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Effect of creatine monohydrate diet supplementation on pre-adult fitness in *Drosophila melanogaster*

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Abstract

Diet is one of the most significant environmental factors influencing an organism's growth, health, ability to reproduce, and ability to survive. Different food types have different composition of nutrient, therefore the current study has been undertaken in *Drosophila melanogaster*. Flies from control and creatine monohydrate-treated medium were used in the current study to explore the effect of creatine monohydrate on pre-adult fitness in *Drosophila melanogaster*. The present study revealed that *D. melanogaster* larva maintained in media supplemented with creatine monohydrate required more time to pupate than larva maintained in wheat agar media (control). It was observed that dietary supplementation with more CrM slowed the rate of development from larva to pupa. It was also discovered that the rate of development from pupa to adult was delayed with increased creatine monohydrate supplementation. Further, percentage hatchability from larva to pupa was high in the control media compared to the creatine monohydrate supplemented media. However, the percentage hatchability from pupa to adult shown no significant different between control and creatine monohydrate supplemented media. Creatine monohydrate delayed the rate of development of *D. melanogaster* from larva to pupa and from pupa to adult. Further, high percentage pupation was recorded in control media than creatine monohydrate treated media but percentage hatchability was high in 5% and 10% creatine monohydrate supplemented media thus showing varied effect in rate development and percentage hatchability in *D. melanogaster*.

Keywords: Creatine monohydrate, Rate of development, hatchability, Larva, Pupa

Introduction

The nutritional impacts brought on by the difference in food type availability are the most obvious way that environmental variation may affect body condition and fertility. Diet can be regarded as a crucial factor that may have an impact on all aspects of a life history because food provides animals with energy and nutrients (Sterner and Schulz, 1998; Taylor *et al*., 2005). The investigation of how organisms modify their use of energy has been greatly aided by the experimental manipulation of animal diets (Chown and Nicolson, 2004; Cruz-Neto, Bozinovic, 2004). The quantity and quality of nutrients that organisms consume have a significant impact on life-history features as illness susceptibility, development, fertility, reproduction, longevity, and stress resistance (Hoffmann and Parsons, 1991; Rion and Kawecki, 2007; Lee *et al.*, 2008). In their natural environment, many species struggle to achieve their supplemental dietary needs for somatic and reproductive growth (Raubenheimer and Simpson, 1999). Body tissues always need a certain amount and ratio of nutrients to grow and function at their best during development (Bauerfeind and Fischer, 2005). Characteristics like growth and reproduction can be affected by a lack or imbalance of fat, carbohydrates, or protein. In Drosophila melanogaster, protein deficit lowers fertility and growth (Wang and Clark, 1995), and in fruit-feeders, protein is frequently a limiting macronutrient (Mattson, 1980; Adams and Gerst, 1991; Hendrichs, 1991; Markow, 1999; Markow, 2001).

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). The organic fruits watermelon and chikku have a significant impact on pre adult fitness, according to past studies on Drosophila species (Geeth and Krishna, 2015). However, their study did not have benefits for D. melanogaster's pre-adult development (Alexander *et al.*, 2018). The avocado and yogurt had demonstrated a favorable influence on larva to pupa, pupal to adult viability, and rate of development in D. melanogaster. Sowza and Krishna (2015) observed that consuming an alternative natural energy drink aided in pre-adult growth when researching the impact of energy drinks on the development of *D. melanogaster*.

Creatine monohydrate (CrM) is a nutritional supplement that enhances muscle performance during brief, high-intensity resistance exercises that rely on the phosphocreatine shuttle for adenosine triphosphate (Hall, 2013). It is a popular ergogenic aid among sports populations. Additionally, the body of research demonstrating the therapeutic advantages of creatine supplementation for a wide range of clinical uses in both adults and children keeps expanding (Jagim and Kerksick, 2021). Creatine monohydrate supplement use has suddenly increased in the present since it is widely asserted that combining it with intense resistance exercise enhances physical performance, lean body mass, and muscle morphology (Volker et al., 1999). Despite the substantial research demonstrating the effectiveness and safety of creatine supplements. It is yet unclear how creatine monohydrate supplement might impact rate of development since most studies mainly concentrate on muscle formation and sports performance. The purpose of this study is to find the effect of CrM on the rate of development by using *D. melanogaster* as a model organism.

Materials and Methods

Experimental stock

The experimental stock was created using *D. melanogaster* strain Oregon K, which was obtained from the Drosophila stock center, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysuru. Wheat cream agar media (100g of jaggery, 100g of wheat flour, 10g of Agar, and 1000ml of boiled distilled water) was utilized to culture the flies. 7.5 ml of propionic acid was added to the media to prevent fungal growth. The culture bottles were kept in a laboratory conditions under 12:12 photoperiod (dark and light cycles) conditions with a humidity of 70% and temperature of 22ºC ± 1ºC.

Control flies were cultured on wheat cream Agar-agar media. The wheat cream Agar-agar media was added with varied amounts of Creatine monohydrate (2.5%, 5%, and 10%) to provide the basis of the experimental diets. The Creatine monohydrate supplement used in the study was purchased India from Synergy Supplement Store in Mysore, Karnataka, India. Twenty flies (10 males and 10 females) were transferred separately to culture bottles containing wheat cream agar media and the creatine monohydrate supplemented media. The culture bottles were kept in lab conditions as previously indicated. For this experiment, flies collected from the culture bottles were utilized.

Effect of creatine monohydrate on rate of development of *D. melanogaster*

Twenty flies (10 males and 10 females) were transferred separately to the wheat cream agar media and the media that had been treated with different concentration of creatine monohydrate. The flies were allowed to breed and lay eggs for a period of 24 hours and then there were removed from the culture bottles. 1st instar larvae were collected from the culture bottles by scooping out the media. From the control diet and the experimental media, ten (10) 1st instar larva were transferred to vial consisting same diet while keeping 5 replicates for each diet (control, 2.5%, 5% and 10%). The time of transference of larvae was noted and developmental phases of each larvae were monitored. Stage of development larvae was checked regularly. Every stage of each larva was timed (1st instar to pupa, pupa to adult) for the estimation of the rate of development.

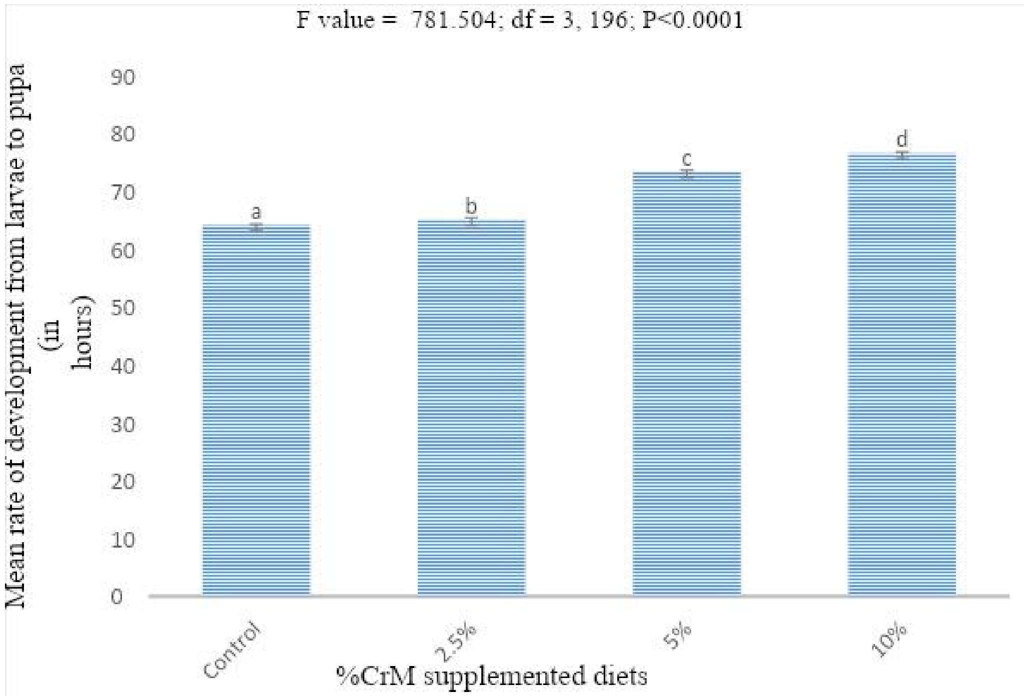
Results and Discussion

Effect of creatine monohydrate on rate development of *D. melanogaster* from larvae to pupa

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. Consequently, the current study has been undertaken to determine how CrM affects the rate of development of *D. melanogaster*. **Fig. 1** represent the mean ± standard error values of the rate of development from 1st instar larva to pupa of flies maintained in wheat-cream agar as well as flies maintained in respective creatine monohydrate supplemented media. It was observed that the larva maintained on 10% CrM treated media become pupa later when compared to larva maintained on 5% and 2.5% creatine monohydrate supplemented media. Larva maintained in wheat-cream agar media (control) took the least mean time to become pupa compared to larva maintained in the respective creatine monohydrate supplemented media. It was further discovered that rate of development from larva to pupa decreased with increased supplementation of CrM in the diet. One-way ANOVA analysis of the data, followed by the Tukey's post hoc test, revealed a significant variation in the rate of development from larva to pupa between control and CrM-treated media.

In *Drosophila*, the larva is the feeding stage among the pre adult stages of growth. Therefore, the observed results means that the quality, quantity of nutrients available and its allocation in the creatine monohydrate supplemented diet were less efficient in promoting the rate of development from larva to pupa when compared to the control diet.

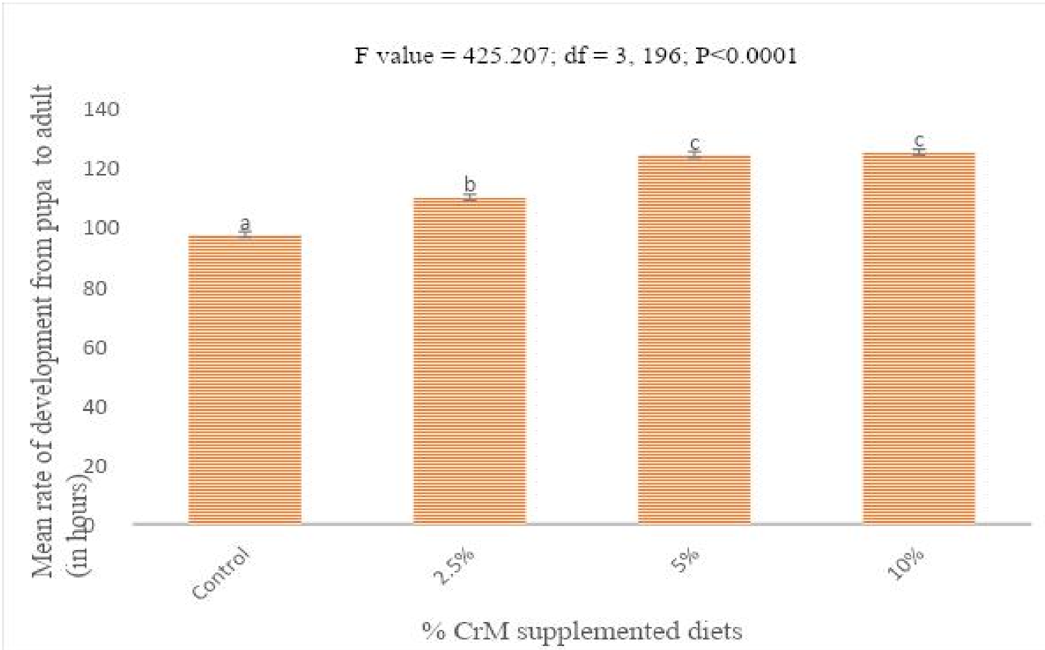
Figure 1: Effect of CrM on the rate development of *Drosophila melanogaster* from larvae to pupa. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.



Effect of creatine monohydrate on the rate development of *D. melanogaster* from pupa to adult

All living things must have nutrients in order to meet their energy needs for growth and proper operation. The mean ± standard error values of the rate of development from pupa to adult of flies maintained in wheat-cream agar as well as flies maintained in the respective creatine monohydrate enriched medium are shown in **Fig. 2**. When compared to pupa maintained on 2.5% CrM supplemented media and the control, it was shown that the development rates of pupa maintained on 5% and 10% CrM treated media were nonsignificant, and they both took longer to become adults. When compared to pupa maintained in media enriched with creatine monohydrate, pupa maintained in control media developed pupa in the shortest amount of time on average. Further research revealed that dietary CrM supplementation led to a slower rate of pupa to adult transition. One-way ANOVA analysis of the data, followed by the Tukey's post hoc test, revealed a significant variation in the rate of development from pupa to adult between control and CrM-treated media however pupa from 5% and 10% CrM treated media were grouped together showing no significant variation. The circadian rhythms of adult *Drosophila* eclosion are hypothesized to be controlled by two coupled oscillators: the master clock, which is temperature- and light-sensitive, and the slave clock, whose period is temperature-sensitive and whose phase is reflected in the overt behavior (Abhilash, 2019). In the present study, the flies were subjected to similar temperature and light conditions hence the observed differences in the rate of development from pupa to adult can be attributed to the effect of CrM. During pupa stage, *D. melanogaster* do not feed hence development relies on nutrient accumulated during larval stage which is a feeding stage thus pupa to adult development had a similar trend of development rate as the larva to pupa phase.

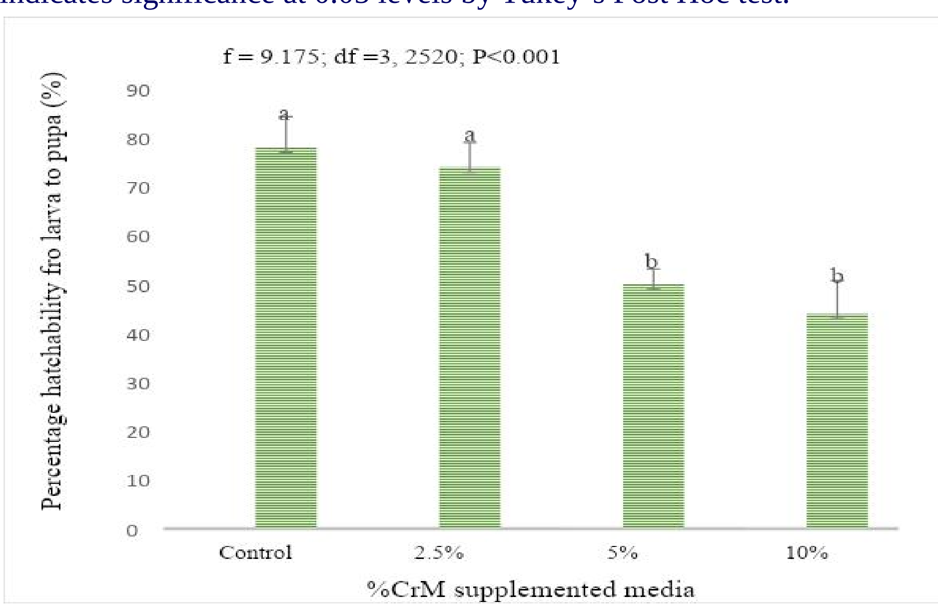
Figure 2: Effect of CrM on the rate development of *D. melanogaster* from pupa to adult. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.



Effect of creatine monohydrate on percentage hatchability from larvae to pupa of *D. melanogaster.*

Our data revealed that larva maintained on control media had higher percentage hatchability compared to larva raised on different concentration of CrM treated media. Larva to pupa hatchability decreased with increased CrM supplementation. **Fig 3**. presents the effect of creatine monohydrate on percentage hatchability from larvae to pupa of *D. melanogaster*. One-way ANOVA analysis of the data, followed by the Tukey's post hoc test, revealed a significant variation in the percentage hatchability from larva to pupa between control and CrM-treated media.

Figure 3: Effect of CrM on percentage hatchability of *D. melanogaster* from larva to pupa. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

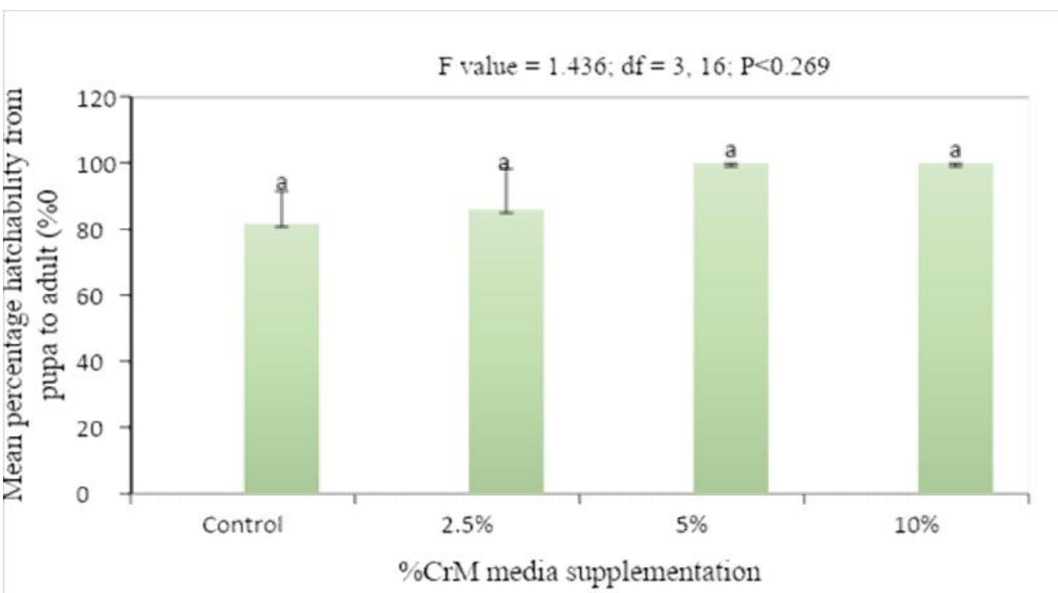


The low percentage hatchability observed from larva maintained in CrM treated media can be attributed to poor growth of the larva resulting from the quality and quantity of nutrients and its allocation for development. Previous research revealed that the amount and kind of nutrients consumed in the diet affected the pre-adult fitness of the *D. melanogaster*. D'Souza and Krishna (2015) discovered that alternative natural drinks were advantageous in pre-adult fitness when compared to synthetic juice and this was due to the difference in nutrient composition.

Effect of creatine monohydrate on percentage hatchability of *D. melanogaster* from pupa to adult.

In present study, it was discovered all the larva the developed into pupa developed into adult in 5% and 10% CrM supplemented media marking a hundred percent (100%) hatchability from pupa to adult. However some of the pupa did not develop into adult in control media and 2.5% CrM tread media with control recording lower percentage hatchability. One-way ANOVA analysis of the data, followed by the Tukey's post hoc test, revealed no variation in the percentage hatchability from pupa to adult between control and CrM-treated media. The effect of creatine monohydrate on percentage hatchability of *D. melanogaster* from pupa to adult is shown in **Fig. 4.** The results demonstrated that CrM could not effect variation of pupa to adult hatchability in *D. melanogaster.*

Figure 4: The effect of creatine monohydrate on percentage hatchability of *D. melanogaster* from pupa to adult



Conclusion

D. melanogaster's rate of development from larva to pupa and from pupa to adult was slowed down by creatine monohydrate. Additionally, higher percentages of pupation were observed in control media than in media treated with creatine monohydrate, however, the percentage hatchability of pupa to adult was insignificant.

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