption of mixed diet media was shown beneficial effect on the rate of development and % of hatchability of the *Drosophila melanogaster*.

Keywords: Diet, *Drosophila melanogaster*, Ensure[®], rate of development

Introduction

In general there are two types of diets: quantitative (food availability) and qualitative (food consumption). Of the two, the quantitative impacts are more obvious since food provides animals with energy and other nutritional requirements. As a result, food availability and organism fitness are positively correlated across a range of natural settings. Conversely, qualitative consequences are sometimes classified into two groups, particularly nutrient deficiency and nutritional deficiency (Pough, 1989; Sibly, 1991) [13, 15]. The whole growth, development, and reproduction of an organism are influenced by both internal and external stimuli that are known to affect all biochemical, physiological, and developmental changes that occur in an organism (Sterner and Schulz, 1998; Taylor *et al.*, 2005). The interaction between food intake, digestion, and the allocation of recently obtained energy among diverse functions such as maintenance, growth, and reproduction determine the degree of balance (Karasov, 1986) [9]. Therefore, experimental food modifications for animals have greatly aided research into how organisms control their energy consumption and how this affects preadult development in creatures.

It was demonstrated in past research on *Drosophila* species (Geetha and Krishna, 2015) [5] that the organic fruits watermelon and chikku had a major impact on preadult fitness. In *D. melanogaster*, the avocado and yogurt had a

favourable impact on the pace of development, pupal to adult viability, and larva to pupa (cleona alexander and Krishna, 2018) [11]. Further Alwyn's D Sowza and Krishna (2015) who while studying in *D. melanogaster* have also found that the consumption of alternative natural energy drink was beneficial in preadult development. In order to enhance survival and reproduction, organism must adapt to changing circumstances by changing their developmental growth, metabolism and behaviour. This adaptation depends on the organisms capacity to detect and react to changes in the external and internal environment. Complex sensing of temperature, light, oxygen and nutritional circumstances is required for this. All phases of animal development use this knowledge and modify their metabolism and behaviour to make the most of available resources and preserve homeostasis (Koyama *et al.*, 2020). Consuming soft drinks and other sweetened drinks for a long time leads to type II diabetes and obesity (Odegaard, 2009).

However, the effect of Ensure[®] on preadult fitness have not found. Therefore, the present study has been undertaken. 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). Due to its numerous health benefits, Ensure[®] is now widely used by individuals as a nutritional supplement. Numerous studies demonstrate that consumption of Ensure[®] has a good effect on enhance bone and muscular development, immunological function, and overall health. However, it's effect on pre-adult fitness (rate of development and % of hatchability) has not been studied.

Materials and Methodology

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

Establishment of Stock

Experimental 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 wheat 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 $22^{\circ}\pm 10^{\circ}\text{C}$.

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 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 under the same laboratory conditions as mentioned above. These flies were used to study the rate of development and percentage of hatchability in *D. melanogaster*.

Experimental Procedure

To analyse, the effect of Ensure[®] on the rete of development around 20 flies in which 10 males and 10 female flies raised in control media, mixed media and test media were collected. The collected flies were introduced into media bottles (control, mixed and test media) and allowed them for 24 hours. Later, the flies were removed from the media bottles and allowed the media bottles as such for 24 hours to obtain 1st instar larvae from the eggs laid by the flies. Total fifteen 1st instar larvae were collected by scooping out the different media i.e., control, mixed, test media. And transferred into its respective media, were kept under control environmental condition to analyse the rate of development.

For hatchability of larva to pupa and pupa to adult, total sixty

1st instar larva were observed and % of hatchability of larva to pupa and pupa to adult were also recorded.

Result Analysis

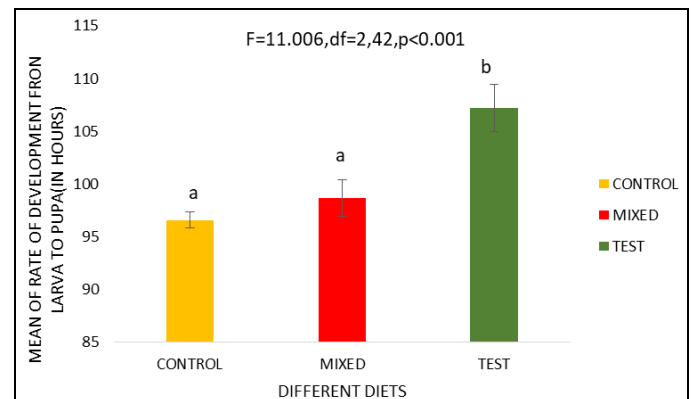


Fig 1: Effect of the Ensure[®] on the rate of development larva to pupa of the *Drosophila melanogaster*

The Different Letter on the Bar Graph are Indicates the Significant Variation in the Different Diet by the Tukey's Post hoc Test at 0.05 Level

Figure 1 showed the mean and standard error value of larva to pupa rate of development in *Drosophila melanogaster* flies which are cultured in the different diet (wheat cream agar, mixed and test). According to data obtained showed that the rate of development was slower in the test media (Ensure[®] compared to the flies fed with wheat cream agar and mixed media.

The experimental data was subjected to the one way ANOVA, followed by the Tukey's post hoc test which reveals the significant variation in the rate of development from larva to pupa between the control and test diets. And insignificant between control and mixed media. According to Tukey's post hoc test the greater significant variation was noticed in the larva fed in the test media it takes longer duration for development from larva to pupa compared with larva fed with wheat cream agar media and mixed media.

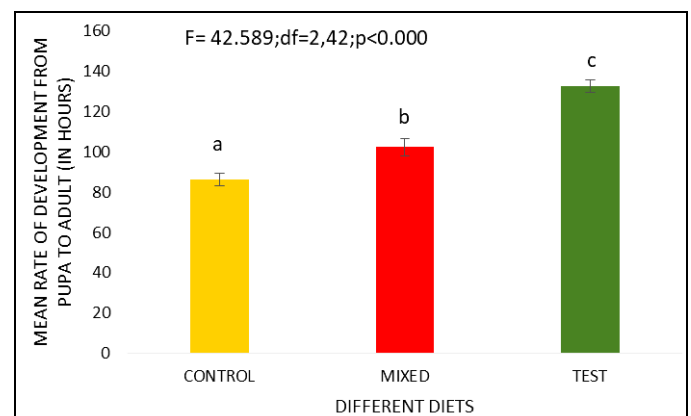


Fig 2: Effect of Ensure[®] on the rate of development pupa to adult of the *Drosophila melanogaster*

The Different Letters on Bar Graph Indicate the Significant Variation between the Different Diets by Tukey's Post hoc Test at 0.05 Levels.

Figure 2 showed the mean and standard error value of pupa to adult rate of development in *D. melanogaster* flies which are cultured in the different diets (wheat cream agar, mixed and test). According to data obtained showed that the rate of

development from pupa to adult was slower in the test media (Ensure[®] followed by mixed media and wheat cream agar). The experimental data was subjected to the one-way ANOVA, followed by the Tukey's post hoc test which reveals the significant variation in the rate of development from pupa to adult between the different diets. According to Tukey's post hoc test the greater significant variation was noticed in the larva fed in the test media it takes longer duration for development from pupa to adult compared with larva fed with wheat cream agar media and mixed media.

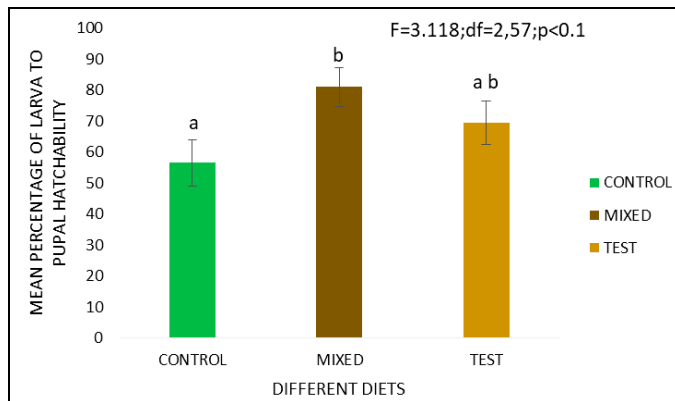


Fig 3: Effect of Ensure[®] on the % of hatchability of larva to pupa in the *Drosophila melanogaster*

The Different Letters on Bar Graph Indicate the Significant Variation between the Different Diets by Tukey's Post hoc Test at 0.05 Levels

Figure 3 shows the mean and standard value of larva to pupa % of hatchability in *D. melanogaster* flies which are cultured in the different diets (wheat cream agar, mixed and test). According to data obtained showed that % of hatchability from larva to pupa was greater in the mixed media followed by test media and wheat cream agar.

The experimental data was subjected to the one-way ANOVA, followed by the Tukey's post hoc test which reveals the significant variation in the % of hatchability from larva to pupa between the different diets. According to Tukey's post hoc the in significant variation was noticed in the larva fed in the mixed media had greater % hatchability of larva to pupa compared with larva fed with wheat cream agar media and test media.

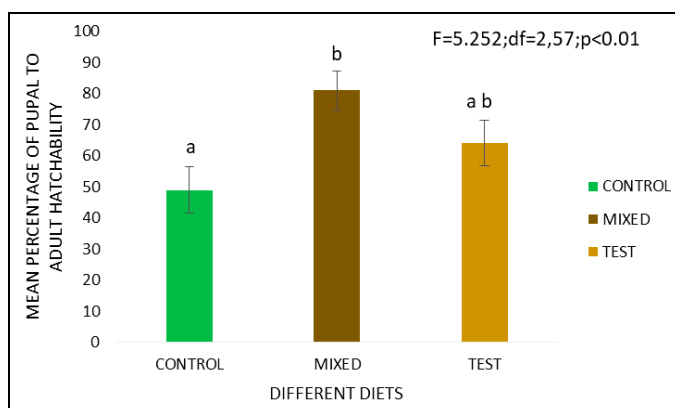


Fig 4: Effect of Ensure[®] on the % of hatchability of pupa to adult in the *Drosophila melanogaster*

The Different Letters on Bar Graph Indicate the Significant Variation between the Different Diets by Tukey's Post hoc Test at 0.05 Levels

Figure 4 shows the mean and standard value of pupa to adult

% of hatchability in *D. melanogaster* flies which are cultured in the different diets (wheat cream agar, mixed and test). According to data obtained showed that % of hatchability from pupa to adult was greater in the mixed media compared to test media and wheat cream agar. Wheat cream agar media shows least % of hatchability from pupa to adult.

The experimental data is subjected to the one-way ANOVA, followed by the Tukey's post hoc test which reveals the significant variation in the % of hatchability from pupa to adult between the different diets. According to Tukey's post hoc the greater significant variation was noticed in the larva fed in the mixed media it had significantly greater % of hatchability from pupa to adult compared with wheat cream agar media. And the test media had the non-significant variation with mixed and control diets.

Discussion

The diverse food environment in which growing, developing organisms need to adjust affects a wide range of life history traits, including survival, developmental time, final body size and form, lifespan, and fertility (Simpson and Raubenheimer, 2012). One of the key environmental factors affecting an organism's ability to endure, reproduce, grow, and develop is its nutritious food. To examine the positive and negative impacts of the Ensure[®] on the fitness of developing adults, including the percentage of hatchability and development rate in *D. melanogaster*. The Ensure[®], wheat cream agar, and mixed diet were given to the flies for ingestion. 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.

Preadult fitness in *Drosophila* is determined by the combination of its genotype, environment, nutrition, and competing members of the population. Important preadult measures of fitness include egg to adult rate of development, hatchability, or viability. Using three different diets wheat cream agar, Ensure[®] and mixed. In this study we examined the rates of development from larva to pupa and pupa to adult. The development rates from larva to pupa and pupa to adult among the three diets, test diet showed a substantial variance, as shown by Figures 1 and 2. The rate of development from larva to pupa in test media was demonstrated to be much slower than in mixed and wheat cream agar media. And mixed and control media shows more or less similar range of rate of development. As well as the pupa to adult rate of development is significantly slower in the test media compared to mixed and wheat cream agar media.

Finally, from the obtained results of developmental duration from larva to pupa and pupa to adult we can explain that the larva fed with Ensure[®] media takes larger time for molt from larva to pupa and pupa to adult. It suggests that the nutrients of the diet may affect and alter the physiology that in turn leads to delayed the larval to pupal and pupal to adult development compared to mixed and control diet. Mixed diet fed larva, took more or less similar duration of control diet fed larva to develop from larva to pupa and pupa to adult. This shows that dietary contents of the mixed media even though able to accelerate the larval to pupal development compared to test media. Finally, the rate of development from the larva to adult as follows i.e., Ensure[®] media < mixed media < wheat cream agar media. This indicates that Ensure[®] with its protein and carbohydrates rich diet provide the excess nutrients hence

flies spend more period to absorb the excess nutrition that cause the slower rate of development compared to mixed and control diet. The earlier studies also showed that the developmental delay occurred in the larval stage, possibly in the TGP (Layalle *et al.*, 2008) and Khushboo Kasya *et al.*, (2016), also shown that high protein diet (casein) impact on the longer larval duration in *D. melanogaster* Guler *et al.*, (2014) also stated that the restricted protein has an important role in larvae to pupae developmental time due to different stress signals.

Rodrigues *et al.*, (2015), also shown that the larval developmental time was reduced in diets with intermediate protein to carbohydrates ratio. The decreased amount of yeast in the larval diet also delayed the pupal eclosion. And also works revealed that the while working on the organic fruits Chikku and watermelon (Geetha and Krishna, 2015) [5], who while studying the *D. melanogaster* also shown that faster rate of pre-adult development. As well as the Energy drinks (Alwyn Dsouza and Krishna, 2015) also shown that Alternative natural drinks had greater pre adult development than the synthetic drinks in *D. melanogaster* further study on the organic fruits by Chabin *et al.*, (2013), they also found that rate of pre adult development is increase in organic fruit than nonorganic fruits as well as some works on the fruits and vegetables also revealed that the beneficial effects on the rate of development and preadult fitness in *D. melanogaster* Similarly, study by Cleona alexander and Krishna, (2018) [1], who while studying the *D. melanogaster* showed that yoghurt had the beneficial effect on the rate of development i.e., increases rate of the pre adult development All of these studies suggest the nutrient amount quantity and quality in the diet and fruits are responsible for the for the pre adult development in the *D. melanogaster*.

The percentage of hatchability also the important components which also enhances the pre-adult fitness in the study, we studied the percentage of hatchability of larva to pupa and pupa to adult to understand the effect of Ensure® on the hatchability of in the *D. melanogaster*. The Figure 3 and Figure 4 was showed that significant variation in the larval to pupa and pupa to adult percentage of hatchability between the diets. The flies fed on the mixed media had the greater significant of larval to pupa and pupal to adult % of hatchability than those flies fed with the wheat cream agar media and Ensure® diet. it suggests that mixed diet provides the greater optimal quality and quantity of the nutrients and energy required for the eclosion from egg to adult stage where as the nutrients in the wheat cream agar media not enough to enhance the hatchability and may have not provided the sufficient energy for eclosion and the mixture of Ensure® and wheat cream agar in mixed diet due to combine effects of the nutrients content may provide the nutrition and energy for hatchability. Our study also supported by the Sisodia and singh (2015), who noticed that increased egg to adult viability in the carbohydrate rich diet and decreases in egg to adult viability in protein rich media and also several researches reveals that the alternative energy drinks and organic fruits like Chikku and watermelon have the increased egg to adult viability or hatchability. Alwyn Dsouza and Krishna, (2015) who while studying in the *D. melanogaster* also found that increased pre adult viability in the alternative natural energy drinks than the synthetic drinks and in contrast to our study, Cleon alexander and Krishna, (2018) [1] who also found that avocado and yoghurt supplemented had the negative or detrimental effect on the egg to adult hatchability in *D. melanogaster* and Rodrigues *et al.*, (2015) also showed that

survival from egg to pupation were increased in high- protein, low-carbohydrate diets These earlier studies suggests that dietary nutrients present in the food and drinks had the significant effect on the pre adult hatchability in *D. melanogaster*.

The earlier several researches also shown that variation in the physical environment like temperature and light also responsible for the variation in the developmental rate and the viability but we conducted the experiments in by providing same temperature and light but larva were raised in the different diets. Hence observed variation in the experimental results was due to the influence of variation in the amount, quality and quantity of nutrient in the dietary food.

From our study in *D. melanogaster* we can say that Nutritional diet is one the key factor which influence on the rate of development in the *D. melanogaster*. The faster rate of development observed in both mixed and control diet more or less equal and greater percentage of the hatchability was observed in the mixed media compared to wheat cream agar and test media. The rate of development of *D. melanogaster* in our study follows that control diet> mixed diet>test diet. Further percentage of hatchability of *D. melanogaster* in our study follows that mixed diet>test diet> wheat cream agar diet. it suggests that the consumption the mixed nutrition of Ensure® and wheat cream agar media had the beneficial effect on the pre-adult fitness of the *D. melanogaster*.

Conclusion

From this experiment we can conclude that Nutritional diet is one the key factor which influence on the rate of development in the *D. melanogaster*. The faster rate of development observed in both mixed and control diet more or less equal and greater percentage of the hatchability was observed in the mixed media compared to wheat cream agar and test media. The rate of development of *D. melanogaster* in our study follows that control diet> mixed diet>test diet. Further percentage of hatchability of *D. melanogaster* in our study follows that mixed diet>test diet> wheat cream agar diet. it suggests that the consumption the mixed nutrition of Ensure® and wheat cream agar media had the beneficial effect on the pre-adult fitness of the *D. melanogaster*.

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