



# **The whey protein reduces the longevity in *Drosophila melanogaster***

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## **Abstract**

Nutrition has long been recognized as an important factor for influencing both the health span and lifespan in a variety of animals, including flies. Diet is important in preserving long term health. Variation in the dietary protein: carbohydrate ratio, in particular, can promote longevity. In the present study the flies of *Drosophila melanogaster* flies were cultured in the wheat cream agar media, whey protein media and mixed media to understand the effect of whey protein on the longevity. The results revealed that flies fed with whey protein were significantly lived shorter compared to the wheat cream agar and mixed diet fed flies which showed average life span. Flies fed with mixed diet showed the greater longevity compare to other two media. Mated flies lived longer compared to the virgin flies, among the sexes, females showed the longer life span than the males in all the three diets. Hence this study suggests that the consumption of whey protein reduces the life span in *D. melanogaster*.

**Keywords:** Nutrition, *Drosophila melanogaster*, longevity, whey protein

## **Introduction**

An organism's diet is an important extrinsic factor that affects its growth, development and survival. (Sisodia and Singh, 2012). Food is a source of nourishment as well as delight, comfort, fuel and protection. Lifespan is a quantitative trait that is impacted by a number of variables, including sex, age, genetic make-up (epigenetics), and environment (Paaby and Schmidt, 2009).

In *Drosophila*, dietary restriction (DR) through food medium dilution can increase longevity. A state brought on by DR is distinguished by a decrease in fertility, an increase in starvation resistance, and a rise in total lipid. Gender differences in lifespan and aging are known across species. Sex differences in longevity within a species can be useful to understand sex-specific

aging. *Drosophila melanogaster* is a good model to study the problem of sex differences in longevity since females are longer lived than males. There is evidence that stress resistance influences longevity. While a large amount of data indicates that the primary dietary factor influencing *Drosophila* longevity is protein, newer studies contend that carbs also have a significant impact. There is growing interest in the connections between nutrition and longevity. Dietary restriction may increase longevity, as seen by the long lifespan of flies fed on diluted food sources (Partridge *et al.*, 2005, Piper and Partridge, 2007). Dietary restriction (DR) can impact an animal's longevity in a number of ways, including high carbohydrate and low protein consumption (Fontana *et al.*, 2010).

It is well recognized that dietary antioxidants are crucial for preserving oxidative balance. When endogenous and diet-derived antioxidants function together, they create a very potent defensive system against oxidative damage. The major energy resource of animals is fat, stored as triacylglycerols (TAG). TAG is deposited in intracellular lipid droplets of specialized organs like mammalian adipose tissue or fat body in *Drosophila*. These organs, together with the digestive tract and the central nervous system form an integrated molecular communication network. According to Finch (1990) and Charlesworth (1994) aging is the steady functional decrease of an organism that results in death. Although a number of hypotheses have been put up to explain the evolutionary pressures involving fitness and reproduction that affects a species' longevity (Hughes and Reynold, 2005).

Whey protein use a high-quality protein powder from cow's milk. Milk has two proteins: casein constitutes approximately 80% and whey protein constitutes approximately 20%. Whey contains less than 1% proteins comprising mainly - lactoglobulin (-LG), -lactalbumin (-LA), bovine serum albumin (BSA), immunoglobulins and proteaseptone, as well as several minor proteins including lactoferrin, lactollin, glycoproteins, lactoperoxidases and transferrin.

Now a days people enormously using Whey protein to boost muscle protein synthesis and to build lean muscle mass. Muscle Asylum Premium Whey protein contain 24g protein, 5.2g BCAAs, low carbohydrate with no sugar per 40g (per serve). It also contains digestive enzymes and flavour (banana). It assures to provide essential amino acids like lysine, methionine, phenylalanine, isoleucine, leucine, valine, threonine, EAAs, SEAAAs, tryptophan, histidine, and glutamic acid. All studies show how it is helpful in health but there is no evidence documented about how it affects longevity. Therefore, the study under taken to address the effects of The Whey protein on the longevity in *D. melanogaster*.

## Materials and Methods

The whey protein powder was obtained from Amazon app online.

### Establishment of stock

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

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 by adding 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 (Whey protein treated) media**: whey protein media was prepared by adding 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+ whey protein (1:1) media)**: Mixed media is prepared

by adding 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 longevity experiments in *D. melanogaster*.

## **Experimental procedure**

### **Longevity for mated flies**

From the wheat cream agar, whey protein (treated), mixed media bottle, virgin females and unmated flies were isolated within 3 hours of eclosion from their respective media. These flies were aged for five days. Virgin female and unmated males were individually aspirated into mating chamber. After mating, these mated pairs were transferred to vial containing their respective media, once in seven days until their death. And note down the number of flies died every day. A total of thirty flies were observed separately for each of the wheat cream agar, whey protein treated and Mixed media.

### **Longevity for unmated flies**

Five days old Unmated male and female flies from wheat cream agar, mixed and whey protein media were taken to conduct longevity experiments. Five unmated male flies and five unmated female flies from each media were taken in separate vials containing respective media. On seventh day, these flies were transferred to other vials containing respective media until the death

of each fly. Every day the number of flies were noted and the number of dead flies were noted. A total of ten flies were observed separately for each of the wheat cream agar media, mixed media and whey protein media. Separate experiment was carried out for virgin female, mated female, mated male and unmated male in *D. melanogaster*.

## **Results and Discussion**

### **Analysis of survival curve**

Survival curve was calculated for longevity of males and females. Two functions that are dependent on time are of particular interest: The survival function and Hazard function. The survival function  $S(t)$  is defined as the probability of dying at time  $t$  having survived until that time. The graph of  $S(t)$  against  $t$  is called the survival curve. The Kaplan- Meir method was used to estimate this curve from observed survival times without assuming an underlying probability distribution. Two survival curves were compared using a statistical hypothesis test called the log-rank test, which is used to test null hypothesis that there is no difference between survival curves, i.e., the probability of an event occurring at any point of time is for each media 20 trials were made for each of the Wheat cream agar, Mixed diet and Whey protein diet. Separate experiment was carried out for virgin female, mated female, mated male and unmated male in *D. melanogaster*. The results revealed that the females fly lived longer than the male flies in all the three diets. Further the whey protein diet flies showed that the less life span compared to wheat cream agar and mixed diet.

Figure 1a: Survival curve of mated male flies

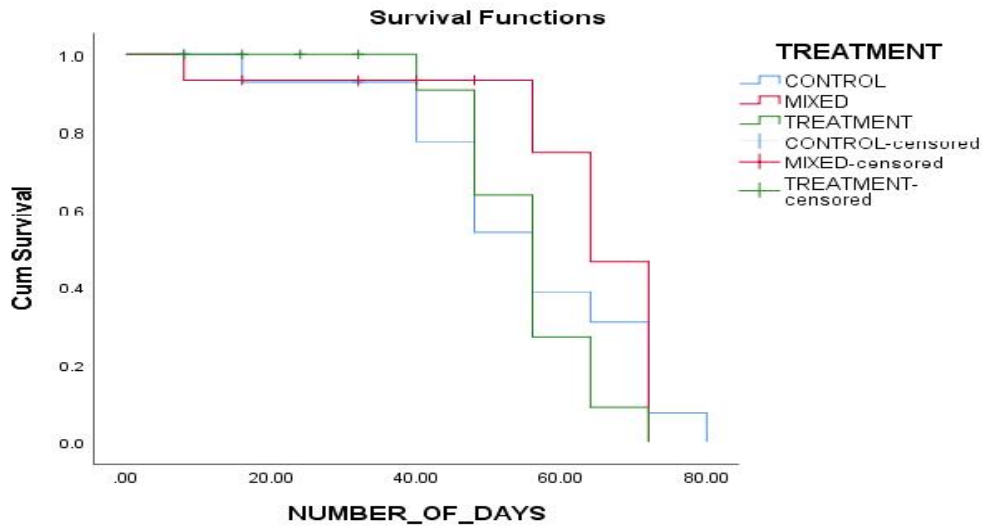
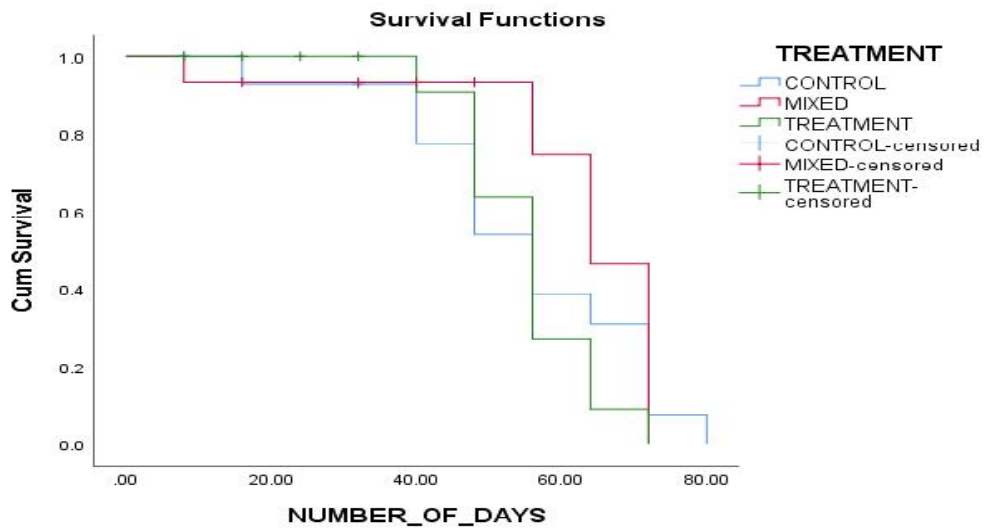
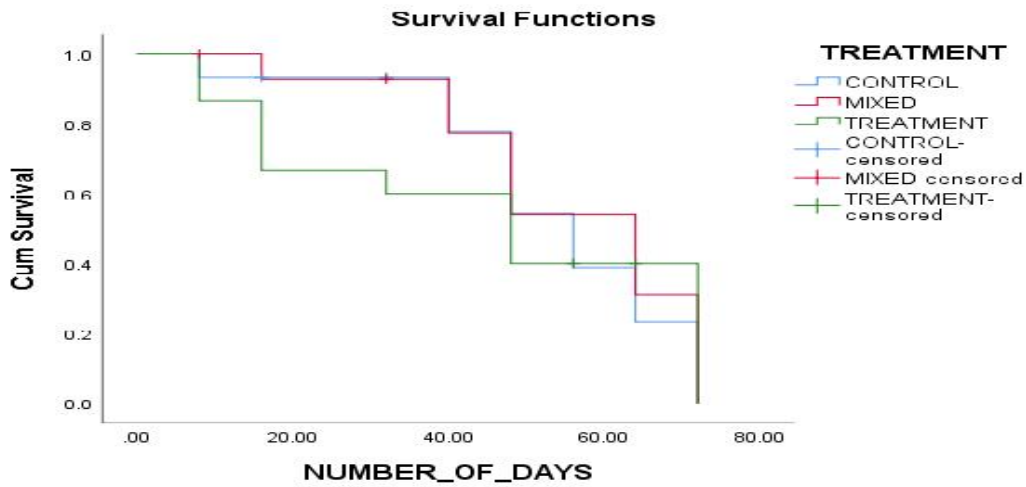


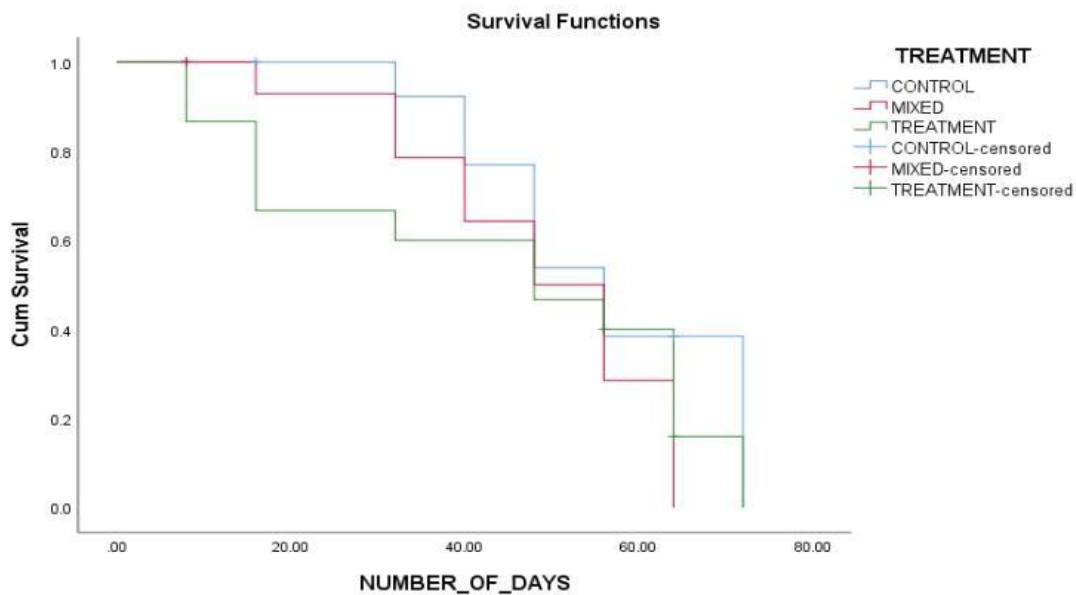
Figure 1b: Survival curve of mated female flies



**Figure 1c:** Survival curve of unmated male flies



**Figure 1d:** Survival curve of unmated female flies



Aging is a complex biological process characterized by a gradual decline of biological functions. Elucidation of the molecular mechanisms, pathways and physiological processes implicated in longevity are of primary importance for medical and biological research. Lifespan is a quantitative trait influenced by various factors including sex, age, genetic background (epigenetics) and environment (Paaby and Schmidt, 2009) and also the diet. Extended lifespan in *Drosophila* is frequently associated with a variety of characteristics, including

resistance to environmental stressor (starvation, desiccation and cold), lipid content, development time, body size, biochemical defence, and so on, which correlate either positively or negatively (Vermeulen and Loeschke, 2007).

**Figures 1a to 1d** showed the lifespan variation in *D. melanogaster* depending on the Wheat cream agar media, mixed media and whey protein media. It was found that the flies fed with mixed media showed the greater longevity compared to whey protein and wheat cream agar media.

This suggests that quantity and quality of nutrients present in the wheat cream agar, whey protein, mixed diet is responsible for the variation in the longevity lifespan in the *D. melanogaster*. This variation in longevity indicate *Drosophila's* nutritional requirements and length of life, making them important life history feature (Wafa and Krishna, 2015; Alwyn and Krishna, 2015).

Our study also supports the study by Brooke *et al.*, (2021) that the high protein and carbohydrate ratios reduced the longevity in *Drosophila*. According to Theodore *et al.*, (1999), that nutrition should be neutral for selecting life span in *Drosophila* and also proved that the lifespan is decreased under conditions of high nutrition as nutrition increases, lifespan decreases. In most insects, dietary restriction, and in particular a reduced protein intake, increases lifespan (Simpson *et al.*, 2012). All these studies support our result that both carbohydrate rich wheat cream agar media and protein rich whey protein media reduced life span compared to mixed media.

In this study, it was discovered that there was sex difference in longevity of *D. melanogaster* whereby the females had higher longevity than males. In both mated male and mated female (**Figure 1a and 1b**), more longevity observed in mated female flies. Among the mated flies, flies fed with mixed media shows more longevity compared to flies fed with other two diets. Among the virgin flies (**Figure 1c and 1d**), females showed more longevity than males and greater observed in the flies fed on the mixed media. This confirms results by earlier studies that, there are difference in average lifespan of the sexes in many animals, and females frequently last longer than males (Lints et al. 1983; Austad and Fischer, 2016).

Alwyn Dsouza and Krishna (2015), also found that flies grown on natural energy drink had consumed greater quantity of food and lived significantly longer than those flies grown on synthetic energy drink which showed the least longevity and normal media. Several studies including effect of Jeeni millet (Kiran and Krishna, 2023), Spirulina (Shreejani *et al.*, 2023)

and Creatine monohydrate (Mamba and Krishna, 2023) also showed that longevity varies with respect to dietary composition in *D. melanogaster*.

Hence from our study in *D. melanogaster* we can conclude that the nutrition is one of the key factors which influenced on the longevity of the organism. The flies raised on the whey protein media showed the least longevity than the wheat cream agar and mixed diet suggesting that the consumption of whey protein reduces the life span in *D. melanogaster*. The females showed higher longevity than males. Further, virgin female flies lived longer than those of mated females.

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