



## Efficacy of two non-nutritive sugars on overall fitness of *Drosophila melanogaster*: I-a comparative study

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### Abstract

The consumption of food and beverages containing non-nutritive sugar has dramatically increased over the past few decades. There may be many reasons, this could be either growing concerns about health and quality of life to prevent overweight, obesity, cardiovascular issues and diabetes. Extensive advertisement of different company products may also attract people towards the same. Unknowingly, consumed by the consumers without the prior knowledge on these through sweets, beverages, food products etc. The main objective of this study is to understand the efficacies of Artificial Sugar Aspartame (AS) and Natural Sugar Stevia (NS) on the fitness and physiology. *D. melanogaster* being a better model to understand all biological process and alternative mammalian model to study toxicity, the Normal, AS and NS based food medias were used in different concentrations to grow *Drosophila melanogaster* flies and they were subjected to analyse, overall fitness, and physiology. Our investigation on *Drosophila* has likewise bolstered positive advantages of the Aspartame (AS) in few parameters and negative consequences for a few and same in Stevia (NS).

**Keywords:** aspartame, stevia, lipid content, gustatory, *D. melanogaster*

### 1. Introduction

Usage of sweetener is an ancient practice in many types of food and beverages. In recent decades the increased concern of health, diabetes, overweight, among population made people to think of an alternative non-nutritive sugar to prevent from the craving for food. Of the several; synthetic non-nutritive sugars substitute aspartame is one of the most widely used sweetener and also approved in many types of food products. Several studies have proved that replacing sugar with aspartame may have favourable effects on body weight, blood glucose level, insulin resistance, no elevation in craving behaviour, less fat accumulation and lower lipogenic effects. <sup>[1, 2]</sup> On the other hand, adverse effects of aspartame has been shown by using rat models, when lifetime feeding of aspartame on rats increased risk for lymphoma, leukaemia and transitional cell carcinoma of pelvis, ureter, bladder in dose dependent manner. <sup>[3, 4]</sup> Further Abhilash *et al* are of the opinion that long term consumption of aspartame lead to the imbalance in antioxidant/peroxide status in the brain of rats exposed to 100mg/kg body weight <sup>[5]</sup>. An interesting study by Fowler *et al* on large number of volunteers showed increased waist circumference after consuming diet coke containing artificial sweetener <sup>[6]</sup>. Further, many studies have shown the effect of these artificial sweetener on the gut micro biota population and there is a reduction in the few types of micro biota or alteration in the gut micro biota upon consumption of aspartame <sup>[7, 1]</sup>. Recently, the introduction of a natural sweetener extracted from the *Stevia rebaudiana Bertoni* plant a native of Brazil and Paraguay attracted growing interests among the population. There are two active stevia

glucosides namely Stevioside and rebaudiosides are known for its rich sweet taste 150 times more than the sucrose <sup>[8]</sup>. Several studies on its positive effects has been done to understand stevia being low-caloric sweetener not stimulate appetite, craving behaviour hence not incurring caloric intake and not promoting weight gain and lipid accumulation as in case of aspartame <sup>[9, 10]</sup>. Further, treating stevia to rats has shown that significant improvement in the ameliorated reduction in body weight besides lesser intake of food in addition decreased levels of blood glucose, total lipids, total cholesterol, triglycerides and LDL and increased HDL <sup>[10]</sup>. This indicates the beneficial effects of Stevia among the mammalian models. But the comparative analysis of both artificial and natural sweetener is not done extensively on the organisms. Hence the present study is an attempt to understand the comparative study on these two sugars for their beneficial/adverse effects on *Drosophila melanogaster* being potential model to study food toxicity and its long term effects can be tested and recorded. The overall growth, development and reproduction of an organism are influenced by both intrinsic and extrinsic factors known to affect all biological, physiological and developmental changes <sup>[11, 12]</sup>. In many holometabolous insects the feeding larval phase is followed by a non-feeding 'wandering' phase prior to pupation, the larvae cease feeding site in search of a suitable pupation site <sup>[13]</sup>. In the laboratory, *Drosophila* larvae typically wander and pupate on the walls of the container because the bottom of the rearing vial or bottle is entirely covered by food <sup>[14]</sup>. The distance that larvae pupate from the surface of the food, 'pupation height' is a polygenic trait that responds

effectively to bidirectional selection [15]. The definition of stress is exposure to any environmental factor that acts to reduce the fitness of an organism [16]. One important cause of stress is shortage of nutrition and certainly most species experience periodical starvation [17]. Lifespan measurement is the basic method used to determine the effects of genetic and non-genetic factors involved in aging [18], diet is a major environmental factor that has a huge impact on lifespan in *Drosophila* and many other species [19] and further proper regulation of carbohydrate homeostasis including one of the major energy storage molecule such as lipids and its metabolism are critical for maintaining normal physiology. These molecules serve a variety of purposes, including providing an essential energy source through glycolysis, substrates for bio-synthetic reactions in growing animals and energy storage in the form of glycogen.

Since Stevia and Aspartame are low-caloric sweeteners - fitness parameters like pre - adult development, pupal height, starvation and biochemical parameters such as carbohydrate and lipid estimation helps to know energy dynamics obtaining from stevia to organisms to perform different daily activities. Hence, in the present investigation the effects of stevia and aspartame on the energy distribution to perform different functions along with reproductive ability and longevity have been studied.

## 2. Materials and methodology

### 2.1 Establishment of stock

The experimental stock of *Drosophila melanogaster* wild type flies was collected from the stock centre, University of Mysore. The flies obtained from the culture bottles were redistributed to twenty different normal culture bottles containing wheat cream agar media. The flies per culture bottle were maintained at 22°C with a relative humidity of 70% in a 12 h dark; 12 h light cycle. The virgin flies were isolated in the pupa stage and cultured in the test media. The test media containing 1%, 2%, 3% Natural sweetener (Stevia), artificial sweetener (Aspartame) based media and the flies grown in normal wheat cream agar media were used as control.

### Quantification of Food intake in Larvae and adult using dye method

Second instar larvae (10 No.) obtained from normal and test media were used to study feeding behaviour. Each larva was placed in a Delcour cup containing normal and 1%, 2%, 3% Natural sweetener [stevia] and artificial (Aspartame) treated media with 2.5% (w/v) blue food dye (FD & C Blue Dye no. 1). The larvae were starved for an hour and later allowed to feed on the dye treated normal and test media for 15 minutes. Then they were transferred to 2ml clear tubes and frozen. These frozen larvae were homogenized by adding 200 µl of distilled water and 800 µl of distilled water was added to make up the volume to 1000 µl. The absorbance was measured at 629 nm using colorimeter. The larvae which were not treated with blue dye were used as blank. The amount of food taken was measured from the standard graph made from serial dilution of the blue dye.

## 2.2 Fitness Parameters

### 2.2.1 Larval resistance to starvation

larvae grown in respective treated media were placed in 1% Non-nutritive agar media for the duration of days the larvae were able to survive in non-nutritive food was recorded by

observing the vials at 2 hour intervals till all the larvae had perished. A total of 5 replicates (10×5=50 larvae) were run separately for each media grown larvae.

### 2.2. Mated/Unmated flies resistance to starvation

Adult unmated flies and mated flies were obtained separately from individual treated groups, were placed in 1% Non-nutritive agar media, Males and females were considered separately for this experiment. The duration of days the flies were able to survive without food was recorded by observing the vials at 2 h Intervals till all the flies had perished. A total of 5 replicates (5×5=25 flies) were run separately for each group and data is subjected to statistics.

## 2.3 Biochemical parameters

### 2.3.1 Determination of total carbohydrate

0mg of starved flies and larvae obtained from the respective treated groups and homogenized with 0.5ml of 2.5N HCL in 2ml clear tubes. It was hydrolyzed by keeping in a boiling water bath for three hours at room temperature. It was then neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 10ml and centrifuged. The supernatant was collected, 0.5 and 1ml aliquots were taken for analysis. Standards were prepared by taking 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard. Zero served as blank. The volume was made up to 1ml in all the tubes including the sample tubes by adding distilled water. Then 4ml of Anthrone reagent was added. The tubes were heated for eight minutes in a boiling water bath. It was cooled rapidly and the green to dark green colour was read at OD= 630nm. The standard graph was drawn by plotting concentration of the standard on X axis versus absorbance on Y-axis. From the graph the amount of carbohydrate present in the sample was calculated.

### 2.3.2 Estimation of lipids

10mg of starved flies and larvae obtained from the respective treated groups were homogenized with 0.2ml of distilled water in 2ml clear tubes. 0.2ml of Neutral solvent was added to this along with a few drops of phenolphthalein. To this 0.1N potassium hydroxide was added in µl. The contents were shook constantly until a pink colour which persisted for fifteen seconds was obtained. The amount of lipids present in the sample was calculated by the given formula

$$\text{Amount of free fatty acids present} = \frac{\text{Vol. of KOH used} \times \text{Normality of KOH} \times 56.1}{\text{Weight of the sample}}$$

### 2.3.3 Statistical Analysis

Mean, standard error, One-way ANOVA, and Turkey's Post-Hoc test were carried out for all the parameters on the data obtained using IBM SPSS V.22.0. 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 10 trials were made for each of the treated groups.

**3. Results**

The amount of food intake by the larvae is depicted in fig.1 Larvae preferred to feed on control media more compared to other two sugars but difference between the concentration groups is non-significant.

Larval resistance to starvation is reported in Fig.2 starvation resistance observed to be more in control group compared to AS and NS. NS treated larvae and adult showed least starvation resistance compared to AS. There is significant difference between concentration groups of starvation resistance. Starvation resistance of Adults depicts that both

mated and unmated adult flies has good resistance in control media. Whereas the Unmated flies fed on AS show more starvation resistance compared to unmated NS fed groups. Mated flies show less resistance to starvation compared to unmated flies. No significant difference between sexes.

Carbohydrate concentration in larva and adult fed with two sugars along with control presented in the figure 4. The larvae and adult carbohydrate concentration is more in the NS fed group compared to other two. Comparison of larval group is non-significant but adult is significant with  $F=39.10$   $df$  6. More carbohydrate concentration in adults observed in 1% AS and less in higher concentrations.

The total lipid concentration of larva and adult flies depicted in Fig.5 low lipid observed in larvae compared to adult 3% AS group showed more lipids whereas lowest lipid recorded in the NS group in all concentrations. Control group with maximum lipid accumulation compared to AS and NS.

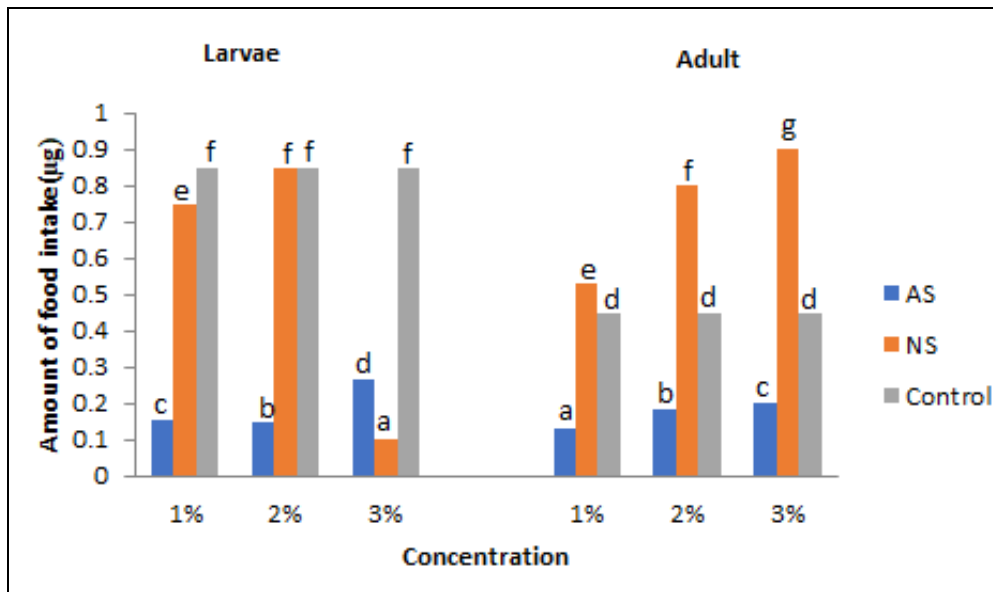


Fig 1: Effect of natural sweetener and artificial sweetener on the food intake by larvae and adult of *D. melanogaster* using dye method

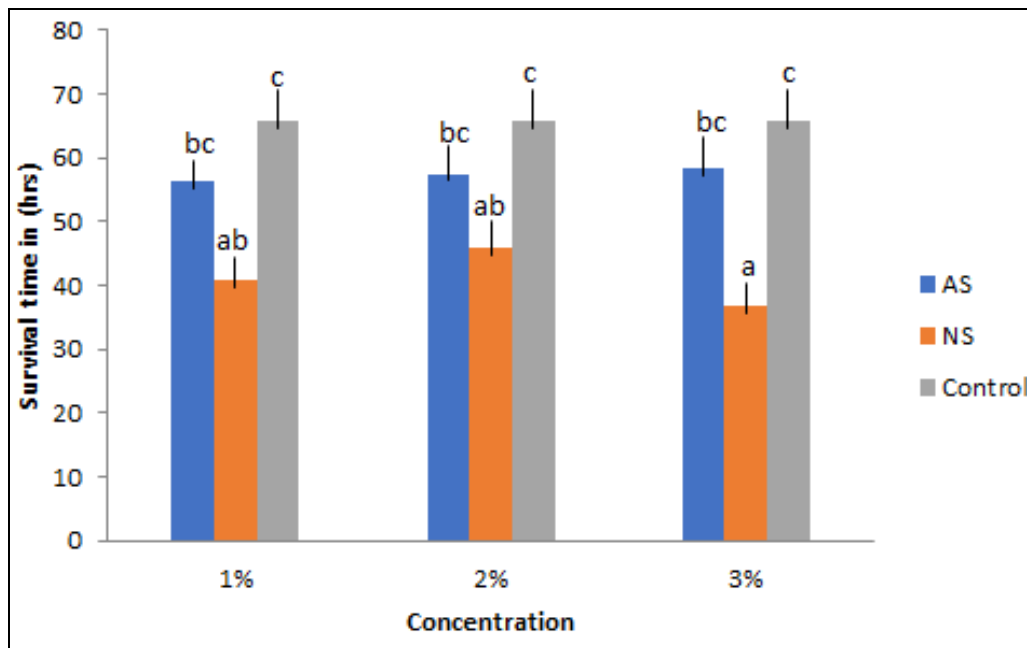


Fig 2: Effect of natural sweetener and artificial sweetener on resistance to starvation of *D. melanogaster* third instar larvae

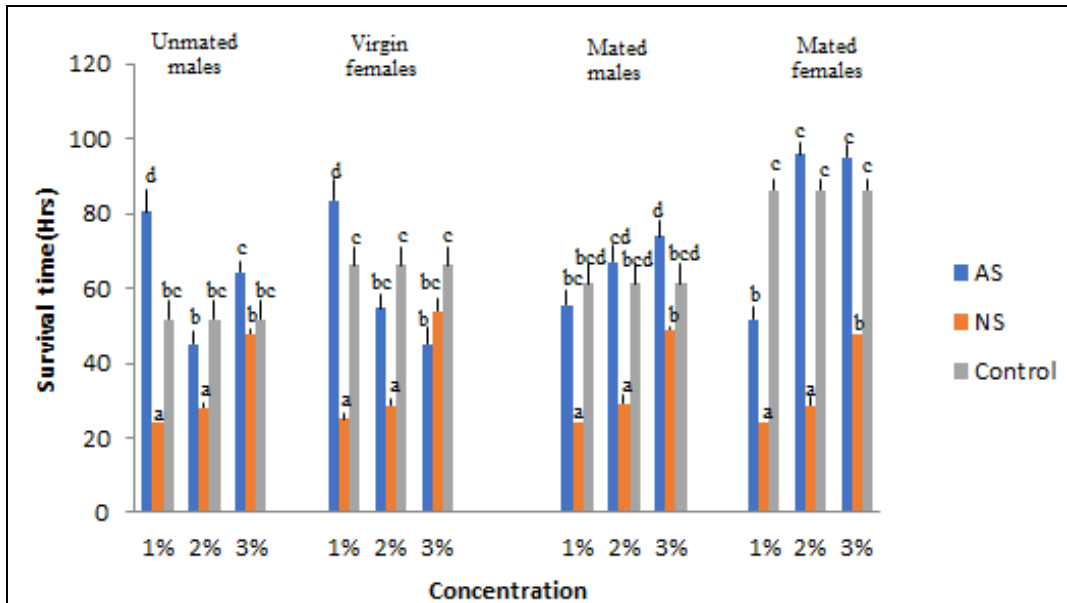


Fig 3: Effect of natural sweetener and artificial sweetener on the resistive capability to starve of *D. melanogaster* mated and unmated flies

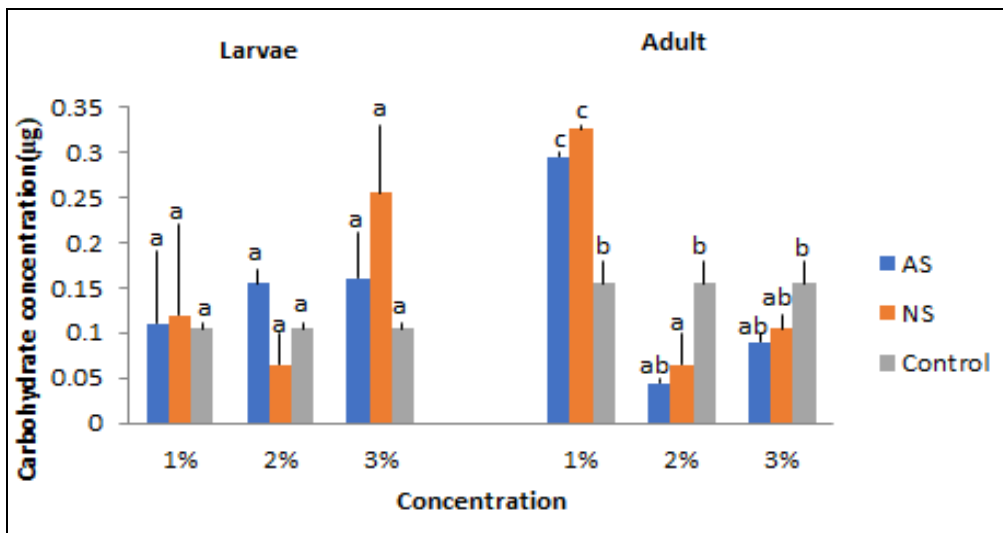


Fig 4: Estimation of carbohydrate concentration on the larvae and adult of *D. melanogaster* cultured on natural sweetener and artificial sweetener

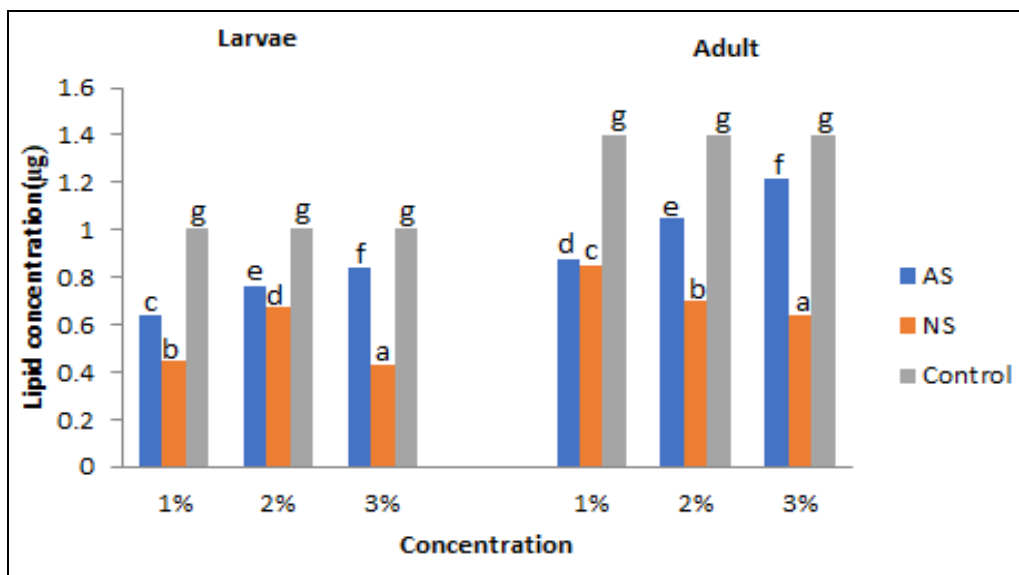


Fig 5: Estimation of lipid concentration on the larvae and adult of *D. melanogaster* cultured on natural sweetener and artificial sweetener

#### 4. Discussion

In spite of the controversy over whether synthetic non-nutritive sweetener usage poses health risks or beneficial the continuous use of the sweetener for beverages sweets and other food products. Many studies on aspartame showed positive effects when it is used short term. On the other hand animal studies have convincingly proven that artificial sweetener cause body weight gain <sup>[20]</sup>. However the studies on natural sweetener like stevia have beneficial effects without side effects <sup>[21]</sup>. Our study on *Drosophila* has also supported positive benefits of the Aspartame on certain parameters and negative effects on some. No inhibition by the larvae for other two AS and NS. The food fed may be utilized in high energy consuming process such as egg production, locomotion and other physiological function <sup>[22]</sup>. Starvation resistance is an important parameter depicts the tolerance levels of the flies under different nutrition. Flies fed with NM is more tolerant than AS and NS. AS fed flies have more starvation resistance compared to NS fed. There is a significant difference between starvation resistance of mated and unmated flies. Unmated flies with AS and NS resistance is better than mated. More starvation resistance of Aspartame groups indicate that the more fat storage compared to stevia. As an alternative NS found better by showing least starvation resistance with less fat accumulation, this also further confirmed by the less total lipid in NS fed flies and larvae. The quality of food intake by an organism plays an important role in the maintenance of health and fitness. Overall growth development and reproduction of an organism are influenced by both intrinsic and extrinsic factor known to affect all biological, physiological and developmental changes <sup>[23, 24]</sup>. In the present study, No doubt, the performance of flies is better in all parameter in Normal nutritive media used for culture, comparatively both non-nutritive sugars (Aspartame and Stevia). Though the AS has little advantage over the NS in some parameters definitely the overall performance and advantages is towards the non-nutritive stevia compared to aspartame. Overall fitness longevity, survival is enhanced by NS. In addition less lipid content may be beneficial to reduce the risk of obesity. On the other hand AS has more starvation resistance and more lipid content indicating the risk for obesity if there is continuous consumption. Comparatively, the starvation resistance is more in AS, still stevia can be a better non-nutritive alternative as they improve overall fitness and survivability. Several studies in small mammals, when rats fed with 25,250,500, 1000mg/body wt. showed decreased body weight may also be low calorie intake by rats with stevioside. Further, Park and cha (2010) found total cholesterol concentration were lower in rats fed with stervioside 1mg/Kg per day with high fat diet compared to rats fed with high fat diet with water <sup>[25]</sup>. They are of the opinion that stevia is beneficial in decreasing the levels of blood glucose, total lipids, total cholesterol, triglycerides and LDL, increases the HDL. Further, Roberts and Wright 2010, have revealed that stevia will not stimulate appetite, thereby may not increase calorie intake and may not promote weight gain. On the other hand, aspartame has show beneficial if in maintaining the blood glucose level, become disadvantageous when continues to use, promote weight gain <sup>[26]</sup>.

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