



The effect of whey protein on pre-adult fitness in *Drosophila melanogaster*

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Abstract

Diet is one of the important extrinsic factors which has an influence on growth, reproduction and survival of an organism based on the quality and quantity of nutrition present in it. The present study demonstrates the effect of the Whey protein on the rate of development and percentage (%) of hatchability in *Drosophila melanogaster* which is cultured in Wheat cream agar media, Mixed media and Whey protein media. The results suggest that, the rate of development from larva to pupa was average and rate of development from pupa to adult was faster than wheat cream agar media which had faster rate of development from larva to pupa and average rate of development from pupa to adult and mixed media which had slower rate of development from both larva to pupa and pupa to adult. Overall rate of development from larva to adult was faster in whey protein media, average in wheat cream agar media and least in mixed media.

Further, the % of hatchability of larva to pupa and pupa to adult was greater in mixed media compared to average in wheat cream agar media and least in whey protein media. Thus, whey protein provides nutrition to increase rate of development meanwhile it decreases the % of hatchability.

Keywords: diet, Whey protein, *Drosophila melanogaster*, rate of development, % of hatchability

Introduction

Intrinsic and extrinsic factors influence the overall growth, development and reproduction of an organism by having an effect on all biochemical, physiological and developmental variations in an organism. In an organism, food is one of the crucial extrinsic factors which provides nutrition

and energy and have influence on its life history (Stern and Schulz, 1998; Taylor *et al.*, 2005). The quality and quantity of food has an influence on development, health, reproduction, longevity and stress resistance (Hoffmann and Parsons, 1991; Rion and Kawecki, 2007; Lee *et al.*, 2008). In a natural condition range, the availability of food (quantity) and organism's fitness show a

positive relation, which is obvious because animal get their energy and other requirements from diet. On the other hand, qualitative effects of food are often classified into two categories: nutritional deficiency and inhibitory metabolites (which causes disorders). For many animals, meeting their supplemental nutritional requirements for somatic and reproductive growth can be challenging in natural conditions (Raubenheimer and Simpson, 1999). Organisms must modify their developmental growth, metabolism, and behaviour to adapt to changing environments and improve their chances of survival and reproduction. The ability of the organism to recognize and respond to alterations in its internal and external surroundings is necessary for this adaptation. This requires sophisticated monitoring of light, temperature, oxygen, and nutritional conditions. Using this knowledge, animals in all stages of development adapt their metabolism and behaviour to maximize available resources and maintain homeostasis (Koyama *et al.*, 2020).

For optimal growth and function, body tissue continuously needs a certain combination and ratio of nutrients during development (Bauerfeind and Fischer, 2005). Protein, carbohydrates or fat deficiencies or imbalances might have an impact on traits like development and reproduction (Wang and Clark, 1995). The experimental alterations of animal diets enormously supported research on change in energy consumption of an organism (Chown and Nicolson, 2004; Cruz-Neto and Bozinovic, 2004). These studies show that an equilibrium between energy intake and expenditure is crucial for the survival, growth and fitness of an organism (Pough, 1989; Sibly, 1991). This balance is dependent on how food is consumed, how it is digested, and how newly acquired energy is allocated to various activities. Therefore, the study of how organisms modify their energy allocation and their effects on preadult development in creatures has benefited greatly from experimental diet adjustments for animals. The interplay of matter intake, digestion, and the distribution of obtained energy across multiple processes, including upkeep, development, and reproduction, determines this equilibrium (Karasov, 1986).

Therefore, nutrition is one of the major aspects for the development of an organism. It is more useful when it contains different proteins, carbohydrates, vitamins and minerals etc. there are various studies have been conducted using different diets such as study of energy drinks (D'souza and Krishna, 2015), organically grown fruits (Geetha and Krishna, 2015), Spirulina (Sheejani *et al.*, 2023) and other natural resources with fruits and vegetables have shown positive effect on pre adult fitness on *D. melanogaster* and also studies on the Avocado and Yoghurt (Alexander and Krishna, 2018), Jeeni millet traditional mix (Kiran and Krishna, 2023), prebiotics (Shresta and Krishna, 2023) and Creatine monohydrate (Mamba and Krishna, 2023) have shown negative effect on pre adult fitness on *D. melanogaster*. However, there are no studies have been done using Whey protein, so the present study was undertaken to know the effect of the whey protein on pre-adult fitness of the *D. melanogaster*.

Whey protein is being used by people these days to increase the synthesis of muscle protein and to gain lean muscle mass. Whey protein is a high quality cow's milk protein powder. Casein makes up around 80% of the protein in milk, whereas whey protein makes up about 20%. Less than 1% of proteins are found in whey, primarily in the forms of immunoglobulins, α -lactoglobulin (α -LG), β -lactalbumin (β -LA), bovine serum albumin (BSA), lactoferrin, lactollin, glycoproteins, lactoperoxidases, and transferrin. The Muscle Asylum Premium Whey protein have the following nutritional value: 24g of protein, 5.2g of BCAAs, no sugar, and minimal carbs. It also has flavour (banana) and digestive enzymes per serving (40g). There are various studies shows the health benefits of Whey protein but there is no evidence documented on the effect on pre-adult fitness. Therefore, the study was undertaken to address the effect of the Whey protein on the pre-adult fitness in *D. melanogaster*.

Materials and Methods

Muscle Asylum Premium Whey protein, Banana flavor was purchased from online platform supplied from H2H Innovations Pvt.Ltd. distributors.

Establishment of stock

Oregon K strain of *D. melanogaster* were collected from *Drosophila* stock centre, Department of studies in Zoology, University of Mysore, Manasagangotri, Mysuru. Experimental stock was cultured in bottles having wheat cream agar media (100g of jaggery, 100g of rava powder, 10g of agar in 1000ml boiling distilled water and 7.5ml of propionic acid to avoid fungal growth). These flies were maintained in laboratory condition like humidity around 70% RH and $22^{\circ}\pm 1^{\circ}\text{C}$ temperature under 12:12 dark and light cycles.

Establishment of experimental stock

The obtained flies from stock were used to establish experimental stocks with different media using different diets.

Wheatcream agar media (Control media) was prepared by adding 100g of jaggery, 100g of rava powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

Whey protein media (Treated media) was prepared by adding 100g of Muscle Asylum Premium Whey protein powder, 100g of jaggery and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

Mixed media was prepared by adding 50g of rava powder, 50g of Muscle asylum Premium Whey protein powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid]. These flies were maintained under the laboratory condition mentioned above and used to study pre-adult fitness (rate of development and percentage of hatchability) in *D. melanogaster*.

Rate of development

To study effect of Whey protein on rate of development, twenty flies (both virgin males and females) of Wheat cream agar media, mixed and whey protein media were taken and transferred into bottles (plugged with cotton) containing each media respectively. After 24 hours, the flies were removed. Thirty first instar larvae were collected by scooping out media from media bottles and transferred to different media bottles containing respective media. Time was noted for rate of development from larva to pupa and pupa to adult in hours. A total of sixty flies were observed for each wheat cream agar media, mixed media and whey protein media maintained in laboratory condition mentioned above.

Percentage of hatchability

To study % of hatchability, thirty first instar larvae were collected by scooping out media from media bottles and transferred to different media bottles containing respective media. The number of larvae which hatches into pupa and the number of pupae which hatches into adult were noted and the percentage was calculated for each wheat cream agar media, mixed media and whey protein media.

Results

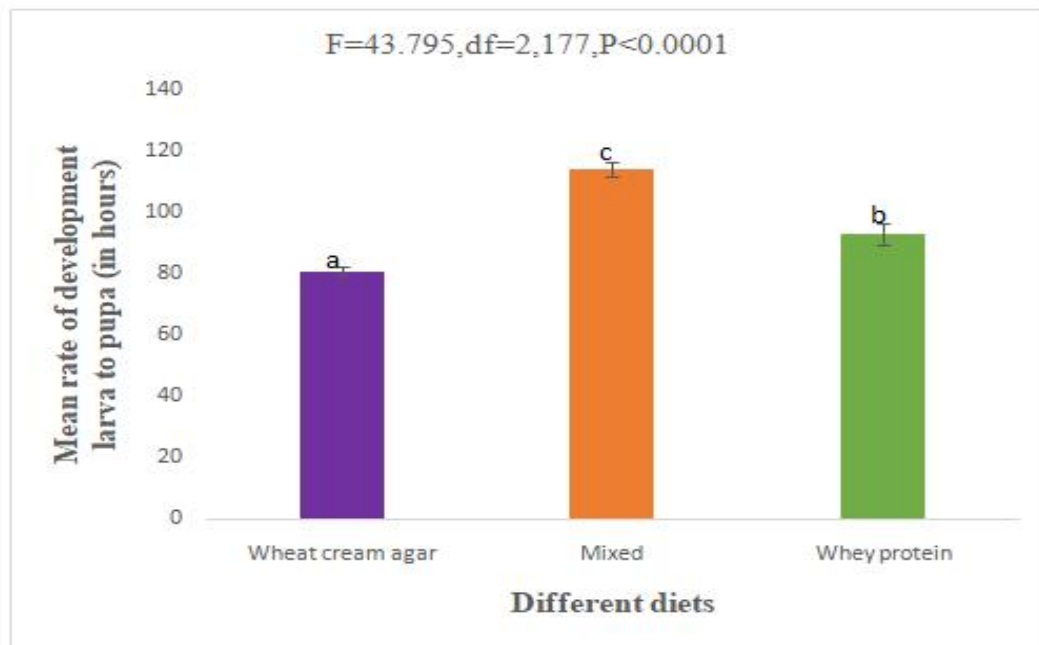
Effect of the Whey Protein on The Rate of Development from larva to pupa of *Drosophila melanogaster*.

Figure-1 showed the mean and standard error value of larva to pupa rate of development in *D. melanogaster* larvae which were cultured in the wheat cream agar media, mixed media and Whey protein media. This data showed that, larvae cultured in wheat cream agar media had greater rate of development when compared to Whey protein media which was average and mixed media which was least.

This data is subjected to One-way ANOVA, followed by the Tukey's post hoc test which showed the significant variation in the rate of development from larva to pupa between the different diets. According to Tukey's post hoc test

showed that Wheat cream agar media showed significant variation in rate of development which took shorter duration to become pupa from larva compared to other two diets (whey protein and mixed media).

Figure 1: Effect of wheat cream agar diet on rate of development from larva to pupa in *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

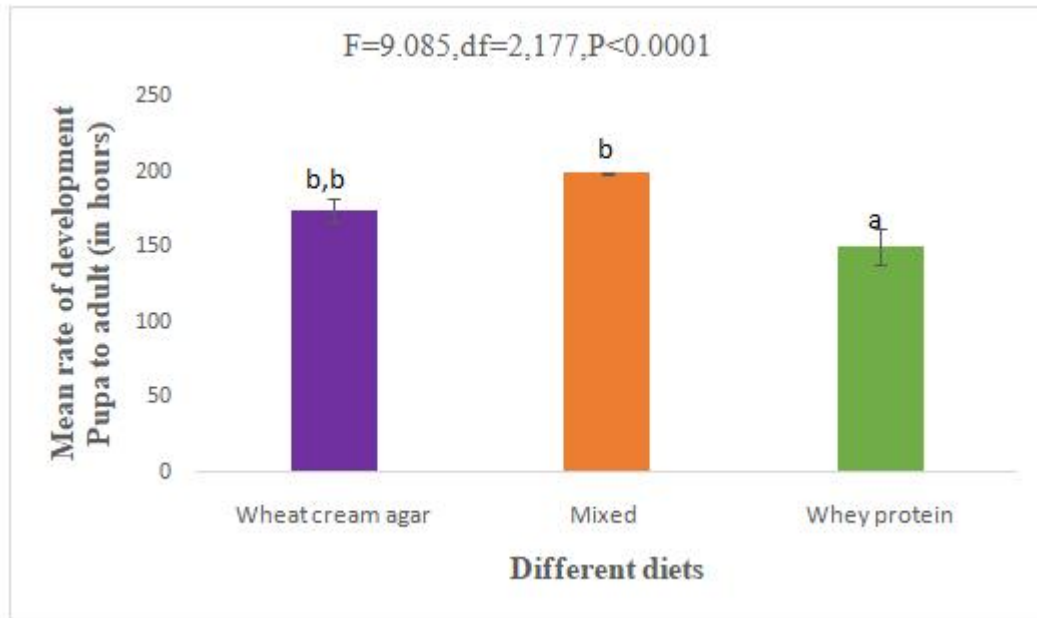
Effect of the Whey Protein on The Rate of Development from pupa to adult of *Drosophila melanogaster*.

The mean and standard error value of pupa to adult rate of development in *D. melanogaster* pupa cultured in wheat cream agar media, mixed media and whey protein media were showed in **Figure-2**. According to this data, pupae cultured in whey protein media had shown greater rate of development compared with wheat cream agar

media which showed average and mixed media which showed least.

This data is subjected to One-way ANOVA, followed by the Tukey's post hoc test which showed the significant variation in the rate of development from pupa to adult between the different diets. According to Tukey's post hoc test showed greater significant variation in Whey protein media which showed greater rate of development from pupa to adult compared to other two diets.

Figure 2: Effect of wheat cream agar diet on rate of development from pupa to adult in *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

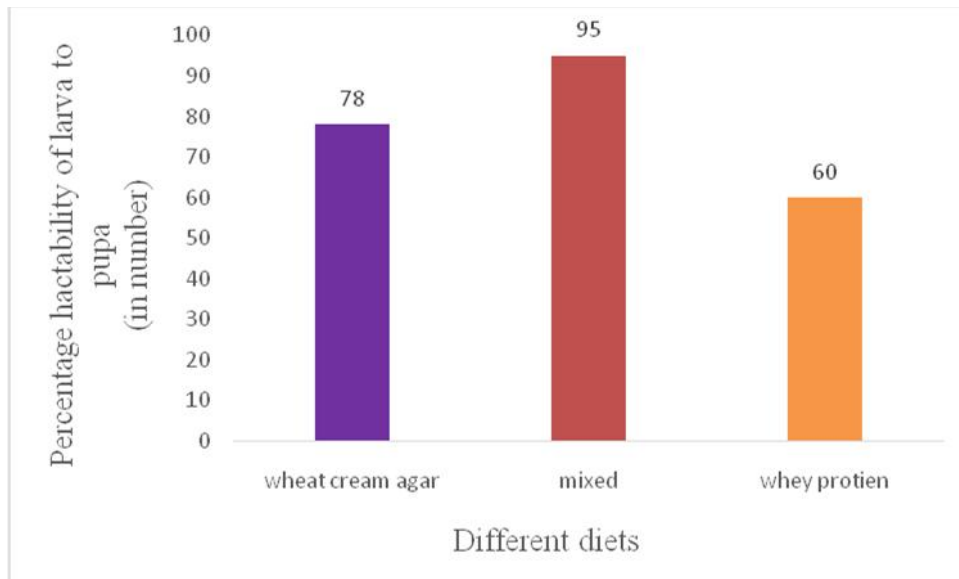
Effect of the Whey Protein on Percentage of hatchability from larva to pupa of *Drosophila melanogaster*.

In **Figure-3**,percentage of hatchability from larva to pupa in *D. melanogaster* larvae which were cultured in the wheat cream agar media, mixed media and Whey protein media was represented. According to this data obtained, larvae obtained from mixed media had greater % of hatchability when compared to wheat cream agar media which

had average and whey protein media which had least.

This data is subjected to One-way ANOVA, followed by the Tukey's post hoc test which showed the significant variation in the % of hatchability from larva to pupa between the different diets. According to Tukey's post hoc test showed that larva cultured in whey protein media had shown significant variation by having least % of hatchability from larva to pupa.

Figure 3: Effect of wheat cream agar diet on % of hatchability from larva to pupa in *Drosophila melanogaster*



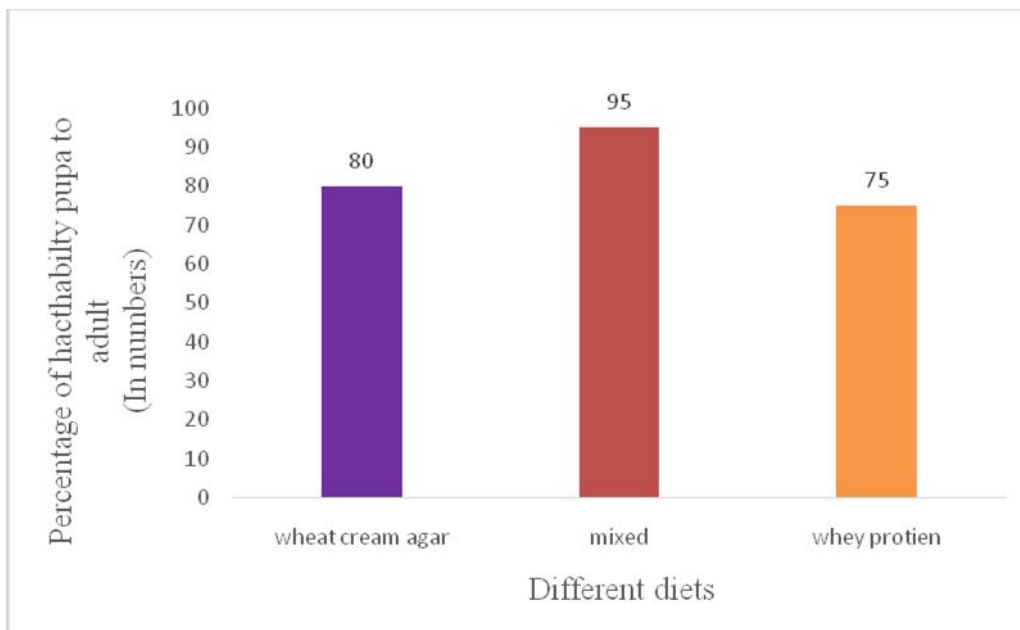
The different numbers on the bar graph are indicates the % of hatchability.

Effect of the Whey Protein on Percentage of hatchability from pupa to adult of *Drosophila melanogaster*.

The % of hatchability from pupa to adult in *D. melanogaster* larvae which were cultured in the

wheat cream agar media, mixed media and Whey protein media was showed in **Figure-4**. According to the obtained data, pupae from mixed media showed greater % of hatchability than wheat cream agar media which had average and whey protein media which had least.

Figure 4: Effect of wheat cream agar diet on % of hatchability from pupa to adult in *Drosophila melanogaster*



The different numbers on the bar graph are indicates the % of hatchability.

Discussion

Food is one of the important factors which can affect the growth, reproduction, development and survival of an organism. Nutrients are essential for all living organisms to meet their energy requirements for growth and proper operation. Nutrition in a diet varies with respect to quality and quantity. Therefore, the present study has undertaken to investigate positive and negative effects of Whey protein on the pre-adult fitness (Rate of development and % of hatchability) in *D. melanogaster*.

Rate of development and percentage of hatchability from egg to adult are the crucial parameters of pre-adult fitness in *Drosophila*. Interaction between genotype, environment, diet and competition with the population results in the pre-adult fitness. The obtained results (**Fig. 1-4**) reveal the significant variation between the larval to pupa and pupa to adult rate of development between diets (wheat cream agar, mixed and whey protein media).

The result (**Figure-1**) reveals that the rate of development from larva to pupa was significantly greater in wheat cream agar media which showed faster growth. Meanwhile larva fed in whey protein media showed an average rate of development which took more time compared to wheat cream agar media to develop into pupa. Further, larva from mixed media showed very slower rate of development which took more time than those of other diets. Larva is the feeding stage of *Drosophila* among its pre-adult stages of development. This suggests that the nutrients in the different diets were responsible for the variations in larva to pupa development. The results from **Figure-2** shows the significant variation of pupae fed with whey protein media which showed faster rate of development by consuming less time to become adult. On the other hand, pupae from wheat cream agar media took more time than those of pupae from whey protein media. Meanwhile, pupae from mixed media took more time by showing slower rate of development than other two diets.

From these two results (**Figure 1& 2**), overall rate of development (larva to pupa and pupa to adult) is faster in Whey protein even though it took average time to moult into pupa, it took least time to eclose into adult. This suggests that nutrition in whey protein media might have affected and altered the physiology which lead to the average rate of development from larva to pupa and might have accelerated the rate of development from pupa to adult. Larvae cultured with wheat cream agar media took shorter time to become pupa but took more time to become adult by showing average overall rate of development from larva to adult. This suggests that the nutrients in diet doesn't enough to speed up or enhance pupa to adult eclosion. Larvae fed with mixed media showed slower rate of development from larva to pupa as well as pupa to adult. In both the cases, nutrition was not sufficient enough to enhance the rate of development. Along with this there are several studies have done to examine pre-adult fitness in *D. melanogaster* by providing different diets. Chhabra *et al.*, (2013) studied on organic fruits and vegetables which had shown positive effect on pre-adult fitness. Similarly, Geetha and Krishna (2015), showed that organic fruits like chikku and watermelon and study by Shreejani *et al.*, (2023), on the spirulina also have positive impact on pre-adult development. In contrast to this, studies on the Avocado and Yoghurt (Alexander and Krishna, 2018), Jeeni millet traditional mix (Kiran and Krishna, 2023), prebiotics (Shresta and Krishna, 2023) and Creatine monohydrate (Mamba and Krishna, 2023) have shown negative effect on pre-adult fitness on *D. melanogaster*. All these shows that pre-adult fitness is dependent on quality and quantity of nutrition in diet.

Two coupled oscillators are thought to regulate the circadian rhythms of adult *Drosophila* eclosion: the temperature- and light-sensitive master clock and the temperature-sensitive slave clock, whose phase is reflected in overt behaviour (Abhilash *et al.*, 2019).

In the present study, larvae and pupae were maintained in laboratory condition with uniform temperature ($22^{\circ}\pm 1^{\circ}\text{C}$) and light (12:12 dark:light). Hence observed variations are due to different nutrition in diets provided.

From the results (**Figure-3& 4**), larvae fed with whey protein media had significant variation by having least percentage of hatchability from larva to pupa and pupa to adult. Meanwhile, mixed media had shown greatest % of hatchability and wheat cream agar media had shown average % of hatchability. This suggests that larvae and pupae fed with mixed media showed greatest % of emergence than other two diets suggesting that nutrition in mixed media i.e., mixture of both carbohydrates and proteins thereby it provides required amount of nutrition and energy which is required for the emergence and eclosion from larva to pupa and pupa to adult. Wheat cream agar media doesn't provide much nutrition which results in slower eclosion % which resulted in average % of hatchability. Meanwhile, larvae and pupae treated with whey protein might have taken longer time to convert the high protein substrates into its metabolites causing least % of hatchability. In *Drosophila*, the rate of development slow down as the temperature increases (Gilbert and De Jong, 2001; Hartwell *et al.*, 2011). Several researches also shown that variation in extrinsic (physical) environment like light and temperature also have impact on variations in % of hatchability. But the present study was conducted by maintaining larvae and pupae in uniform laboratory conditions and by varying diets. There are several studies like energy drinks (D'souza and Krishna, 2015), organically grown fruits like chikku and watermelon (Geetha and Krishna, 2015), Avocado and Yoghurt (Alexander and Krishna, 2018), Jeeni millet traditional mix (Kiran and Krishna, 2023), prebiotics (Shresta and Krishna, 2023) and Creatine monohydrate (Mamba and Krishna, 2023), spirulina (Sheejani *et al.*, 2023) and others have done experiments on *D. melanogaster* by varying the diets and maintaining uniform laboratory condition showing that quality and quantity of nutrition in

diet is responsible for the variation in % of hatchability. Hence, the observed results were not due to physical factors but due to variation in nutrition in different diets.

Conclusion

From this study, we can conclude that the quality and quantity of nutrition available in the diet is responsible for variations in the rate of development and % of hatchability in *D. melanogaster*. Faster rate of development and low % of hatchability can be seen in larvae fed with whey protein. The rate of development from larva to adult follows whey protein > wheat cream agar > mixed. And % of hatchability from larva to adult follows mixed > wheat cream agar > whey protein.

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