



DIETARY CALABASH CHALK INDUCES DECLINE IN REPRODUCTIVE FITNESS IN HARWICH STRAIN OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

Calabash chalk, a naturally occurring mineral, is widely consumed by expectant mothers seeking relief from morning sickness. This study aimed to investigate the potential impact of dietary CCH on fecundity, eclosion, and filial generation output in *Drosophila melanogaster* (Harwich strain). The lethal concentration (LC50) of CCH was determined as 1800 mg per 10 g of standard corn meal diet. The experiment involved five groups: a control group fed a 10 g diet, and four experimental groups fed varying doses of CCH (225 mg, 450 mg, 900 mg, and 1800 mg in 10 g standard corn meal diet) for 30 days. Results revealed a significant reduction ($p < 0.05$) in fecundity among flies fed 900 mg and 1800 mg CCH compared to the control, alongside decrease in filial generation output and filial fecundity. This suggests that CCH consumption negatively impacts reproductive fitness (fecundity and filial output) in *D. melanogaster* (Harwich strain), suggesting potential harm at high doses and advocating for caution, particularly among women of reproductive age and during pregnancy.

Keywords: Calabash Chalk, *Drosophila melanogaster*, eclosion, fecundity, filial generation

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INTRODUCTION

In the ceaseless pursuit of health, longevity, and remedies, humanity has delved into the depths of our immediate environment in search of solutions. Among the intriguing discoveries lies calabash chalk—a substance both naturally occurring and artificially produced, reminiscent of the phenomenon of pica [1]. This peculiar material holds communal significance among the rural and tribal populations of Sub-Saharan African nations, including Nigeria [2], alongside its recognition in therapeutic practices in regions as distant as Australia [3]. The ancient tradition of geophagia—the deliberate consumption of earthy materials—is a practice shared between humans and animals [4], transcending boundaries of age, gender, and geography. Pregnant women, in particular, often turn to earth materials to alleviate morning sickness or vomiting, a practice prevalent across nations and epochs [5, 6].

Calabash chalk, known by a multitude of names across cultures such as Calabash clay, “Calabar stones”, “poto”, “la craie”, or “argile” in French, “nzu” by the Igbos, “farin kasa” by Hausas, “ndom” by the Efiks/Ibibios of Nigeria, as well as “mabele” by the Lingala of Congo, is commercially available in various forms, from blocks to powdered, and is believed to possess diverse ethnobotanical uses [7]. Calabash chalk is made up of aluminum silicate hydroxide ($\text{Al}_2\text{Si}_2\text{O}_5\text{OH}_4$), a member of the

kaolin clay group. It consists of metalloids and metals like lead (which forms a high percentage of its constituent), arsenic, iron, aluminum, potassium, zinc, titanium, manganese, barium, chromium, copper, nickel, rubidium, and tin respectively [8]. Pregnant women, driven by perceived physiological advantages, frequently incorporate calabash chalk into their diets to combat morning sickness and satisfy heightened appetites [9]. However, lurking beneath its cultural significance lies the potential for contamination by microorganisms or heavy metals, posing risks exacerbated by processing and storage methods. While studies on its composition remain limited, available data suggest a complex makeup, including aluminum silicate hydroxide and an array of metals whose qualitative and quantitative compositions vary with geographical origin [10].

Drosophila melanogaster is a remarkable model organism with profound implications for toxicological studies. Sharing fundamental biological mechanisms with humans [11], the fruit fly emerges as a promising avenue for exploring the effects of calabash chalk consumption. With its high fecundity, short lifespan, ease of rearing, available genetic tools and databases, combined with good knowledge of the biology, physiology, development, and behavior of this species, the model offers an unparalleled insight into the

biological processes, impact of pollutants, and human diseases [12 - 15].

In this study, we aim to uncover the impact of calabash chalk consumption by utilizing the adaptable fruit fly, *Drosophila melanogaster*, as our

MATERIALS AND METHODS

Experimental animals

Harwich strain of *Drosophila melanogaster* was obtained from *Drosophila* and Neurogenetics laboratory, Department of Zoology, Ahmadu Bello University, Zaria, and reared at $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ on 12:12 hours light: dark cycle under standard fly rearing conditions.

Cornmeal (standard) diet

The cornmeal diet was used as a control due to its rigorous use as a standard diet in many studies. To prepare 850 ml of diet, according to the modified method of Markow and O'Garday [16], 850 ml of distilled water was measured in a measuring cylinder. 500 ml of the water was transferred to the pot and brought to quick 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 (10 g). Agar (8 g) was added to the pot of boiling water, and it was stirred properly to avoid lumps. It was left to cook for 10 minutes. The dissolved corn flour (50 g) was added next and was stirred properly. It was left to cook for another 10 minutes. The dissolved yeast was added and left to cook for 15 minutes. The diet was left to cool for a bit and nipagin (1 g), diluted with ethanol (1ml) was added to the diet [17].

Experimental procedure

Blocked-form calabash chalks were purchased from vendors in Sabon-Gari market, Zaria, Kaduna State, Nigeria. The chalks were pulverized into powder form using sterile mortar and pestle under laboratory conditions. The powder was kept in a dry and airtight rubber container. Each treatment vial containing 10 g of dispensed feed medium, was mixed with the various doses of the calabash chalk as shown below, the research experimental design consists of five groups namely:

Group I: Fed with 10 g *Drosophila* standard diet (Control).

Group II: Fed with 225 mg of calabash chalk per 10 g of diet.

Group III: Fed with 450 mg of calabash chalk per 10 g of diet.

Group IV: Fed with 900 mg of calabash chalk per 10 g of diet.

Group V: Fed with 1800 mg of calabash chalk per 10 g of diet.

model organism. Our goal is to investigate any potential health risks associated with long-term consumption of calabash chalk, thus enriching our understanding of health within the context of ancient practices.

Elemental analysis

Pulverized dried Calabash chalk was passed through a 2 mm sieve, weighed (1g) accurately, and quantitatively transferred to a 250 mL conical flask. Next, 10 mL of the digestion acid mixture (volume ratio 1:2:2 of perchloric, nitric, and sulfuric acids) was added to the sample and gently heated on a hot plate in a fume hood until a white fume was observed which signified that digestion was complete (HNO_3 : 69%, BDH Laboratory Supplies, Poole, England; H_2SO_4 : 98%, BDH Laboratory Supplies, Poole, England; HClO_4 : 70%, Sigma-Aldrich, USA). The digest was allowed to cool and 20 mL of distilled water was added to bring the minerals into the solution, thereafter, filtered using ashless Whatman filter paper into a calibrated volumetric flask, and made up to mark with distilled water. The sample digests were subsequently analyzed for Ba, Ca, Co, Cr, Cu, Fe, K, Mg, Ni, and Pb using a flame atomic absorption spectrophotometer (200A, Buck Scientific, Norwalk, CT, USA). Arsenic (As) was analyzed by Continuous Hydride Generation Atomic Absorption Spectroscopy (Model: AA 7000, Shimadzu, Japan) with HVG (Hydride Vapor Generator, Model: HVG-1, Shimadzu, Japan) [18, 19, 20].

Grape plate preparation

2 g of agar was dissolved in 100 ml of water. In another container, 100 ml of grape juice was mixed with 10 g of sucrose and was properly stirred to dissolve completely. All the contents were combined in a pot and brought to a boil for five minutes. The mixture was allowed to cool down a bit, and 1 g of Nipagin dissolved in ethanol was added to it. The media was transferred into sterile petri plates and allowed to solidify. A pinch of yeast was smeared on top of the media [21].

Fecundity assay

A newly eclosed female was transferred to an egg-laying chamber daily. The number of eggs laid were counted daily for 5 days. Three replicate plates were set up for each group [21; modified].

Filial output assay

Three newly eclosed Harwich strains of *Drosophila melanogaster* adult male and six adult female flies were placed in vials containing respective diets. This generation of flies was recorded as the parental (P) generation. Parental flies were removed after 48 hours 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) [22].

RESULTS

The elemental analysis of the calabash chalk (CCH) used in the study (Table 1) revealed the following elements and concentrations: lead (1.37 ± 0.11) mg/kg, arsenic (0.67 ± 0.03) mg/kg, cobalt (0.0012

Data analysis

Data were analyzed and presented as Mean \pm SEM (Standard Error of Mean). Using Graph-Pad 8 version Prism software, two-way analysis of variance (ANOVA) was used to compare between groups, followed by *Tukey's post hoc* test for individual comparison, Values of $p < 0.05$ were considered statistically significant.

± 0.01) mg/kg, copper (0.003 ± 0.00) mg/kg, nickel (0.004 ± 0.02) mg/kg, iron (1500 ± 450.00) mg/kg, magnesium (120 ± 32.00) mg/kg, potassium (240 ± 140.00) mg/kg, calcium (340 ± 280.00) mg/kg, copper (0.003 ± 0.00) mg/kg.

Table 1: Elemental analysis of the Calabash chalk used for the study.

Elements analysed	Concentration of elements (mg/kg \pm SD)
Lead (Pb)	1.37 ± 0.11
Copper (Cu)	0.003 ± 0.0031
Cobalt (Co)	0.0012 ± 0.012
Nickel (Ni)	0.004 ± 0.021
Arsenic (As)	0.67 ± 0.03
Iron (Fe)	1500 ± 450
Calcium (Ca)	340 ± 280
Potassium (K)	240 ± 140
Magnesium (Mg)	120 ± 32
Chromium (Cr)	130 ± 10

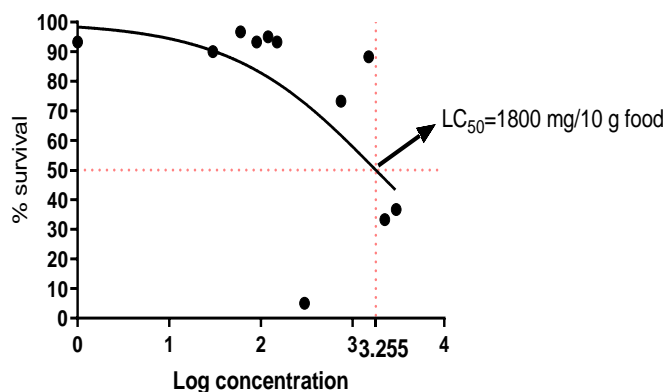


Figure 1: The Lethal Concentration (LC_{50}) of Calabash chalk diet in *Drosophila melanogaster*.

The mean lethal concentration (LC_{50}) of CCH in *D. melanogaster* (Figure 1) in this study was calculated to be 1800 mg/10g diet.

Determination of LC_{50}

The LC_{50} of the calabash chalk for this study was determined using probate analysis where the data was transformed using a probate function to achieve

linearity, and then a regression analysis was performed to estimate the LC_{50} . as 1800 mg/10 g of standard corn meal diet, the value was to calculate the various doses used in the experimental groups 2 to 5.

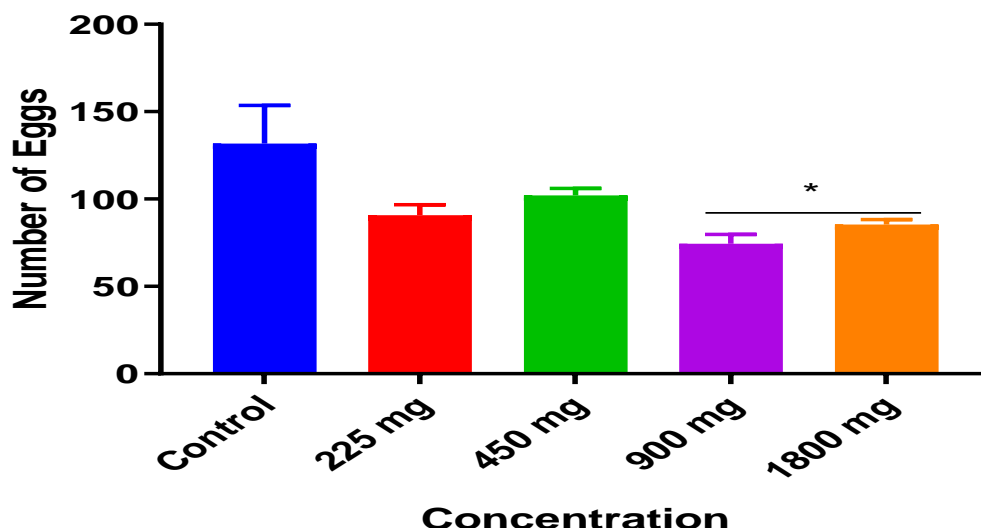


Figure 2: Fecundity of fruit flies, *D. melanogaster* fed with different concentrations of calabash chalk diet (* $p < 0.05$ vs control).

The number of eggs laid by *D. melanogaster* fed with CCH (Figure 2) ranged from 74.33 ± 5.5 to 131.7 ± 21.9 . The highest number of eggs laid was recorded in the control (132 eggs) while the lowest was

recorded in the 900 mg group (74 eggs). The 900 mg (74 eggs) and 1800 mg CCH (85 eggs) groups had significantly lower ($p < 0.05$) number of eggs laid compared to control (132 eggs).

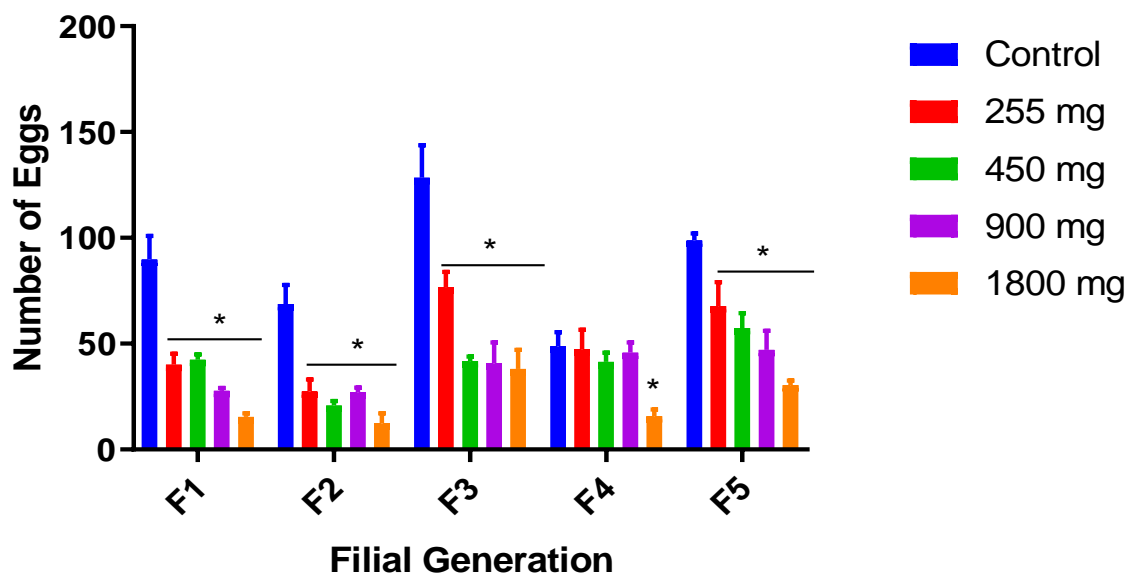


Figure 3: Filial Fecundity of *D. melanogaster* (* $p < 0.05$, compared to control)

The filial fecundity (Figure 3) shows a significant decrease in fecundity ($p < 0.05$) in the F₁-F₅ generation when compared to the control, especially for the 450 mg, 900 mg, and 1800 mg of CCH diet. From F₁- F₅

generation, the average number of eggs laid in the groups are control (67 eggs); 225 mg CCH (51 eggs); 450 mg CCH (40 eggs) 900 mg CCH (37 eggs), and 1800 mg CCH (20 eggs).

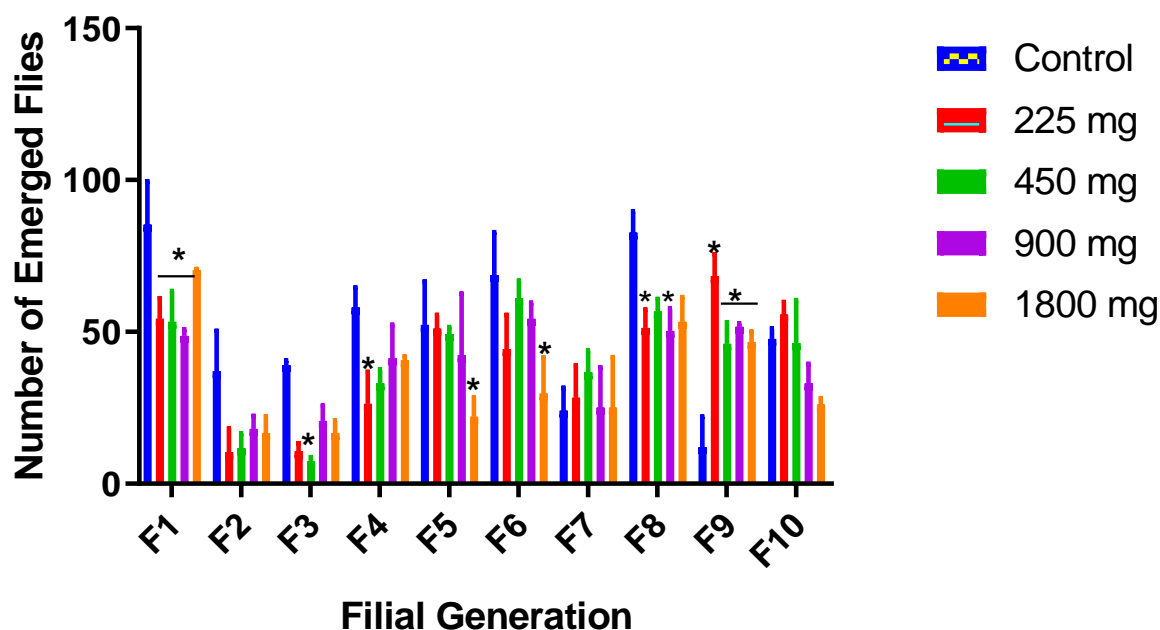


Figure 4: Filial generation output of *D. melanogaster* fed with different concentrations of calabash chalk diet. * $p < 0.05$ vs control.

In Figure 4 above, a significant decrease ($p < 0.05$) in the number of emerged flies (F₁- F₁₀) was observed especially in the 900 mg/10g and 1800 mg/10g of CCH diet concentration compared to the control.

DISCUSSION

The minerals found in CCH were reported to be beneficial to both plants and animals [23, 24]. Expectedly, the presence of these mineral elements also justifies the consumption of CCH by some women [25]. These heavy metals found in the calabash chalk used in our study agreed with that of previous findings with little variation in the composition of the metallic elements [26]. In addition, the concentration of the heavy metals in CCH turns out to be above the tolerable maximum permissible level and maximum admissible level (0.010 - 0.015 mg/L) as recommended by the World Health Organization (WHO). However, the concentration levels of the CCH elements in this study could probably be due to the geographical location where the CCH was sourced from. However, this did not contaminate the clay totally, because some essential elements like calcium, magnesium, and potassium are necessary elements found in nearly all living organisms and could serve as a supplement in those who are deficient in them [27]. The elemental analysis for the heavy metals in our study of CCH found a high concentrations of metal like lead; WHO tolerable limit: 0.010 mg/L: CCH:1.37 mg/kg; Arsenic; WHO tolerable limit: 0.010 mg/L: CCH :0.67 mg/kg; Chromium: WHO tolerable limit: 0.05 mg/L: CCH:130 mg/kg. The difference between our study and the previous ones reported could be due to

differences in the environment where both CCH were obtained [28]. This usually affects the constituent of the CCH. Clay of sedimentary origin is suggested as better geophagic clays compared to those of primary origin because they usually contain low concentrations of elemental impurities [29]. Furthermore, all the heavy metallic elements found in the study were above the permissible limit for consumption [30, 31]. The median lethal concentration (LC₅₀) of CCH in mice was determined by Ekong *et al.* [32] to be 5000 mg/kg body, weight. However, there is a paucity of literature on the mean lethal concentration (LC₅₀) of CCH in *D. melanogaster*. Hence this study has filled the gap as LC₅₀ was calculated to be 1800 mg/10 g diet. The LD₅₀ of 5000 mg/kg reported in mice [32] indicates that CCH has a low acute toxicity hazard. The difference in LC₅₀ between this study and that of Ekong *et al.* [32] may be because insects were used as the experimental model in the present study instead of mammals. However, the LC₅₀ of 1800 mg/kg diet obtained in this study still suggests that the CCH used has a relatively low acute toxicity effect. It is worth noting that CCH could be classified under 'Hazard Category 5' in the Globally Harmonized Classification System for Chemical Substances and Mixtures (GHS) as suggested previously [33, 34, 35]. The assessment of eggs laid is a marker of fertility or reproductive fitness in fruit flies [36].

It is worth noting that the toxic nature of some metal elements contained in the CCH used in this study may have interfered with egg production and consequently, with the oviposition behavior of the female flies. This decrease in eggs laid could be a

result of the effect of lead (Pb) which is part of the constituents of CCH. This agrees with the finding of [37], who observed declined reproductive fitness with altered homeostasis of essential elements in *Drosophila melanogaster*. This finding is in agreement with that [38], who revealed a dose-dependent decrease in implantation sites and decreased foetal output in pregnant Wistar rats exposed to calabash chalk. The study also corroborates with the findings of Bahadorani & Hilliker [39], who reported a decrease in eggs laid by *Drosophila melanogaster* exposed to heavy metals in adults and larvae. The finding is also in agreement with that of Oyewopo *et al.* [40], who reported a decrease in ovarian function in adult female Wistar rats fed with a high dose of calabash chalk diet. In related studies, the high levels of lead (Pb) in calabash chalk were linked to altered reproductive function [41, 42]. This finding also corroborated with [43], who reported deleterious effects of heavy metals on the reproductive organs and systems of mammals. Furthermore, several studies on the deleterious effects of heavy metals on the reproductive dysfunction agrees with our finding [44, 45, 46]. The possible mechanism involved in causing this toxicity could be by displacement of essential metal ions from biomolecules, which occurs with different types of heavy metals [47].

CONCLUSION

The higher concentration of calabash chalk diet had lowered the fecundity and filial output in the harwich strain *Drosophila melanogaster*, therefore long-term consumption of calabash chalk in high concentration should be discouraged. However further studies should be carried out on the effect of calabash chalk on the several traits of *Drosophila melanogaster* strains.

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