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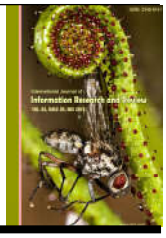

Research Article

EFFECT OF ENERGY DRINKS' (NATURAL AND SYNTHETIC) ON OVARIOLE NUMBER AND BODY SIZE OF *D.MELANOGASTER*

Alwyn D'souza and *Krishna, M.S.

Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore - 560006. Karnataka, India

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ABSTRACT

Dietary composition is one of the primary experimental factors that affects many aspects of animal physiology including life span, general health, and reproductive potential hence present study has been undertaken in *Drosophila melanogaster* to study effect of synthetic and natural energy drink based media on ovariole number and wing length, In the present study the flies of *Drosophila melanogaster* reared in synthetic and natural energy drink based media were used the results showed that the flies fed on natural energy drink had consumed significantly greater quantity of food compared to flies fed on synthetic energy drinks as well as normal media, It was also noticed that the ovariole number and wing length were significantly greater in flies reared in natural energy drinks than those flies reared in natural energy drinks than those flies reared in synthetic and normal, Thus these studies suggests that the dietary composition of natural energy drinks had increased female fitness in *Drosophila melanogaster*

INTRODUCTION

Dietary compositions are known to have profound effect on many aspects of animal physiology, including life span, general health, reproductive potential and also body size of an organism (Anderson, 1994) Studies have shown in animals that body size is an important trait associate with the reproductive success,(Santosh.*et.al.*1992), It is generally believed that larger the individuals greater is the fitness i.e. larger females can carry more ovarioless than small females, Thus fitness in general is believed to be an increasing function of body size in animals, particularly among insects (Krishna and Hegde, 2003).

Female reproductive success depends on the number of eggs laid by female in her life span, whereas male reproductive success depends on number of females he could inseminate in his life span, (Anderson,1994; Krishna and Hegde, 2003), In species of *Drosophila* it was shown that ovarioles number is one of the important life history trait and it is positively correlated with female fecundity (Krishna.*et.al.,* 2012; Sisodia and Singh, 2012). Numerous factors are known to affect ovarioles number in species of *Drosophila* such as temperature, diet etc (Sisodia and Singh, 2012). However the effect of synthetic and natural energy drinks on ovarioles number and body size has not been studied in *D.melanogaster,* Hence the present study has been undertaken in *D.melanogaster* to evaluate the effect of natural and synthetic energy drinks on ovariole number and body size.

Corresponding author: Krishna, M.S.
Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore - 560006. Karnataka, India

MATERIALS AND METHOD

Establishment of Stock

Progenies of 105 naturally inseminated females of *D.melanogaster* collected at Chamundi hills, Mysore, India. Was used to establish experimental stock, 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

To study larval feeding rate ten Second instar larvae obtained from normal media were used. 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 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 wing length and ovariole formation in *D.melanogaster*

Four day old virgin females were etherized. The abdomen of these flies was dissected out using a pair of fine dissection needles in physiological saline under a binocular stereomicroscope. The ovarioles of both ovaries were separated and the total number of ovarioles in either the left or right ovary was noted. The wings of the same fly were measured under a micrometer with a correction factor of 0.0165 using the procedure of Krishna and Hegde (1997).

RESULTS

Figure 1 shows Food intake by a larva was measured using dye method. It was found that the larvae which were grown in natural energy drink based media have consumed more amount

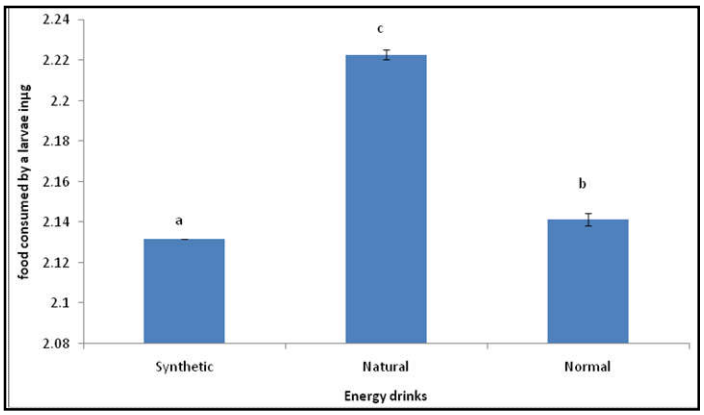

Figure 1. Effect of synthetic and natural energy drink on feeding behavior in larvae of *D. melanogaster.* [Different letters on the bar graph indicates significance at 0.05 level by Tukey's Post Hoc test]

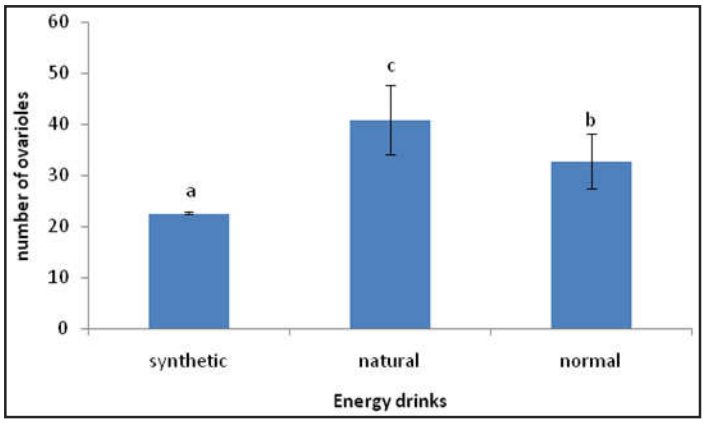

Figure 2. Effect of synthetic and natural energy drink on ovariole number of *D. melanogaster.*. [Different letters on the bar graph indicates significance at 0.05 level by Tukey's Post Hoc test]

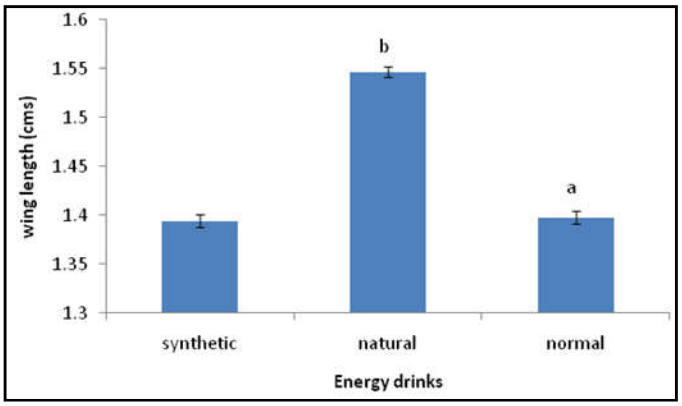

Figure 3. Effect of synthetic and natural energy drink on wing length of *D. melanogaster.* Different letters on the bar graph indicates significance at 0.05 level by Tukey's Post Hoc test

of food compared to larvae which were 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 variation in feeding rate (Table 1). Figure 2 shows ovariole numbers found in flies grown in Synthetic, Natural energy drink based media and Normal media.

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

Dependent Variable	Energy drink	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. One way ANOVA of ' Synthetic and Natural energy drink' effect on overole number and wing length in *D melanogaster*

Dependent Variable	Energy drink	Source	Sum of Squares	Df	Mean Square	F-Value
Overrule number	Synthetic and Natural	Media	16824.89	2	8412.443	307.9749**
		Error	8112.66	297	27.31535	
		Total	24937.55	299		
Wing length	Synthetic and Natural	Media	1.511991	2	0.755996	192.4736**
		Error	1.166553	297	0.003928	
		Total	2.678544	299		

** significant at 0.0001 level

It was noticed that ovariole number was found to be highest in flies grown in natural energy drink based media, the sequence of ovariole numbers in different media as follows, natural energy drink > normal and normal media > synthetic energy drink based media flies. The above data was subjected to one way ANOVA followed by Tukey's Post-Hoc test showed significant variation in ovariole number between flies grown in different media used, Tukey's Post-Hoc test showed that ovariole number was found to be significantly greater number in flies grown in natural energy drink than those flies grown in synthetic energy drink and in normal media. Mean wing length of flies grown in synthetic, natural energy drink based media and normal media are provided in figure 3, It was noticed that mean wing length of flies grown in natural energy drink based media was found to be larger compared to flies grown in Synthetic energy drink based media as well as normal media. One way ANOVA followed by Tukey's Post-Hoc test carried out on mean wing length data showed significant variation in mean wing length between flies grown in different energy drinks based media. Tukey's Post-Hoc test also revealed that mean wing length of flies grown in natural energy drink based media had significantly longer wings compared to flies grown in other media.

DISCUSSION

The Figure 1 and Table 1 reveal that the larval feeding rate of *D.melanogaster* was varied between Natural, Synthetic energy drinks and normal media. 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 stages shows an inhibition threshold when consuming a new or foul tasting foods (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. Figure 2 and table 2 revealed that flies grown on natural energy drinks had significantly greater number of ovarioles than those flies grown on synthetic energy drinks and in normal media.

This suggests that diet of female had significant influence on female fitness in *D.melanogaster.* This result also confirms earlier studies of dietary effect on growth development, and fitness in species of *Drosophila*(Sisodia and Singh, 2012; Alwyn and Krishna, 2015). Further Sisodia and Singh, (2012). Who while studying in *D.melanogaster* have also shown that ovariole number was found be significantly greater in females fed on protein rich media when compared to flies fed on carbohydrates rich diet suggesting that quality of the diet (nutrients) found in the food had significant effect on the female fitness. In our study synthetic energy drinks had various additives such as caffeine, taurine etc where as natural energy drinks has calcium, vitamins, proteins, antioxidants etc therefore greater number of ovarioles in the natural energy drinks could be due to the nutrients present in it, Further larvae fed on natural energy drinks had also consumed greater quantity of food this also had contributed occurrence of greater number of ovarioles. In animals compelling evidence suggests that fitness of an organism is directly related to body size, "Bigger is Best" hypothesis has also been proposed suggesting that larger females always carry greater number of ovarioles than small females (Krishna and Hegde, 1997).

In species of *Drosophila* wing length is used as an index of body size, larger the wing length larger the body size (Partridge *et.al.,* 1987; Krishna, 1997). Therefore in the present study wing length of flies grown in synthetic and natural energy drinks had been measured Figure 3 and table 2 reveled that flies grown on natural energy drinks had significantly larger body size than those flies grown in synthetic energy drinks and in normal media. A positive association between body size and ovariole number has also been noticed in species of *Drosophila* (Krishna and Hegde, 1997; Sisodia and Singh, 2012). In present study also positive association has been found between ovariole number and female size. Thus these studies suggests that

natural energy drinks had greater female fitness in *D. melanogaster* and increases female reproductive success.

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