Original Research Article

Growth Performance of Albino Rats Orally Administered with Honey from *Apis mellifera adansonii* and Refined Sugar at Varying Levels

ABSTRACT

The purpose of this research was to find out the growth performance of albino rats orally administered with honey from *Apis mellifera adansonii* and refined granulated sugar at varying quantities. Twenty-five (25) healthy albino rats used in the study were grouped into five treatments based on the dose of natural honey and granulated sugar as follow: T1 (1.02g of honey kg BW), T2 (1.40g of honey kg BW), T3 (1.02g of granulated sugar kg BW), and T4 (1.40g of granulated sugar kg BW). However, rats in T5 were not administered honey and refined granulated sugar hence served as the control. The data on fructose and micronutrients concentrations of natural honey and refined sugar as well as data on the weight gain and feed intake of the albino rats were determined after 28 days. According to the findings of this study, the fructose concentration was higher in natural honey (195.78mg/ml) while refined sugar (30.225mg/ml) recorded the least value. Also, copper (0.15ppm) and manganese (0.05ppm) had the highest mean concentration in natural honey, while iron (0.04ppm) and zinc (0.07ppm) were higher in refined sugar. The study's findings revealed that the highest feed intake by albino rat was recorded in T1 (148.16g) while T5 (140.27g) had the least. There were no significant differences in albino rat feed intake among the five treatments ($p>0.05$). The highest weight gain was recorded by the albino rat in T1 (69.84g) while T5 (32.28g) had the least. There were no significant differences in the weight gain of the albino rats among the five treatments. This study's findings led to the conclusion that the doses of natural honey used did not significantly increase the weight gain of albino rats.

Keywords: Growth Performance, Albino Rats, Natural Honey, Refined Sugar

INTRODUCTION

Sweeteners when used provide a sweet taste to food and beverages. They can be used in food preservation, fermentation, baking, and the browning and caramelization of foods. Natural sweeteners may be both nutritive and flavorful, making them popular as both food and flavoring agent (Mendoza-Pérez *et al*., 2021). The utilization of sweeteners has been shown to increase the metabolism rate in the human body. Furthermore, sweeteners contain essential nutrients which can be utilised for body growth, development, and maintenance of the body system (Archibald *et al*., 2018).

The nutritional requirements of the human body are obtained through the consumption of essential nutrients and supplements found in many food products (Folayan *et al*., 2020). Lack or excess of certain nutrients can affect the body's homeostasis and can hinder metabolic activities in the body of an organism. The energy that drives body metabolism comes mainly from foods rich in carbohydrates such as sugars (Shlisky *et al*., 2017). However, man's modern-day lifestyle involves a high intake of 'junk' and "fast foods", with added sugars mainly sucrose and also fructose; and natural sweeteners such as natural honey. The metabolism of these nutrients takes

place within body cells and tissues, excess of these nutrients, depending on the source may be undesirable and consequently may alter body homeostasis (Dolezal and Toth, 2018).

Natural honey (NH) is a sweet, viscous liquid food produced in the honey sacs of various bees, particularly *Apis mellifera* (a common species of honeybee found in Nigeria), from the nectar of flowers (Abdullahi *et al*., 2011). The flavor and color of the nectar are solely determined by the flower from which it is gathered. The nectar is converted into honey through the inversion of the majority of its sugars, levulose (fructose) and dextrose (glucose), as well as the removal of excess moisture (Britannica, 2021). Natural honey is primarily composed of sugars and water, with minor amounts of a variety of vitamins and minerals, particularly B vitamins. Honey also contains amino acids, antibiotic-rich inhibine, proteins, phenol antioxidants, and micronutrients (Dolezal and Toth, 2018). Because the sugars in honey are sweeter and provide more energy than regular sweeteners, fructose (the sweetest natural sugar) is the most abundant sugar in honey. This substance is extremely beneficial to the human body in terms of nutrition and health. Niacin, riboflavin, and pantothenic acid (also known as vitamin B5) are among the vitamins found in honey, as are minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium, and zinc (Zulkhairi-Amin et al., 2018).

Honey is widely accepted by people of all ages, and its use transcends religious, cultural, and ethnic boundaries (Dunne *et al*., 2021). Natural honey has been used as food and medicine by humans since time immemorial. Honey is also very likely the world's most ancient sweetener, having been used for millennia throughout the world (Daiana *et al*., 2019). It contains major components of a meal, micronutrients that will enhance the digestion and absorption of dietary essentials, as well as non-essentials required for the metabolic activities and the proper functioning of the human body (Dolezal and Toth, 2018). Natural honey, which contains up to 17 mg of carbohydrates per tablespoon consumed, has been linked to improved physical performance and provides much-needed energy, making it an inexpensive substitute for refined sugar (Kadri *et al*., 2017). These enzymes (amylase and D-glucose) found in natural honey aid in food digestion in the human body system (Lim *et al*., 2019). The advantage of eating natural honey as a source of energy over commonly used refined sugar is that the sugars contained in honey are in a very simple pre-digested form, which allows them to be directly absorbed by the body (Wright *et al*., 2018). Refined sugar, on the other hand, must be broken down in our bodies into simpler forms before it can be absorbed. Natural honey has nutritional advantages over refined sugar because it contains micronutrients that aid digestion in the human body (Shlisky *et al*., 2017).

Refined sugar is a sweet-flavoured substance that is classified as carbohydrate foods and composed of carbon, hydrogen, and oxygen with the molecular formula of $C_nH_{2n}O_n$ (where n is between 3 and 7) (Lu *et al*., 2017). In Nigeria, the per capita consumption of refined sugar in 2019 is about eight kilograms (United States Department of Agriculture, USDA 2019). There has been a tremendous rise in the intake of refined sugar in recent times, due to its inclusion in cooking and baking, as well as in the production of sweets, canned fruits, jams, jellies, dairy foods, carbonated beverages, and other sweetened drinks (Sholeye *et al*., 2018). In addition to the global menace of refined sugar, its consumption in excess has been associated with various health problems. A high refined sugar intake induces insulin resistance and contributes to obesity and other deleterious metabolic changes (Folayan *et al*., 2020). Diets high in refined sugars, such

as those found in sweetened beverages, candy, baked goods, and sugary cereals, contribute to weight gain and chronic health conditions such as obesity, heart disease, and diabetes. There are several studies on the effect of refined sugar on animal health (Adegoke, 2019; Shamsi-Goushki *et al*., 2020) and natural honey (de Oliveira Cécere *et al*., 2020; Nnaji and Ekpe, 2018).

There is an increasing demand for a healthy substitute to refined sugar, this has become important to curb the menace of metabolic syndrome (John-Isa *et al*., 2019). The World Health Organization (WHO) recommends limiting refined sugar consumption to less than 10% of daily calories and, specifically, choosing beverages with no added sugars. The dietary recommendation according to the work of Olatona *et al*. (2019) as adapted from American Heart Association (AHA, 2019) reported that there should be "no added sugar for children younger than age two, no more than 100 sugar calories from added sugar per day for children older than age two and most women and no more than 150 calories from added sugar a day for most men. Natural honey undoubtedly is a good source of natural sugars and thereby can be used as a substitute for refined sugar. Natural honey was reported to improve the growth and performance of the body when consumed with feed without any side effects (Kadri *et al*., 2017). However, there is scarce literature on the effect of natural honey and refined sugar, on albino rats in Awka, Anambra State Nigeria. Hence the aim of this study was to ascertain the effect of refined sugar and natural honey on albino rats in Awka Anambra State.

MATERIALS AND METHODS

Study Area

The study was carried out in the Animal House of the Department of Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria. The experimental site lies between latitude 6°15′18.06″N and longitude 7°06′41.37″E. Awka is located at latitudes 6°9'19"N 7°07'12"E and stretches 8 kilometers East-West along the Enugu-Onitsha expressway and about 5 kilometers North-South. Awka has a land area of 1,207,800m^2 (12,007 ha). Ecologically, Awka lies in the Guinea Savanna and has experienced 1798.52mm of rain annually between 1977 and 2019. It experiences two seasons: the dry and the wet seasons with a spell of harmattan between December and January (Abajue and Ewuim, 2020).

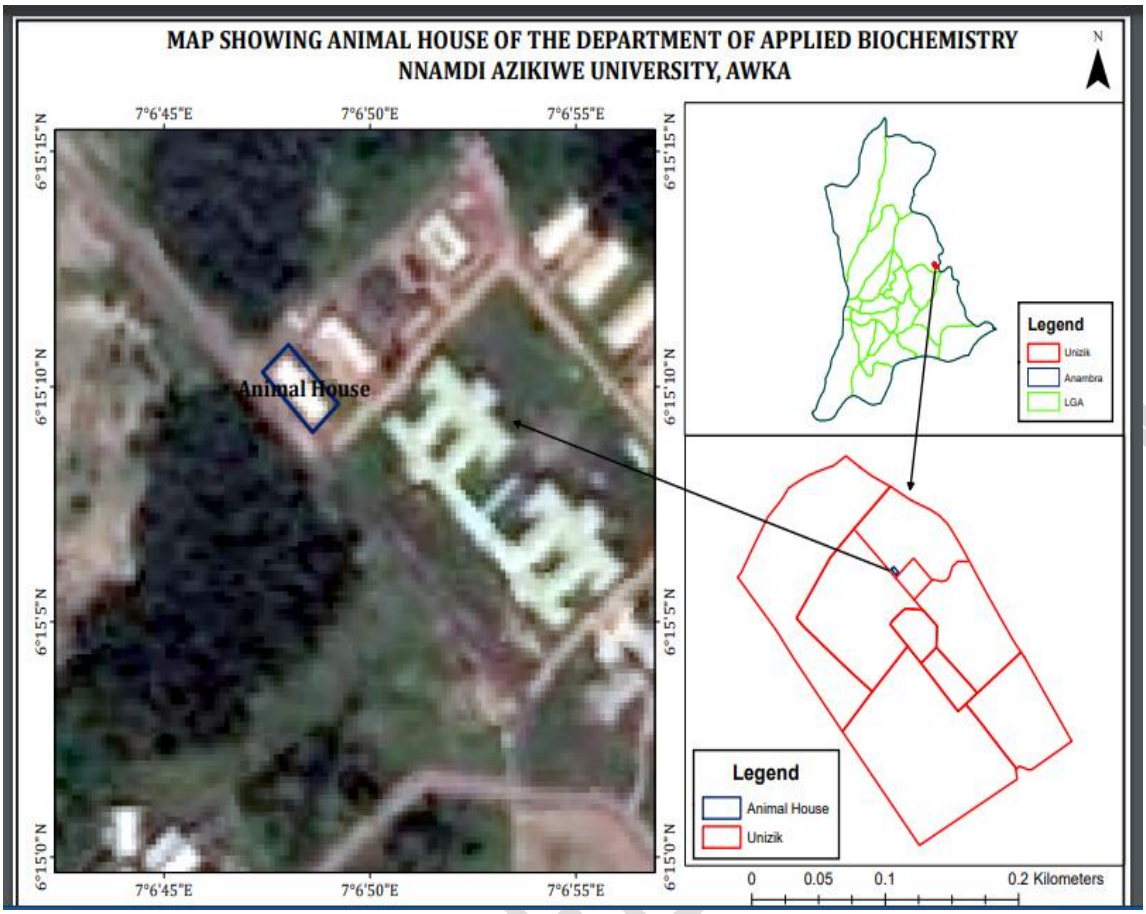

Figure 1: Map of NnamdiAzikiwe University Awka Showing Animal House
(Source: Cartography Lab, Dept of Geography and Meteorology, NnamdiAzikiwe NAU, 2021).

Sources of Experimental Materials

Twenty-five (25) healthy albino rats with mean weights of 41.21±1.769 g, were purchased from Mr. Onyewuchi farms in Awka, Anambra State. The albino rats were carefully transferred to the Animal House of the Department of Biochemistry, Nnamdi Azikiwe University, Awka, where the experimental animals were housed for the whole period of the experiments. The refined sugar (Dangote granulated sugar) used for this study was purchased from Eke-Awka market, Awka Anambra State. However, the honeycombs freshly harvested by the apiarists at the Uzoben Integrated Services, Awka Anambra State were processed and used for this study.

Experimental Design

The albino rats were housed in cages with wire lid to allow proper ventilation in the animal house. They were allowed to stay for one (1) week days to acclimatize at room temperature (27±3°C) before the commencement of the experimental feeding procedures. The initial weights of the rats were recorded after acclimatization. For easy identification, the experimental rats were marked at the head, abdomen, centre, neck, and tail then labeled H, Ab, C, N, and T respectively. The albino rats were grouped into five treatment groups based on the dose of natural honey and granulated sugar namely: T1(1.02g of honey kg BW), T2(1.40g of honey kg BW), T3(1.02g of granulated sugar kg BW), T4(1.40g of granulated sugar kg BW) while rats in T5 was not

administered with honey or granulated sugar hence was served as control. The experiment was set up in a completely randomized design, each treatment comprised five albino rats.

Management of Experimental Animals

The albino rats were intensively managed in an improvised metal cage with dimension length 1m, height, 1m, and breadth, 0.5m which was used to house the albino rats. The sides of the cages were covered with wire to allow proper ventilation and the floor was made of tin metal covered with sawdust. During the experiment, the albino rats were subjected to similar sanitary conditions so that the only source of variation was the dosage of sweeteners orally administered.

Fructose and Micronutrient Analysis

The fructose and some micronutrients (copper, iron, manganese and zinc) concentrations of the honey and refined sugar were determined according to the method described by Edewor and Theresa, (2013) and Tuzen *et al*. (2007) respectively.

Determination of growth performance rate

1. Weight Gain

The initial weights of the animals were recorded after a measurement at the end of the acclimatization period. After acclimatization, the weights of the animals were measured on a weekly basis. The albino rats were grouped according to their doses of sweeteners administered to them. The weight gains of the rats were determined using the formula presented below:

Weight Gain = body weight at the end of the period – body weight at the beginning of the previous period (Oke *et al*., 2016)

2. Feed Intake

The daily feed intake of the albino rats was recorded for the whole period of the experiment. The rats had continuous access to water. Feeding was continued up to 28 days. Feed intake was monitored daily and expressed on a dry weight basis. The rats were fed on standard rat chow and water *ad libitum* during the experiment

Statistical Analysis

The data collected from the fructose and micronutrient concentrations of honey and refined granulated sugar was subjected to Independent T-test while data on weekly weights and feed intake of the albino rats was subjected to a One-way analysis of variance (ANOVA). The sample means were statistically separated using Tukey Honestly Significant Difference test. Graph of fructose concentration of honey and refined granulated sugar was plotted with Microsoft Office Excel (2016 version). Complete data was analysed using IBM SPSS statistics version 23 at 5% significance.

RESULTS

The fructose concentrations of natural honey and refined sugar are presented in table 1. The result revealed that the fructose concentration was higher in natural honey (195.78±142.991 mg/ml) than in refined granulated sugar (30.225±33.001 mg/ml). However, there was no

significance difference in the mean fructose concentration of honey and refined granulated sugar (P>0.05).

Table 1: Fructose concentration of Natural Honey and Refined Granulated Sugar

Sweeteners	Mean Fructose Concentration ±SD (mg/ml)
Natural honey	195.78±142.991
Refined sugar	30.225±33.001

The micro-nutrients found in natural honey and refined sugar are presented in Table 2 showed that the highest mean concentration of copper was higher in natural honey (0.15±0.19 ppm) than in refined sugar (0.00±0.004 ppm). It was also showed that refined sugar recorded high concentration of iron (0.04±0.013ppm) than natural honey (0.01±0.019 ppm). Similarly, it showed that manganese concentration was high in natural honey (0.05±0.071 ppm) than refined granulated sugar (0.04±0.024 ppm). Also, the concentration of zinc was high in refined granulated sugar (0.07±0.073ppm) than natural honey (0.05±0.011 ppm). Statistical analysis showed that there was no significant difference in the mean concentration of copper, iron, manganese and zinc between the natural honey and refined granulated sugar examined (P> 0.05).

Table 2: Mean Concentration of Micro Nutrient in Natural Honey and Refined Sugar

Sweeteners	Copper concentration (ppm)	Iron concentration (ppm)	Manganese concentration (ppm)	Zinc concentration (ppm)
Natural honey	0.15±0.19	0.01±0.019	0.05±0.071	0.05±0.011
Refined granulated sugar	0.00±0.004	0.04±0.013	0.04±0.024	0.07±0.073

The weekly feed intake of albino rats fed with natural honey and refined sugar are presented in table 3. The result of the study revealed that the highest feed intake by albino rat was recorded in T1 (148.16±3.675g) followed by T2 (142.21±15.590g), followed by T4 (140.79±18.423g) and T3 (140.79±18.423g) while the least was T5(140.27g). However, ANOVA result revealed that there were no significant differences between the feed intake of albino rat fed with the five treatments (p>0.05).

Table 3: Weekly feed intake of albino rat fed with natural honey and refined sugar

Treatment	Week 1	Week 2	Week 3	Week 4	Total	Mean feed intake ±SD (g)
T1	142.65	150.00	150.00	150.00	592.65	148.16^{a}±3.675
T2	118.82	150.00	150.00	150.00	568.82	142.21^{a}±15.590
T3	113.15	149.99	150.00	150.00	563.14	140.79^{a}±18.423
T4	116.26	148.00	150.00	150.00	564.26	141.07^{a}±16.564
T5	111.08	150.00	150.00	150.00	561.08	140.27^{a}±19.460

Table 4 showed that highest weight gain was recorded by the Wistar rat fed with treatment T1(69.84g) followed by T3 (61.9g) while the least is those fed with treatment T5(32.28g). The result revealed that there were no significant differences between the weight gain of Wistar rat fed with the five treatments (P>0.05).

Table 4 weekly weight gain of albino rat fed with natural honey and refined sugar for a period of 4 weeks

Treatments	Week 1	Week 2	Week 3	Week 4	Weight Gain (g)
T1	47.27^{b}±1.769	66.7^{a}±9.218	81.2^{a}±14.874	117.11^{a}±15.871	69.84^{b}±16.028
T2	43.85^{ab}±1.78	78.18^{a}±8.189	82.46^{a}±11.149	97.29^{a}±22.639	53.44^{ab}±23.196
T3	45.58^{b}±3.438	68.88^{a}±7.522	72.88^{a}±9.775	107.55^{a}±15.023	61.97^{ab}±17.674
T4	43.69^{ab}±2.031	79.99^{a}±11.488	84.61^{a}±11.015	106.01^{a}±14.64	62.32^{ab}±13.220
T5	41.21^{a}±1.247	65.48^{a}±5.435	78.53^{a}±24.268	73.5^{a}±20.033	32.28^{a}±20.169

Columns sharing similar superscripts are not significantly different (P>0.05)

DISCUSSION

The study showed that natural honey had higher fructose concentration than refined sugar. This is in line with the study of Sohaimy *et al*. (2015) who reported that Dextrose (glucose) and laevulose (fructose) are the main sugars in honey. These are the building blocks for the more complex honey sugars and account for about 85 percent of the solids present in honey. Also, Adekanmbi *et al*., (2019) reported that honey is rich in fructose, glucose, and minerals such as magnesium, potassium, calcium, phosphate, sulfur, ferrous, and sodium chloride as well as vitamins such as B1, B2, B5, B6, and C.

The analysis of micronutrients of refined sugar and natural honey showed that copper was obtained in natural honey (0.15±0.19 ppm) while the least value was obtained in refined sugar (0.00±0.004 ppm). The high content of copper and manganese present in honey could be attributed to the fact that some honey according to Nigussie *et al*. (2012) is richer in minerals, and due to their mineral content, they are said to be less suitable for storage in the winter. However, it was observed that refined sugar recorded high concentration of iron. The reduce iron content of honey could be as a result of the plant visited by honey.

The weekly feed intakes of albino rats fed with natural honey and refined sugar revealed that the highest feed intake by albino rat was recorded in T1 (148.16g), while the least was T5 (140.27g). The finding of this study was in line with the Atangwho *et al*. (2020); on their study on albino rats fed with honey and a sugar free diet, reported that the feed intake of albino rats was significantly higher in rats fed with honey.

The effect of honey and refined sugar on weekly weight gain of albino rat at varying level showed that the highest weight gain was recorded by the Wistar rat fed with treatment T1 (69.84g) followed by T3 (61.97g) while the least is those fed with treatment T5 (32.28g). The findings were similar to the work carried out in Nigeria which showed enhanced body weight gain by experimental rats fed blossom honey and honeydew honey in several different studies (Samat *et al*., 2017). Furthermore, in a study carried out by Atang *et al*. (2020) on the effect of long-term feeding of the natural honey and table sugar-sweetened diets on obesity and pro-inflammatory biomarkers in rats, showed that both honey and sugar incorporated diets produced significant body weight gain in the female animals. Their findings further specified that the effect which was higher in treatment with honey than sugar, depended on the level of each sweetener used as well as feeding duration. Chepulis and Starkey (2009) fed honeydew honey to 8-week-old rats for 52 weeks to assess weight gain. These workers show that the growth influence of honey in rodents is due to increased bone growth and mineralization (Minhas and Dhaliwal, 2018), probably due to calcium content of honey.

Conclusion

This study showed that natural honey increases the feed intake and weight gain of albino rats. Hence it can be concluded that natural honey invariably increased the growth performance of albino rat. Since natural honey contained substantial amount of fructose which is a main source of energy, and a reasonable amount of micronutrient (such as iron, manganese and zinc), it was recommended that natural honey should be consumed regularly by humans for efficient performance of the body.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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