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EVALUATION OF THE EFFICACY OF DIETARY *Canarium schweinfurthii* OIL INCLUSION ON GROWTH PARAMETERS IN MALE ALBINO WISTAR RATS

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ABSTRACT
40 male wistar rats between 7-8 weeks old and weighing between 100 and 120 kg were used in a 28-day trial to examine the effect of supplementing different levels of Canarium schweinfurthii oil on growth rate of male wistar rats. The rats were randomly distributed into groups of ten (10) using a completely randomized design in four treatment groups with one serving as control. Control group was fed using standard growers mash as diet without oil, while the remaining treatment groups were fed diet supplemented with 2.5%, 5.0%, and 10.0% Canarium schweinfurthii oil. ANOVA was used to analyse the data generated and the results indicated that weight gain of rats differed significantly (p<0.05). Feed intake also differed significantly (p<0.05) among the rats receiving 10% level of Canarium schweinfurthii oil supplementation. Proximate analysis study revealed high ethyl extracts, protein and calcium levels in this particular level of supplementation. The findings in this present study suggest that dietary Canarium schweinfurthii oil supplementation at the appropriate levels can offer improved nutritional outcomes.
Keywords: Canarium Schweinfurthii; Dietary supplementation; Growth; health promotion; proximate analysis

INTRODUCTION

Child undernutrition is a major public health problem, especially in many low-income and middle- income countries (UNICEF, 2015). It adversely affects the productivity of nations as well as creating economic and social challenges among vulnerable groups. Poor nutrition is associated with suboptimal brain development, which negatively affects cognitive development, educational performance and economic productivity in adulthood (Leroy *et al*., 2014).

Child growth is the most widely used measure of children's nutritional status. The first 1000 days of life (0-23months) is a very critical phase in a child's life during which rapid physical and mental development occurs (Onis & Branca 2016). Undernutrition during this critical phase can have irreversible consequences on the child's growth leading to an increased risk of morbidity and mortality in children. Undernutrition is commonly assessed through the measurement of a child's anthropometry (height, weight), as well as through screening for biochemical and clinical markers (Black *et al*., 2013). Wasting, stunting and underweight are expressions of undernutrition and the anthropometric indicators for the assessment of a child's nutritional status.

Undernutrition is the underlying cause of child mortality in about 45% of all deaths reported for children under-5 years of age (WHO, 2010). In 2015, globally about 7.7% of children were wasted, 24.5% were stunted and 15% were underweight. The African region and South-East Asia have reported the highest prevalence of undernutrition, with the former accounting for about 39.4% of the stunted, 24.9% of the underweight and 10.3% of the wasted children under-5 years of age (UN, 2015). According to the 2015 Millennium development goal (MDG) report, sub-Saharan Africa (SSA) accounts for one third of all undernourished children globally, highlighting that malnutrition still remains a major health concern for children under 5 years in the sub-region, thus buttressing the need for urgent intervention (UN, 2015).

There have been individual studies reporting the burden and determining factors of childhood undernutrition in SSA (Bain *et al*., 2013). These individual studies have varied in design and geographic operationalization, making it difficult to make regional comparisons and put in place regional initiatives to meet global agendas such as the MDGs.

"Atili" (*Canarium schweinfurthii bursaraceae*) is the fruit of the perennial tree plant also called "atili"' tree. In Nigeria, the fruit is called 'ube okpoko' in Ibo and "atili" in Hausa. The fruit is commonly found in large quantity in Pankshin, Plateau State of Nigeria and is also produced in similar quantities in other states of the Northern and South-Eastern Nigeria.

The plant produces its fruits in the rainy season (usually) between the months of April and September. The flowers grow in clusters at the end of the twigs and are small and dark green in color. The fruits which are of two varieties-long spiral and short round in shape develop from the flowers. The fruits contain single triangular-shaped seed with small projections at the three edges. The seeds are embedded in a purplish green pulp with a desirable sweet but not too sugary taste similar to that of avocado pear. The pulp is of oily consistency and edible with a weight which ranges from 3.5 to 9 g with a predominant average weight of about 5.3 g. The fruit is very hard, the seed is cooked and yields an oil, sometimes used as a substitute for shear butter (Kochar, 1981). The fruit store best under cold storage thus, preventing moisture loss that may result in shrinkage of the fruit surface thus reducing its aesthetic value and also prevent microbial growth (Looney, 1985). The importance of lipids in human diet cannot be over emphasized of which in normal diet, about 20-25% of the caloric intake consist of fats and oils which are the most concentrated form of energy in human diet and when metabolized produce about 9.5 kcal/g (Okaka *et al*, 2002).

Crude "atili" oil from both long and short varieties are rich in fat, protein, carbohydrate, water and in spite of its rich nutrient content, it is cheaper than groundnut oil especially during the hot season when they are locally produced more abundantly, it is used in frying different kinds of foods such as meat, bean cake and fish. It is rich in flavor and is sometimes sprinkled on food like cooked rice and smoked fish before consumption. Crude "atili" oil in addition to containing natural flavors, free fatty acids, pigments, moisture, trace element [metal], pro-vitamins, vitamins also contain naturally occurring antioxidants and enzymes. The fruits have served man for centuries as snack and oil from the fruits have served man for domestic, pharmacological and industrial purposes. The study intends to determine the growth and feeding rate potential of *Canarium schweinfurthii* oil supplemented diets on male wistar rats.

MATERIALS AND METHODS

Plant Identification and Collection

Canarium schweinfurthii fruit was purchased in Bukuru, Jos, Plateau State, Nigeria. The fruits were identified at the Heberium of the Biological Science Department Ahmadu Bello University Zaria, Kaduna State, Nigeria where a Voucher Number 7232 was issued.

Preparation of the Oil Extract

The fruit was first washed, and then 1.5 litres of water was boiled at a temperature of 87^0C after which an equal volume of water at room temperature was then added bringing the temperature down to 55^0C and then it was poured into a bucket containing the fruit to make it tender. The fruit pulp was then removed from their seeds, sun-dried outdoors for three (3) days and then milled in a locally made wooden mortar and pestle, after which it was then defatted using the Soxhlet technique adopting the method described by Association of Official Analytical Chemist (1980) using n-hexane.1.5 kg of the powdered fruit pulp were packed in muslin cloth, and inserted into the soxhlet extractor and subjected to continuous reflux action for 10 hrs using n-hexane as the solvent. n-hexane was recovered using a rotary evaporator model number SRC14. Exactly 6 kg *Canarium schweinfurthii* fruit was defatted and the residual oil was placed in a hot water bath at 45^0C for one hour for traces of n-hexane to evaporate. The oil was then stored in glass jars and stored in a refrigerator at 4^0 C until required.

Test Animals

Forty Wistar rats, between 7-8 weeks old and weighing between 100 and 120 kg were used for the experiment. They were purchased from the Rat Colony at the Faculty of Pharmacy, Ahmadu Bello University Zaria, Kaduna State, Nigeria. The animals were housed in standard rat cages and were fed *ad libitum* with growers mash acquired from Vital feeds produced by Grand Cereals in Bukuru, Jos Plateau State, Nigeria and tap water.

Experimental Animals/Groupings

Group 1: Normal control

Group II: Fed with growers mash and 2.5% *C. schweinfurthii* oil.

Group III: Fed with growers mash and 5% *C. schweinfurthii* oil.

Group IV: Fed with growers mash and 10% *C. schweinfurthii* oil.

Diet Supplementation

Wistar rats in Group 1 were fed standard growers mash, however the wistar rats in Groups 2-4 were fed growers mash supplemented with varying levels of *Canarium schweinfurthii* oil for six weeks.

Feed Intake Measurement

Feed intake was measured using a battery operated weighing balance by subtracting the left-over of the feed remaining in the bag of feed at the end of the week from the original quantity of the feed kept in the bag which is 1kg. The value so derived indicated the volume of feed consumed by the rats per week.

Body Weight Measurement

The wistar rats were weighed using a battery operated weighing balance at the commencement of the experiment and subsequently once a week during the course of the study experiment.

Proximate Analysis of *Canarium schweinfurthii* Oil

Determination of Total Protein

Protein in the sample was determined by Kjeldahl method described by Chang (2003). 1.0 g of dried samples was taken in digestion flask and then 10-15 ml of concentrated H_2SO_4 was then added to make up at least 8 g of digestion mixture i.e. K_2SO_4: CuSO 4 (8: 1). The flask was swirled in order to mix the contents thoroughly and then placed on a heater to start digestion till the mixture become clear (blue green in color) this procedure took approximately 2 hrs to complete after which the digest was cooled and transferred to 100 ml volumetric flask and the volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markam Still Distillation Apparatus (Khalil and Manan, 1990) in which 10 ml of digest was introduced in the distillation tube and then 10 ml of 0.5 N NaOH was gradually added through the same way for a further 10 min and NH_3 produced was collected as NH_4OH in a conical flask containing 20 ml of 4% boric acid solution with few drops of modified methyl red indicator. During distillation yellowish color appears due to NH_4 OH of which the distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink color. A blank was also run through all steps as above after which percent crude protein content of the sample was calculated by using the following formula:

$$N\ (g\%) = \frac{(mL\ 0.1N\ HCl\ sample - mL\ 0.1N\ HCl\ blank) \times 0.0014 \times N\ HCl \times 100}{Weight\ of\ sample}$$

Determination of Total Fat

This was determined by solvent extraction gravimetric method described by Kirk and Sawyer (1980). Sample was blended until homogenous and then 5 g of homogeneous sample or 10 ml was weighed in duplicate, into a container (W1) in which some glass beads were placed which was then connected to an air condenser and refluxed with gentle boiling for 30 min after which the residue produced was then washed with warm water until the filtrate was deemed free from acid. The filter paper containing the residue was dried in an oven at 60^0C for 6h and then transferred into an extraction thimble which was then placed in a reservoir part of a soxhlet apparatus. A round flat bottom flask was then dried in an oven at 100^0C for 1 h and this was then cooled in a desiccator and weighed (W2) after which 50 ml of diethyl ether was then added into the pre-weighed round flat bottom flask and placed into the fat extraction system. Sample was extracted in the thimble by immersing it in warmed solvent for 30 min after which the solvent was evaporated in each round flat bottom flask on a water bath in a fume hood. The flask was then dried in an oven at 100 ^{0}C for 30 min and cooled in a desiccator and then re-heated and weigh again every 30 min until constant weight was obtained (W3), (Horwitz 2000).

$$Total\ Fat\ (g/100\ g) = \frac{W_3 - W_2}{W_1} \times 100$$

where: W1 = Weight of sample
W2 = Weight of dried extraction cup before fat extraction
W3 = Weight of dried extraction cup after fat extraction

Determination of Crude Fiber

This was done by furnaces incineration gravimetric method described by James (1995). Sample (5.0g) was dried in an oven at 150^{o}C for 1 h. Then the sample was allowed to cool in a desiccator and weighed (W_1) after which the sample was kept in crucibles in a muffle furnace at 55^{o}C for 4 hrs after which the samples were then cooled in a desiccator and weighed again (W_2). Calculations were done by using the formula:

$$\%\ Crude\ Fiber = \frac{W1 - W2}{Weight\ of\ sample} \times 100$$

Determination of Total Ash

This was done by furnaces incineration gravimetric method described by James (1995) and AOAC (1984). Clean empty crucibles were placed in a muffle furnace at 600^{o}C for an hour, cooled in desiccator and then the weight of empty crucible was noted (W_1).

One gram of each sample was taken in crucible (W_2) and the sample was ignited over a burner with the help of blowpipe, until it is charred. Then the crucible was placed in muffle furnace at 550^{o}C for 4 h after which appearances of gray white ash were observed which indicated complete oxidation of all organic matter in the sample. After ashing furnace was switch off, the crucible was cooled and weighed (W_3) and percent ash was calculated by following formula:

$$\%\text{Ash} = \frac{\text{Difference in Wt. of Ash} \times 100}{\text{Wt. of sample}}$$

Determination of Cation (Calcium)

The digested sample was analyzed for mineral contents by Atomic Absorption Spectrophotometer (Hitachi model 170-10). The equipment was run for standard solution using specific electrodes of the mineral before and during determination to check that it is working properly given that the dilution factor for calcium was 100, however further dilution of the original solution was done by using 0.5 ml original solution and enough distilled water was added to it to make the volume up to 100 ml. About 1.0 ml lithium oxide solution was added to the original solution to unmask Ca from Mg. The concentrations of the mineral was recorded in terms of "ppm" and converted to milligrams (mg) of the mineral by multiplying the ppm with dilution factor and dividing by 1000, as follows:

$$MW = \frac{\text{absorbency (ppm)} \times \text{dry wt.} \times D}{\text{Wt. of sample} \times 1000}$$

Statistical Analysis

The data generated from the study were analyzed using Microsoft Excel 2013 and SPSS 20 applications. ANOVA was used to analyse the data obtained and all results are expressed as ± standard deviation.

RESULTS

In the proximate analysis (Table 3c) the 10% *C. schweinfurthii* oil supplemented diet significantly ($p>0.05$) higher values for dry matter, ethyl extract, crude fibre and calcium compared to the normal control and the other supplemented diet groups.

Table 3a.Proximate Analysis for *Canarium Schweinfurthii* Oil

Supplementation level	% Dry matter	% Ash	% Ethyl extract	% crude fibre	% Nitrogen	% Crude protein	Calcium (mg)
2.5% *Canarium schweinfurthii* oil (100g)	93.69 ± 1.11	4.83 ± 0.93	15.41 ± 1.41	7.20 ± 1.05	3.88 ± 0.05	24.25 ± 1.98	0.19 ± 0.01
5% *Canarium schweinfurthii* oil (100g)	93.75 ± 1.01	4.76 ± 1.02	16.16 ± 1.30	9.45 ± 1.51	3.58 ± 0.64	22.38 ± 1.78	0.20 ± 0.00
10% *Canarium schweinfurthii* oil (100g)	93.80 ± 1.00	4.44 ± 1.20	21.28 ± 0.67	22.41 ± 0.75	3.67 ± 0.55	22.94 ± 1.56	0.21 ± 0.01

Values are presented as mean ± Standard Deviation.

The body weight change and feed efficiency data generated during the period of the study are presented below.

In the group fed 10% *C. scheinfurthii* oil supplemented diet (Fig. 3a) the weight change and (Fig 3b) cumulative weight change were significant ($p>0.05$) when compared to the weight change and cumulative weight change observed in the normal control and the other supplemented diet groups.

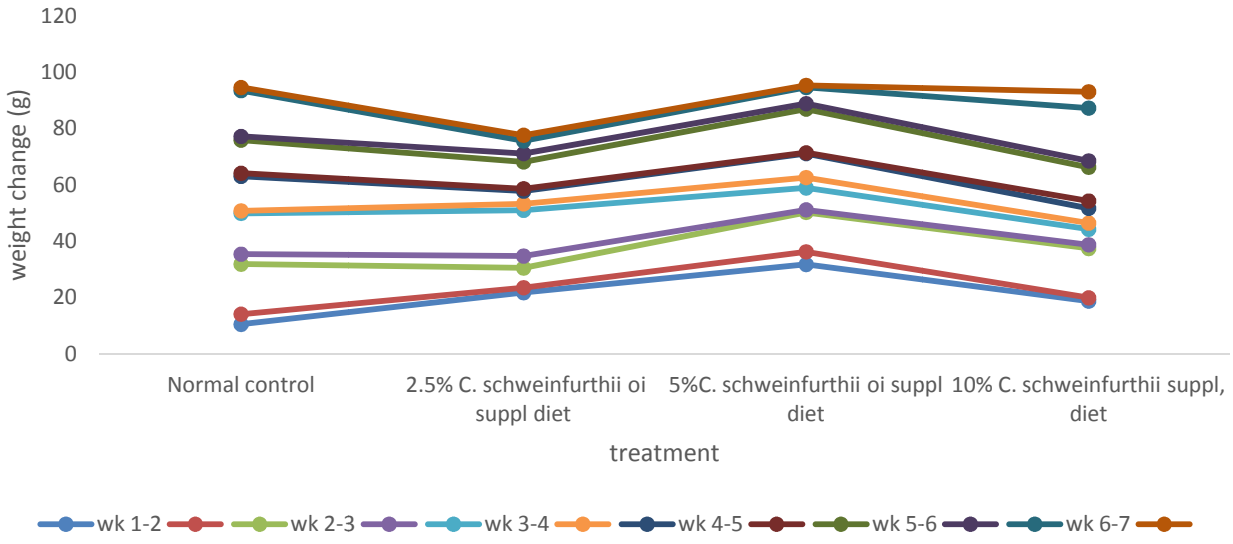

Figure 3a. Effect of *Canarium schweinfurthii* Oil Diet Supplementation on Biweekly weight change of Male Wistar Rats.

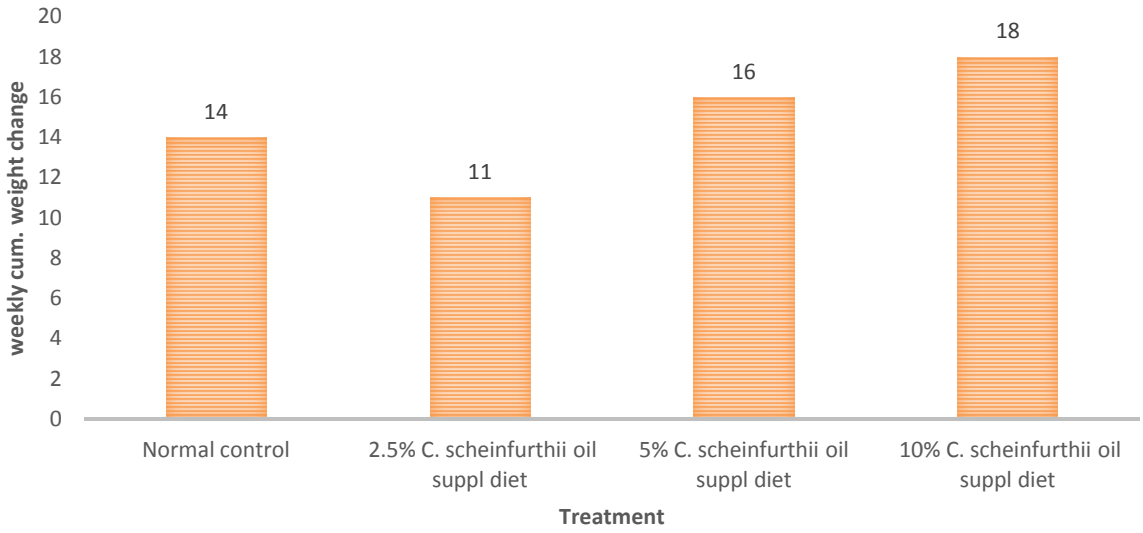

Figure 3b. Effect of *Canarium schweinfurthii* Oil Diet Supplementation on cumulative weight change of Male Wistar Rats.

In the normal control group fed basal diet and 10% *C. scheinfurthii* oil supplemented diet (Table 3b) only there was a significant ($p>0.05$) increase in feed intake and cumulative feed intake during the six weeks of the study compared to the feed intake observed in the other supplemented groups. However in the feed efficiency ratio (Fig. 3d) there was no significant difference ($p<0.05$) between the normal control and the supplemented diet groups.

Table 3b. Effects of *C. schweinfurthii oil* Supplementation on Biweekly Feed Intake in Male Wistar Rats

Treatment	Wk 1-2	Wk 2-3	Wk 3-4	Wk 4-5	Wk 5-6	Wk 6-7
Normal control	680 ± 20.11	700±21.89	651±31.11	667±34.7	674±25.64	681±34.7
2.5% *C. schweinfurthii* oil suppl diet	540 ± 15.89	554±23.67	562±18.34	581±27.12	573±21.44	584±27.12
5% *C. schweinfurthii* oil suppl diet	650 ± 18.33	620±22.78	624±25.11	652±29.35	641±28.77	654±29.35
10% *C. schweinfurthii* oil suppl diet	736 ± 22.34	600±25.56	775±30.43	864±37.39	902±35.62	925±37.39

Values are presented as mean ± Standard Deviation.

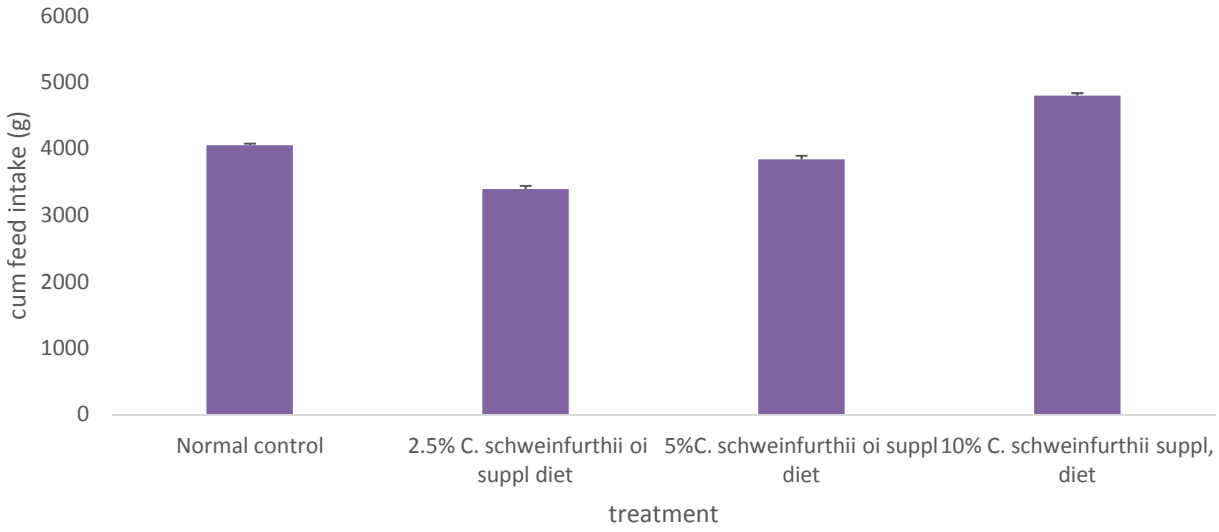

Figure 3c. Effect of *Canarium schweinfurthii* oil Diet Supplementation on cumulative feed intake of Male Wistar Rats

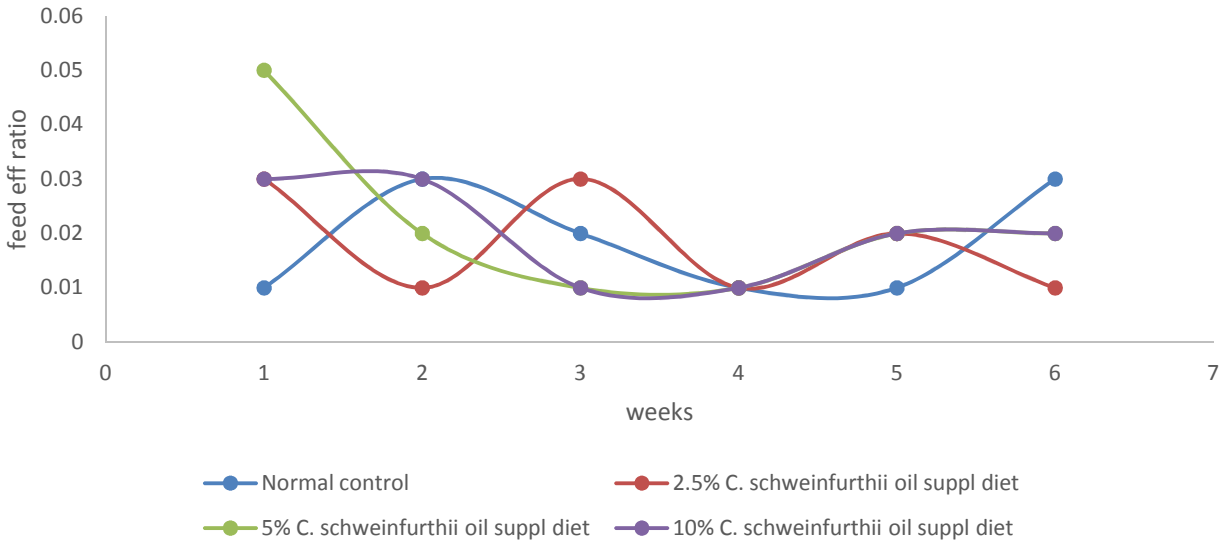

Figure 3d: Effect of *Canarium schweinfurthii* Oil Diet Supplementation on feed efficiency ratio of Male Wistar Rats.

Table 3c. Effects of *C. schweinfurthii* oil Supplementation on Cumulative Feed Efficiency Ratio in Male Wistar Rats

Treatment	Cumulative feed efficiency ratio
Normal control	0.05 ± 0.01
2.5% *C. schweinfurthii* oil suppl diet	0.05 ± 0.00
5% *C. schweinfurthii* oil suppl diet	0.08 ± 0.01
10% *C. schweinfurthii* oil suppl, diet	0.10 ± 0.01

DISCUSSION

Weight gain of rats received different dietary levels (%) of *Canarium schwerfurthii* oil is given in Table 2.4. Weight gain of rats differed significantly ($p<0.05$) among the treatments at 2nd week of age. The weight gain of 10% *Canarium schwerfurthii* oil group was higher ($p<0.05$) than that on 5% dietary oil at 2nd week of age. At the 5th week weight gains of the normal control and 10% oil almost similar. From the results of *Canarium schwerfurthii* oil supplementation on weight gain of rats it as clear that supplementation up to 5% had considerable non-significant ($p>0.05$) positive effect on this parameter of the rats. Results also showed that at the 1st week of age of rats, there was no significant effect of oil supplementation and it might be the adjustment period in the utilization of oil which perhaps has been minimized to show significant ($p<0.05$) increasing weight gain of the rats in the subsequent weeks. This result coincides with the findings of Nwoche *et al*. (2003) who found that 10% inclusion level of *Canarium schwerfurthii* oil showed the highest ($p<0.05$) body weight gain. Hake *et al*. (2005) reported that *Canarium schwerfurthii* oil has positive effect on weight of rats. Figure 3b shows that rats fed on different levels of *Canarium schwerfurthii* oil gained cumulative weight similar to 0% oil. The results of this study indicated that weight gain of 0% and 10% dietary *Canarium schwerfurthii* oil group are similar but lower than the rest of the groups.

Differences in feed intake among Normal control, 2.5, 5 and 10% *Canarium schwerfurthii* oil groups were found significant ($p<0.05$) at 2nd week of age. Rats that received 10% dietary *Canarium schwerfurthii* oil consumed the highest amount of feed compared to others. Highly significant ($p<0.01$) differences in feed intake among different groups were also found at 4th week of age, but there were no significant differences among different groups at 3 and 5th week of the trial. Cumulative feed intake was ($p<0.05$) higher on 10% dietary *Canarium schwerfurthii* oil group during 4th week (Fig.3c).

Supplementation of energy from *Canarium schwerfurthii* oil gave an interesting result as regards feed intake of rats. At the 1st week, feed intake was similar ($p>0.05$) in all supplemented group compared with without oil. It is interesting that all other lower levels of supplementation (2.5 and 5%) showed lower feed intake than that of the normal control. Among the lower level of supplementation, 5% showed the highest feed intake. This result is consistent with the findings of Nwoche *et al*. (2003), who found that feed intake was highest ($p<0.05$) at 5% inclusion level of *Canarium schwerfurthii* oil in rats diet and also observed. Total feed intake of all treated groups was higher than that of the normal control except 10% oil group. This was probably for increased level of energy in the diet as supported by Franco *et al*. (1995). Olorede and Longe (1999) reported that supplementation of *Canarium schwerfurthii* oil in rats diet improved feed intake which is relevant to the present study.

Feed efficiency ratio under different dietary treatments are presented in Figure 3d. The feed efficiency ratio differed significantly ($p<.0.05$) among the treatments at 2nd week of age. The best weekly FER was observed in 5% dietary *Canarium schwerfurthii* oil supplemented group at 1, 2 and 3rd week and rats on 10% *Canarium schwerfurthii* oil had better FER than 2.5% and 5% oil supplementation groups at 4 and 5th week of age. However, no significant differences were found throughout the experimental period. The differences observed among the normal control and 2.5, 5 and 10% *Canarium schwerfurthii* oil diets were not significant. Cumulative FER of rats is also given in the Table 3c showing no significant difference among dietary treatments but it was observed that increasing level of *Canarium schwerfurthii* oil resulted in better FER than the normal control in most of the cases.

The stimulation for efficient utilization of diets supplemented with *Canarium schwerfurthii* oil has been reported by different researchers. *Canarium schwerfurthii* oil promotes the intestinal uptake of amino acids, even when the amino acids are present as a mixture, by modifying the intestinal epithelium for better uptake Abaelu *et al*. (1991). In terms of absorbed amino acids, *Canarium schwerfurthii* oil improves the metabolism of the sulphur containing amino acids Umoh *et al*. (1983). As a result, growth rate as well as FER tends to be improved.

From the study we can infer that wistar rats whose diets were supplemented with 10% *Canarium schweinfurthii* oil exhibited high growth rate and hence when supplemented in human diets can make them nutritionally dense thereby improving human nutrition.

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