

# Assessment of Body Weight Change, Locomotion and Exploration in Mice Exposed to Plantain Diet

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## Abstract

Body weight change was investigated in three groups of Adult Swiss mice weighing 20.2kg –27.4kg and consumed normal rodent chow (0% plantain), 50% plantain diet or 100% plantain (*Musa paradisiaca*) diet for 30 days. Food and water intake was measured daily, while weight change was monitored at every three day interval using a sensitive electronic scale. Locomotors and exploratory activities were investigated by Open field maze. Then Brain serotonin level was estimated by high performance liquid chromatography. The result shows that mean food intake varied significantly ( $p < 0.05$ ) among the different experimental group of mice; control >50% plantain >100% plantain. The mean water intake was increased significantly ( $p < 0.05$ ) in the 100% plantain diet fed-mice than control. But the mean water intake in the 50% plantain diet group did not differ significantly from control at  $p < 0.05$ . The body weight change in the 100% plantain group was significantly ( $p < 0.05$ ) higher than control and 50% plantain diet group. The frequency of line crosses, walling and rearing was significantly ( $p < 0.05$ ) lower in the plantain groups than control. Serotonin concentration was significantly ( $p < 0.001$ ) higher in the 100% plantain diet fed-mice than 50% group and control. These observations suggest an increased weight gain margin in plantain diet fed-mice; with higher tendency for water intake but reduced locomotor and exploratory activities that may implicate serotonin.

**Keywords:** Plantain; Water; Body weight; Locomotion; Exploration

## Introduction

Weight gain and loss are physiological indices of global concern that have been accepted largely as indicators of health status. Weight gain which may be an increase in muscle mass, fat deposits, or excess fluids such as water can also be correlated to activity/inactivity of individuals. The effect that eating has on weight gain can vary greatly depending on some factors that include: energy (calorie) density of foods, amount of water intake, amount of salt contained in the food, time of day eaten, age of individual, exercise regimen and individual's overall stress level including activity or sedentary lifestyle and sleep [1,2].

Gaining weight can cause increase in body fat percentage, muscle mass and body hydration levels. Increase in breast size and in more extreme cases a noticeably larger stomach is also sign of weight gain.

Researchers investigating how diet may have other effects besides providing nourishment for the body have shown relationship between body weight and some indicators of disease or its development.

If enough weight is gained by way of increased body fat deposits, one may become overweight or fat (having more adipose tissue than is optimally healthy); predisposing persons to diseases [3]. In regards to adipose tissue increases, a person generally gains fat-related weight by increasing food consumption, becoming physically inactive, or both. When energy intake exceeds energy expenditure (when the body is in positive energy balance), the body can store the excess energy in a dense, high-energy form as fat. One pound of fat stores about 3500 calories of energy.

So over time, excessive energy intake and/or lack of exercise can contribute to fat gain and obesity. On the other hand, when people desire to watch weight gain margin, they more often than not resort to exercise regimen. The need to keep weight gain within reasonable margin is a common opinion people require adequate scientific information about; the genetic predisposition to overweight or underweight, environmental/life style factors, choice of diet, activity/inactivity, state of the mind and stress-related factors [4].

It is an established fact that some diet can contribute to weight gain more rapidly, while others have less tendency depending on their caloric values. Plantain is one crop that is vastly resourceful for man all through history, perhaps due to the usefulness of all its parts (such as the roots, stem, leaves, flower, fruit or pulp, peel and the sap) to different people for varying purposes including nutritional, domestic, medicinal and industrial use such as clothing, tools, shelter, furniture and paper [5-7].

More recently, plantain has been reported to have effects on some neurobehaviour such as; improved learning and memory, attenuation of fear and anxiety, suppression of spontaneous pain perception, all in mice. It is also shown to increase brain levels of serotonin [8,9].

Since plantain diet increases serotonin concentration in mice brains and serotonin is known to have varying influences on some physiological parameters that include neurobehavioral; the effect of long term consumption of plantain (*Musa paradisiaca*) diet on weight gain, locomotion and exploratory activities was investigated in mice, alongside water intake.

## Materials and Methods

### Animals

Thirty Adult Swiss mice weighing between 20.2 kg-27.4 kg were used for the study. Mice were housed singly in 28 cm by 12 cm by 16 cm cages, under control room temperature ( $28 \pm 2^\circ\text{C}$ ) and humidity ( $85 \pm 5\%$ ).

The animal room was properly ventilated. The animals were kept in a normal light/dark cycle and allowed to acclimatize for 2 weeks before any experiments were started. All animals had access to rodent chow (Grower's mash, Vital Feed Company, Calabar, Nigeria) and clean drinking water ad libitum.

### Preparation of plantain diet

Bunch of plantain properly identified by the Department of Botany, University of Calabar as *Musa paradisiaca* was purchased from the central market in Calabar, Nigeria. The peels were removed and the pulp washed, chopped into slices and oven dried at  $40^\circ\text{C}$  and 55% humidity for 24 h. The dehydrated slices were then grinded into powder in a grinding mill, in line with previous method [10].

This was the 100% plantain diet. The 50% plantain diet (mixed diet) was prepared from plantain powder (flour) by measuring equal gram weights of plantain flour and rodent chow and mixing thoroughly.

### Measurement of food intake

Food was measured daily into a container of known weight and weighed using electronic weighting balance, and the weight of the food was obtained from the weight difference between the empty container and the container when the food was in it. The food intake was determined by measuring the difference between the remnant food and the served food. The weight change was monitored at every three day interval using a sensitive electronic scale.

### Measurement of water intake

A known quantity of water was measured daily into the water container, using clean syringe. The water intake was measured as the difference between the remnant water and the served water.

### Measurement of body weight change

The weight change was monitored at every three day interval. A sensitive electronic scale was used to weigh an empty scooping container. Then the mouse placed inside the container was weighed. The difference in weight between the plastic scooper and the scooper, when the mouse was inside it was recorded as the weight of the mouse.

### Measurement of locomotion and exploratory activities

The open field maze test which provides simultaneous measurement of locomotion, exploration and anxiety was used [11]. Mice were

carried to the test room in their home cages and tested one at a time for 5 minutes each. A video cassette recorder (VCR) was turned on to record and the mouse's number indicated before testing. Mice were scooped up in a small plastic container from their home cage and placed in the center square of the open field.

They were then allowed to explore the apparatus for 5 minutes while the line crosses, walling and rearing behaviour were scored manually and VCR served as a backup for rescoring behavior [12].

### Neurochemical analysis

Established method was used to estimate the concentration of serotonin in the brains of all the mice as follows; Animals were anaesthetized with ethyl chloride; brains were removed, weighed and snap frozen on dry ice.

The frozen tissues were homogenized on lyses buffer (containing 10  $\mu\text{M}$  ascorbic acid and 18% perchloric acid); centrifuged for 30 minutes at 20,000 g,  $4^\circ\text{C}$  and the supernatant was used for HPLC analysis. Sample separation occurs at  $20^\circ\text{C}$  on C 18 Reversed-phase column using a 10  $\mu\text{M}$  potassium phosphate buffer, pH 5.0, containing 5% methanol and at a flow rate of 2 ml/min.

Fluorescence of 5-Hydroxytryptophan (5-HTP) and serotonin (5-HT) is excited at 295 nm and measured at 345 nm. Amount of 5-HTP and 5-HT were normalized to wet tissue weight for statistical analysis and calculation of substance levels was based on external standard values [13].

## Results

The results for the body weight change in the groups of mice as recorded in Table 1, shows that there was weight gain in all three groups of mice when considering the initial and final body weights as well as cumulatively.

Figure 1 showing moving averages of food intake, indicates that the daily food intake was not consistently increasing or reducing, hence the graph is not linear. Results of the comparison of food intake among all the groups as seen in Figure 2 shows that the 100% plantain diet group had significantly ( $p < 0.05$ ), the least food intake, followed by 50% plantain diet group and then control that had the highest intake.

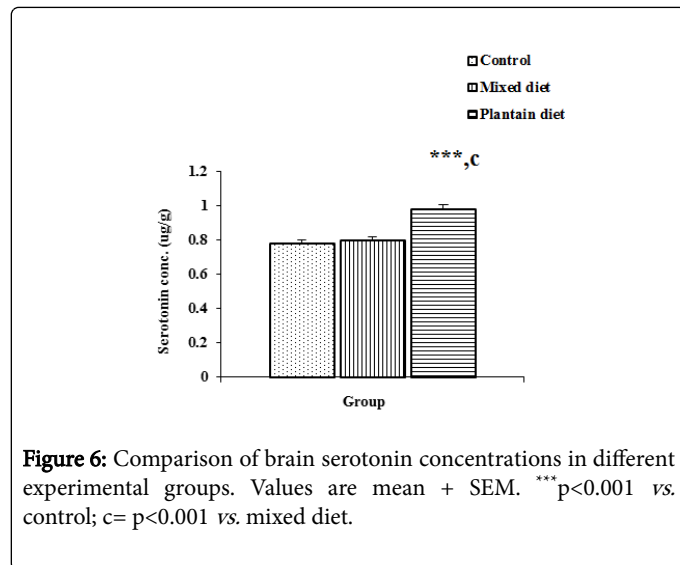
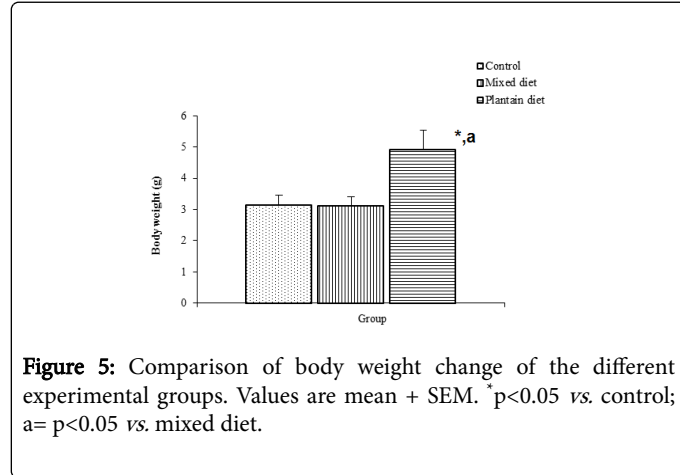
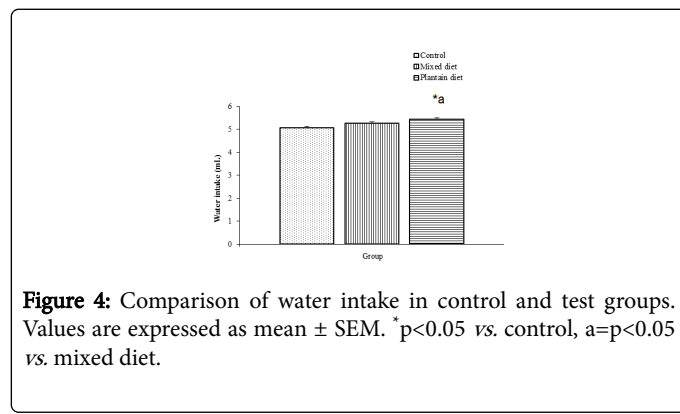
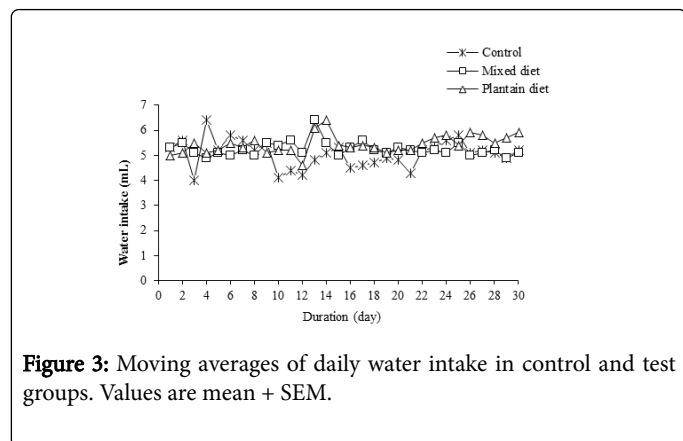
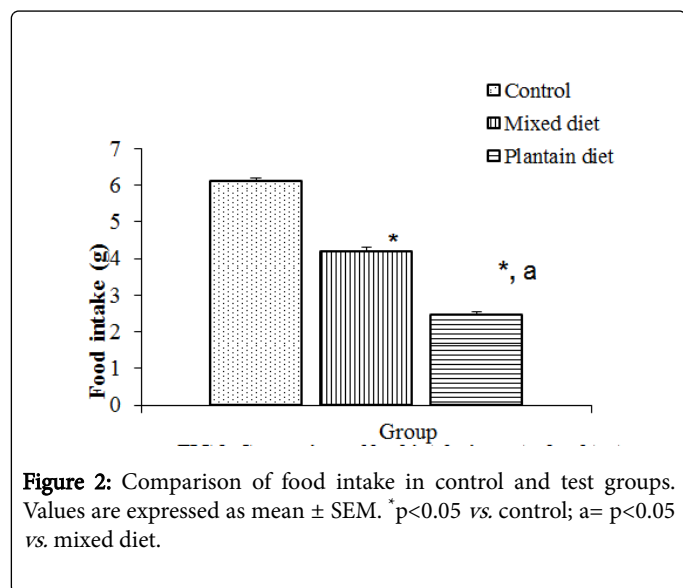
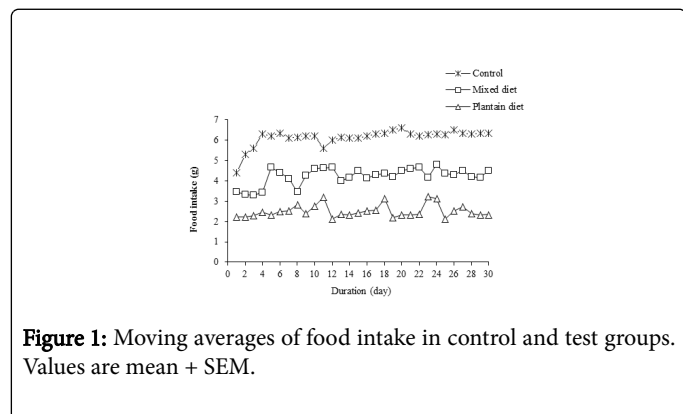
Similarly, the moving averages of water intake (Figure 3) was not linear in all the groups, but the comparison in Figure 4 shows that water intake in 100% plantain group was significantly ( $p < 0.05$ ) higher than 50% plantain group and control. The comparison of weight change in all the experimental groups in Figure 5, shows that 100% plantain diet fed-mice had significantly ( $p < 0.05$ ) higher weight gain or change than 50% plantain group and control.

When serotonin concentration in the brains of the experimental groups was compared as shown in Figure 6, the 100% plantain diet fed-mice had significantly ( $p < 0.001$ ) higher brain serotonin levels than the 50% plantain group and control.

	Mean Initial	Mean Final	Cumulative Mean	Mean Weight
	Body Weight	Body Weight	Body Weight $\pm$ SEM	Change $\pm$ SEM
Control	27.37	30.51	29.85 $\pm$ 0.31	3.14 $\pm$ 0.31

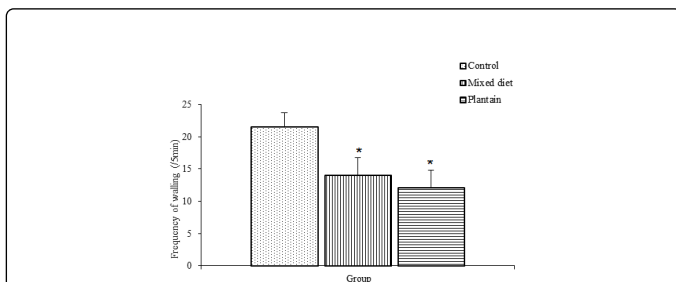
Mixed Diet	26.48	29.59	27.95 ± 0.30	3.11 ± 0.30
Plantain Diet	20.21	25.11	21.68 ± 0.63	4.90 ± 0.63

**Table 1:** Mean initial, final, cumulative body weights and weight change.

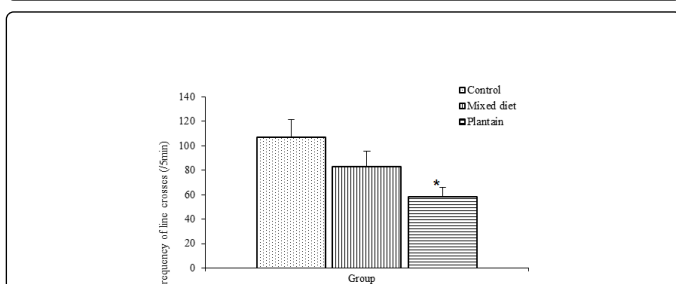


The results of the comparison of parameters that measure locomotion and exploratory activities in mice, using the Open Field

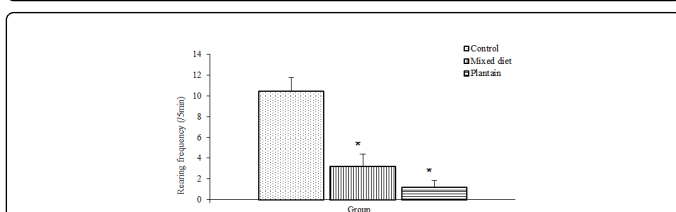
Maze test are shown in Figures 7-9. Figure 7 indicates that frequency of line crosses was significantly ( $p < 0.05$ ) lower in the 100% plantain diet fed-mice than the control group. The frequency of walling was significantly ( $p < 0.05$ ) lower in the 100% plantain group than control (Figure 8). In the same vein, frequency of rearing was significantly ( $p < 0.05$ ) lower in the 100% and 50% plantain diet fed-mice than control as shown in Figure 9.



**Figure 7:** Comparison of frequencies of line crosses in the open field maze test in different experimental groups. Values are mean + SEM,  $n=9$ . \* $p < 0.05$  vs. control.



**Figure 8:** Comparison of frequency of walling in the open field maze test in the different experimental groups. Values are mean + SEM,  $n=9$ . \* $p < 0.05$  vs. control.



**Figure 9:** Comparison of frequency of rearing in the open field maze test in the different experimental group. Values are mean + SEM,  $n=9$ . \* $p < 0.05$  vs. control.

## Discussion and Conclusion

Generally, food and water intake in rodents have long been shown to be regulated by the hypothalamus. Bruce and Kennedy reported an experiment which indicated that the tuberal nucleus is involved in regulation of food intake while the supraoptic region controls water intake. It is expected that a positive correlation exists between food intake/water intake and body weight change. Food and water intake may be influenced by certain conditions and can be a sign to detect the health status in animals [14].

In the present study, the mean daily food and water intake of three groups of mice were compared. The results showed that mice that consumed 100% plantain diet had the least food intake, followed by the 50% plantain diet group. On the other hand, mice that consumed 100% plantain diet recorded the highest water intake, followed by the 50% plantain diet group.

These observations may be due to preference that the mice had for their normal rodent chow over plantain diets or that plantain diet causes satiety while stimulating water intake. It is also possible that since serotonin was increased in the brains of the plantain diet fed-mice, it may have modulated dopamine action, thereby decreasing appetite. And if this were the case, it corroborates the report of Stahl et al., that serotonin activates 5-HT<sub>2C</sub> receptors on dopamine-producing cells to halt dopamine release which normally increases appetite [15]. All the groups of mice gained weight in the course of their exposure to the different diets. However, when the mean weight change was assessed, the result indicated that the mice that consumed 100% plantain diet had the highest weight change. This observation could be due to rich caloric value of plantain and or increases in water intake, since weight gain could also be contributed by fluids intake. Moreover, the results from the open field maze for locomotor and exploratory behaviour may explain the increase in weight following consumption of 100% plantain diet. Locomotion and exploratory activities were reduced in the plantain diet fed-mice in such a way that implies plantain may have slowed activity in the mice; it is possible that since locomotion and exploration were reduced, the animals were sedentary and did not lose a lot of calories possible, through metabolic activity during explorations/locomotion thereby conserving and gaining more weight. The preliminary neurochemical analysis shows that the brain's level of serotonin was significantly higher in the plantain diet fed-mice. Perhaps serotonin contributed to the sedentary state of those mice. This may give credence to the report of Foy and Parrat stated that plantain contains tryptophan and 5-HTP that encourage the production of serotonin [16]. Serotonin is involved in the regulation of sleep, and this can be affected in two ways, first through its metabolite, melatonin which induces sleep and secondly by counteracting the wakefulness, arousal mechanisms of brain nor epinephrine and dopamine to prevent over arousal [17]. It is possible that plantain diet increased the biosynthesis of serotonin in the brain of the mice which in turn caused the reduced locomotor and exploratory activities.

## Statistical Analysis

All data were collated using standard statistical methods. Analysis was done with computer SPSS Version 17.0 The variance within and among samples were analyzed using one way analysis of variance (ANOVA TEST), while the statistical difference between groups of two were analyzed by post-hoc least significance difference (LSD) test. The results were recorded as mean  $\pm$  SEM (standard error of the mean) shown in Table 1, graphs and/or bar charts with the 50% group as mixed diet and 100% as plantain diet.

## Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" were followed. All experiments had been examined and approved by the appropriate ethics committee (Ethical committee on research, University of Calabar).

## Acknowledgement

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## Conflict of Interest

We declare there is no conflict of interest regarding this research.

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