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Nutritional Composition of Semolina Jaggery Diet and its Effect on Reproductive Fitness of Harwich strain *Drosophila melanogaster*

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ABSTRACT: Nutrition plays a pivotal role in the development of an organism. *Drosophila melanogaster* is an important model for nutritional research. The nutritional composition of semolina-jaggery diet on fecundity and filial generation output of Harwich strain *D. melanogaster* was studied using standard methods. Semolina- jaggery diet, which are product of wheat endosperm and cane sugar respectively are rich in carbohydrate, protein, and dietary sugars. Semolina-jaggery diet was prepared in 5%, 10% and 15% with corn flour diet as the control group. 15% semolina-jaggery had the highest percentage carbohydrate (15.12±0.30) while highest protein content was recorded in control diet (3.46±0.08). Fecundity of *D. melanogaster* reared on semolina-jaggery diet varied across days with 5% SJ having the highest mean number of eggs. The 10% SJ recorded the highest mean number of offspring across five generations but was lower to the offspring output of control group in all the five generations. The nutritional composition in the varying percentages of semolina-jaggery diet had significant effect on the egg laying and offspring output of Harwich strain *Drosophila melanogaster*.

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The continuity of an organism relies on its adept utilization of nutrients for various essential aspects including growth, reproduction, longevity, behavior, metabolism and microbiome composition and function (Brookheart and Duncan, 2016; Stefana *et al.* 2017; Davies *et al.* 2018; Erkosar *et al.* 2018). *Drosophila melanogaster* commonly referred to as the vinegar fruit fly is one of the most studied organisms in biomedical research and a powerful model in the study of metabolic disorders (Chattopadhyay *et al.* 2015). The suitability of the fruit fly as a model stem from its short generation time, cheap maintenance, high fecundity, 60% conserved genes with humans and its ease in genetic manipulations (Chattopadhyay *et al.* 2015). Dietary nutritional composition is key to dietary restriction with protein to carbohydrate (P: C) ratio known as an important factor influencing life history traits (Lee *et al.* 2008; Jang and Lee, 2018). *Drosophila melanogaster* is typically reared in the laboratory on diet composed of agar, yeast, sugar source, and cornmeal. The dietary components, its source and quantity used varies greatly across laboratories thereby limiting the use of the terminology "standard fly diet" and certain level of reproducibility in nutritional research among laboratories (Lesperance and Broderick, 2020). The uncooked semolina is rich in carbohydrate (70.9%) and protein content (12.3%) with a P:C of 0.17. Jaggery made from cane sugar is a good source of dietary sugar comprising of 50% sucrose, 20% invert

sugar, 20% moisture and 10% protein, ash and bagasse. The worldwide production of semolina and jaggery, its healthy palatability due to its low glycemic response, its medicinal properties, its cost effectiveness, and the ease in diet preparation makes semolina and jaggery a potential medium in bulk rearing of fruit flies (Chattopadhyay *et al.* 2015). The need to investigate the nutrient composition including the holidic medium of several fly diet to aid in the standardization of fruit fly diet can't be overemphasized. This study, therefore, aimed at determining the nutritional composition of semolina-jaggery diet and its implications on fecundity and filial output traits of the fruit fly.

MATERIALS AND METHODS

Harwich strain of *Drosophila melanogaster* was obtained from *Drosophila* and Neurogenetics laboratory, Department of Zoology, Ahmadu Bello University Zaria and reared at 25°C ± 1°C on 12:12 hours light: dark cycle under standard fly rearing conditions. Semolina (Plate I) and jaggery (Plate II) were purchased at Tudun wada-Zaria market, Kaduna state. (Lat 11°5'17.600"N and Long 7°42'8.920"E).

Plate 1: Packed semolina

Plate 2: Jaggery

Semolina-jaggery (SJ) diet: The varying percentages of SJ diet (5, 10 and 15%) were prepared according to the procedure described by Chattopadhyay *et al.* (2015). To prepare one litre of the SJ diet, jaggery was completely dissolved in 500ml of water followed by addition of semolina. The mixture was boiled for 10 minutes with continuous stirring to obtain a viscous consistency. Agar was dissolved in 100ml of water and added to the mix. Final volume was made by addition of water and the mix cooked for another 10 minutes with continuous stirring. Care was taken to stir continuously during preparation, as uncooked semolina tends to clump at the bottom. When the temperature reached 70°C, Nipagin was added, and the meal was immediately dispensed into vials.

Cornmeal (standard) diet: The cornmeal diet was used as control due its rigorous use as a standard diet in many studies. To prepare 850ml, according to the modified method of Markow and O'Gardy (2006), 850ml of distilled water was measured in measuring cylinder. 500ml of the water was transferred in the pot and brought to boil, 350ml of water was left to dissolve the corn flour and rinse out the remnants. A scoop of hot water from the pot was used to dissolve the yeast (10g). Agar (8g) was added to the pot of boiling water, and it was stirred properly to avoid lumps. It was left to cook for 10mins. The dissolved corn flour (50g) was added next and was stirred properly. It was left to cook for another 10mins. Yeast was added and left to cook for 15mins. The diet was left to cool for a bit and niacin (1g), diluted with ethanol (1ml) was added to the diet.

Grape plate preparation: 2g of agar was dissolved in 100ml of water. In another container, 100ml of grape juice was mixed with 10g of sucrose and was properly stirred to dissolve completely. All the contents were combined in a pot and brought to boil for few minutes. The mixture was allowed to cool down a bit, 1g of Nipagin dissolved in ethanol was added to it. The media was transferred into sterile petri plates and allowed to solidify. Little amount of yeast was smeared on top the media (Sabat *et al.* 2015).

Nutritional analysis of semolina-jaggery diet: Percentage moisture, ash, fiber, crude protein, lipid was determined using the AOAC (2005) procedures while percentage carbohydrate was estimated by subtracting the sum of all values from 100.

Fecundity assay: A newly eclosed female was transferred in an egg-laying chamber daily. The number of eggs laid were counted daily for 5days. Three replicate plates were set up for each group (modified from Chattophadhyay *et al.* 2015).

Filial output assay: Three newly eclosed Harwich strain of *Drosophila melanogaster* adult male and six adult female flies were placed in vials containing respective diets. This generation of flies were recorded as parental (P) generation. Parental flies were removed after 48hrs to ensure synchronous larval growth. The larvae were allowed to rear and emerge as F_1 flies. The F_1 generation flies were counted and recorded. From the F_1 generation, 3 male and 6 female flies were selected randomly and transferred into fresh diets, to give rise to second filial generation (F_2). This procedure was repeated to the 5th filial generation (F_5) (Chattophadhyay *et al.* 2015).

Data analyses: The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one-way analyses of variance (ANOVA) and t-test were used to determine the significant differences among and between concentrations. P-Values less than 0.01 were considered as significance.

RESULTS AND DISCUSSIONS

Significant difference was observed in percentage carbohydrate with 15% SJ having the highest percentage carbohydrate (15.12±0.30), lowest crude protein (3.12±0.01) and P:C ratio of 0.21 while control had the least carbohydrate (2.02±0.48), highest crude protein (3.46±0.08) and P:C ratio of 1.71 (Table 1). The higher percentage carbohydrate observed in all concentrations of SJ diet confirms the statement of Oladummoye *et al.* (2014), although the nutritional values are lower compared to their findings on the uncooked semolina. The reduction in the nutritional content observed could be due to the break down and loss of nutrients by thermal processing as studies have shown that thermal processing reduces the nutritional, anti-nutritional and vitamin contents of various foods (Arinola and Adesina, 2014; Iyenagbe *et al.* 2017). The protein content was lower to the reported lower (6.33) value of standard (cornmeal) diet by Lesperance and Broderick, (2020). The corn variety, processing time, source and quantity of diet components could be the reasons for the observed variations. The diets are considered rich based on the P:C scale of 0.05 – 0.80 (Lesperance and Broderick, 2020), cornmeal having the highest P:C value in this study (1.71). Reproduction is an indicator of an organism's health (Chattophadhyay *et al.* 2015). The mean number of eggs laid per day varied across days. Flies reared on 5% SJ diet laid significantly higher number of eggs in day 5 and the least was recorded in 10% SJ diet (Figure 1a). When the total number of eggs laid for 5 days by females reared on 5% SJ diet (12.80 ± 4.12) was compared with those control diet (11.53 ± 0.81), there was no significant difference at $p \leq 0.01$ (Figure 1b). Studies have shown that fruit flies fed high-protein and low-carbohydrate (P:C) diet produces higher number offspring at the expense of the fly's lifespan (Bowman and Tatar, 2016; Zanco *et al.* 2021).

Table 1. Nutritional composition of semolina-jaggery diet

Treatment	Moisture content (%)	Ash content (%)	Lipid content (%)	Protein content (%)	Crude fibre (%)	Carbohydrate (%)	P:C
Control	881.3±0.47^{a}	0.20±0.00^{a}	6.19±0.07^{a}	3.46±0.08^{a}	0.00±0.00	2.02±0.48^{d}	1.71
5%	83.57±0.29^{c}	0.06±0.01b	1.41±0.10^{d}	2.93±0.15^{c}	0.00±0.00	12.03±0.19^{b}	0.24
10%	86.57±0.29^{b}	0.27±0.04^{a}	1.76±0.01^{c}	3.29±0.00^{ab}	0.00±0.00	8.12±0.33^{c}	0.41
15%	79.25±0.21^{d}	0.17±0.06^{ab}	2.16±0.07^{b}	3.12±0.01^{bc}	0.00±0.00	15.12±0.30^{a}	0.21
P-value	0.000	0.021	0.000	0.011	NA	0.000	

Values are means ±SEM of triplicate samples

This could be the reason for the observed higher number of eggs laid by flies fed with cornmeal diet. Chattophadhyay *et al.* (2015), observed higher fecundity in flies reared on 10% SJ diet. Figure 2a and 2b depict the effect of different concentrations of SJ diet on filial generation output. Among the Semolina-jaggery diet concentrations, 10% recorded the highest number of emerged flies in most generations (Figure 2a).

Control diet however recorded highest number of eclosed flies across all generations, which was significantly different from all SJ concentrations (Figure 2b). Fly development is affected by its microbiota, which is influenced by the P:C ratio. Little shift in protein has been shown to have significant biological effect of a diet (Lesperance and Broderick, 2020).

This could be the reason for the high emergence of fruit flies recorded in cornmeal and 10% Semolina-jaggery diet, as it is a well-known fact that protein is vital to the developmental processes of an organism. Control diet favored the development of fruit flies across five generations supporting its suitability as a medium for mass rearing of Harwich strain *Drosophila melanogaster*. This is contrary to the report of Chattophadhyay *et al.* (2015), who reported higher emergence of fruit flies when reared on 10% SJ diet against the standard diet. Again, the source of diet components and strains of the fruit flies could be a factor for the observed difference in both studies.

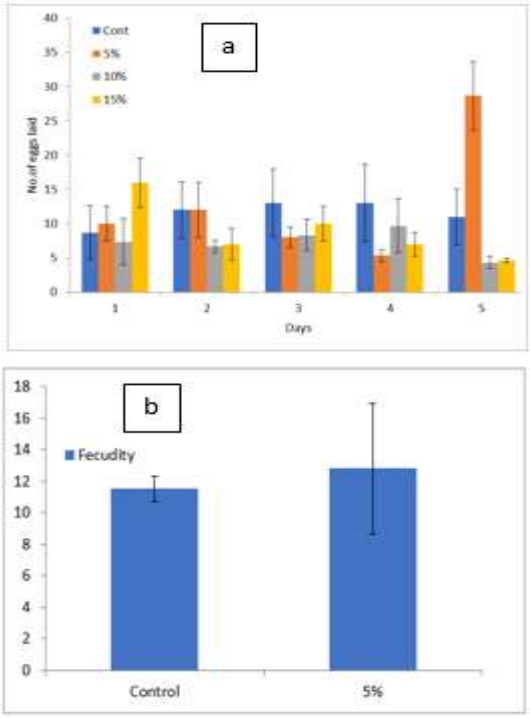

Fig 1. Effect of semolina-jaggery diet on female fecundity**.** *Data presented as mean number of eggs laid by a female per day for five days with error bars denoting standard deviation (a). Unpaired t-test for total number of eggs laid in five days (control diet vs. 5% SJ diet). Three replicate egg laying plates were set up for each diet, with a female in each replicate (b).*

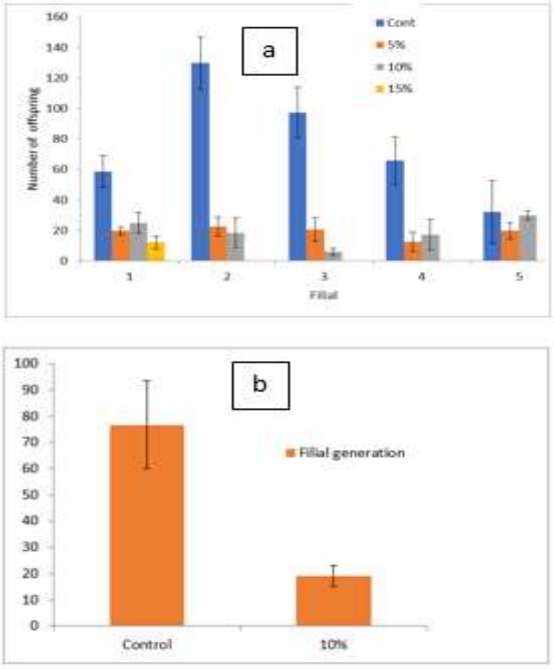

Figure 2. Effect of semolina-jaggery diet on filial output. *Data presented as mean number of eggs emerged adult flies per generation for five generations with error bars denoting standard deviation (a). Unpaired t-test for total number of eclosed offspring in five generations (control diet vs. 10% SJ diet). Three replicate vials were set up for each diet, with 3 males and 6 females (b).*

Conclusion: The various concentrations of semolina-jaggery diet had lower protein: carbohydrate ratio compared with the cornmeal diet. Harwich strain reared on semolina-jaggery diet had lower fecundity and filial generation output. Therefore, the use of cornmeal diet in mass rearing of Harwich strain *D. melanogaster* is encouraged. However, further studies should be carried out on the effect of SJ diet on several traits of *Drosophila melanogaster* strains.

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