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AN ASSESSMENT OF THE IMPACT OF KETOGENIC DIET ON WEIGHT, SOME BIOCHEMICAL AND HAEMATOLIGICAL PARAMETERS IN WISTAR RATS

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ABSTRACT

An increasing number of Nigerians are embracing the controversial ketogenic diet (KD) which has shown to be quite effective in reducing body weight and the risk factors for various chronic diseases. The study assessed the impact of locally sourced ketogenic food materials in Jos, Nigeria, on weight, some haematological and biochemical parameters in Wistar rats. The proximate nutrient composition of KDs was determined using standard procedures while elemental analysis was determined using Atomic Absorption spectrometry. Wistar rats were fed hyper-calorie diets for rapid weight gain prior to treatment with KD(s) and normal laboratory feed (control). Weight, some biochemical and haematological parameters were measured to evaluate the suitability of formulated diet as tool for weight management. Furthermore, histological assessment of the intestine was carried out to determine safety of the diets. Analysis of data was done using analysis of variance. The results showed significant weight loss expressed in grams by Awara/African spinach (A/S): 131.33±4.90 to 106.73±10.68, Fish/Cucumber (F/C): 124.73±4.90 to 117.98±10.68 for day 5 to 30 of experiments. For the biochemical parameters analyzed, the effects of treatments on total cholesterol and total protein were significant for A/S (1.48±0.09 mmol/l, 66.70±2.64 g/l) and F/C (1.90±0.09 mmol/l, 64.25±2.64 g/l). Results for all haematological parameters checked except haemoglobin was not significantly different. Haemoglobin results for base line (BL): 141.33±4.0 significantly differed with control (CTRL): 123.33±4.0, A/S: 123.67±4.0 and F/C: 121.33±4.0 g/l. Histological results showed little structural changes in the small intestine. In conclusion, this study reveals that, weight management is achievable with the formulated KD(s) available and affordable.

KEYWORDS: ketogenic diet, weight management, histological assessment

INTRODUCTION

Ketogenic diet is a high-fat, moderate-protein, low-carbohydrate diet initially used in the 1920's for treatment of epilepsy. KD became recognized for its use in treatment of obesity in the 1960's and years after [1]. During recent years, there has been increasing evidence in support of KD as an effective tool for weight management. A number of Nigerians are embracing the ketogenic diet as a dietary tool for weight loss. The success story of the KD is overwhelming especially for people struggling with weight management for long [2].

Ketogenic diet is considered to share physiological similarities with fasting or starvation. In both cases, the body resorts to fatty acid oxidation to meet its energy needs [3]. KD which is characterized by decrease carbohydrate allows the body to move from glucose to fat-based metabolism which results in the production of water soluble ketone bodies [4]. Thus, ketone bodies produced are transported to extra-hepatic cells for energy.

Studies with animal models have shown that animals placed on a high-fat, low-carbohydrate diet (KD) loss weight [5, 6]. In addition to weight loss, animals fed KD showed improve glucose control [6, 7]. The study also shows statistically non-significant change in HDL-cholesterol, LDL-cholesterol triglycerides and significant fall in serum albumin levels [8].

MATERIALS AND METHODS

Biological Assay

Adult wistar rats weighing an average of 120g were obtained from the animal house, University of Jos were used. The diets (hyper calorie and ketogenic diets) were formulated using food items readily available in Jos, Plateau State. This is to allow for the use of local food items that are readily available and affordable as ketogenic diets. Normal laboratory feed was used as control diet. The nutrient composition of the formulated ketogenic diets were determined by proximate and elemental analysis respectively. The proximate food composition was determined by the [9, 10, 11] method while the elemental analysis was determined using atomic absorption spectrophotometer.

The rats were randomly distributed into three (3) groups of 5 rats each kept in metabolic cages at the start of the experiment. The rats were allowed to stabilize on the normal laboratory feed for 3 days and starved for one day before feeding with hyper-calorie diet commenced. Animals were

fed the hyper-calorie diet for 14 days to enhance rapid weight gain such that the animals were overweight. After treatment with hyper-calorie diet, animals were fasted overnight prior to feeding with experimental diets (ketogenic diet and normal laboratory feed) with one animal randomly selected from each group and sacrificed for baseline study. Rats were allotted the diets as follows:

Group 1: Awara : Spinach (diet 1)

Group 2: Fish: cucumber (diet 2)

Group 3: Normal Laboratory Feed as control group (diet 3)

Just before every feeding, known quantity of each food material was mixed as follows:

- Herring fish (shawa) and cucumber in a ratio 3:1
- Locally made soya bean cheese (awara) and African spinach, in a ratio 3:1
- Normal laboratory feed with water.

Rats were fed 10-30 grams of diet per day (5 gram per 100 gram body weight) and water given ad libitum for 30 days. Fasting blood glucose was measured on day 1, 15, and 30 in addition to records of the weight of rats was taken after every 5 days.

At the end of the feeding period, rats were anaesthetized with diethyl ether and blood collected via the jugular vein. Whole blood was collected (1ml) from each rat into sample bottles containing EDTA (1mg/ml) for parameters that required the use of whole blood. The remaining blood samples were collected into anticoagulant-free bottles and allowed to clot for 20 minutes, before centrifuging at 3000rpm for 15 minutes in a refrigerated centrifuge, (to obtain serum for biochemical parameters). Serum was carefully transferred with pasteur pipettes into clean, dry labeled light-shielded sample bottles and stored frozen until required.

Furthermore, the small intestine was harvested, weighed and preserved in 10% formal saline for histological test.

Biochemical Assay

The following biochemical parameters were evaluated in the blood serum: total cholesterol using the CHOD-PAP method [12], HDL-cholesterol using the combined method of Lopes-Virella *et al* [13] and Allain *et al* [12], triglycerides by methods of Esders and MIchrina [14], LDL-cholesterol by the Friedwald formula,total protein by the Biurette method, total albumin by Bromocresol Green method and fasting blood glucose using Oncall-plus glucose test strips.

Haematological Assay

The following hematological parameters in whole blood were determined using an automatic method (automatic cell counter) Vet haematological analyzer: white blood cells, red blood cells, haemoglobin, platelet count and pack cell volume.

Histological Test

The small intestines, after washing in physiologic saline solution, were immediately preserved in 10% neutral buffered formal saline and allowed to fix for about 72 hours. The tissues were dehydrated in ascending grades of isopropyl alcohol by immersing in 50%, 70%, 80%, 90%, 95%, and 100% for 1 hour each. The dehydrated tissues were cleared in two changes of xylene, 1 hour each. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax. The paraffin blocks were mounted and cut with rotary microtome at 5 micron thickness. The sections were floated on a tissue floatation bath at 40°C and taken on glass slides that had been previously smeared with equal parts of egg albumin and glycerol. The sections were then driedin an incubator at 60°C and after 5 min the sections were allowed to cool [15].

Tissue Staining

Haematoxylin and Eosin Staining

The sections were deparaffinised by immersing in two changes of xylene for 10 mins each in horizontal staining jar. The deparaffinised sections were hydrated in descending grades of isopropyl alcohol (IPA) for 2 mins each and taken to water, after which it was stained in Ehrlich's hematoxylin for 10 min in horizontal staining jar. After staining in hematoxylin, the sections were washed in tap water and dipped in acid alcohol to remove excess stain (1% HCl in 70% alcohol). The sections were then placed in running tap water for 10 min for blueing (slow alkalization). The sections were counter stained in 1% aqueous eosin (1 gm in 100 ml tapwater) for 1 min and the excess stain was washed in tap water and the sections were allowed to dry. Complete dehydration of stained sections was done by placing the sections in the incubator at 60°C for 5 min. When the sections were cooled, they were mounted in DPX mountant having the optical index of glass (the sections were wetted in xylene and inverted on to the mount and placed on the cover slip). The tissue morphology was observed with low power objective under light microscope. The cell injury and other aspects were observed under high power dry objective [15].

Statistics

The spss, version 23, was used for statistical analyses with a random block design according to the animals' weights. The two-way variance analysis (ANOVA) was used to determine the value for P with a probability of 5%.

Results

The macro-nutrient composition of formulated ketogenic diets is as presented in figure 1. The mixture of awara/spinach had high protein (8.56), moderate carbohydrate (4.23) and slightly low lipid (2.88) content. While the mixture of fish/cucumber had higher lipid (10.24), slightly high carbohydrate (9.67) and protein (9.22). When the non-digestible carbohydrate portion (crude fibre)

is subtracted from the total carbohydrate in both diets, the amount of absorbable carbohydrate becomes less than fats and protein content.

The mineral element composition of formulated ketogenic diets is as presented in figure 2. This showed that fish + cucumber had higher quantity of Fe (45) and Ca (85) as compared to that of awara + spinach. While awara + spinach had higher quantity of Zn (21.3) and Mg (27.4) as compared to that in fish + cucumber.

Result of food intake of rats is shown in figure 3. Rats fed control diet consumed higher quantity of food probably due to its carbohydrate content while rats fed formulated ketogenic diets ate less quantity of diet than the control groups with fish/cucumber groups having the least quantity for food consumed.

Results of the weight gain of rats fed formulated ketogenic diets and normal laboratory feed are shown in figure 4. This showed that the effect of treatments on body weight of Wistar rats were not significantly different ($P \geq 0.05$) at day 1 and 5 but significantly ($P \leq 0.05$) different from day 10 to day 30 of data collection. The result equally showed a decrease in body weight for rats fed Awara + Spinach (137.73 to 106.73) and fish and cucumber (139.18 to117.98) while rats fed control diet increased in weight (136.53 to 180.83) over the 30 days period.

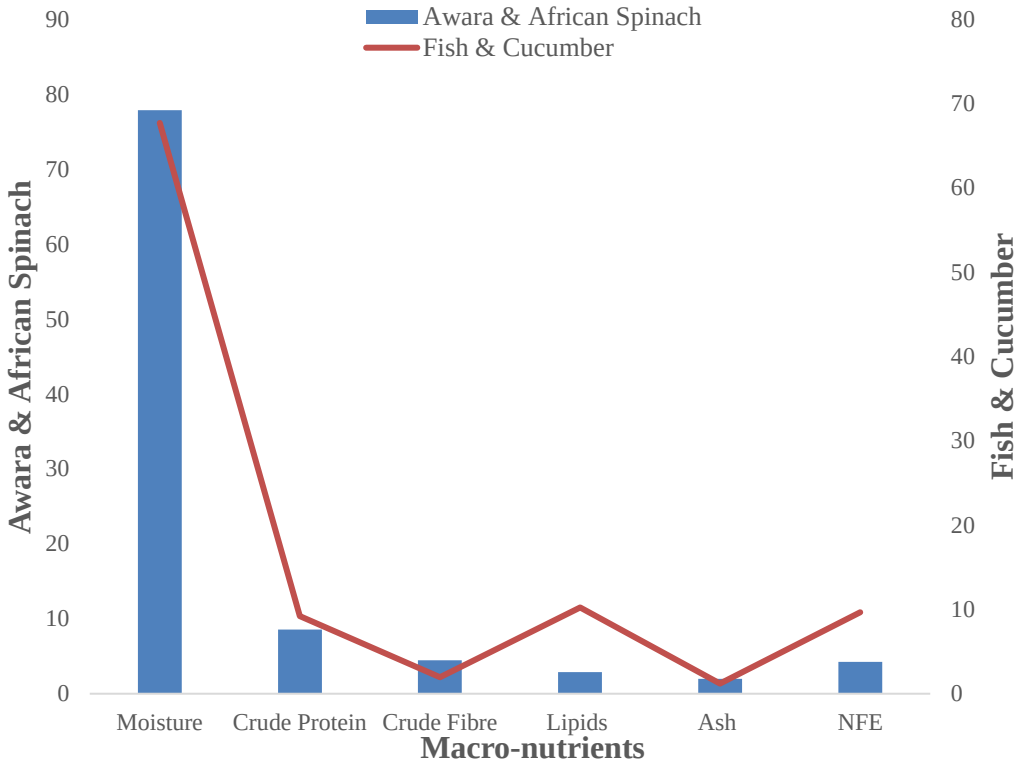


Figure 1: Proximate Nutrient Composition of Formulated Ketogenic Diets (3:1) in g/100

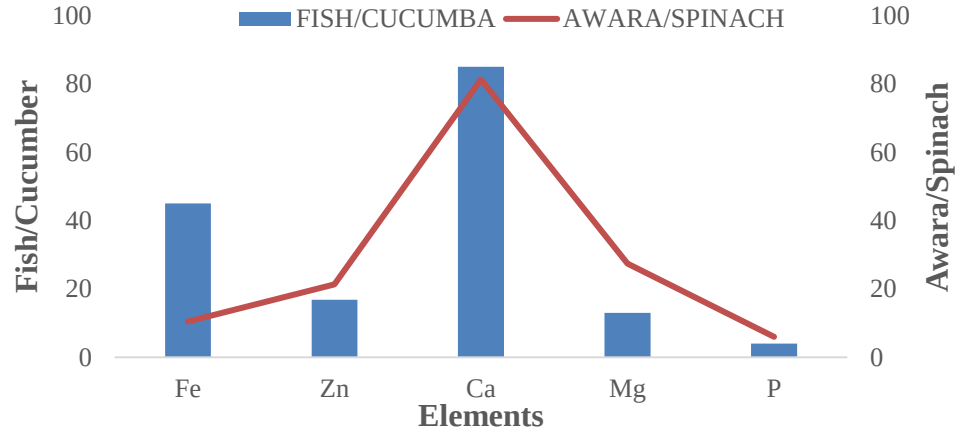


Figure 2: Elemental Composition of Formulated Ketogenic Diets (mg/100g)

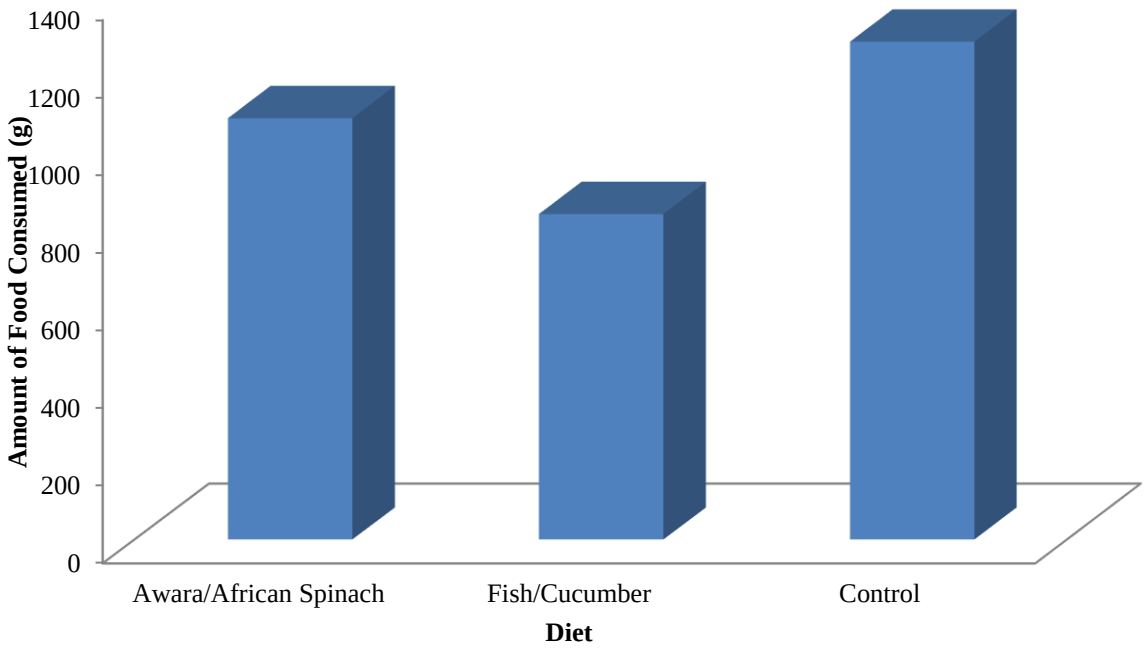


Figure 3: Food Consumed by Rats Fed with the Experimental Diets for 30 Days

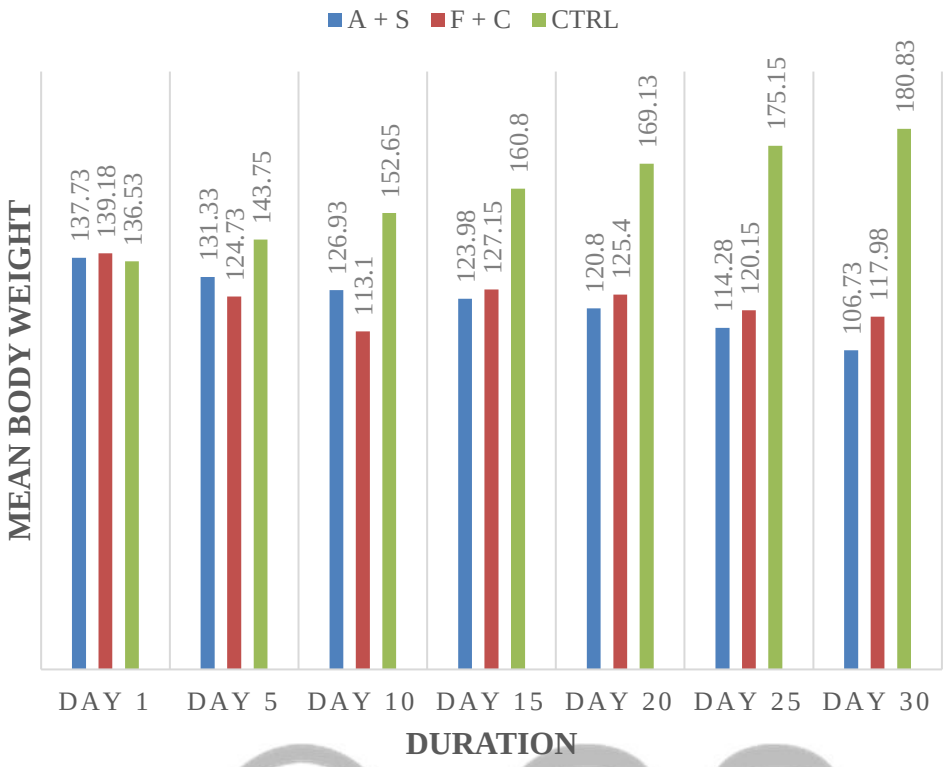


Figure 4: Effects of Formulated Ketogenic Diets on Body Weight of Wistar Rats

Table 1 shows results of total cholesterol, HDL-cholesterol, triglyceride, LDL-cholesterol, total protein and albumin in rats fed with experimental diets. It therefore revealed statistically non-significant decrease in total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol levels of rats fed formulated ketogenic diet as compared to results from base line groups fed hyper-calorie diet at the beginning of the study. Rats fed formulated ketogenic diets showed statistically non-significant change in HDL-cholesterol, LDL-cholesterol and triglyceridelevels as compared with control groups. Rats fed awara/spinach had low level of mean total cholesterol compared to rats fed normal laboratory feed (control) while rats fed fish/cucumber had slightly higher total cholesterol than the control. Result for serum total protein and albumin was significantly higher for the formulated ketogenic diets as compared to control.

Table 2 shows fasting blood glucose of Wistar rats fed experimental diets taken on day 1,15and 30 and it revealed that the effects of formulated Ketogenic diets on mean fasting blood glucose were not significantly different ($P \geq 0.05$). The result thus shows a decrease in mean value between day one and fifteen but a slight increase on day 30. At day 1 and 15, the highest values obtained were noticed with rats treated with Awara + Spinach (day1=100.5; day15=85.25), this was followed in the order of performance as Awara + Spinach (day1=100.5; day15=85.25)> Fish + Cucumber(day1=97.00; day15=81.50)> Control(day1=96.00; day15=79.50), while at day 30, the highest value was obtained from rats treated with Fish + Cucumber (day30=95.50), and the order of performance was Fish + Cucumber(day30=95.50)> Control(day30=93.50)>Awara + Spinach(day30=86.50).

Table 1: Mean Effects of Treatment on Biochemical parameters

Treatment	BL	CTRL	A + S	F + C	SE
TC (mmol/L)	2.03[a]	1.65[bc]	1.48[c]	1.90[ab]	**0.09**
HDL(mmol/L)	0.63[a]	0.55[a]	0.55[a]	0.58[a]	**0.06**
TG(mmol/L)	0.90[a]	0.50[b]	0.53[b]	0.75[ab]	**0.11**
LDL(mmol/L)	0.53[a]	0.60[a]	0.40[a]	0.58[a]	**0.09**
TP (g/L)	66.50[a]	54.55[b]	66.70[a]	64.25[a]	**2.64**
ALBUMIN(g/L)	29.00[a]	23.33[b]	28.50[a]	26.50[ab]	**1.55**

Means on the same column with the same letter do not differ significantly from each other (P = 0.05).

Where: BL = Baseline; A + S = Awara + Spinach; F + C = Fish + Cucumber; CTRL = Control (where no treatment was applied); TC = Total cholesterol; HDL = High-Density Lipoprotein Cholesterol; TG = Triglyceride; LDL = Low-density Lipoprotein cholesterol; TP = Total Protein; SE = Standard error.

Table 2: Mean Effects of Ketogenic Diets on Fasting Blood Glucose of Wistar Rat

FASTING BLOOD GLUCOSE OF WISTAR RAT (g/dl)

Treatment	A + S	F + C	CTRL	SE
DAY 1	100.50[a]	97.00[a]	96.00[a]	**6.72**
DAY 15	85.25[a]	81.50[a]	79.50[a]	**7.37**
DAY 30	86.50[a]	95.50[a]	93.50[a]	**5.45**

Means on the same column with the same letter do not differ significantly from each other (P = 0.05).

Where: A + S = Awara + Spinach; F + C = Fish + Cucumber; CTRL = Control (where no treatment was applied); SE = Standard error

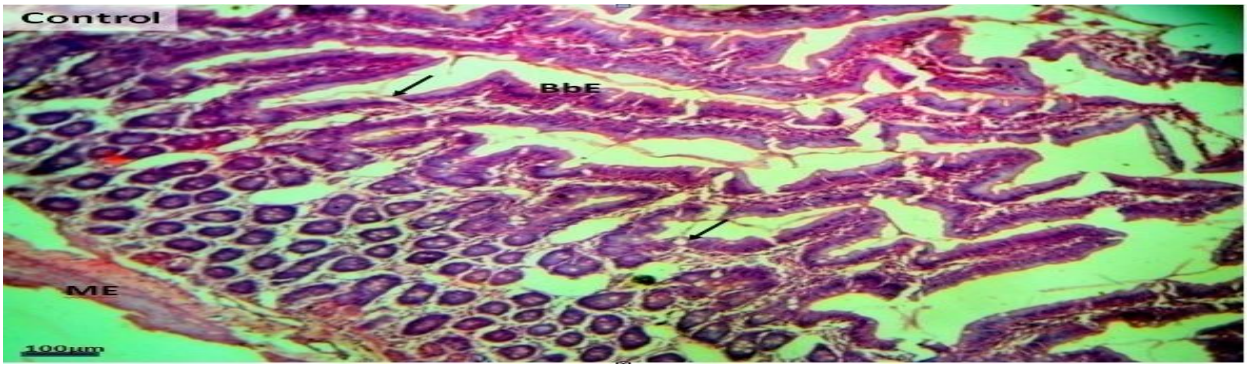
Table 3 shows haematological parameters taken in rats fed experimental diets. And it revealed that the effects of treatments on Packed Cell Volume (PCV), Red Blood Cell (RBC), white blood cell (WBC) and platelet count were not significant ($P \geq 0.05$)butwas significantly different ($P \leq 0.05$) for Haemoglobin. The results obtained for PCV showed non-significantly low values by fish/cucumber (44.33) and anon-significantly high value by awara/spinach (45.33) compared to values from control. On the other hand, results for RBC, WBC and platelet showed non-significantly high value by formulated ketogenic diet compared to control. Results for haemoglobin showed non-significantly higher value by awara/spinach and non-significantly low value by fish/cucumber than control.

Table 3: Mean Effects of Treatment on Haematological Parameters

Treatment/Haematological Parameters	BL	CTRL	A+S	F+C	SE
PCV (%)	46.33[a]	44.67[a]	45.33[a]	44.33[a]	**0.83**
RBC ($\times 10^{12}$ cells/l)	8.03[a]	6.43[a]	6.96[a]	6.91[a]	**0.57**
WBC($\times 10^{9}$ cells/l)	6.57[a]	5.47[a]	6.81[a]	5.52[a]	**0.72**
HGB(g/l)	141.33[a]	123.33[b]	123.67[b]	121.33[b]	**4.00**
PLATELET($\times 10^{9}$ cells/l)	636.67[a]	643.33[a]	653.33[a]	640.00[a]	**41.83**

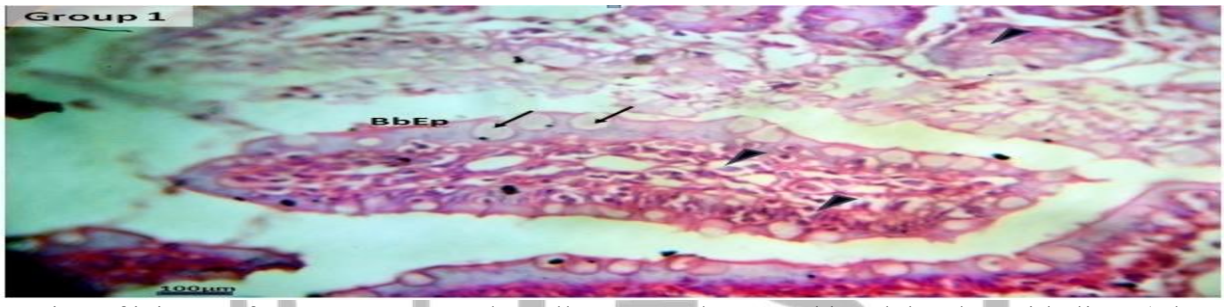
Means on the same column with the same letter do not differ significantly from each other (P = 0.05).

Where: BL = Baseline; A + S = Awara + Spinach; F + C = Fish + Cucumber; CTRL = Control (where no treatment was applied); PCV = Packed Cell Volume; RBC = Red Blood Cell; WBC = White Blood Cell; HGB = Haemoglobin; SE = Standard error



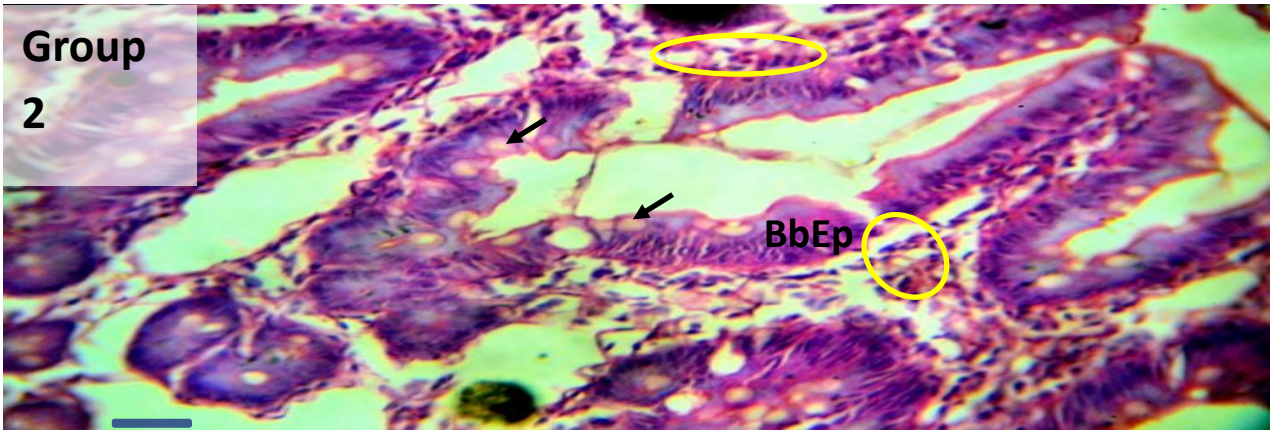
Sections of jejunum from the *Control* group showing normal appearing luminal mucosa and sub-mucosa with intact brush border columnar epithelium (BbEp) interspersed by secretory goblet cells (arrows). An intact muscularisexterna (MEx) is also observed.*H&E Stain*

Plate 1: Effects of Normal Laboratory Feed on Haematoxylin and Eosin (H&E) tissue sections of Rat intestinal tissue.



Section of jejunum from *Group 1*reveals well-preserved mucosal brush border epithelium (BbEp). There is increased secretory goblet cell population (arrows) and vacuolation (arrowheads) of submucosalcells.*H&E Stain*

Plate 2:Effects of Awara + Spinach on Haematoxylin and Eosin (H&E) tissue sections of Rat intestinal tissue.



Sections from *Group 2*show well-preserved jejunal epithelium with full brush border. There is also a slight inflammatory cell infiltrate (circles). Fewer secretory goblet cells (arrows) are seen along the epithelial brush border (BbEp). *H&E Stain*

Plate 3: Effects of Fish + Cucumber on Haematoxylin and Eosin (H&E) tissue sections of Rat intestinal tissue.

Discussion

Ketogenic diet (KD) is a diet that is designed to bring about ketosis, by the breaking down of body fat into ketones, which allows the body to run majorly on ketones rather than glucose. There are a number of ways in which ketosis can be brought about and also a number of different variants of ketogenic diet. Because the goal of these diets are the same, the different types of ketogenic diet usually share a number of similarities, notably in being low in carbohydrate and high in dietary fat(diabetes.co.uk). In this study, the ketogenic diets formulated using locally available food materials that are affordable also shares similarities with the different variants of ketogenic diet notably in being low in carbohydrate and high in dietary fat. This is particularly of importance in Nigeria as a result of the rising cases of obesity and overweight as presented in the 2017 Global Nutrition report which are considered major risk factors for developing chronic disease such as

type 2 diabetes mellitus, hypertension and cardiovascular diseases. These diets formulated in a ratio (3:1) were assessed quantitatively and further fed to rat models to determine their efficacy in weight management.

The result of mineral composition of the formulated KD's is comparable to previously published studies that show deficiency of KD's in micronutrients [17]. The present study shows the inadequacy of some mineral content of the formulated ketogenic diet, particularly calcium (Ca), magnesium (Mg) and phosphorus (P) out of the five minerals analyzed when compared to their recommended dietary allowances (RDAs). On the contrary, iron and zinc are present in sufficient amount in the formulated ketogenic diets when compared to their RDA's. Thus, there may be need for supplementation of deficient micro nutrient to meet up daily nutritional requirements.

Studies on ketogenic diet from various laboratories have shown that a high-fat diet rich in polyunsaturated fatty acids (ketogenic diet) is quite effective in reducing body weight [16]. One study showed that 88% of subjects placed on low-carbohydrate KD loss weight compared to 34.6% of subjects placed on low calorie diet [5]. In the present study, it was observed that rats fed awara/spinach and fish/cucumber gained less weight, shedding the excess weight gained from feeding with hyper-calorie diet. This could be attributed to the decrease in carbohydrates, which allows the body to metabolize fats rather than carbohydrates for energy [4] and also to the protein composition which increases satiety and in turn reduces food intake. On the other hand, rats fed control diet (normal laboratory feed) gained more weight.

Analysis of the lipid profile showed statistically non-significant decrease in total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol levels of rats fed ketogenic diet as compared to results from base line groups fed hyper-calorie diet at the beginning of the study. Rats fed formulated ketogenic diets showed statistically non-significant change in HDL-cholesterol, LDL-

cholesterol and triglyceride levels where lipid profile results were reported statistically non-significant when compared to control group. Rats fed awara/spinach had low level of mean total cholesterol compared to rats fed normal laboratory feed (control) while rats fed fish/cucumber had slightly higher total cholesterol than the control. [8]showed significant fall in serum albumin levels with development of hypoproteinemia with pedal edema which responded to increase in protein intake. Result for serum total protein and albumin was significantly higher for the formulated ketogenic diets as compared to control. Therefore, there may be no incidence of hypoproteinemia on consumption of these formulated ketogenic diets.

The Mean values for fasting blood glucose of rats from present study fed formulated ketogenic diets showed improvement over the 30 days period. This showed that consumption of the formulated ketogenic diets results in blood glucose control [7].

This study examined some iron-dependent parameters and some other blood parameters such as haemoglobin, packed cell volume (PCV), red blood cell (RBC), platelet, and white blood cell (WBC). The values obtained on PCV, RBC, WBC and platelet count was not significant. The result for haemoglobin was significant with the baseline group having the highest haemoglobin concentration (141.33 g/l) and fish/cucumber the least (121.33 g/l). This is indicative of low bioavailabilty of iron present in the formulated diets which may be due to poor absorption. Dietary iron absorption from a meal is determined by iron status, heme- and non heme-iron contents, and amounts of various dietary factors that influence iron absorption [19].

Jejunum and ileum are similar in structure and function, and are both involved in digestion of food which also entails absorption of nutrients. These roles are greatly enhanced by the epithelial lining of the digestive tract with a selectively permeable membrane that enhances transport and digestion of food. The cells in the semi permeable layer generate mucus that lubricates and protects the

intestinal wall [20]. From the histograph results, intestinal tissue sections from the control group of rats in general, showed a normal appearing, well-preserved mucosal epithelium with brush border. Sub-mucosa and muscularis externa were also intact. Tissue sections from groups 1 and 2 generally showed a well preserved epithelial brush border with adequate goblet cell population. The group 1(awara + Spinach) in particular had a few portions of submucosal cell vacuolation while the group 2 (fish + cucumber) had a few portions of inflammatory cells infiltration of the submucosa. In all groups the muscularis externa remained intact, only thinning out a little where there was lymphoid hyperplasia. In this experiment, Consumption of formulated ketogenic diets caused little structural changes to the intestinal tract, which are not degenerative. Degenerative changes have been reported to result in cell death, which is of two types, namely apoptotic and necrotic cell death. These two types differ morphologically and biochemically [21]. Pathological or accidental cell death is regarded as necrotic and could result from extrinsic insults to the cell such as osmotic, thermal, toxic and traumatic effects. The process of cellular necrosis involves disruption of membrane's structural and functional integrity [21]. Therefore, the formulated ketogenic diets are considered non-toxic to the small intestine.

Conclusion

The research work showed that affordable ketogenic diets can be formulated using our locally available food items which can be effective in weight management. In terms of health benefits, the formulated diets improved some blood parameters particularly fasting blood glucose, showed histological results comparable to the control indicating the safety of the diet to the small intestine. The formulated diets were found to be deficient in some micronutrients notably Ca, P and Mg. Therefore, fortification and or supplementation may be necessary to meet the recommended dietary allowances.

Finally, there are varieties of local food materials that can be exploited to formulate indigenous ketogenic diets with proper selection and mixture.

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