has adapted well to environment with intense natural radiation, as observed in Lajes Pintadas. Such adaptation may indicate a given degree of radioresistance acquired recently, since the species' arrival in the last years in the region, in what should be more thoroughly investigated in future studies.

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Feeding behavior and nutrients act concertedly in determining fecundity and lifespan in *Drosophila nasuta nasuta.*

Neethu, B.K.[1], and B.P. Harini[1*]. [1]Department of Zoology, Bangalore University, Bangalore-560 056, Karnataka, India; e-mail: bpharini@ yahoo.co.in

Abstract

Feeding behavior is an important element in insects which involves the detection, the initiation of ingestion, and the consumption of food. The larval and adult stages of *Drosophila nasuta nasuta* were fed in combination of restricted glucose with varied concentrations of methionine. These enriched nutrients of restricted glucose and methionine showed greater influence on the fecundity of flies, and subsequently was less affected with respect to lifespan along with control and single concentrations of methionine. The gustatory feeding assay was performed in larval and adult stages with combination of restricted glucose and varied methionine concentrations and single methionine concentrations along with control. It revealed that the former diet has led to increased mortality, while decreased mortality with only methionine fed diet (*i.e.*, 0.03% g/L) in absence of glucose and were significant with control. Thus, the present study indicates that the feeding behavior and nutritional composition act concertedly to determine fecundity and lifespan. In addition, the flies fed *ad libitum* are capable of restricting their feeding behavior in response to their nutritional state.

Introduction

Dietary restriction (DR), the reduction of nutrient intake short of malnutrition, appears to improve measures of human health and extends the lifespan of various organisms ranging from yeast to primates (Fontana *et al.,* 2009). Dietary restriction (DR) refers to a moderate reduction of food intake that leads to extension of lifespan beyond that of normal, healthy individuals. Many organisms face a challenge of meeting their optional nutritional requirement for somatic and reproductive growth under natural conditions. Nutrition plays prominent roles in aging, health, metabolism, and disease (Min and Tartar, 2006). Unlike mammals, where the foetus develops in the mother's womb, fertilized eggs of insects develop outside the female body (Starr *et al*., 2008). All insects go through a larval phase of development that precedes metamorphosis (Aguila *et al*., 2007). Soon after hatching, larvae forage for nutrients in their vicinity and eventually find a favorable niche that contains an adequate supply of food. This phase of insect development is dedicated to feeding voraciously, and fulfils the much-needed food requirements for the subsequent non-feeding pupal phase. Ingested nutrients are stored as fat bodies (Tian *et al*., 2010), which serve as potential energy reservoirs for synthesis of macromolecules that are essential for cellular growth during larval and pupal stages (Chapman, 2013).

Carbohydrates are important dietary components for many omnivorous and herbivorous animals, including both humans and livestock. Carbohydrates provide energy for many reactions and processes flowing inside cells. Most organisms can tightly adjust their metabolism according to the availability of dietary components, including carbohydrates. Physiological effects of carbohydrates depend on their type and dosage, as well as on the physiological state of an organism (Wheeler and Pi-Sunyer, 2008). However, the effect of carbohydrate diets, and particularly the type of carbohydrate, as well as the protein-to-carbohydrate ratio on life span and reproduction, are poorly investigated. They are generally studied in comparatively simple organisms like *Drosophila melanogaster*, which is intensively used as a model for nutritional studies. Over the last decade, several studies explored the effect of diet on life span, reproduction, behavior, and adaptation of fruit flies (Vigne and Frelin, 2010). In mammals, this manipulation, which is often called dietary restriction (DR), not only increases lifespan but also imparts a broad-spectrum improvement in health during aging.

Nutrient imbalance, particularly amino-acid imbalance, also received attention with regard to its influence on physiological function and modulation of aging. It was reported that disproportionate levels of amino acids may be associated with the incidence of adverse effects in a rat model. In particular, Met was defined as the most toxic amino acid when used in excess (Harper *et al*., 1970). Restricted use of amino acids was suggested to be responsible for the lifespan extension caused by protein restriction (Min *et al*., 2006); on the other hand, Met restriction was reported to be ineffective in extending lifespan of *Drosophila (*Grandison *et al*., 2009). This sensitivity to methionine may result from impairment of one or more aspects of eukaryotic methionine metabolism. These include the synthesis of polyamines, cysteine and glutathione, and the methylation of DNA, lipid, hormones and enzyme substrates (Finkelstein, 1990). Furthermore, comparing the effects of glucose restriction and methionine restriction might provide insight into generalizable pathways that modify longevity across species. Feeding behavior in many insects involves the detection of food, the initiation of ingestion, and the consumption of discrete meals (Bernays, 1985). The present study was undertaken to ascertain the various concentrations of dietary glucose and methionine content while holding all other nutrients constant so as to determine the effect of dietary methionine and restricted glucose on fecundity and lifespan in *Drosophila nasuta nasuta***.**

Materials and Method

Drosophila *strain, media, and culture conditions*

Drosophila nasuta nasuta stocks were maintained in an uncrowded culture condition at 22±1ºC, 70% humidity and 12h: 12h light and dark cycles in standard wheat cream agar medium. From the stock the virgin females and unmated males were collected within 6 hours of eclosion and were aged for 2days. On the third day a single virgin female and an unmated male were transferred to a fresh food media vial (25 × 100 mm) for egg laying. Likewise, three successive changes were made every alternate day. The said experiments were carried out by feeding different concentrations of methionine and restricted glucose concentration 30 g/L, (Bass *et al*., 2007) along with single concentration of methionine.(0.01 g, 0.02 g, 0.03 g/L) (Pletcher *et al*., 2002).

Fecundity assay

Fecundity was assayed by counting number of eggs laid. Flies were successively transferred into fresh vials containing media every alternate day for 6 days. Eggs were allowed to hatch till pupation. Further, the same sets of vials were assessed for the emergence of the adult flies and likewise the fertility was recorded for the total productivity (Harini, 2011).

Gustatory feeding assay

The method described by Lee *et al*. (2010) was adopted for gustatory assay. Larvae and adult flies were reared in media supplemented with experimental diet. A basal media control was also maintained. To perform a feeding assay, after starving the larvae and flies for 2 h, from each experimental group were transferred into the vials containing the specific diets with bromophenol blue dye (0.05% wt/vol). The absorbance of 100 times diluted supernatant was measured at 595 nm using a spectrophotometer.

Lifespan assay

Synchronous cultures of 2–3-day-old files were obtained as described earlier and transferred into vials containing basal medium supplemented with experimental and control. Each group including the control had 10 vials each with 14–20 flies per vial with equal sex ratio. Flies were transferred to fresh media every third day. Dead flies were counted and removed daily throughout the experiment.

Statistical Analysis

Mean fecundity and lifespan were subjected to one-way ANOVA, Tukey's HSD by using SPSS 20.0.

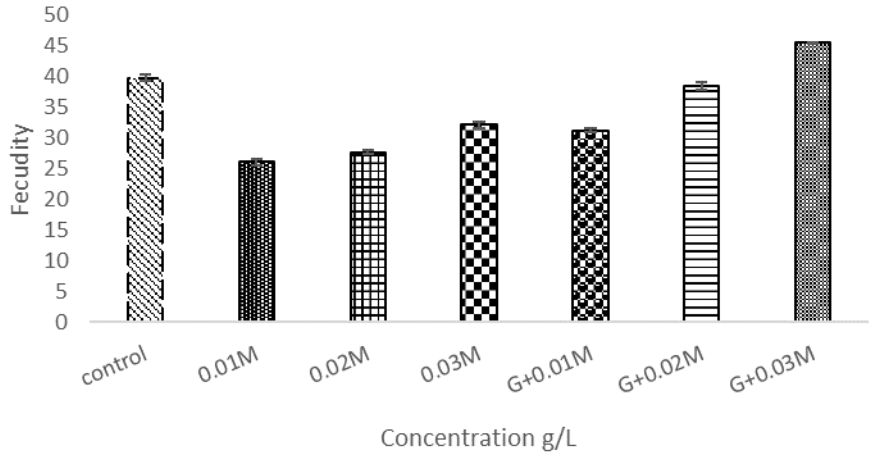

Figure 1. Mean (±SE) fecundity on exposure to single methionine concentrations and restricted glucose (G) with varied concentration of methionine (M) in *Drosophila nasuta nasuta*.

Results

Nutrients are important factors in determining the reproductive success and life-span of flies. In the present study it is reported that restricted glucose concentrations and methionine (*i.e*., G+0.03Mg/L) has enhanced the fecundity when compared with all the other concentrations of the fed experimental diet as shown in Figure 1. The analysis of variance for fecundity showed significant values in all the single concentrations of methionine (*i.e*., 0.01%, 0.02%, 0.03% g/L) and mixed concentrations of glucose and methionine with that of control ($p < 0.000$) as shown in Table. 1. The differences were insignificant in single concentration of methionine fed diet, *i.e*., 0.01% and G+0.01Mg/L concentration ($p > 0.05$). The lifespan of male and female on exposure to restricted glucose with methionine diet concentrations has not extended lifespan, whereas significant changes were observed in single concentrations of methionine ($p < 0.000$) (Figure 2). To ensure this observed change in lifespan of *Drosophila nasuta nasuta*, gustatory feeding assay was performed in both larvae and adult flies for varied dietary concentrations (Figure 3). The mean food intake in larvae decreased as the methionine concentrations increased, *i.e*., 0.01% to 0.03% (OD 595 nm = 0.80 (0.01 g/L), 0.69 (0.02 g/L), and 0.49 (0.03 g/L), and 30 g/L glucose restriction with varied methionine concentrations was recorded for values of OD 595 nm, *i.e*., 0.5, 0.3, and 0.05, respectively. In the adult flies, both male and female when fed with different dietary concentrations showed similar results in both single

concentration of methionine and mixed concentration of 30 g/L restricted glucose with methionine diet for male (*i.e*., OD 595 nm = 0.1 (0.01 g/L), 0.03 (0.02 g/L), 0.03 (0.03 g/L), 0.03, 0.01, and 0.07) and with further reduction in food taken by the adult female (*i.e*., OD 595 nm = 0.1 (0.01 g/L), 0.03 (0.02 g/L), 0.03 (0.03 g/L), 0.03, 0.01, and 0.06). Thus the data obtained reveal significant increase in the lifespan of females compared to males Table.1.

Table 1. Results of one-way ANOVA of mean fecundity and lifespan of *Drosophila nasuta nasuta* feeded with different concentration of experimental diets with control.

concentrations	N	Fecundity	Lifespan(Number of days)	
			Male	Female
Control	30	39.08 ± 0.13**a**	73.23 ± 0.08**a**	77.13 ± 0.04**a**
0.01 Methionine(M) g/L	30	26.10 ± 0.15**c**	73.23 ± 0.60**a**	77.26 ± 0.60**b**
0.02 Methionine(M) g /L	30	27.80 ± 0.12**b**	73.20 ± 0.06**a**	82.10 ± 0.02**c**
0.03 Methionine(M) g/L	30	32.76 ± 0.13**d**	76.60 ± 0.20**b**	86.90 ± 0.04**d**
30% glucose(G) + 0.01 Methionine(M) g/L	30	31.05 ± 0.05**b**	73.21 ± 0.50**a**	77.41 ± 0.30**a**
30% glucose(G) + 0.02 Methionine(M) g/L	30	38.02 ± 0.20**a**	73.02 ± 0.36**a**	77.05 ± 0.36**a**
30% glucose(G) + 0.03 Methionine(M) g/L	30	45.02 ± 0.50**c**	73.10 ± 0.36**a**	79.50 ± 0.06**b**
ANOVA		F = 354.626 d.f = 6,203 P = 0.05	F = 9.830 d.f = 6,203 P < 0.000	F = 61.90 d.f = 6,203 P < 0.000

Note: Mean in each column followed by different alphabitical letter with in the same life stage were significantly different by Tukey's HSD test (P < 0.05) where a, b, c, d repesents the significancant difference between diets.

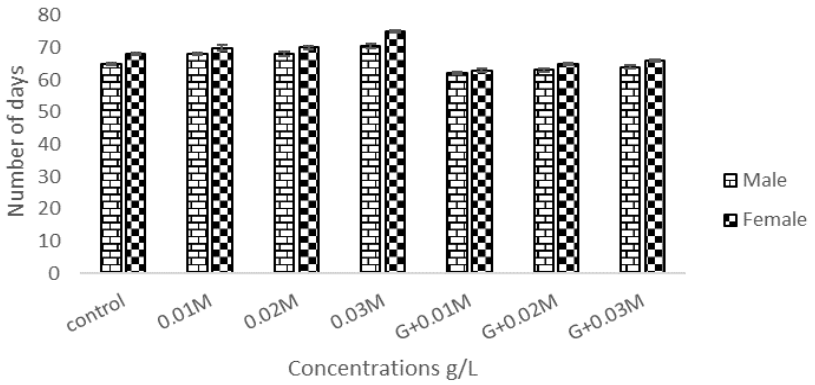

Figure 2. Mean (±SE) lifespan of *Drosophila nasuta nasuta* on exposure to single methionine concentrations and restricted glucose (G) with varied concentration of methionine (M).

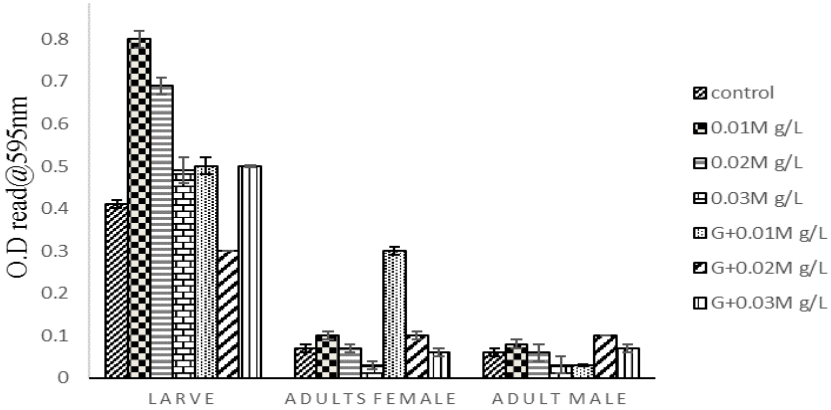

Figure 3. Gustatory feeding assay with single methionine concentrations and restricted glucose (G) with varied concentration of methionine (M) in *Drosophila nasuta nasuta*.

Discussion

"Dietary restriction", the experimental restriction of food and nutrient intake compared with *ad libitum* feeding, is a reliable means of extending lifespan in model organisms (Weindruch and Walford, 1988). The repeated observation that dietary restriction retards aging in phylogenetically diverse species ranging from yeast to primates is the cornerstone of a fertile working hypothesis that diet regulates lifespan and aging through a universal mechanism that has been conserved throughout evolution. Commonalities between life-extending mutations in several species suggest a plausible account of how genes and diet might regulate growth and aging by converging on energy and nutrient-responsive pathways. In view of this, the present study has found that the fecundity and lifespan of *Drosophila* can be modified by varying the dietary content of restricted glucose with methionine and single methionine concentrations.

Studies using more tractable insect models have shown that poor nutrition during development generally results in detrimental fitness effects including decreased size, fecundity, and life span (Dmitriew and Rowe, 2011). The most obvious way by which environmental variation may influence body condition and fecundity is via nutritional effects resulting from variability in food type availability. In general terms, diet effect can be classified as either quantitative (*i.e*., food availability) or qualitative (*i.e*., food composition). The quantitative effects are evident, since animals obtain energy and other nutritional requirements from food. Thus, under a natural range of conditions there is a positive correlation between food availability and body condition or fecundity. Qualitative effects often are divided into two categories: namely, nutritional deficiencies and inhibitory metabolites. In our study females fed with enriched diet have shown increased fecundity in restricted glucose with methionine diets, *i.e*., G+0.03M g/L and fecundity decreased in lower methionine (0.01M g/L and 0.02M g/L) with restricted glucose (30 g/L) and single methionine concentrations along with control showing significant ($P < 0.05$) with only the high methionine and restricted glucose. However, of the three methionine concentrations used, the higher concentration of methionine, *i.e*., 0.03%M g/L was optimal with regard to lifespan and showed significance with the control.

Demographic modelling has suggested that dietary restriction in *Drosophila* acts by lowering age-independent mortality rather than by slowing the accumulation of senescent damage (Partridge *et al*., 2005). Dietary restriction (DR) - restriction of one or more components of intake (typically macronutrients) with minimal to no reduction in total caloric intake – is another alternative to CR. While research suggests that neither carbohydrate restriction nor lipid restriction extend life (Sanz, 2006), protein restriction increases maximum lifespan by roughly 20% (Pamplona and Barja, 2006). This extension of life may be solely due to the reduction of the amino acid methionine (Caro *et al*., 2009). In accordance to the above study the flies fed with the high methionine concentration and restricted glucose diets ingested relatively less food in both males and females showed increased fecundity. A low intake of diet supplemented restricted glucose with varied methionine diet did not abrogate the lifespan. We also found that restricting calories merely by limiting glucose intake had only modest benefit for longevity and increase lifespan in single concentration of methionine. Thus the feeding behavior and nutrients have a sole impact on the productivity and longevity.

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A modified method to assay the effects of ethanol on the behavior of *Drosophila melanogaster.*

Badrinarayanan, Saishree[1], Saranya Balasubramanian[2], and V. Deepa Parvathi[1].
[1]Department of Human Genetics, College of Biomedical Sciences, Technology & Research, Sri Ramachandra University, Chennai – 600 116, INDIA; [2]Department of Biotechnology, Sathyabama University, Chennai-600119, INDIA.

Abstract

For over a long period of time, many scientists have frequently studied the fruit fly *Drosophila melanogaster* as a model organism to help elucidate the complex mechanisms that govern development and behavior. Recent advances in scientific research have enabled us to discover that certain strains of *Drosophila* are not only tolerant towards concentrations of alcohol, but also display many ethanol-induced behaviors resembling intoxication (*e.g*., loss of motor control). Hence, *Drosophila* makes an ideal model to study the effects of alcohol and deduce the neural circuitry involved in producing its intoxicating and rewarding effect. In the present study varying concentrations of ethanol were administered and its effect on the larval locomotion (Larval crawling assay), adult climbing ability (RING assay), and courtship behavior (Courtship and Mating assay) were assessed. It was found that after short term exposure of ethanol, larvae were found to have a decreased locomotor activity at 10% ethanol concentration; adult flies had shown a biphasic reaction towards the effect of ethanol. Mating behavior was affected by ethanol, with a reduction in the Courtship Index for flies that had been exposed to 20% ethanol (loss of postural control and instability in balance was observed). Most of the circuits governing these behaviors involve the inhibition and excitation of certain neurotransmitters, which are conserved between humans and flies. These results indicate that studies using *Drosophila* as a model system may help in understanding how ethanol influences behavior, which is vital to decipher the mechanisms of action of ethanol and alcoholism. Keywords: *Drosophila melanogaster*; alcohol; behavior; larva.

Introduction

The human society has resorted to the use of alcohol for a variety of reasons. For more than a thousand years, documentation of its use as a part of food production, medicine, mood changers, and also as an intoxicant has been kept. Along with their uses, the adverse effects of alcohol have also been documented to as far as written records have existed. However the mechanisms responsible for alcohol related behavior and alcohol addiction are still poorly understood.

Complex genetic and environmental factors contribute to a predisposition to drug addiction. The ability to modulate the genetic conditions, which are likely multigenic and heterogeneous make the process of identifying specific genes responsible for addiction a difficult task.

As a model organism *Drosophila* has been instrumental in providing insights into the various molecular (Ulrike, 2000) and neural mechanisms underlying addiction and intoxication (Devineni, 2000).