



(RESEARCH ARTICLE)



## The effect of the whey protein supplement on the sex ratio in *Drosophila melanogaster*

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### Abstract

Dietary composition is one of the important external environmental factors that affects many aspects of animal physiology, including growth and development, stress resistance, survivability, reproductive fitness and also alters the sex ratio. The present study aims to investigate the impact of whey protein on the sex ratio in *Drosophila melanogaster* flies by culturing the flies in wheat cream agar, whey protein treated media, and mixed media. The results showed that the female offspring are produced more than the male offspring in all three diets. The mixed diet produced more female offspring compared to the other two diets. There is a much greater difference in sex ratio observed in wheat cream agar media compared to treated and mixed media, where an average difference is observed. Thus, suggests that the whey protein increases the female offsprings suggesting nutritional dietary components in the food alters offspring sex ratio in *D. melanogaster*.

**Keywords:** Nutrition; *Drosophila melanogaster*; Whey protein; Sex ratio

### 1. Introduction

The term "sex ratio" itself frequently implies the proportion of male to females in a population (Skalski, 2005; Bailey, 2004). Animal sex ratios are known to be impacted by a variety of causes, including environmental and genetic influences. The study of sex ratio in insects has been one of the important topics in evolutionary biology (Leigh *et al.*, 1985). In many organisms with the sexes are separate, females are produced in approximately equal numbers with males (Hardy, 2002). Therefore, in many organisms, such as insects, the sex ratio is 1:1 (Prakahs, 2008) and this 1:1 sex ratio generally indicates stabilising selection on males and females (Schowalter, 1996). This ratio maximizes the availability of males to females, and hence maximizes genetic heterogeneity (Schowalter, 2016). The proportion of females indicates reproductive potential of a population. Modification of offspring sex ratios in response to parental quality is predicted when the long-term fitness returns of male and female offsprings differ. However, several environmental factors ultimately affect sex ratios. The environmental factors affecting sex ratio can from physical, chemical, or biological factors (Schowalter, 2016; Hardy, 2002). Many species exhibit multiple changes in physiology, behaviour, and gene expression following mating. Responses to mating are predicted to be sex specific, and these differences can be attributed to the divergent reproductive roles of the sexes. For example, mating induces significant changes in female insects including: elevated egg-laying rate, increased food consumption, reduced immunity, and reduced receptivity to courting males (Chapman *et al.*, 1995; Liu & Kubli, 2003; Rolff & Siva-Jothy, 2002).

However, it is still largely unknown what factors and underlying mechanisms cause these alterations. A population's ability for reproduction is indicated by the ratio's female gender composition (Schowalter, 2016). The significance of the sexual mating system and other information related to the past, present, and future of a population are also reflected in the sex ratio, along with other life history qualities (Skalski *et al.*, 2005). The organism was chosen in this study was

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*D. melanogaster* because it regularly produces a large number of progenies and it has a brief life cycle (Neethu *et al.*, 2014).

Whey protein use a high-quality protein powder from cow's milk. Milk has two proteins: casein (approximately 80%) and whey protein (approximately 20%). It contains less than 1% proteins comprising mainly  $\beta$ -lactoglobulin ( $\beta$ -LG), immunoglobulins,  $\alpha$ -lactalbumin ( $\alpha$ -LA), bovine serum albumin (BSA), and protease peptone, as well as several minor proteins (lactollin, glycoproteins, lactoperoxidases, lactoferrin, and transferrin). Whey protein is widely used to boost muscle protein synthesis and to build lean muscle mass. Muscle Asylum Premium Whey protein contains 24g protein, 5.2g BCAAs, low carbohydrate with no sugar. It also contains digestive enzymes and flavour (banana) [for 40g]. These studies show the effect of whey protein to health but there is no evidence documented about how it affects sex ratio. Therefore, the study under taken to address the effects of the Whey protein on the sex ratio in *D. melanogaster*

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## 2. Materials and methods

The whey protein powder was obtained from Amazon app online.

### 2.1. Establishment of stock

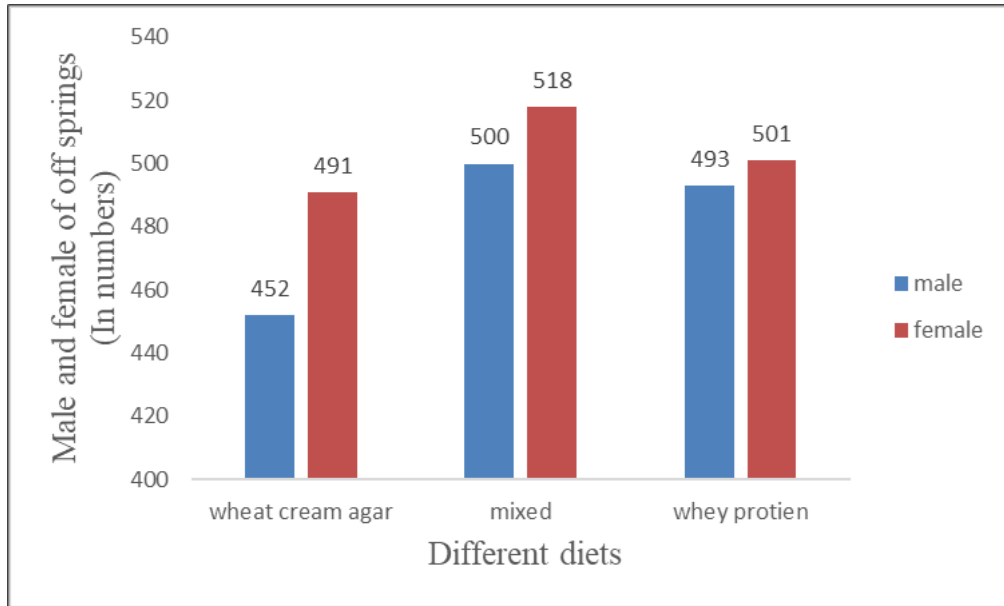
*D. melanogaster* of Oregon K strain used in the study was obtained from *Drosophila* stock centre, Department of Zoology, University of Mysore, Mysore, and this stock was cultured in bottles containing wheat cream agar median (100g jaggery, 100g wheat powder, 10g agar was heated in 1000ml distilled water and 7.5ml of propionic acid was added). Flies were kept in lab conditions with a humidity of 70% RH, 12-hour dark:12-hour light cycles, and temperature of  $22^{\circ} \pm 1^{\circ}\text{C}$ .

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 100g of jaggery, 100g of wheat rava powder, 10g of agar boiled in 1000ml distilled water and 7.5 ml of propionic acid added to it.; Whey protein (treated) media: whey protein media was prepared from 100g of jaggery, 100g of whey protein powder, 10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it; Mixed (Wheat cream agar+ whey protein (1:1) media): Mixed media is prepared from 100g of jaggery, 50g of wheat cream powder and 50g whey protein powder, 10g of agar boiled in 1000ml of distilled water and 7.5 ml 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 and were used to study the sex ratio experiments in *D. melanogaster*.

### 2.2. Sex ratio experiments

The virgin male and female flies were collected from the wheat cream agar, whey protein (treated) and mixed diets. Allowed to mate and after mating, these mated pairs were transferred to other vials containing their respective media, once in seven days until their death and the number of male and female offspring were recorded. A total of fifteen pairs were observed separately for each of the wheat cream agar, whey protein (treated) and mixed media. Results and discussion

Figure 1 showed the mean and standard error value of sex ratio of *D. melanogaster* cultured in Wheat cream agar media, Mixed media and Whey protein media. This data showed that, the number of female offsprings were greater when compared to those of male offsprings in all three diets. The greater difference in sex ratio observed in wheat cream agar media compared to treated and mixed media, where an average difference is observed.



The different letters on the bar graph shows the number of offspring

**Figure 1** The graphs show the sex ratio between the male and female progenies of *Drosophila melanogaster* raised on the different diets (wheat cream agar, whey protein and mixed).

**Table 1** Sex ratio of *D. melanogaster* progenies from wheat cream agar, whey protein treated and mixed diet.

Treatment (diets)	No. adults	No. females	No. males	F:M ratio
Wheat cream agar	943	491	452	1:1.08
mixed	1018	518	500	1:1.03
Whey protein	994	501	493	1:1.01

**Table 2** Sex ratio of *D. melanogaster* progenies from wheat cream agar, whey protein treated and mixed diet.

Treatment(diets)	No. adults	No. females	No. males	M:F ratio
Wheat cream agar	943	491	452	1:0.92
mixed	1018	518	500	1:0.96
Whey protein	994	501	493	1:0.98

**Table 3** Sex ratio of *D. melanogaster* progenies from wheat cream agar, whey protein treated and mixed diet and Chi square analysis value

Treatment (diets)	No. adults	F:M ratio	Chi square analysis for female	Chi square analysis for male	Significance male and female
Wheat cream agar	943	1.08:0.92	0.848	0.806	P>0.05
Mixed	1018	1.03:0.96	0.159	0.159	P>0.05
Whey protein	994	1.01:0.98	0.032	0.032	P<0.05

The nutritional diet is one of the important external environmental factors which effects the growth and development, stress resistance, survivability, reproduction and also alters the sex ratio. The several studies reveal that the physical factors temperature, photoperiod effects on the Sex ratio in insects including *D. melanogaster*. Higher temperatures in some species result in more females, whereas lower temperatures result in more males (Sharkey and Schrader 2006; Eppley, 1972). At each temperature, each genotype appeared to have a unique sex ratio, which frequently differed significantly from a 1:1 ratio. Temperatures can alter the sex ratio by altering male or female survival rates from the larval to imago stages (Precht *et al.*, 1973). In the present study, the flies were maintained in uniform temperature indicates the no effect of temperature on sex ratio.

In the present study, results (Figure 1) revealed that female off spring are produced more than the male off springs. This suggests that the quality and quantity of the diet is influenced on the variation in the sex of the offspring. Several studies have been demonstrated that quality and quantity influence maternal reproductive output and sex ratio in organisms. According to the Yazgan (1972), the increase in amino acid in the diet increases the number individuals of *Pimpla turionella*. Furthermore, the aging of *Drosophila* males caused a shift in the sex ratio, favouring a larger prevalence of females. Offspring sex ratios were strongly affected by maternal diet. In general, females fed the high-quality diet produced female biased sex ratios and those on the low-quality diet produced male-biased sex ratios (Warner *et al.*, 2007). The current study illustrates the change in the sex ratio between different sexes as well as the varied diets. The flies that were given three different diets produced a greater number of female progenies. The mixed diet produced more female offspring compared to the other two diets. There is a much greater difference in sex ratio observed in wheat cream agar media compared to treated and mixed media, where an average difference is observed. The flies fed with the whey protein (treated) and mixed diet produced more female progeny and less male offspring compared to the wheat cream agar media. The study found that the Jeeni millet diet (Kiran and Krishna, 2023), and also the Spirulina-treated diet (Shreeraksha *et al.*, 2023) produce more female offspring than male offspring compared to wheat cream agar and a mixed diet. These three studies demonstrate that diet had a significant impact on the organism's sexual ratio. Several studies have shown that the variation in environmental elements such as temperature and light variation in age also affects the sex ratio of some insects, including *D. melanogaster*. However, in our experiments, we used the same aged flies raised on different diets that were kept under the same environmental conditions to study the sex ratio, so the observed variation in experimental results is due to differences in the quality and quantity of nutrients in the diet.

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### 3. Conclusion

Hence from our study in *D. melanogaster* we can conclude that the nutrition is one of the important factors that influences the sex ratio of the organism. Further, there is a greater production of female offspring than male offspring in all the three diets studied. Hence, whey protein increases production of the female offsprings.

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### Compliance with ethical standards

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#### Disclosure of conflict of interest

No conflict of interest to be disclosed.

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