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CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 14, Issue, 08 (A), pp. 4051-4054, August, 2023

ISSN: 0976-3031

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

INCREASED CREATINE MONOHYDRATE DIET SUPPLEMENTATION REDUCE REPRODUCTIVE FITNESS IN *DROSOPHILA MELANOGASTER*

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DOI: http://dx.doi.org/10.24327/ijrsr.2023.1408.0763

ARTICLE INFO

Article History:

Received 6th May, 2023
Received in revised form 15th June, 2023
Accepted 12th July, 2023
Published online 28th August, 2023

Keywords:

Drosophila Melanogaster

ABSTRACT

The ability of a species to pass on its genes to future generations determines its survival. This study was undertaken to ascertain the effect of creatine monohydrate on the reproductive (fitness mating latency, copulation duration and fertility) of *D. melanogaster.* In the current study, we discovered that mating latency increased with increasing CrM concentrations in the media (2.5%, 5%, and 10%) and was lowest in flies maintained on control media versus flies maintained on creatine monohydrate treated media. The copulation data revealed that flies fed control media had a lower copulation duration than flies fed CrM supplemented media, and the mean copulation time rose as CrM concentration increased. Furthermore, fertility data demonstrated that flies fed control media produced a greater number of progeny than flies fed creatine monohydrate-treated media. With increasing CrM dosage, overall progeny production dropped (5% and 10%). As a result, the control mated faster, copulated shorter, and produced a greater number of progeny, whereas mating latency, copulation duration, and fertility of flies kept on 2.5% CrM enriched medium were not significantly different from the control. This means that 2.5% CrM supplementation in media had no influence on *D. melanogaster* reproductive fitness. Flies kept on 5% and 10% CrM supplemented medium had significantly reduced fertility but significantly higher mating latency and copulation length. It can be concluded that more 2.5% CrM supplementation on diet of D. melanogaster result in reduced reproductive fitness.

INTRODUCTION

The most evident way that environmental variation might affect physical health and reproductive performance is through nutritional impacts caused by changes in food type and availability. Dietary effect can be characterized as quantitative (i.e. food availability) or qualitative (i.e. food composition) in general. The quantity and quality of nutrients consumed by organisms have a significant influence on life-history features such as disease sensitivity, fertility, reproduction, longevity, and stress resistance [9, 13, 20].

Nutritional impact studies frequently test the physiological and morphological reactions of individuals exposed to varying quality and amount of foods. A wide range of male characteristics are known to influence female mating decisions. One male trait that has gotten a lot of attention as a possible cue is reproductive fitness. Unstable settings can result in variable mating preferences, hence it is critical to understand how constantly changing environmental factors, such as nutrition availability, influence mate choice [16]. Animal fitness is affected by environmental factors such as nutrition, and nutrient availability has been demonstrated to influence sexual selection and mate choice [10, 12, 29]. The optimal macronutrient ratio varies by sex and species, but an ideal diet enhances lifelong fertility [13, 15, 19, 25]. If unbalanced diets reduce reproduction, we can predict that healthy animals will find these mates.

D. melanogaster consumes rotting fruit colonised by yeast in the wild [5]. Fruit macronutrient composition varies according to genetics, location, and season, and because yeast carries protein and fats, the level of colonisation will also contribute to nutritional variety in a natural landscape. Due to the dispersion ability of adult *D. melanogaster* [6], sexually mature adults that have evolved on substrates of different quality are likely to gather and mate on new food sources. Condition-dependent discriminating of mates of varied quality may be relevant in this circumstance for maximising fitness. Creatine (Cr), a natural substance in the human body, is synthesized both endogenously and exogenously from food, particularly meat

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and fish. It is a nitrogenous organic acid that is synthesized endogenously, primarily in the liver, from glycine, arginine, and methionine. Oral creatine monohydrate ingestion has been shown to raise plasma Creatine concentrations (Munoz *et al*., 2018) and total creatine concentrations [7], which is mostly stored as creatine phosphate (CrP) inside skeletal muscle. It is essential for delivering fast energy during muscle contraction by transferring the N-phosphoryl group from phosphorylcreatine (PCr) to ADP in a reversible mechanism catalyzed by phosphorylcreatine kinase (PCK) [8]. Creatine's primary job is to transmit energy from mitochondria to cytosol.

There has been a significant growth in the number of people who are interested or obsessed with sport performance, body building, or physical fitness. Many people have been enticed to use oral dietary supplements to improve their performance or exercise potential as a result of this. Creatine monohydrate is one of the oral dietary supplements that are flying off the shelves of supplement stores these days. With the increased use of supplements, there is an urgent need for a better knowledge of these supplements, notably creatine monohydrate, and its effect on aspects other than the desired physical fitness. Therefore the present study has been conducted to ascertain the effect of creatine monohydrate in reproductive fitness (mating latency, copulation duration and fertility) of *D. melanogaster*.

MATERIALS AND METHODS

Establishment stocks

Oregon-K strain of *D. melanogaster* flies were obtained from the Drosophila Stock Centre, Department of Zoology in the University of Mysore, Karnataka, India. These flies were bred for two generations to establish the experimental stock. The flies were kept on culture bottles containing wheat cream agar media. The culture bottles were kept in the laboratory condition with temperature around 22^0C ± 1^0C degrees Celsius and a relative humidity of 70%.

Wheat cream Agar-agar media was utilized to culture control flies. The experimental diets were based on CrM which is supplemented at different percentages (2.5%, 5% and 10%) to the wheat cream Agar-agar media. The CrM supplement was procured from Synergy Supplement Store, Mysore, Karnataka, India. Twenty flies were transferred separately to of wheat cream agar media and creatine monohydrate treated media. The treated flies were maintained in laboratory condition as mentioned above. The flies obtained from the culture bottles were used for the reproductive fitness experiment.

Effect of creatine monohydrate on reproductive fitness

Virgin females and unmated male flies from control and creatine monohydrate treated media were isolated within 3 hours of their eclosion and were maintained in the laboratory condition as mentioned above until they attained sexual maturity. When the flies were aged 5-6 days, they were gentle aspirated individually (one male and one female) into mating chamber and observed for 1 hour (7am to 8am). If mating doesn't occur within an hour, pair was discarded. The pair that mated had their mating latency (times elapsed between introduction of male and female into the mating chamber until initiation of copulation) were recorded. These mated pairs were placed into their respective media and these pairs were changed into new vial of same media once in week. Total number of progeny produced by each recorded as fertility. A total of twenty trials were made separately for each of control and creatine monohydrate treated flies.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software version 20 was used for analysis of statistical data. Analysis of variance (ANOVAs) followed by Tukey's post hoc test was used on mating latency, copulation duration and fertility data.

RESULTS

Fig. 1 represent the mean values of mating latency in wheat-cream agar as well as flies maintained in creatine monohydrate based media. It was observed that the flies maintained on 10% creatine monohydrate based media had higher mean mating latency when compared to both those of maintained on 5% and 2.5% creatine monohydrate based media. Flies maintained in wheat-cream agar media (control) showed lowest mean mating latency and it was not significantly different to that of flies maintained on 2.5% creatine monohydrate supplemented media. One way ANOVA followed by Tukey's post hoc test variation between the creatine monohydrate based media used. Tukey's Post Hoc Test showed significant variation (Fig. 1).

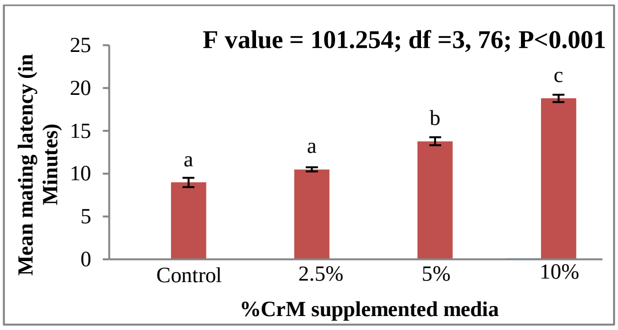

Figure 1 Effect of creatine monohydrate on mating latency of *D.melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

Copulation duration of flies reared in wheat-cream agar as well as creatine monohydrate based media is represented in **Fig. 2**. It was observed the mean copulation duration of flies maintained on 5% creatine monohydrate and those maintained 10% creatine monohydrate were not significantly different and they both had higher mean copulation duration compared to flies reared on 2.5% creatine monohydrate. Flies maintained on control media showed shortest copulation duration than those maintained on creatine monohydrate supplemented media. Data subjected to one way ANOVA followed by Tukey's post hoc test showed significant variation in copulation duration between control and CrM treated flies.

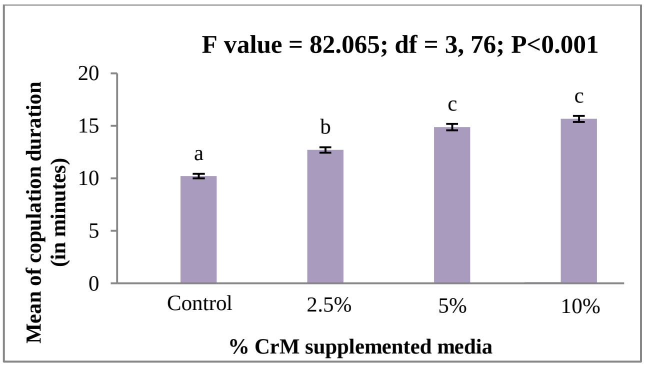

Figure 2 Effect of creatine monohydrate on copulation duration of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

The results of progeny production of flies maintained on control and those maintained on respective concentration of CrM are provided in Fig. 3. Results obtained showed that the mean number of progeny produced by flies that were maintained on 5% CrM supplemented diet and 10% CrM supplemented diet were not significantly different. The mean progenies of 5% CrM supplemented diet and 10% CrM supplemented diet were observed to be lower than the mean progenies of both flies maintained on 2.5% CrM supplemented diet and the control diet. There was no significant difference in the mean number of progenies of flies maintained on 2.5% CrM supplemented diet and flies maintained on control diet. One way ANOVA followed Tukey's post hoc test showed significant variation in progeny production between control and CrM treated flies. However, there was no variation between flies fed control media and flies fed 2.5% CrM supplemented media.

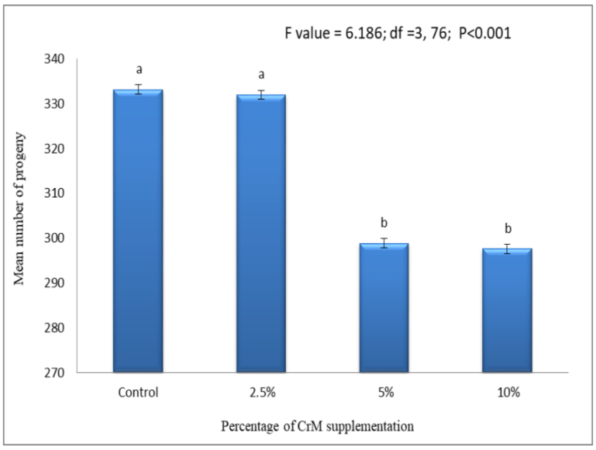

Figure 3 Effect of creatine monohydrate (CrM) on fertility of *D. melanogaster*. Different letters on the bar graphs indicates significant variation at 0.05 levels by Tukey's Post Hoc test.

DISCUSSION

Nutrition is necessary for an organism's growth, development, reproduction, ability to withstand starvation, and overall survival. The organism's ability to reproduce will, however, depend on the food's quality and quantity [24]. In the present study, the effect of creatine monohydrate on reproductive fitness such as mating latency, copulation duration and fertility has been studied on *Drosophila melanogaster*. In species of *Drosophila*, courtship behavior involves series of male and female activities which culminates in copulation duration [26]. Further, during copulation duration mating males transfers accessory gland proteins to the mated females [21]. This materials helps in post physiological changes in mated females and they are also responsible for production of eggs and fertile offspring.

In the present study, we found that mating latency increased with increasing CrM concentration (2.5%, 5% and 10%) in the media and was lowest in flies maintained on control media compared to flies maintained on creatine monohydrate treated media (Figure 1). The copulation data showed that flies fed control media had a shorter copulation duration compared to flies maintained on CrM supplemented media and the mean copulation duration increased with increased CrM concentration (Figure 2). Further, the fertility (Figure 3) revealed that control media fed flies produced high number of progeny compared to flies maintained on creatine monohydrate treated media. The overall progeny production decreased with increased CrM supplementation. Therefore, control flies mated faster, copulated shorter and produced high number of progeny whereas among the CrM treated media flies, mating latency, copulation duration and fertility of flies maintained 2.5% CrM supplemented media was not significantly different to the control. Meaning ±2.5% CrM supplementation in media has no effect on the reproductive fitness of *D. melanogaster*. On the other hand, flies maintained on 5% and 10% CrM supplemented media recorded significantly lower fertility yet they showed significantly high mating latency and copulation duration. The observed relation between copulation duration and fertility was consistent with the findings of Sears *et al*[23] who discovered that sperm transmission occurs relatively early during copulations hence even with short copulation duration control media flies produced greater progeny. The observed variation in mating latency, copulation duration and fertility between control media flies and CrM treated media was due to the variation in quantity and quality or availability of nutrients. This supports earlier studies of dietary effect on mating behavior and reproductive fitness on species *Drosophila* [11, 1]. They also found that variation in protein to carbohydrates ratio affects reproductive parameters such as mating latency, copulation duration and fertility. Further they found that flies fed with protein rich diet mated faster, copulated longer and produced greater number of progenies. These studies suggest that animal fitness depend upon condition and can be influenced by environmental factors such as diet, and nutrient availability in the food.

According to Arnqvist and Rowe [2] copulation duration is a shared reproductive feature that develops as a result of the interaction between males and females. Further, Bretman *et al* [4] *suggested* that males exert control over the duration of extended matings in response to the potential level of sperm competition but the inputs from females also play an important role in the duration of mating itself. Prolonged mating period in *D. melanogaster* is linked to significant fitness benefits for males (i.e. increased paternity in both competitive and non-competitive contexts), which are at least partially mediated by the transfer of higher seminal fluid protein concentrations [4, 28]. Our data can be explained as follows; the flies maintained control media with its faster mating, latency and short copulation duration produced high progeny size because of their ability to transfer high quantity of sperm and accessory gland proteins within a short duration compared to the flies maintained on CrM treated media. Male flies fed on CrM supplemented media needed more time to transfer sperm and accessory gland proteins to the females hence the observed increase in mating latency and copulation duration while showing less fertility. From all this studies it was found out that creatine monohydrate decreases reproductive fitness in *D. melanogaster* thereby it showed detrimental effect on reproductive fitness.

Acknowledgements

The authors extend their gratitude to the Chairman, Department of studies in Zoology, National Drosophila Stock Center, University of Mysore, Manasagangotri campus for providing equipment to enable efficient completion of the study. Much appreciation to all lecturers and support staff in the Zoology department.

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How to cite this article:

Sithembiso Sebastian Mamba and Mysore Siddaiah Krishna.2023, Increased Creatine Monohydrate Diet Supplementation Reduce Reproductive Fitness in Drosophila Melanogaster. *Int J Recent Sci Res.* 14(08), pp. 4051-4054.

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