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RESEARCH ARTICLE

EFFECT OF ENERGY DRINKS ON RESISTANCE TO STARVATION OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

Organisms require energy for growth, development, reproduction, resistance to environment variables. In the present study effect of synthetic and natural energy drinks on resistance to starvation has been studied using *D.melanogaster*. It was noticed that larvae fed on natural drink consumed significantly greater quantity of food compared to larvae fed on synthetic energy drink and normal media, Further larvae and flies grown on natural energy drink based media had significantly greater resistance to starvation than those larvae and flies grown in synthetic energy drink and normal media, furthermore larvae and flies first treated with sub lethal temperature (37˚C for 15min) and cold treatment (-4˚C for 15min) then subjected to resistance to starvation also yielded similar results, Females had greater resistance to starvation than unmated . In mated flies males had significantly greater resistance to starvation than females while the reverse trend was noticed in unmated flies. Thus, these studies suggest that natural energy drink has beneficial over synthetic energy drink in *D. melanogaster*

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INTRODUCTION

A variety of factors may known to effect organisms stress tolerance. Through physiological as well as behavioral changes. Which inturn affect life history traits such as fecundity, fertility, longevity and stress resistance, it was also shown that climatic changes met by an organism may also cause physiological changes such as, hardening process, coma, production of metabolites, making an organism tolerate to temperature extremes (Sørensen, Nielson *et al.,* 2005; Lalouette, Kostal *et al.,* 2007). Further diet restriction or mild starvation can also increase tolerance to stress, such as Thermal stress (Wenzel, 2006; Smith, Hoi *et al.,* 2007) Recently, studies have shown that amount and quality of nutrients intake by organisms have a strong impact on life history, traits such as disease vulnerability, fertility, reproduction, longevity and stress resistance (Prasad *et al.,* 2003; Jenkins *et al.,* 1997; Bijlsm *et al.,* 1996) The bulk of studies on physiological and evolutionary responses to nutrients deficiencies focus on reproduction and fecundity (Bauerfeind *et al.,* 2005; Markow *et al.,* 1999; Sisodia and Singh, 2012)

Nowadays there are growing evidences suggesting that the people of all the age groups prefers many of the Synthetic energy drinks, which often consists of many synthetic additives which may have deleterious effect on the development of an organism example they cause obesity, diabetes, cancer. Therefore, there is a need to have a natural energy drink which should not contain any synthetic additives. Therefore there is a need to evaluate the benefit of natural energy drinks over synthetic energy drinks, present study has been undertaken in *D.melanogaster* to understand the effects of synthetic energy drinks and natural energy drinks on resistance to starvation

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MATERIALS AND METHODS

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 Twenty 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) 20 flies per culture bottle were maintained at 22˚C with a relative humidity of 70%in a 12 hrs dark;12 hrs 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) and 100 eggs were placed separately for normal media/ 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). Flies obtained from these eggs were used in the present experiment.

Quantification of Food intake in Larvae using dye method

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

Energy drinks (synthetic and Natural) effect on larval resistance to starvation of *D. melanogaster*

Ten larvae (grown in Normal media/Synthetic energy drink based media/Alternative natural energy drink based media) were placed in 10% Non nutritive agar media (12g of agar and 2g of p-hydroxybenzoic acid is boiled with 23ml of ethanol in a liter of water and placed in 22± 1˚c, 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 3 replicates (3×10=30 larvae) were run separately for each of Synthetic/ natural/ normal media grown larvae. One way ANOVA followed by Tukey's post hoc test has been carried out on the above data

Energy drinks (synthetic and Natural) effect on mated/unmated flies resistance to starvation of *D.melanogaster*

Four days old ten adult unmated/Ten days old mated flies obtained separately (grown in Wheat cream agar media/Synthetic energy drink based media/ natural energy drink based media) were placed in 10% Non nutritive agar media (12g of agar and 2g of p-hydroxybenzoic acid is boiled with 23ml of ethanol in a liter of water and placed in 22± 1˚c, the duration of days the flies were able to survive without food was recorded by observing the vials at 4 hrs intervals till all the flies had perished. A total of 3 replicates (3×10=30 flies) were run separately for each of Synthetic/ natural/ Normal media grown flies. Data was subjected to two way ANOVA followed by Tukey's post hoc test

Energy Drinks' (Synthetic and Natural) Effect on larval resistance to Thermal stress of *D.melanogaster*

Ten larvae (grown in Synthetic/Natural/Normal based media) were placed in a sub lethal temperature of 37˚c. The vials were plugged with a damp cotton roll which was moistened using saline or PBS to prevent desiccation and to prevent the larval crawling freely. Following treatment these larvae were transferred to vials containing non nutritive media and their survival was recorded at 2hrs interval until these larvae perish. A total of 3 replicates (3×10=30 larvae) were run separately for each of the three different media used in the experiment. One way ANOVA followed by Tukey's Post Hoc test was carried out on the above data

Energy drinks' (synthetic and Natural) effect on unmated/mated flies for resistance to starvation after thermal treatment in *D.melanogaster*

Four days old unmated and mated flies were taken separately (grown in Synthetic/Natural/Normal media) These flies were treated with sub lethal temperature of 37˚c for 15mins. The vials were plugged with a damp cotton roll, which was moistened using saline or PBS to prevent desiccation. These treated flies were transferred to non-nutritive media and placed in 22±1˚c and their survival was recorded by observing the vials at 2 hour intervals till all the flies had perished. A total of 3 replicates (3×10=30 flies) were run separately for each of Synthetic/Natural/Normal media grown flies. Two way ANOVA followed by Tukey's Post Hoc test was carried out on the above data

Energy Drinks' (Synthetic and Natural) effect on larval resistance to starvation after Cold stress in *D.melanogaster*

Ten larvae (grown in Synthetic/Natural/Normal media) these larvae were treated with (-4)˚c for 15 minutes in an empty culture vials which were pre-chilled to (-4)˚c. The vials were plugged with a damp cotton roll which was moistened using saline or PBS to prevent desiccation and to prevent the larval crawling freely. Following treatment the larvae were transferred to non-nutritive media and placed in 22±1˚c. and their survival was recorded until all the larvae perished at 2hrs intervals. A total of 3 replicates (3×10=30 larvae) were run separately for each of Synthetic/Natural/Normal media grown larvae. One way ANOVA followed by Tukey's Post Hoc test was carried out on above data.

Energy drinks' (synthetic and Natural) effect on mated and unmated flies resistance to starvation after Cold stress in *D.melanogaster*

Four days old adult unmated flies/ mated flies were taken separately (grown in Synthetic/Natural/Normal media) these flies were treated in (-4)˚c for 15 minutes in an empty culture vials which were Pre-chilled to (-4)˚c. The vials were plugged with a damp cotton roll which was moistened using saline or PBS to prevent desiccation. Following treatment the flies were transferred to non-nutritive media and placed in 22±1˚c.and their survival in non nutritive media was recorded at 2hrs intervals. This mortality assay was continued until all the flies were perished. A total of 3 replicates (3×10=30 flies) were run separately for each of Synthetic/Natural/Normal media grown flies. Two way ANOVA followed by Tukey's Post Hoc test was carried out on the above data.

Statistical Analysis

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

RESULTS

Figure 1 shows Food intake by a larvae was measured using dye method. It was found that the larvae which were grown in Natural energy drink based media have consumed more amount of food compared to larvae which were grown in Synthetic energy drink based media and wheat cream agar media.

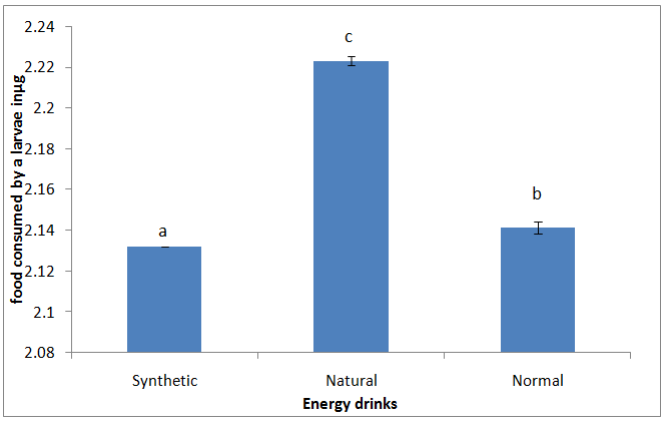

Figure 1. Effect of synthetic and natural energy drink on feeding behavior in larvae of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

One-way ANOVA followed by Tukey's Post Hoc test carried out using SPSS version 14.0 on the above data showed significant variation in feeding rate (Table 1).

Mean data of larval resistance to starvation to different fruit based media (Figure 2) It was observed that larvae fed on Natural energy drink based media survived longer when compared to those fed on synthetic as well as normal media based larvae , Two-way ANOVA followed by Tukey's post hoc test Table 2. Showed significant difference in resistance to starvation between different media. Tukey's Post Hoc test showed larval resistance to starvation was significantly greater compared to larvae grown on synthetic energy drink or normal media

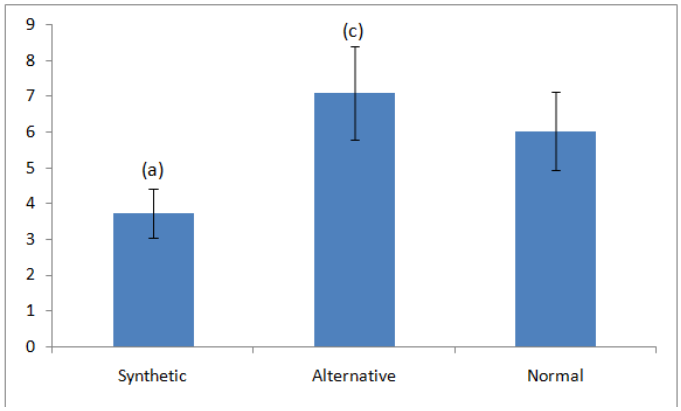

Figure 2. Effect of synthetic energy drink and Natural natural energy drink on effect of starvation resistance in third instar larvae of *D. melanogaster* Different letters on the bar graph indicates significance at 0.05 level by Tukey's Post Hoc test

Figure 3 shows, adult resistance to starvation. It was observed that flies grown on natural energy drink based media survived maximum number of days when compared with flies grown on synthetic energy drink and normal media based flies. Two-way ANOVA followed by Tukey's post hoc test carried out for above data showed Tukey's Post Hoc test showed significant variation among mated and unmated flies (Table 2)

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

Dependent Variable	Energy drinks	Source	Sum of Squares	Df	Mean Square	F-Value
Larval feeding in (µg)	Synthetic	Media	1.12848	3	0.37616	1684.48**
		Error	0.025904	116	0.000223	
		Total	1.154383	119		
	Alternative	Media	0.150647	3	0.050216	295.6858**
		Error	0.0197	116	0.00017	
		Total	0.170347	119		

** significant at 0.0001 level

Table 2. Two way ANOVA of ' Synthetic and Alternative energy drink' effect on Starvation resistance in *D melanogaster*

Dependent Variable	Energy drink	Source	Sum of Squares	Df	Mean Square	F-Value	Sig
Starvation Resistance in larvae and Adult flies of *D.melanogaster*							
Starvation resistance in Larvae of *D.melanogaster*(hrs)		Media	0.150279	2	0.07514	505.4546	1.27E-48
		Error	0.012933	87	0.000149		
		Total	0.163212	89			
		Gender	10.75556	1	10.75556	0.128936	0.719973
		Source	5048.711	2	2524.356	30.26166	5.27E-12
		Gender*Source	32.17778	2	16.08889	0.192872	0.824764
	males	Error	14514.67	174	83.41762		
		Total	80400	180			
		Gender	8.888889	1	8.888889		
Starvation resistance in adult flies of *D.melanogaster* (hrs)						0.112832	0.737348
		Source	7093.333	2	3546.667	45.01984	1.75E-16
		Gender*Source	214.0444	2	107.0222	1.358494	0.25976
	females	Error	13707.73	174	78.78008		
		Total	75104	180			

Table 3. Two way ANOVA of ' Synthetic and Alternative energy drink' effect on Thermal shock resistance and Cold shock resistance in *D melanogaster*

Dependent Variable	Energy drink	Source	Sum of Squares	Df	Mean Square	F-Value	Sig
Thermal stress Resistance in larvae and Adult flies of *D.melanogaster*							
Effect of Thermal stress in larvae		Media	1207.822	2	603.9111	13.2055	9.81E-06
		Error	3978.667	87	45.7318		
		Total	5186.489	89			
Effect of thermal stress in adult flies of *D.melanogaster*	males	Gender	142.2222	1	142.2222	2.16721	0.142788
		Source	2579.733	2	1289.867	19.6552	2.01E-08
		Gender*Source	470.5778	2	235.2889	3.58538	0.029793
		Error	11418.67	174	65.62452		
		Total	51936	180			
	females	Gender	1456.356	1	1456.356	22.7664	3.86E-06
		Source	2184.533	2	1092.267	17.0748	1.69E-07
		Gender*Source	284.4444	2	142.2222	2.22328	0.111319
		Error	11130.67	174	63.96935		
		Total	50336	180			
Cold stress resistence in larvae and Adult flies of *D.melanogaster*							
Effect of cold stress in larvae		Media	483.2889	2	241.6444	12.8346	1.3E-05
		Error	1638	87	18.82759		
		Total	2121.289	89			
Effect of cold stress in adult flies of *D.melanogaster*	males	Gender	1868.889	1	1868.889	25.7555	9.88E-07
		Source	235.5111	2	117.7556	1.62281	0.200314
		Gender*Source	282.9778	2	141.4889	1.94989	0.145386
		Error	12625.87	174	72.56245		
		Total	120816	180			
	females	Gender	3748.023	1	3748.023	64.1180	1.65E-13
		Source	1411.584	2	705.7921	12.0741	1.23E-05
		Gender*Source	875.2933	2	437.6466	7.48689	0.000761
		Error	10112.73	174	58.45507		
		Total	126112	180			

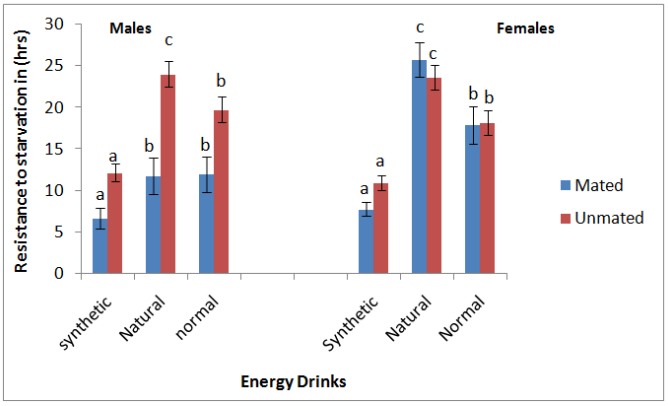

Figure 3. Effect of synthetic and natural energy drink on resistance to starvation in mated males and females of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

Data of larval resistance to starvation after thermal treatment is provided in Figure 4. It was observed that larvae fed on natural energy drink based media survived longer when compared to those larvae grown on synthetic Energy drink as well as normal media based larvae, above data was subjected to ANOVA, followed by Tukey's Post Hoc test showed significant difference between different traits

Figure 5. Represents the mean days survived when unmated/mated flies were subjected to resistance to starvation after thermal stress, It was observed that unmated flies grown on natural energy drink based media survived maximum number of days when compared with synthetic energy drink and normal media based files. In case of mated flies males survived longer than females, whereas in unmated flies females survived longer compared to males.

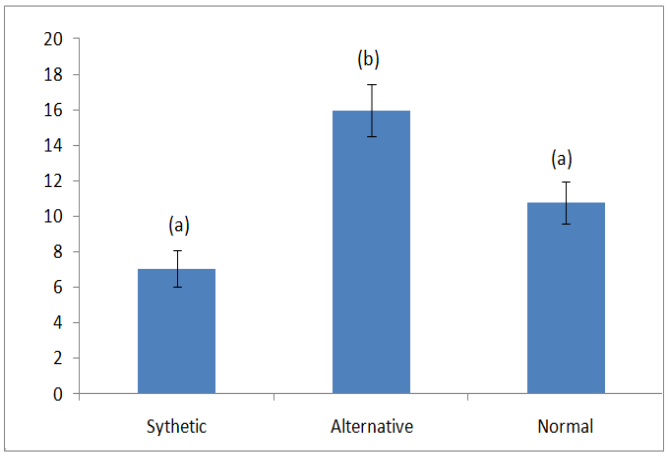

Figure 5. Effect of synthetic energy drink and natural energy drinks on resistance to starvation after the thermal treatment of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

Two-way ANOVA test followed by Tukey's Post Hoc test showed significant variation between mated and unmated flies between different media.

Figure 6. Shows mean larval resistance to starvation after cold treatment. It was observed that larvae fed on natural energy drink based media survived longer when compared to those larvae grown on synthetic Energy drink as well as normal media based larvae Two way ANOVA followed by Tukey's Post Hoc test showed that significant difference between different media

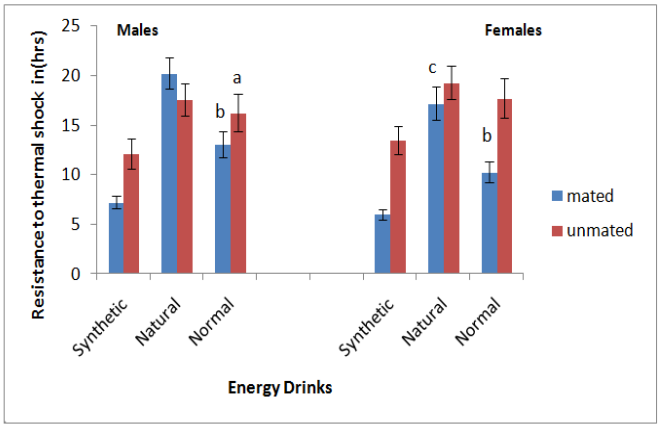

Figure 6. Effect of synthetic and natural energy drink on Adult resistance to starvation of Thermal treatment of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

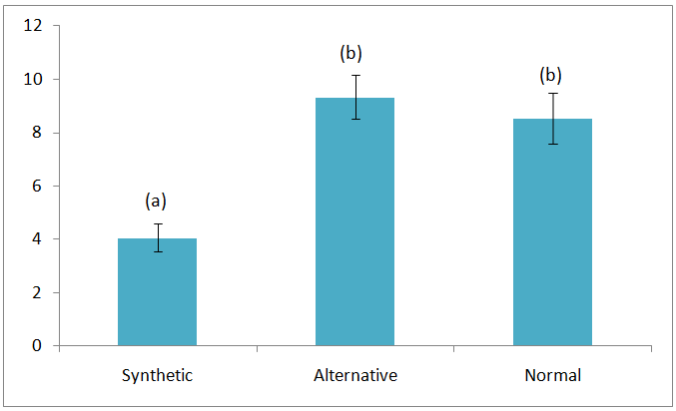

Figure 8. Effect of synthetic energy drink and natural energy drinks on resistance to starvation after cold treatment of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

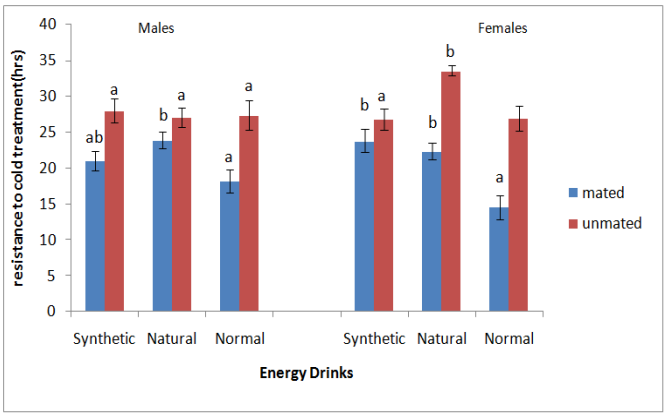

Figure 9. Effect of synthetic and natural energy drink on Cold shock resistance in mated males and females of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

Mean data of unmated/mated flies to resistance to starvation after cold treatment it was found that, flies grown on natural energy drink based media survived longer than those flies grown synthetic energy drink and normal media based flies. Two-way ANOVA test followed by Tukey's Post Hoc test showed significant variation in different media as well as mated and unmated flies

DISCUSSION

Variation in stress related traits in insects in other organisms has been widely studied because it underlies the ability of insects to adopt and counter the effects of changing climatic conditions, for instance in Drosophila a high level of desiccation resistance is associated with adaption to and habitats while a high level of cold resistance is linked to adaption of high latitudes. The Figure 1 and Table 1 reveal that the feeding rate of *D.melanogaster* was found to be different, vary between Natural energy drink and Synthetic energy drinks among three the feeding rate was found to be highest in Natural energy drink followed by Normal 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 Natural energy drink when compared to Synthetic energy drinks as well as Normal media since the rate of larval feeding was highest among larvae fed on Natural energy drinks.

Fig. 2 and Table 2. Reveal that larval resistance to starvation was found to be significantly greater in larvae grown in natural energy drinks, based media compared to larvae grown in normal and synthetic energy drink based media. This suggests that the quantity and quality of natural energy drinks, based media was found to be beneficial to the organisms to resist starvation, than those nutrients found in normal and synthetic energy drink based media. Even in the adult both mated and unmated flies grown in normal, synthetic and natural energy drink based media showed significant variation in resistance to starvation. Greater resistance to starvation was noticed in flies reared in natural energy drink based media and least resistance was noticed in flies grown in synthetic energy drink based media. This suggests that the use of synthetic energy drink is not beneficial as they contain additive and has less energy support to organisms. On the other hand natural energy drink because of the presence of natural fruits like Pomegranate, Apple, Orange, Banana, They contain proteins, vitamins, calcium, Iron and other minerals they were responsible for its greater resistance to starvation

In the present study resistance to starvation was also assessed even after treating in sub lethal temperature (37˚C, 15min) and cold treatment (-4˚C, 15min). Even in these studies also it was found out that larval and adult both mated and unmated flies, resistance to starvation was significantly greater in flies grown in natural energy drinks than flies grown in normal and synthetic energy drink based media. Our study also support the work of Sisodia and Singh (2012) who while working in *D.ananassae* have found that variation in carbohydrates and protein in the diet can had significant effect on resistance to starvation. Thus, these studies in *D. melanogaster* suggest that use of natural energy drinks is beneficial over synthetic energy drinks

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