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RESEARCH ARTICLE

EFFECT OF CREATINE MONOHYDRATE SUPPLEMENTED MEDIA ON SEX RATIO IN *DROSOPHILA MELANOGASTER.*

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ABSTRACT

Dietary composition is one of the major experimental factors that affects many aspects of animal physiology including life span, general health, reproductive fitness and progeny sex ratio hence the present study has been undertaken in *Drosophila melanogaster* to investigate the effect of creatine monohydrate supplemented media on sex ratio. In the present study, the results showed that flies fed creatine monohydrate treated media produce greater number of female offspring than male offspring while control media produced almost equivalent number of female and male progenies. It was observed that the progenies of flies maintained on 2.5% creatine monohydrate supplemented media had a sex ratio similar to that of control. Thus this study suggests that the increased diet supplementation with creatine monohydrate increases the number of female offspring in *Drosophila melanogaster*.

INTRODUCTION

In many species, when the sexes are separated, about the same number of females as males emerge (Hardy, 2002). As a result, many creatures, including insects, have a 1:1 sex ratio. By increasing the availability of males in comparison to females this ratio optimizes genetic variation (Schowalter, 2016). The sex ratio is a topic that is frequently explored in biological study. According to Skalski *et al*. (2005) and Bailey (2004), the word "sex ratio" itself typically denotes the ratio of males to females in a population. Males to women ratios are frequently depicted using the ratio of males to women (or vice versa) or as a proportion of the population (Skalski *et al*., 2005; Qazi and Qazi, 2006; Prakash, 2008). What causes these changes, however, and the underlying mechanisms are still completely unclear. Insects, reptiles, and birds have all shown altered sex ratios as a result of changes in food availability and other environmental factors (Pienaar and Greeff, 2003; Thuman *et al*., 2003; Freedberg and Wade, 2001; Komdeur *et al*., 2002; Dyson and Hurst, 2004). A population's ability for reproduction is indicated by the ratio's female representation (Schowalter, 2016). The sex ratio also conveys information about a population's past, present, and future, as well as the importance of the sexual mating system (Skalski *et al*., 2005; Schowalter, 2016).

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Following that, a variety of environmental factors have an impact on sex ratios (Schowalter, 2016; Hardy, 2002; Rosenfeld and Roberts, 2004). Environmental sex ratios can be affected by physical, chemical, or biological factors (Skalski, 2005; Schowalter, 2016; Hardy, 2002; Rosenfeld and Roberts, 2004; Wajnberg, 2008). *Drosophila* is a genus of little flies (approximately 3mm long) in the Drosophilidae family, whose individuals are sometimes referred to as "fruit flies." *Drosophila melanogaster*, in particular, has been extensively utilized in genetics research and is a frequent model organism in developmental biology. It is the perfect creature for research studies for a number of reasons: 1) Fruit flies are space-efficient, resilient insects with basic dietary requirements. 2) At room temperature, the reproductive cycle is finished in about 12 days, making test crosses easy to analyze. 3) Fruit flies have a lot of offspring, which makes it possible to gather enough information (Demerec and Kaufman, 1996; Stephenson and Metcalfe, 2013). The flies can be rapidly and readily immobilized for examination, making it simple to collect data and conduct examinations. Creatine monohydrate (CrM) is a dietary supplement that enhances muscle performance during quick, intense resistance exercises that utilize the phosphocreatine shuttle to transport adenosine triphosphate (Hall, 2013). It is a popular ergogenic aid among athletes. Furthermore, a growing body of evidence (Jagim and Kerksick, 2021) supports the therapeutic advantages of creatine supplementation for a range of clinical applications in both adults and children. Since it is widely believed that combining rigorous resistance exercise with creatine monohydrate

supplements improves physical performance, lean body composition, and muscle morphology, the use of these supplements has abruptly expanded in the modern era (Volker *et al*., 1999). Despite the extensive evidence showing that creatine pills are safe and effective. Since the majority of research primarily focus on muscle development and athletic performance, it is yet unknown how a creatine monohydrate supplement may affect the sex ratio. The current study has been conducted to ascertain the effect of creatine monohydrate supplemented diet on sex ratio in *Drosophila melanogaster.*

MATERIALS AND METHODS

Establishment of stocks: The *Drosophila* Stock Centre at the Department of Zoology at the University of Mysore, Karnataka, India, provided the Oregon-K strain of *D. melanogaster* flies. To create the experimental stock, these flies were bred for two generations. The flies were maintained on culture bottles that contained wheat cream agar media. The culture bottles were kept in a laboratory environment with a relative humidity of 70% and a temperature of 22 ^{0}C ± 1 ^{0}C (degrees Celsius). Wheat cream agar media was used to culture the flies that were used as control. The wheat cream Agar-agar media was added with creatine monohydrate at varied percentages (2.5%, 5%, and 10%) for the experimental diets. The supplement of creatine monohydrate was purchased in Mysore, Karnataka, India, at Synergy supplements Store. Twenty flies were divided up and placed in culture vials containing wheat cream agar media and media that had been treated with creatine monohydrate. The cultured flies were kept under laboratory conditions as previously indicated. For this experiment, flies collected from culture bottles were utilized.

Effect of creatine monohydrate supplemented diet on sex ratio in *Drosophila melanogaster:* Unmated male and virgin female flies were separated from the control and creatine monohydrate treated medium within 3 hours after eclosion. These flies were put into mating chamber in pairs and allowed to mate before being moved to a vial containing their respective medium. A pair that did not mate within an hour was discarded. Twenty pairs in all were kept independently for the control and the respective creatine monohydrate treated medium. The mated flies (pairs) were transferred into freshly prepared media once every seven days. Note the males and females that emerged from each vial of the food during the course of the seven-day experiment, which continued until the flies died.

RESULTS AND DISCUSSION

The results revealed that there is no significant difference in the number of female and male offspring produced by flies maintained on control diet and those maintained on 2.5% creatine monohydrate supplemented diet. However, more female offspring than male offspring were observed in 5% and 10% creatine monohydrate supplemented diet. Fig 1 shows the comparison between the male and female adult flies' numbers in *D. melanogaster* raised on the different diets [Control diet- wheat cream agar media; creatine monohydrate supplemented diet (2.5%, 5%, 10% concentration)]. Sex ratios of *Drosophila melanogaster* adult flies that emerged from the control and Creatine monohydrate treated media (2.5%, 5%, 10% concentration) and displayed in table 1 and table 2 respectively.

Proportion of female and male as well as male and female were compared with the expected 1:1 (F:M and M:F) by using Chi-squared test. Table 3 shows the proportion of female and male ratios when compared with the expected 1:1 (F:M) by using Chi-squared test.

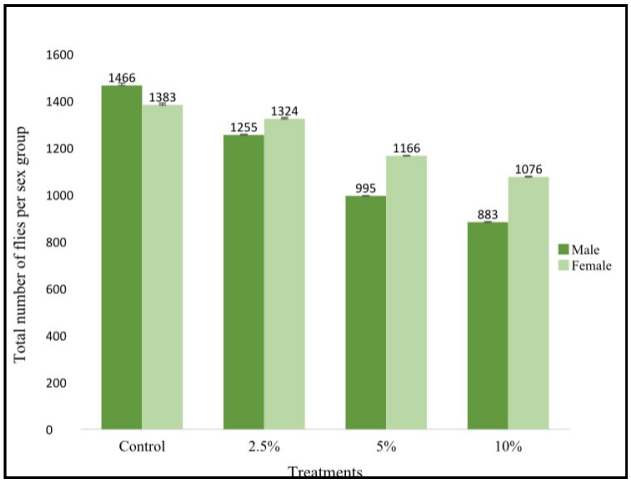

Fig 1: Comparison between the number of male and female progenies of *D. melanogaster* reared on the different diets [Control diet- wheat cream agar media; creatine monohydrate supplemented diet (2.5%, 5%, 10% concentration).

Table 1: Sex ratio of *D. melanogaster* progenies emerged from the control and Creatine monohydrate treated media (2.5%, 5%, 10% concentration). Proportion of female and male were compared with the expected 1:1 (F:M) by using Chi-squared test.

Treatment	No. adults	No. females	No. males	F:M ratio
Control 2.5%	2849	1383	1466	1:1.06
5%	2579	1324	1255	1:0.94
10%	2161	1166	995	1:0.81
	1959	1076	883	1:0.82

Table 2: Sex ratio of *D. melanogaster* progenies emerged from the control and Creatine monohydrate treated media (2.5%, 5%, 10% concentration).

Proportion of male and female were compared with the expected 1:1 (M:F) by using Chi-squared test.

Treatment	Total off spring	No. females	No. males	M:F ratio
Control 2.5%	2849	1383	1466	1 :0.94
5%	2579	1324	1255	1 :1.05
10%	2161	1166	995	1 :1.17
	1959	1076	883	1 :1.21

Table 3: Sex ratio of *D. melanogaster* progenies from control media and Creatine monohydrate treated media (2.5%, 5%, 10% concentration). Proportion of female and male ratios were compared with the expected 1:1 (F:M) by using Chi-squared test.

Treatment	Sex ratio F: M	Chi square value	Significant level
Control 2.5%	0.94:1.06	0.0072	P<0.05
5%	1.05:0.94	0.0061	P<0.05
10%	1.17:0.81	0.0613	P>0.05
	1.21:0.82	0.0765	P>0.05

The physical elements of temperature and photoperiod have an impact on the sex ratio of insects, especially *Drosophila melanogaster* (Sharkey and Eppley, 2006). Insect sex ratios are influenced by temperature in a variety of ways. Higher temperatures in some species result in more females whereas lower temperatures result in more males (Sharkey and Eppley, 2006). The phrase "temperature-dependent sex determination" (TSD) applies to this. Sharkey and Eppley (2006) further divulged that by affecting the survival rate of men and females, temperature indirectly affects the sex ratio in other species. For instance, greater temperatures result in more males towards the end of development in Drosophila melanogaster, but more females at the start. This is because the expression of genes involved in determining sex is influenced by temperature (Sharkey and Eppley, 2006). However, in the current study, the flies were maintained under laboratory conditions hence temperature had no effect on the observed sex ratio thus the obtained result can only be attributed to the effect of creatine monohydrate and its varying concentrations. Since the results of the current investigation showed that the control media and 2.5% CrM treated media produced about equal numbers of male and female offspring, whereas the 5% and 10% creatine monohydrate treated media produced more female offspring than male offspring (fig. 1). This shows that the variation in the sex ratio of the offspring was influenced by the type and amount of the meal. Numerous studies have shown that mother reproductive output and sex ratio are influenced by quality and quantity in organisms. According to Yazgan (1972), the number of *Pimple turionella* offspring increases as the quantity of amino acids in the diet increases. Parents of flies encourage the development of a certain sex in their progeny because it increases the species' chances of surviving by boosting reproduction or lowering competition for resources and mates. The mother's health can occasionally influence the sex of the offspring in addition to these other factors (Trivers and Willard, 1973). Maternal nutrition had a significant impact on the sex ratios of the offspring. In general, females fed the high-quality food created sex ratios that were biased toward women, while those fed the low-quality diet produced sex ratios that were slanted toward men. According to Wiebe and Bortolotti (1992), *Falco sparverius* produces large numbers of female progeny whenever food resources are abundant leading to the diversity in the *Pimple turionellae*'s sex ratio (Mustafa *et al*., 2005). Additionally, a number of studies have shown that insects, including *D. melanogaster*, vary in their sex ratio.

In the present study, it was discovered that one of the major factors affecting the sex ratio of the organisms is diet. In comparison to the control media, the creatine monohydrate-treated media boosts production of female progeny**.**

CONCLUSION

According to the study, *D. melanogaster* reared on media supplemented with increased amount (more than 2.5%) of creatine monohydrate increase female progeny production, indicating that creatine monohydrate has an effect on the sex ratio in this species.

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