

Energy Drinks' effect on Pre Adult development of *Drosophila melanogaster*

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Abstract: Quality of energy drinks consumed by an organism has a significant effect on an organism's fitness. In the present investigation *D. melanogaster* were reared in Alternative natural energy drink based media, Synthetic energy drink based media and Wheat cream agar media. Feeding rate and pre adult development in these energy drinks, based media were studied. Results revealed that flies fed on alternative natural energy drink had consumed significantly greater quantity of food than those flies fed on synthetic energy drink and wheat cream agar media. Egg to larva, larva to pupa and pupa to adult viability was found to be significantly greater in alternative natural energy drink, whereas in larva to pupa viability wheat cream agar media was greater. This suggests that the consumption of alternative natural energy drink was beneficial in pre adult development of *D. melanogaster*.

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Introduction

The overall growth, development and reproduction of an organism is influenced by both intrinsic and extrinsic factor known to affect all biological, physiological and developmental changes that take place in an organism (Sternier and Schulz, 1998; Taylor *et al.*, 2005) In general diet can be classified as either quantitative (i.e food availability) or qualitative (i.e food consumption) out of these two, the quantitative effects are evident since animals obtain energy and other nutritional requirements from food, therefore, under a natural range of conditions there is a positive relation between food availability and fitness of an organism on the other hand qualitative effects often are divided into two categories, namely Nutritional deficiency and Inhibitory metabolites therefore these studies suggest that there is a balance between energy intake and expenditure is very much necessary for the survival, reproduction and fitness of an organism (Pough, 1989; Sibly, 1991). This balance depends on the interplay between matter intake, digestion and allocation of acquired energy to various functions such as maintenance, growth and reproduction (Karasov, 1986) so therefore Experimental modifications of animal diet have played a key role in the study of how organisms adjust their energy allocation and their effect on pre adult development in organisms

In modern times the popularity and consumption of many energy drinks and soft drinks is growing at an exponential rate due to a belief that such an energy drink provides significantly greater energy, however the problem with such drinks, they contain high amount of caffeine, herbs and other additives, caffeine are used to supply energy and increased alertness the

stimulant effects are however short term and high doses of caffeine have found to be caused disorders such as Gastric acid secretion, heart diseases and kidney malfunctioning etc. (Noer, 2010). Consuming soft drinks and other sweetened drinks and other sweetened drinks for a long time leads to type II diabetes and obesity (Odegaard, 2009). Thus, there is a need to develop an alternative natural energy drink without caffeine and other additives and study its effect on health benefits in *Drosophila melanogaster*. Since *Drosophila melanogaster* forms a very good model to test this because many of the mechanisms involved in the energy consumptions, digestions and allocations of energy are highly conserved between *Drosophila* and Human beings, therefore, present study has been undertaken in *Drosophila melanogaster* to test the effect of Synthetic and Alternative natural energy drinks on pre adult development.

2. Materials And Method

2.1. Establishment of Stock

The experimental stock of *D. melanogaster* was established from progenies of 105 naturally inseminated females collected at Chamundi Hills, Mysore, India. In each generation flies obtained from these culture bottles were mixed together and redistributed to 20 different culture bottles containing wheat cream agar media (100g of jiggery, 100g of wheat powder, 8g of Agar-Agar was boiled in 1000ml of double distilled water and 7.5ml of propionic acid was added) each with 20 flies were maintained at 22°C with a relative humidity of 70% in a 12 hours dark; 12 hours light cycle. This procedure was carried out for 3 generations to acclimatize flies to lab condition. At

fourth generation eggs were collected using Delcour's procedure (1969) to study pre adult fitness in control (wheat cream agar media) along with different concentrations of natural drink based media (chop the four fruits, apple, pomegranate, orange, banana, juices are prepared separately each of 50ml is mixed together and 10ml of vitamin B12 and 60ml of carbonated water are been added a volume of 270ml is finalized for the further analysis and treatments) and synthetic drink based media (Red Bull).

2.2. Quantification of Food intake in Larvae using dye method

Ten Second instar larvae obtained from normal media were used to study feeding behavior. The larvae were obtained by scooping out from the respective treated media and washed in saline. Each larva was placed in a vial containing normal /Natural/ synthetic energy drink based media treated medium containing 2.5% (w/v) blue food dye (FD & C Blue Dye no. 1). The larvae were allowed to feed for 15 minutes. Then the larvae were transferred to Eppendorf tube and frozen. These frozen larvae were homogenized by adding 200 μ l of distilled water further 800 μ l of distilled water was added. The absorbance was measured at 629 nm using a calorimeter. The larvae which were not treated with blue dye were used as the blank. The amount of food taken was measured from the standard graph made from serial dilution of a blue dye.

2.3. Energy drinks (synthetic and alternative) effect on the Pre adult Fitness of *D. melanogaster*

Eggs were obtained using Delcour's procedure (1969) to study the effect of the synthetic and Natural energy drink treated media on pre Adult development. Eggs (100) were seeded separately into each vial containing 5ml of respective media (wheat-cream agar media/ synthetic energy drink treated media/natural energy drink treated media). A total of 10 trials, was made eggs were observed to viability of eggs into larvae, larvae to pupae and pupae to adult. This were recorded and Separate experiments were performed for wheat-cream agar media/alternative natural energy drink/synthetic energy drink media. Percentage of eggs to larval hatching, larval to pupal viability and pupae to adult eclosion were calculated.

2.4. Statistical Analysis

Mean, standard error, One-way ANOVA and Tukey's Post-Hoc test were carried out on the obtained data using SPSS version 14.0.

Results

Figure 1 Food intake by a larvae was measured using dye method which is shown in (Figure 1) It was found that the larvae which are grown in Natural energy drink based media have consumed more amount of food compared to larvae which are grown

in Synthetic energy drink based media and wheat cream agar media. One-way ANOVA followed by Tukey's Post Hoc test carried out using SPSS version 14.0 on the above data showed significant variations in feeding rate depending on whether Natural or Synthetic energy drinks were used (Table 1). At 25% synthetic energy drink, the food consumed was very less where as in 50% synthetic energy drink, food consumption was more compared to 25% synthetic energy drink where as in 75% synthetic energy drink, food consumption was more compared to 25% synthetic energy drink and 50% synthetic energy drink, but in Alternative natural energy drink there is no such variation among all three concentrations.

Figure 2 shows data of egg to larval viability. It was found that in both 25% and 50% concentrations egg to larval viability was recorded as follows, control was greater than alternative natural energy drink and alternative natural energy drink was greater than synthetic energy drink, whereas in 75% concentration egg to larval viability is as follows Alternative natural energy drink is greater than control and control is greater than synthetic energy drink. Further, it was noticed that in all the concentration used, egg to larval viability was found to be least in synthetic energy drink. The above data was subjected to one-way ANOVA followed by Tukey's Post Hoc test, showed significant variation between different energy drinks. In all the concentrations egg to larval viability was found to be significantly lesser in synthetic energy drink compared to alternative natural energy drink with Tukey's Post-hoc test.

Figure 3 provides data of larvae to pupae viability, It was found that in all three concentrations larvae to pupal viability was recorded as follows control is greater than Alternative natural energy drink and Alternative natural energy drink was found to be greater than Synthetic energy drink. Further, it was noticed that in all concentrations larvae to pupal viability was found to be least in the Synthetic energy drink. Then the above data were subjected to One-way ANOVA followed by Tukey's Post-Hoc, It was shown that there is a significant variation between all the concentrations, It was found to be significantly lesser in Synthetic drink compared Alternative natural energy drink with Tukey's Post-Hoc test.

Figure 4 provides pupae to adult viability it is shown that in all the concentrations larvae to pupal viability was recorded as follows Alternative natural energy drink is greater than control and control was found to be greater than Synthetic energy drink. Further, it was noticed that in all concentrations pupae to adult viability was found to be least in the Synthetic energy drink. Then the above data were subjected to One-way ANOVA followed by Tukey's Post-Hoc, It was shown that there is a significant variation between

all the concentrations. It was found to be significantly lesser in Synthetic drink compared Alternative natural energy drink with Tukey's Post-Hoc test.

Discussion

To study beneficial and detrimental effects of energy drinks on pre adult development of *D.melanogaster*. The fruit flies were fed with synthetic energy drinks (Red Bull) made of fruits and plant extracts in addition to different additives and the Alternative natural energy drink, which was synthesized in our laboratory using a known quantity of Pomegranate, Apple, Orange and Banana these fruits are rich in Antioxidants, Vitamins, Proteins, and calcium as a result they increase longevity, protect against stress and infections.

The flies grown in these energy drinks were then subjected to study their pre adult fitness. The Figure 1 and Table 1 reveal that the feeding rate of *D. melanogaster* was found to be different, vary between different concentrations of Alternative natural energy drink and Synthetic energy drinks among three the feeding rate was found to be highest in Alternative natural energy drink followed by wheat cream agar and followed by synthetic energy drink.

This suggests that there is a significant influence of energy drinks on larval feeding rate. In *Drosophila*, it was shown that it is a larval stage shows an inhibition threshold when consuming a new or foul tasting food (Meleher *et al.*,2007) however such inhibition threshold is not observed in larvae fed on Alternative natural energy drink when compared to Synthetic energy drinks as well as Wheat, cream agar

media since the rate of larval feeding was highest among larvae fed on Alternative natural energy drinks.

Egg to Adult rate of development and viability are the important pre adult parameters of fitness in *Drosophila*, which is the outcome of interaction between its genotype, environment and competing individuals in the population.

The pre adult viability consisting of different stages which involves Egg to Larval viability larvae to pupae viability and pupae to adult viability It was found that from figure 1, figure 2, figure 3 and Table 1 shows there is a significant variation in the pre adult development in flies fed on Synthetic energy drink and Alternative natural energy drink significantly great Egg to larval viability, larvae to pupae and pupae to adult viability was found in flies fed on Alternative natural energy drink than those flies fed on Synthetic energy drinks as well as control that suggests that the alternative energy drink provides greater quantity of nutrients and energy required for the pre adult development of egg to adult stage, whereas the synthetic energy drink do not equally support energy and required nutrients to increase the pre adult fitness, further the Alternative natural energy drink did not contain any additives like caffeine thereby it increases the pre adult fitness, further the Alternate natural energy drink did not contain any additives like caffeine there by it increases the pre adult viability in *Drosophila* our study also support the nutrients found in pomegranate, apple, orange, banana are responsible for greater Pre adult fitness.

Thus we can conclude from the study the Alternative natural energy drink provides greater health benefit compared to Synthetic energy drink.

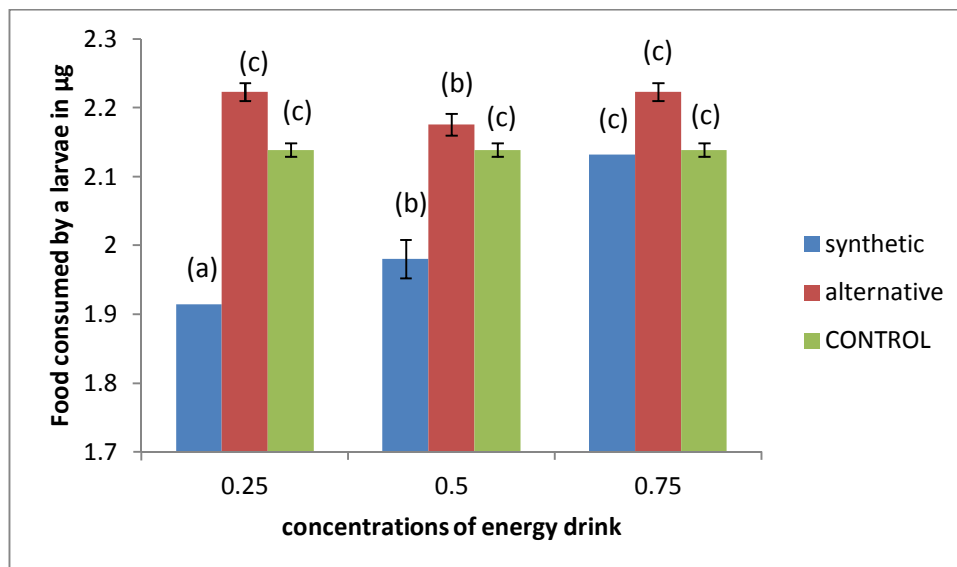


Figure 1. Effect of synthetic and alternative energy drink on feeding behavior in larvae of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

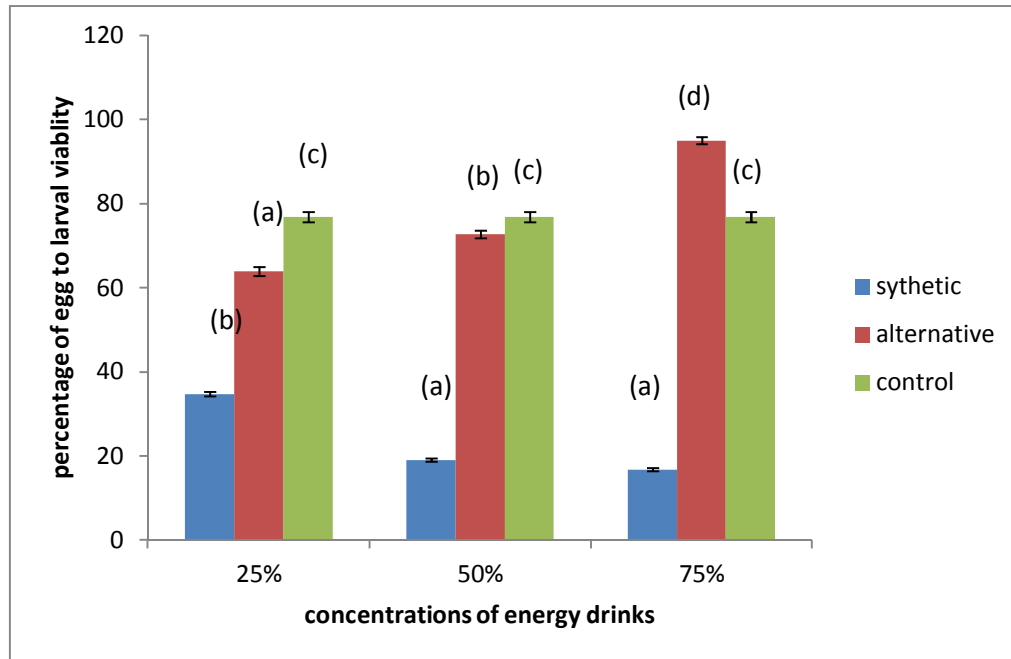


Figure 2. Effect of synthetic energy drink and Alternative natural energy drink on Egg to larval viability of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

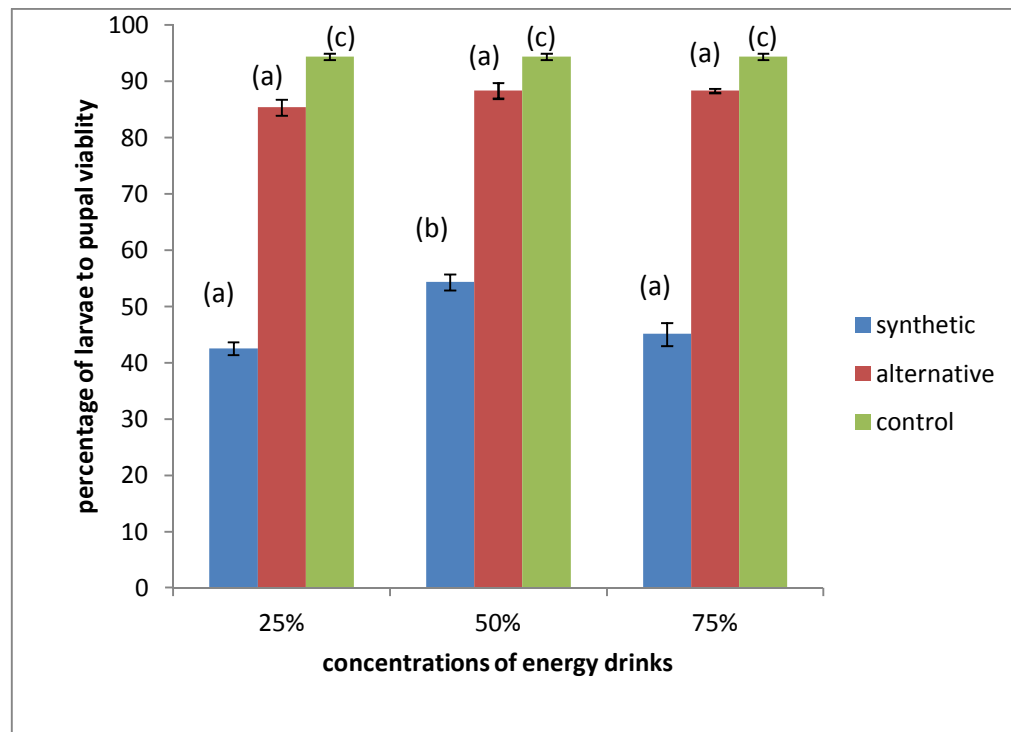


Figure 3. Effect of synthetic and natural energy drink on larvae to pupal viability of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

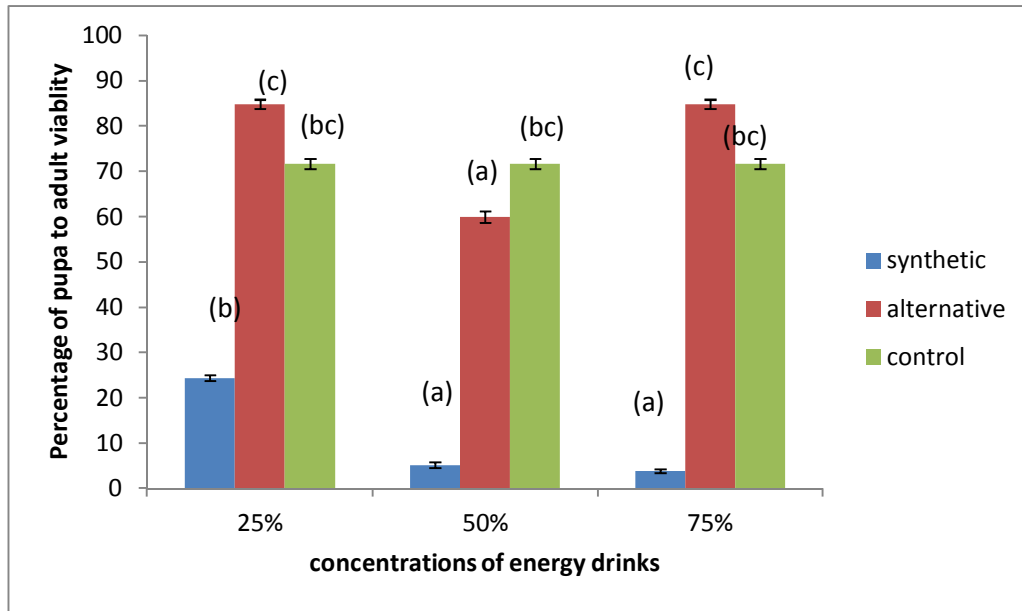


Figure 4. Effect of synthetic and Alternative natural energy drink on pupae to adult viability of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey’s Post Hoc test

Table 1. One way ANOVA of 'Synthetic and Alternative energy drink' effect on larval feeding, pre adult development in *D melanogaster*

Dependent variable	Energy drinks	Source	Sum of squares	Df	Mean square	F-Value
Larval feeding in (μ g)	Synthetic drink	Media	1.12848	3	0.37616	1684.48**
		Error	0.025904	116	0.000223	
		Total	1.154383	119		
	Alternative drink	Media	0.150647	3	0.050216	295.6858**
		Error	0.0197	116	0.00017	
		Total	0.170347	119		
Pre adult development						
Egg to larval viability	Synthetic drink	Media	5141	3	1713.667	163.8131**
		Error	376.6	36	10.46111	
		Total	5517.6	39		
	Alternative drink	Media	23176.9	3	7725.633	1526.47**
		Error	182.2	36	5.061111	
		Total	23359.1	39		
Larvae to pupal viability	Synthetic drink	Media	428.2568	3	142.7523	12.8689*
		Error	399.341	36	11.09281	
		Total	827.5978	39		
	Alternative drink	Media	17385.22	3	5795.074	299.0462**
		Error	697.6268	36	19.37852	
		Total	18082.85	39		
Pupae to Adult viability	Synthetic drink	Media	4300.524	3	1433.508	118.6957**
		Error	434.7783	36	12.07717	
		Total	4735.303	39		
	Alternative drink	Media	30081.47	3	10027.16	1840.854**
		Error	196.0924	36	5.447012	
		Total	30277.56	39		

• Significant at 0.01 level; ** significant at 0.0001 level

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