

# Cassava Starch Supplementation Modulates Behavioural Characteristics and Oxidative Status in *Drosophila Melanogaster*

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**Abstract** - Studies have revealed that adaptive behaviours, life-history and reproductive capacity of an organism are influenced by the amount and quality of nutrients. Furthermore, it has been established that the balance between energy production and utilization is crucial to animal's survival and reproduction vis-a-vis the organism's ability to adjust their developmental, physiological or behavioural response to environmental conditions. In the present study an attempt was made to investigate the effect of cassava starch supplementation on chill-coma recovery, reproductive capacity and some metabolic-induced changes in oxidative status of *Drosophila melanogaster*. *Drosophila melanogaster* was raised on a normal fly diet supplemented with 500, 750, 1000, 1250, 1500 and 2000 mg/mL starch extracted from three cassava cultivars (TMS 92/0326, TMS 30572 and Omu-Aran local sweet cassava). Data generated from this study showed that flies consuming starch-supplemented diet have higher cold resistance, survival rate and egg to adult viability. However, flies raised on starch supplemented diet showed a significantly higher concentration of thiobarbituric acid reactive substances (TBARS) with a compensatory significant increase in antioxidant defense enzymes activities and nitric oxide scavenging capacity compared to fly raised on the normal fly diet. In conclusion, data obtained from this study revealed that starch supplementation to normal *Drosophila melanogaster* diet with cassava starch enhances the organism's survival rate, and adaptive response to cold stress, with concomitant improvement in antioxidant status.

**Key Words:** *Drosophila melanogaster* diet, starch supplementation, adaptive behaviour, oxidative stress

## 1. INTRODUCTION

Studies have revealed that adaptive behaviours, life-history, reproductive capacity and fecundity of an organism are influenced by the amount and quality of nutrients (Sisodia and Singh, 2012). Furthermore, it has been established that the interplay between energy input and utilization is crucial in the determination of an organism's survival and reproduction and to a great extent the capacity of organism to adjust its developmental, physiological or behavioural response to varying environmental conditions.

Dietary adjustment through experimentation have been reported to impact significantly on the capacity of an organism to efficiently allocate their energy resources (Cruz-

Neto and Bozinovic, 2004; Nuñez-Villegas et al., 2014). Reports on the effect of dietary carbohydrates on body physiology have been limited to humans and rodent models. More so, majority of such studies were carried out using pre-established disease models of diabetes, obesity, or hypertension. Thus, studies focused on the effects of dietary carbohydrate on animal survival and reproductive capacity are scanty.

*The use of Drosophila melanogaster as a suitable model for nutritional studies has been widely reported in literature.* Although several studies have explored the effect of diet on life span, reproduction and adaptive behavior of fruit flies (Lee et al., 2008; Catterson et al., 2010; Yurgel et al., 2015) none has specifically focused on the inclusion of dietary starch. Therefore, this study sought to evaluate the effects of starch supplementation on behavioural, reproductive capacity and changes in oxidative status in *Drosophila melanogaster*.

## 2. MATERIALS AND METHODS

### 2.1 Fly stock and husbandry

*Drosophila melanogaster* flies were obtained from the *Drosophila* Laboratory, University of Ibadan, Nigeria. The flies were maintained for more than 10 generations on simple culture medium containing agar-agar, dried yeast, corn flour and nipagin and plain water at 25°C, under a 12:12 light:dark cycle, and 60%–70% relative humidity.

### 2.2 Cassava Samples

Freshly harvested tubers of two different cassava varieties; TMS92/0326 and TMS 30572 were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria and a sweet local cassava (designated local) was also obtained from Omu-Aran community, Kwara State, Nigeria and transported to Landmark University, Omu-Aran Kwara State Nigeria where this study was carried out.

### 2.3 Cassava Starch Preparation

The cassava starch was prepared using standard methods. The cassava roots were washed, peeled, washed again, grated and sieved with substantial quantity of water (10 times the volume of the mash) using a muslin cloth. The

sieved solution was allowed to settle (sediment) after which the supernatant was decanted. The starch obtained was dewatered and then dried in a hot air oven (Gallenkamp Co. Ltd London England) at 70 °C for 4 h. The dried starch was then milled into powder using a milling machine and sieved (sieve aperture, 0.4 mm) to obtain fine flour. The starch sample for each cassava cultivar was stored in an airtight container at 4 °C for further analysis.

## 2.4 Preparation of Normal Diet

The media bottles were washed and rinsed with tap water and then sterilized in hot air oven at 60 °C for 5-6 h. Distilled water (700 mL) was poured into 1000 mL beaker and allowed to boil on hot plate. On boiling, 10 g of yeast and 7.9 g of agar agar was added slowly with incessant stirring for 10 minutes. Corn flour (52 g) dissolved in 750 mL distilled water was added into the mixture on hot plate and stirred for 15 min after which it was allowed to cool to 60 °C. Nipargin (5 g) was added as an antibacterial and antifungal agent. The viscosity of the media was checked before pouring into the media bottles.

## 2.5 Experimental diet

The experimental diet was prepared by adding starch (5%) from the three cassava varieties as extra calorie to normal *Drosophila* diet as described above.

## 2.6 Survival Rate

Fly survival rate was estimated according to the method of Chhabra *et al.* (2013). Briefly, newly eclosed flies (25 males and 25 females/vial) were collected under light anesthesia and randomly assigned into the respective treatment groups. Each treatment was carried out in triplicates. Flies in each treatment were passed every other day and the number of dead flies were counted daily and used to evaluate their survival rate.

## 2.7 Negative Geotaxis

The locomotor performance of the flies fed with control and experimental diet was investigated using the negative geotaxis test, according to Coulom and Birman (2004), with minor modifications. Ten flies from each treatment group were rendered motionless under anesthesia with ice and after which they were placed separately in a vertical glass column (15 cm x 1.5 cm). After 30 minutes of recovery from the anesthesia, the flies were gently tapped to the bottom of the column and allow to climb through the column while monitoring the time lapse to climb to the 8 cm mark. The tests were repeated three time for each fly at 1 min intervals.

## 2.8 Chill Coma Recovery

The fly ability to withstand and recover from chill-induced coma was monitored following the procedures described by Nilson *et al.* (2006).

## 2.9 Biochemical assays

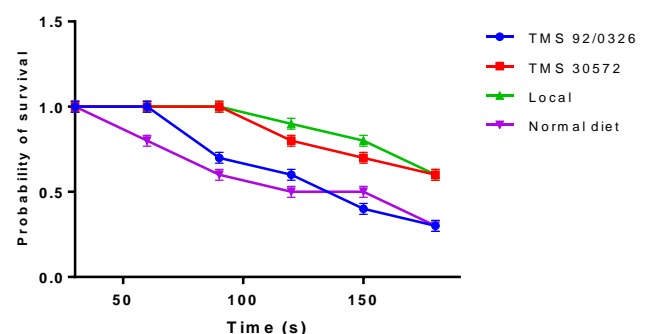
Glucose concentration was determined using the glucose oxidase method as described by Trinder (1969). Lipid peroxidation was determined by the method described by Buege and Aust (1978). Superoxide dismutase (SOD) activity was determined following the method of McCord and Fridovich (1969). Catalase (CAT) and nitric oxide scavenging activities were assayed according to the methods described by Aebi (1984) and Igbinosa *et al.* (2011) respectively. Reduced glutathione (GSH) level was estimated according to the method of Moron *et al.* (1979)

## 2.10 Statistical Analysis

Data are presented as the means  $\pm$  standard error of mean (SEM) of triplicate determinations. Comparison of means was by One-way Analysis of Variance (ANOVA) followed by Turkey's Multiple Comparison using SPSS version 17.  $P < 0.05$  was considered significant.

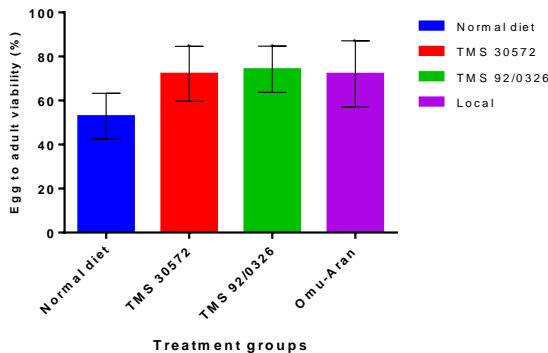
## 3. RESULTS

According to the results presented in Figure 1, fly fed TMS 30572 and local cassava starch supplemented diet had higher chance of survival compared to fly fed TMS 92/0326 cassava starch and normal diets. According to the results presented in Figure 1, fly fed TMS 30572 and local cassava starch supplemented diet had higher chance of survival compared to fly fed TMS 92/0326 cassava starch and normal diets.



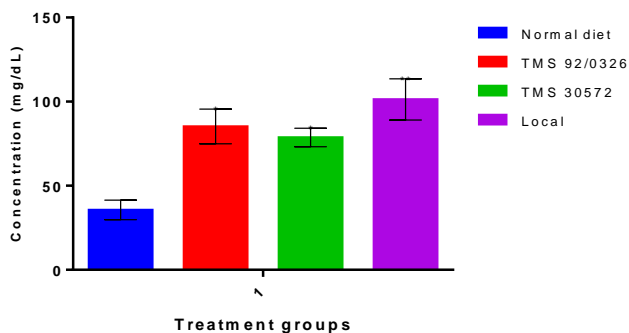
**Figure-1:** Survival curve for chill-coma recovery in *Drosophila melanogaster* flies fed cassava starch supplemented diet. Values are means  $\pm$  SEM of 3 determinations. Note: TMS 30572 and TMS 92/0326 are diets supplemented with starch obtained from improved cassava varieties from IITA (Ibadan, Nigeria), local, diet supplemented with starch obtained from local cassava variety.

Flies fed diets supplemented with the respective cassava starch had greater percentage egg to adult viability compared to flies fed normal fly diet (Figure 2).



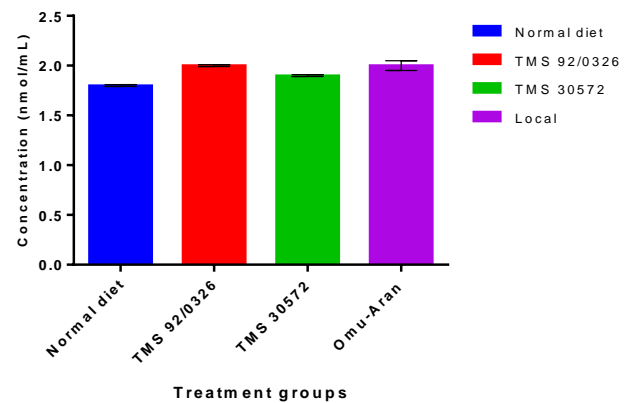
**Figure-2:** Egg to adult viability of flies developing on cassava starch supplemented. Results are means ± SEM of 3 determinations. \* Significantly different from normal diet at  $p < 0.05$ . Note: TMS 30572 and TMS 92/0326 are diets supplemented with starch obtained from improved cassava varieties from IITA (Ibadan, Nigeria), local, diet supplemented with starch obtained from local cassava variety.

Glucose concentration was significantly higher ( $p < 0.05$ ) in flies fed starch supplemented diet compared to normal diet (Figure 3).



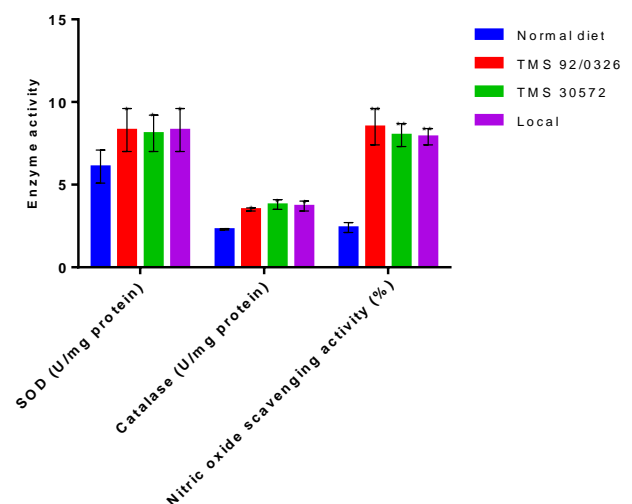
**Figure-3:** Glucose concentration level in *Drosophila melanogaster* flies kept on cassava starch supplemented diet. Results are means ± SEM of 3 determinations. \* Significantly different from normal diet at  $p < 0.05$ ; \*\* at  $p < 0.001$ . Note: TMS 30572 and TMS 92/0326 are diets supplemented with starch obtained from improved cassava varieties from IITA (Ibadan, Nigeria), local, diet supplemented with starch obtained from local cassava variety.

Lipid peroxide level in flies fed starch supplemented diet and normal diet was not significantly altered (Figure 4).



**Figure-4:** Lipid peroxidation level in *Drosophila melanogaster* flies kept on cassava starch supplemented diet. Results are means ± SEM of 3 determinations. Note: TMS 30572 and TMS 92/0326 are diets supplemented with starch obtained from improved cassava varieties from IITA (Ibadan, Nigeria), local, diet supplemented with starch obtained from local cassava variety.

Superoxide dismutase and catalase activities were significantly ( $p < 0.05$ ) higher in flies fed starch supplemented diet compared to flies fed normal diet. In addition, the percentage nitric oxide scavenging activity was significantly ( $p < 0.05$ ) higher in flies fed starch supplemented diet compared to flies fed normal diet (Figure 5).



**Figure-5:** Oxidative status of *Drosophila melanogaster* flies kept on cassava starch supplemented diet. Results are means ± SEM of 3 determinations. \* Significantly different from normal diet at  $p < 0.05$ ; \*\* at  $p < 0.001$ . Note: TMS 30572 and TMS 92/0326 are diets supplemented with starch obtained from improved cassava varieties from IITA (Ibadan, Nigeria), local, diet supplemented with starch obtained from local cassava variety.

#### 4. DISCUSSIONS

The survival rate of flies to a great extent dependent on temperature, diet and other environmental conditions (Driver and Lamb, 1980). In this research flies whose diet was supplemented with cassava starch from TMS92/0326 cultivar showed the highest survival rate.

The ability of an organism to recover from chill-induced coma is greatly influenced by both genetic and environmental factors (Macmillan and Sinclair, 2011). From previous research, *D. melanogaster* has been shown to show improved resistance to cold when fed calorie-laden and protein-enriched diet (Chen *et al.*, 1993). Findings from this study showed that flies fed diet supplemented with cassava starch had higher recovery rate from chill-induced coma compared to those fed normal fly diet. The balance between energy intake and expenditure has been reported to contribute both to the survival and reproductive success of animals (Metcalfe and Monaghan, 2003; Salmon *et al.*, 2001). This balance depends on the interplay between energy intake, digestion and allocation of acquired energy to various functions such as maintenance, growth and reproduction (Baker and Thummel, 2007). Experimental modifications of animal diets have played a key role in the study of how organisms adjust their energy allocation (Chown and Nicolson, 2004; Cruz-Neta and Bozinovic, 2004).

Cassava starch supplementation as demonstrated in this study thus provide extra energy source which could be channeled to various functions such as maintenance, growth and reproduction. This observation could explain the higher survival rate and greater tendency to withstand cold stress. It is also pertinent to note that increase calorie loading may translate to increased metabolic rate and thus higher tendency for generation of free radical as a result of incomplete reduction of molecular oxygen to water. Thus, the observed increase in lipid peroxidation in flies grown on starch supplemented diet could be as a result of the high calorie-induced metabolic abnormality bringing about increased generation of free radicals.

Superoxide dismutase (SOD) and catalase are two of the key enzymes that mop up free radicals from cellular system. The activity of these enzyme is induced following increased generation of reactive oxygen species (ROS). The increased carbohydrate loading as a consequence of starch supplementation in this study may have led to increased generation of ROS as evident in higher lipid peroxidation rate in flies fed diets containing starch supplement and ultimately leading to the induced synthesis of these enzymes (SOD and catalase) (Di Naso *et al.*, 2012; Furukawa *et al.*, 2017). Thus, the observed increase in the activity of the two enzymes in *D. melanogaster* flies fed or grown on cassava starch supplemented diet could have been a resultant effect of the elevated ROS in these flies. Increased oxidative stress in high carbohydrate/calorie diet is an established feature of

metabolic syndromes associated with obesity, because oxidative stress may favour cell damage due to generation of pro-inflammatory substances such as nitric oxide (Lushchak *et al.*, 2011). However, results from this study showed increased nitric oxide scavenging potential in flies fed cassava supplemented diet. Obesity caused by derangement in carbohydrate metabolism and/or increased lipid deposition in adipose tissue has been reported to initiate cellular oxidative stress, culminating in chronic inflammation mediated through enhanced generation nitric oxide by adipose tissue (Di Naso *et al.*, 2012).

Data obtained from this study reveal that supplementation of normal *Drosophila melanogaster* diet with cassava starch enhances the organism's survival rate, and adaptive response to cold stress, with concomitant upregulation of antioxidant enzyme activity.

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