ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Enhanced protein diet accelerates the rate of development -A study in *Drosophila nasuta nasuta*

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Manuscript Info

Manuscript History:

Received: 14 December 2013
Final Accepted: 19 January 2014
Published Online: February 2014

Key words:
Drosophila nasuta nasuta, protein (Brewer's yeast), Dietary restrictions, developmental time, viability

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Abstract

Effects of dietary conditions on many life history traits have been studied for a long time with *Drosophila*. The present study is aimed to show the influence of protein on development time and rate of development. *Drosophila nasuta nasuta* flies were fed on the different concentrations of protein (Brewer's yeast) with sugar (Glucose), showed significant differences in relation to developmental time and rate of development. As observed, the flies reared on high protein diet showed significantly faster metamorphosis and decreased viability

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Introduction:

Animals obtain energy and nutrients from food, so diet can be considered a key factor that potentially affects all life-history components (Taylor et al., 2005). Experimental modifications of animal diets have played a key role in the study of how organisms adjust their energy allocation (Cruz-Neto and Bozinovic, 2004). The intra- and interspecific variability of life history traits can be explained not only by the genetic constitution of species or populations but also by environmental effects (food abundance, heat, etc.), and genotype by environment interaction (James et al., 1997; Gibert et al., 2004; Lazzaro et al., 2008). Stress can be defined as any environmental factor that acts to reduce the fitness of an organism. Thus, almost by definition, environmental stress is one of the most important sources of natural selection, as certified by many specific adaptations evolved to alleviate the consequences of stress (Hoffman and Parsons, 1991). One of the most ubiquitous causes of stress, at least for animals, is shortage or suboptimal quality of food. Many species must cope with periodical malnutrition or starvation, and even those for which food may seem abundant (e.g. herbivorous insects) may be limited by availability of specific nutrients and the need to cope with toxic secondary chemicals (White, 1993).

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. The balance between energy intake and expenditure is necessary to the survival and reproductive success of animals (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).

Dietary restriction (DR) in *Drosophila* is often achieved by dilution of the food medium, and complete records of food intake are needed to determine if flies compensate for the reduced nutritional content of food by increasing the total amount of food they consume. Developmental time, a very important life history trait, is largely affected by environmental conditions (James and Partridge, 1995). *Drosophila* is an organism that breeds and feeds in

ephemeral substrates; therefore, the larval developmental time is a very important trait (Chippindale et al., 1997; Soto et al., 2006; Folguera et al., 2008). Nutritional manipulation is one of the mostly used ways to expose the effects of food as an environmental variable on aging and development of the organisms. Important levels of genetic variation in developmental time occur in natural populations (Cortese et al., 2002; Fanara et al., 2006). There are various DR studies that were focused on the adult stage of *Drosophila*, but only a few studies were conducted to investigate the effects of DR on juvenile stages (Tu and Tatar, 2003).

The *Drosophila nasuta nasuta* subgroup, belonging to the *Drosophila immigrans* species group of *Drosophila* has attracted the attention of taxonomists, cytogenetics, biochemists, molecular and evolutionary biologists (Nirmala,1973; Ranganath, 1975, 1978, 2002; Ramachandra and Ranganath,1988), morphometric, reproductive (Harini and Ramachandra, 2003), and allozyme analysis (Kitagwa et al.,1982) have been extensively studied.

In view of this, the study is mainly focused to imply the stress in the form of variable nutritional composition (protein) and glucose through the food media to assess the impact on the rate of development and viability in *Drosophila nasuta nasuta.*

Materials and method:

Fly Stocks

Drosophila nasuta nasuta stock was obtained from *Drosophila* stock centre, Department of studies in Zoology, University of Mysore and Mysore, India. The stocks were maintained in an uncrowded culture condition at 22±1˚C, 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 third day a single virgin female and an unmated male was transferred to a fresh food media vial (25 X 100mm) for egg laying, likewise three successive changes were made every alternate days. Further, the eggs laid were recorded for hatchability, pupation and adult eclosion (Harini, 2011). Simultaneously, the time taken from egg to adult eclosion was assessed in terms of rate of development in number of days. The said experiments were carried out by feeding different concentrations of protein (Brewer's yeast) i.e 5g/L, 15g/L, 25g/L and fixed glucose (30g/L) Bass *et al.* (2007a) through the food media along with the control as provided in Table 1.

Statistical Analysis

Mean egg-to-adult developmental times and viability (egg laid, larval hatchability, pupation and adult emergence) were subjected to One-way ANOVA, Tukey's HSD by using SPSS 17.

Results and discussion:

The developmental time significantly shortened (by 8 days) on exposure to high protein (Brewer's yeast) concentration of (25g yeast) than in control (9-11days) while the flies on exposure to 5g and 15g of yeast concentration the number of days taken to develop from egg to adult was similar with that of control. The differences were insignificant from egg to larval hatchability, while it was significant from pupa to adult eclosion ($P<0.01$) with that of control (Fig.1). The time taken to develop from egg to adult lasted for 6 days in the flies raised on the high protein diet, while flies which were raised on the low protein diet had a significantly longer metamorphic stage (9-11days). Hence one can conclude that the enrichment of protein concentration would yield faster metamorphosis. Fig.2 signifies the mean viability in terms of hatchability, pupation and adult emergence of *Drosophila nasuta nasuta* on exposure to different concentrations of protein diet. Interestingly, the mean viability significantly varies with for all the observed traits in control and experimental diet. The flies exposed to increased concentration of protein showed decreased percentage of viability (larval hatchability, pupation and fertility).

The analysis of variance (Table 3) indicates significant difference for all the concentration of protein with that of control ($P<0.001$) respectively. The hatchability was significantly different ($P<0.05$) between the groups of different concentrations with that of control, and insignificant between 5g/L and 15g/L of the protein concentration ($P>0.672$), experimental and controlled flies have not shown differences in all concentrations with that of control $P<0.05$ and insignificant between 15g/L and 25g/L ($P>0.955$). The fertility has reduced in all the protein concentration showing high significance difference with that of control ($P<0.05$) and there was no difference found between 15g and 25g of protein concentration. Viability decreased with increase in the concentration of protein from 5g, 15g and 25g were observed. *Drosophila* in the wild consumes fruit material and microbes from fermenting and/or rotting fruit (Spieth, 1974). In the laboratory, *Drosophila* can be maintained on a combination of sugar, yeast, and water (Ashburner and Appendix, 1989). DR is a well-established intervention for extending fly life span. Indeed, the interaction among diet, life span, and fecundity has formed the basis for both practical and theoretical investigations into the possible trade-offs between these life-history traits (Barnes and Partridge 2003). Yeast has

been shown as the most important compound of the food medium in *Drosophila* studies by several researchers (Onder and Yılmaz, 2009).

In the present data flies fed with different concentrations of protein i.e control, 5g/L,15g/L,25g/L has led to larval hatchability with 58.96% 65.12%48.4%63.52% respectively. The percentage of pupation is 88.13%,66.02%,40.02%,72.89% and of fertility with 78.5%,64.01%,49.55% and 39.66% respectively for all concentrations. Development time is affected at-most with yeast restriction as shown in Fig.1 respectively. Fecundity, the number of egg laid by an individual is the major determining factor of female fitness. The egg laying capacity is one of the suitable parameter to compare the performance of different strains of *Drosophila* (Harini and Ramachandra, 2003). Egg laying potentiality is an important attribute, which determines to certain extent the reproductive success of a population determined increasingly at different concentrations of protein. Life history traits like ageing, fecundity, viability and development are directly affected by the levels of yeast used in the food medium. The high yeast level (25 g/L) in the diet enhanced the developmental time and optimum for viability when compared with that of control.

Table 1: Nutritional composition of control and experimental diet

Diet Components	Control media	Enriched media	Enriched media	Enriched media
Water	1000ml	1000ml	1000ml	1000ml
Agar	10g	10g	10g	10g
Wheat cream	100g	100g	100g	100g
Glucose	-----	30g	30g	30g
Brewer's yeast	-----	5g	15g	25g
Propionoic acid	7.5ml	7.5ml	7.5ml	7.5ml
Jaggery	100g	100g	100g	100g

Table.2: Results of one-way ANOVA of mean developmental time of *Drosophila nasuta nasuta* feeded on protien media with the restricted diet.

Concentrations	N	Egg	Larvae	Pupae	Adult eclosed
Control	30	0.86±0.135	1.83±0.145	3.42±0.155a	8.36±0.237a
5grams/l	30	0.80±0.150	1.63±0.208	4.43±0.156b	7.86±0.241a,b
15grams/l	30	0.80±0.126	1.73±0.162	5.20±0.250c	7.10±0.340b
25grams/l	30	0.76±0.133	1.66±0.221	2.60±0.208d	5.90±0.149c
ANOVA		F=0.273 d.f=3,116 P>0.05	F=0.650 d.f=3,116 P>0.05	F=95.830 d.f=3,116 P<0.05	F=52.905 d.f=3,116 P<0.05

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), N=Total number of samples

Table.3: Mean viability (±SE) of *Drosophila nasuta nasuta* on exposure to different concentration of protien.

Concentrations	N	Fecundity	Hatchability	Pupation	Fertility
Control(C)	30	75.9 ±2.08a	43.3 ±1.74a	38.1 ±1.30a	31.1 ±0.66a
5grams/l	30	46.5 ±1.78b	29.8 ±1.50b	20.0 ±0.71b	12.8 ±1.06b
15grams/l	30	57.1 ±2.05c	26.2 ±1.70b	11.6 ±0.53c	5.0 ±0.39c
25grams/l	30	25.3 ±1.62d	15.5 ±1.93c	11.0 ±1.50c	4.6 ±1.04c
ANOVA		F=117.346 d.f =3, 119 P<0.05	F=43.404 d.f. = 3,119 P<0.05	F=140.355 d.f.= 3,119 P<0.05	F=221.284 d.f.= 3,119 P<0.05

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), N=Total number of samples

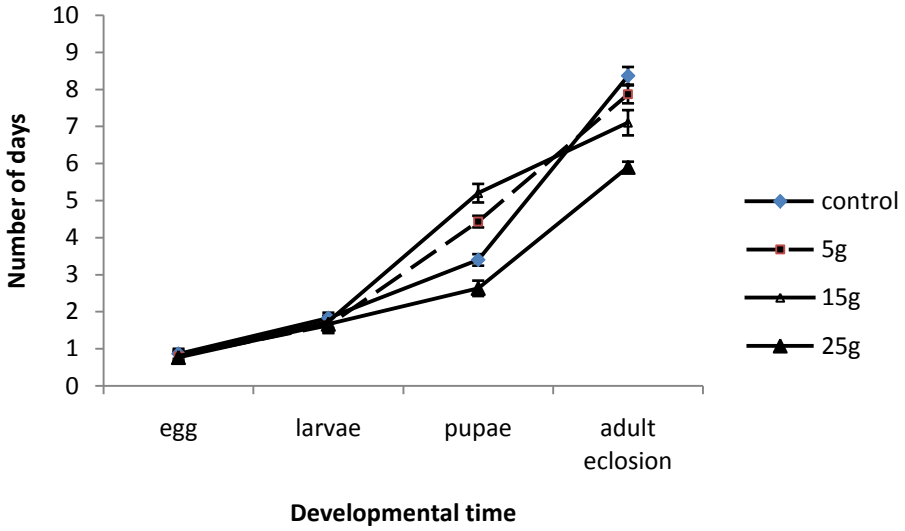

Fig.1: Mean (±SE) developmental time from egg to adult on exposure to different concentration of protein (Brewer's yeast) in *Drosophila nasuta nasuta*.

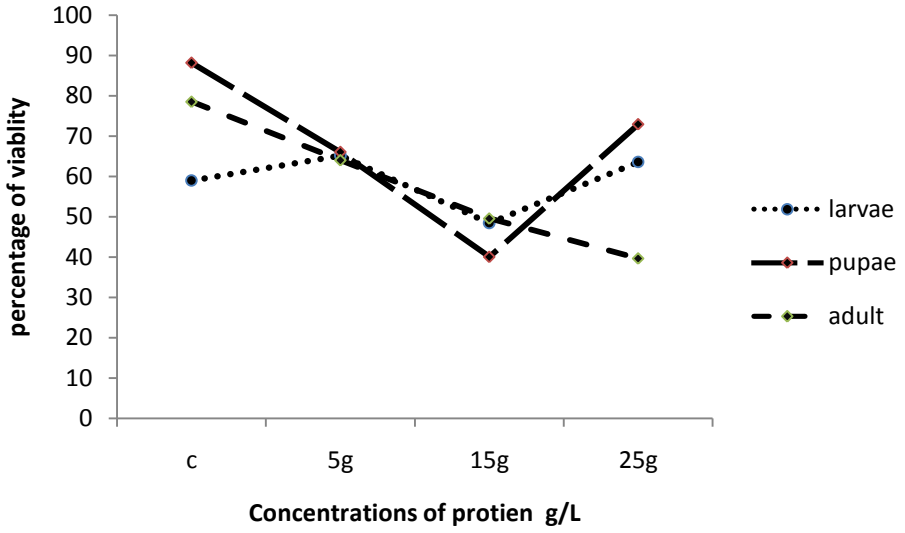

Fig.2 :Percentage of viability of *Drosophila nasuta nasuta* exposed to different concentration of protien (Brewer's yeast).

Conclusion

The mean developmental time was insignificant for egg to larva hatchability as well as from larval to pupal formation for all the cocentration with that of the control. Significant differences were observed in pupae and adult eclosion time i.e the pupation time exceeds in 5g and 15g, while it was reduced in higher concentration(25g) than control. Flies fed with minimal cocentration (5g) has taken more number of days for adult eclosion followed by 15g and 25g with that of the control.The rate of development is faster at 25g than control and the other treated trials,but the percentage of adults eclosed drastically decreases with increased protien concentration, while larval hatchability

and pupation increases with increase cocentrations of 25g/L,but decreases at 5g/L and 15g/L concentration with fixed glucose (30g/L) . Therefore the protein concentration is directly proportional with increased rate of development and decreased viability (in terms of fecundity/hatchability/pupation and adult eclosion) when compared with the control. Thus, the present study reveals that enriched protein diet (Brewer's yeast) enhances the metabolic rate with reduced viability in *Drosophila nasuta nasuta*.

Acknowledgements:

The authors thank DST-SERC and UGC-RGNF New Delhi, India, for providing financial assistance and also Department of Zoology, Bangalore University, Bangalore, to carry out the above work.

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