Weight Change, Haematology and Lipid Profile of Normal Male Wistar Rats Fed on High-Calorie Diet and Vegetables

***Adeoye, B. K.[1] Dada, G. O.[1], Oyerinde, O. O.[2] , Akinlade, A. R. [1], Esiaba, I. [3], Adewole, O. A.[3]**

[1]*Department of Nutrition and Dietetics, Ben. Carson College of Health and Medical Sciences, Babcock University Ogun State, Nigeria.*

[2]*Department of Public Health, Benjamin S. Carson College of Health and Medical Sciences, Babcock University Ogun State, Nigeria.*

[3]*Department of Biochemistry, Ben. Carson College of Health and Medical Sciences Babcock University Ogun State, Nigeria.*

****Corresponding author:*** adeoyeb@babcock.edu.ng +234 8020708806

ABSTRACT

Background: *The decline in vegetable consumption, some of which are gradually getting extinct because of insufficient information on their health benefits, is a major factor for the rise in the incidence of nutritional disorders.*

Objective: *Effect of Amaranthus hybridus, Crassocephalum crepidioides, Senecio biafrae and Corchorus olitorius on the weight, haematology, and lipid profile of rats fed on high-calorie diet.*

Methods: *The study comprised of six groups of male Wistar rats. Positive control (A) was fed on regular feed and water while the negative control (B) was fed a high-calorie diet. The four treatment groups were given high-calorie diet along with 5 % of one of the four vegetables for five weeks respectively. The weights of the rats were obtained before and after the experiment. The feed and water consumption of the rats were recorded daily. The haematology and the lipid profile were determined at the end of the experiment.*

Results: *The composition of the feeds was significantly different ($p<0.05$). The mean weekly water intake (332 ml) and feed consumption (92 g) of the positive control were not significantly different while its mean weight change (22 g) was significant at $p<0.05$. S. biafrae increased non-significantly the PCV (48.25 %), haemoglobin (16.25 g/dl) and platelet count (289×10^9/ L). A. hybridus lowered the total cholesterol (89.93 mg/dl), triglyceride (45.38 mg/dl) and LDL (77.65 mg/dl) while S. biafrae increased the HDL (44.65 mg/dl) level.*

Conclusion: *The effect of the high-calorie diet was better ameliorated by A. hybridus and S. biafrae.*

Keywords: Antioxidant, Functional foods, Haematological properties, Leafy vegetables, Lipidemia

INTRODUCTION

There is a continuous shift towards a diet that is characterized by high calorie in most African countries due to increased urbanisation (1). This consequently results in chronic diseases of lifestyle which include cardiovascular diseases, coronary heart diseases, and various types of cancer. Haemtological parameters and lipid profile are useful in the diagnosis of many of these lifestyle diseases (2). The examination of blood allows for investigating the concentrations of haematology parameters and the lipids which sometimes are not in normal amounts in the human body (3). Haematology parameters and lipid profile are good indicators of physiological, nutrition, and pathological status of an organism (3, 4).

Haematology parameters and lipid profile have been reported in several studies to be positively modulated by consumption of diets rich in vegetables (5,6, 7). However, consumption of vegetables is low and a lot of vegetables that have been used for food over the years have been neglected due to inadequate information (8). Inadequate intake of fruit and vegetable below the recommended daily intake is the cause of about 3.0 % of deaths occurring globally (9). About 85 % of these deaths occur in developing and under-developed countries (10). Incidence of these diseases and death will increase astronomically in Africa if there is no meaningful intervention (11).

Corchorus olitorius leaves (jute mallow) and *Amaranthus hybridus* also known as Amaranth or Pig weed are eaten in different ways in various countries. They are notable for their ability to supply certain nutrients and as precursors of some hormones (12, 13). A *hybridus* has been shown to contain large amount of squalene which has both health and industrial benefits (14). However, *Solanecio biafrae* and *Crassocephalum crepidioides* are not widely consumed and known as *C. olitorius* and *A. hybridus*. S. *biafrae* is used as a cheap healing agent for microbial gastroenteritis, and some other medicinal and physiological disorders (15). While *C. crepidioides* is believed to have antibiotic, anti-inflammatory, anti-diabetic, anti-malaria, and blood regulation properties in addition to its nutritional value (16). However, information about S. *biafrae* and C. *crepidioides* is scanty and some available information about their functional properties has not been scientifically proven.

MATERIALS AND METHODS

Processing of vegetables

The vegetables *(A. hybridus*, *C. crepidioides*, *S. biafrae* and *C. olitorius)* were obtained from Oje market in Ibadan, Oyo State in the Western part of Nigeria. They were transported to the laboratory in plastic baskets and spread out overnight before being processed. The vegetables were picked, washed, and cut into smaller pieces followed by drying in the hot air oven at 40 °C (17) before being ground.

High-calorie diet

Pelletized regular rat feed was procured from Sesco Agro-Allied and Livestock Ventures, Ogun State. The high-calorie diet consisted of high-fat feed and water containing 30 % sucrose. High-fat feed was produced by adding 30 % vegetable oil to the pelletized regular rat feed while table sugar was added to water at a concentration of 30 % (18). Feed and water for the high-calorie diet were prepared daily. The vegetable oil (refined palm olein) is a cooking oil that contains an appreciable amount of monounsaturated fatty acids (38 g) omega-9- fatty acids (38 g), omega-6- fatty acids, vitamins A and E with no cholesterol and less than 1 g trans fatty acids.

Animal study

Thirty male Wistar rats were obtained from Animal Holding Facilities, Babcock University, and were allowed to acclimatize for 10 days before the commencement of the experiment. During the period, they were allowed free access to regular rat feed and water. The rats were randomly separated into six groups, with each group consisting of 5 rats. The groups consisted of 2 control groups and 4 treatment groups.

Positive control A was given regular rat feed and water. While negative control B was given only high-calorie diet. Treatment group C was given high-calorie diet and C. *olitorius* while group D was given high-calorie diet and C. *crepidioides*. Also, the treatment group E was given high-calorie diet and A. *hybridus* while group F was given high-calorie diet and *S. biafrae*. The vegetables were added to the feed at a concentration of 5 % (19). The experiment was terminated at the end of five weeks and the blood sample of the rats was collected by the ocular puncture method (20) for haematological tests and lipid profile. The reference number for the ethical consideration for the protocol is BUHREC786/19 and was obtained from Babcock University Health Research Ethics Committee.

Proximate composition of the feeds

The proximate composition of the regular rat feed and the high-fat feed was determined by the methods described by the Association of Official Analytical Chemists (21). While the energy content was determined using the Atwater system. The Atwater general factor system includes energy values of 4 kcal per gram (kcal/g) (17 kJ/g) for protein, 4 kcal/g for carbohydrates and, 9 kcal/g (37 kJ/g) for fat (22).

Mean weekly feed consumption and water intake

Daily feed and water intake were recorded through the 5 weeks of the experiment. Rats were given water and feed at 24 hours intervals, leftovers were removed and water and feed feeders were cleaned before adding new ones. The quantity of water given to the rats and the leftover was measured with the 500 ml measuring cylinder while a weighing scale was used to determine the weight of the feed to an accuracy of 0.01 g. The mean weekly consumption of rats in each group was determined at the end of the experiment.

Mean change in weight

The rats were marked and the weight of each rat was recorded before the experiment. The rats were weighed again at the expiration of the experiment. The mean change in weight of each group was determined from the average weight gain/loss of the rats in each group.

Haematological test

The blood samples collected for haematological test at the end of the experiment was placed in plain bottles containing 10 % ethylene diamine tetracectic acid (EDTA). The blood samples were analysed using automatic blood analyser to determine the following haematological parameters; packed cell volume (PCV), haemoglobin concentration, white blood cell count and platelet count (23).

Determination of serum lipid profile

Serum total cholesterol, triglycerides, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were estimated using a commercially available kit by Randox Laboratories.

Total cholesterol

Cholesterol reagent (1000 μl) was added to test tubes labelled tests, standard and blank. This was followed by 10 μl of blood samples, reference standard, and distilled water in the respective test tubes. The content of the various test tubes was properly mixed and thereafter incubated for 10 min at 20 to 25 °C. The content of each test tube was transferred into cuvettes and the absorbance of the samples was measured against the reagent blank within 60 min.

The concentration of cholesterol in the sample = Δ in Abs. of the sample/ Δ in Abs. of the standard x concentration of the standard (24, 25).

Serum triglycerides

Triglycerides reagent (100 μl) was pipetted into all the test tubes. After which, 10 μl of the standard solution was pipetted into all the test tubes followed by 10 μl of the various blood samples into the test tubes labelled test and 10 μl of distilled water into the test tube labelled blank. The content of each test tube was properly mixed and incubated at 20 - 25 °C for 10 min. The absorbance of the sample and standard was measured against the reagent blank within 60 min.

Triglyceride concentration (mg/dl) = Abs. sample/Abs. standard x standard concentration (24, 25).

Serum HDL cholesterol

Diluted precipitant for HDL (500 μl) was pipetted into centrifuge tubes followed by 200 μl of the standard solution and blood samples into the tubes labelled standard and test respectively. The content of each test tube was mixed and allowed to sit for 10 min at room temperature. The content of the tubes was centrifuged for 10 min at 4,000 rpm (revolution per minute). The clear supernatant fluid was separated for cholesterol content determination.

Cholesterol content was determined using the CHOD-PAP method. The cholesterol reagent (1000 μl) was pipetted into properly labelled test tubes followed by the addition of 100 μl of the standard supernatant to the test tubes labelled standard. While 100 μl of the samples supernatant was pipetted into their respective test tubes and 100 μl of distilled water was added to the test tube labelled blank. The content of the various test tubes was mixed and incubated for 10 min at 20-25 °C. The absorbance of the sample and standard was measured against the reagent blank within 60 min (24, 25).

Concentration of HDL cholesterol (mg/dl) =

$$\frac{\Delta \text{ Abs.sample}}{\Delta \text{ Abs.standard}} \times \text{conc. of standard}$$

LDL cholesterol

The LDL cholesterol was obtained as follows;

LDL cholesterol = Total cholesterol – Triglycerides/2.2 – HDL cholesterol (24, 25).

Statistical analysis

All analyses were carried out in triplicate and the result was expressed as mean ± standard deviation. T-test was used to determine the significant difference between the feeds while analysis of variance was used to determine the significant differences among the rat groups and means were separated using Duncan's multiple range tests.

Results

Nutrient composition of regular and high-fat feeds

Table 1 shows the nutrient composition of the regular feed (Feed A) and high-fat feed (Feed B). Regular rat feed was higher in protein (17.78 %), carbohydrate (50.03 %), moisture content (7.68 %), and ash content (9.71 %). While the high-fat feed was higher in fat content (26.79 %), fiber (14.83 %), and energy content (422.67 %) but had lower content of protein (13.77%), moisture (6.19%), ash (6.79%), and carbohydrate (31.62%) content. There was a significant difference ($p<0.05$) in the composition of the regular and the high-fat feed.

Mean weekly feed consumption and water intake of rats

The mean weekly feed consumption and water intake of rats in the different groups are presented in Fig. 1. There was no significant difference ($P<0.05$) in the mean weekly feed consumption and water intake of the control groups and the treatment groups. However, the mean feed consumption (92 g) and water intake (332 ml) of the positive control A (regular rat feed) was higher compared to that of the negative control B(fed high-calorie diet), and the treatment groups which ranged from 65-93 g – 75.56 g for feed consumption and 287.29 ml – 305. 43 ml for water intake.

Mean weight change

The mean weight change of the rats in the different groups is presented in Figure 2. The mean change in weight of the positive control A (22.56 g) was significantly ($p>0/05$) higher than what was recorded for the other groups. The mean change in weight of the groups fed vegetables and the negative control B was between -9.10 – 11.09 g with the group fed *S. biafrae* having the highest weight gain. There was a weight loss of -9.10 g in the group fed *C. crepidioides* during the experiment.

Mean haematological parameters

Figure 3 presents the mean values of the packed cell volume (PCV), haemoglobin concentration (HB), white blood cell count (WBC) and platelet count for the Wistar rats in the different groups. There was no significant difference ($p<0.05$) in the four haematological parameters determined. The PCV ranged between 43.5 and 51% and positive control A (51 %) had the highest PCV which was followed by the group fed *S. biafrae* (48.25 %) and the group fed *A. hybridus* had the

Table 1: Nutrient composition of the regular rat feed and high-fat feed

Sample	Feed A	Feed B	T-Value	P-Value
Crude protein (%)	17.78±0.03	13.77±0.08	84.924	0.114
Fat (%)	5.41±0.03	26.79±0.04	-717.107	0.517
Moisture (%)	7.68±0.02	6.19±0.05	51.615	0.218
Fiber (%)	9.38±0.04	14.83±0.10	-88.932	0.113
Ash (%)	9.71±0.05	6.79±0.05	78.040	1.002
Carbohydrate (%)	50.03±0.07	31.62±0.09	274.052	0.527
Energy (kcal)	319.89±0.11	422.67±1.04	-169.715	0.110

Feed A = Regular rat feed; Feed B = High-fat feed

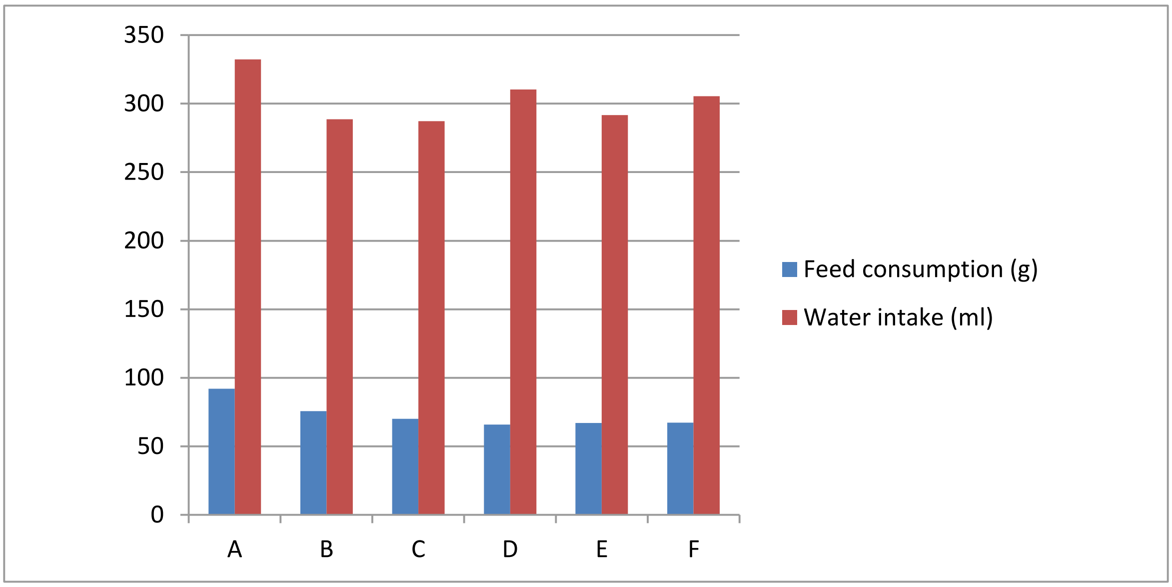

Figure 1: Mean weekly feed consumption and water intake of the rats

A = Positive control group fed regular rat feed and water; B = Negative control group fed high-calorie diet; C = Treatment group fed high-calorie diet and C. olitorius; D = Treatment group fed high-calorie diet and C. crepidioides; E = Treatment group fed high-calorie diet and A. hybridus; F = Treatment group fed high-calorie diet and S. biafrae

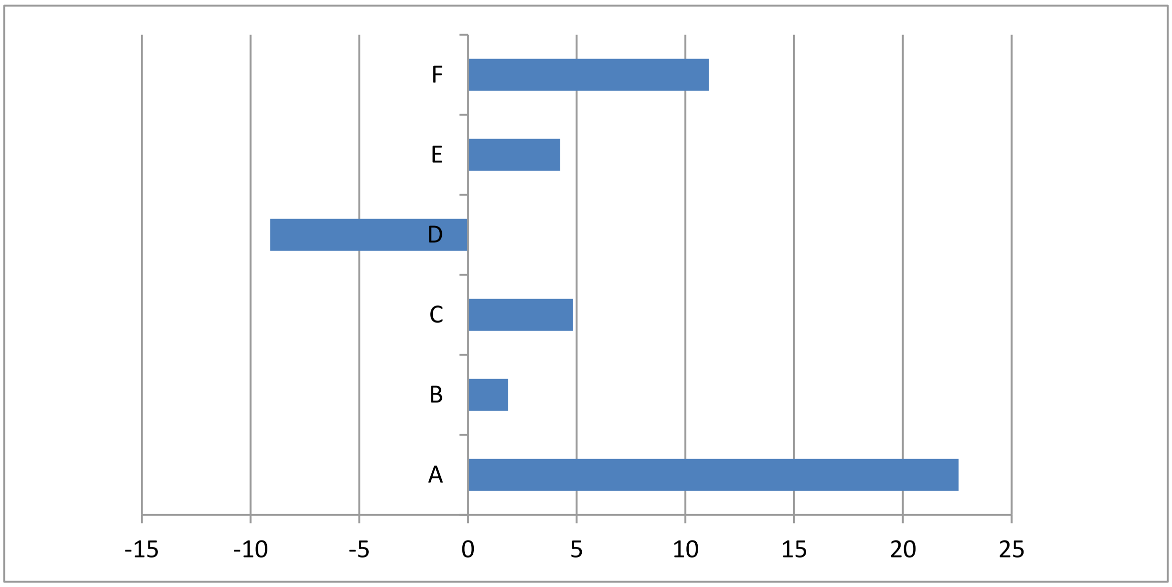

Figure 2: Mean weight change

A = Positive control group fed regular rat feed and water; B = Negative control group fed high-calorie diet; C = Treatment group fed high-calorie diet and *C. olitorius;* D = Treatment group fed high-calorie diet and *C. crepidioides*; E = Treatment group fed high-calorie diet and *A. hybridus;* F = Treatment group fed high-calorie diet and *S. biafrae*

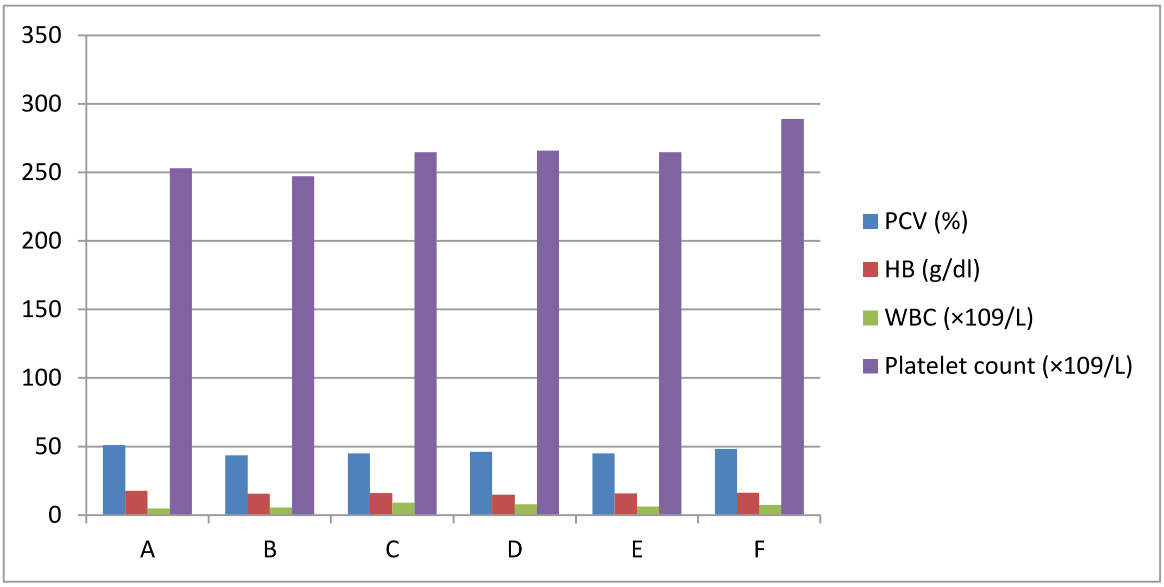

Figure 3: The mean haematological parameters of the rats

A = Positive control group fed regular rat feed and water; B = Negative control group fed high-calorie diet; C = Treatment group fed high-calorie diet and C. olitorius; D = Treatment group fed high-calorie diet and C. crepidioides; E = Treatment group fed high-calorie diet and A. hybridus; F = Treatment group fed high-calorie diet and S. biafrae

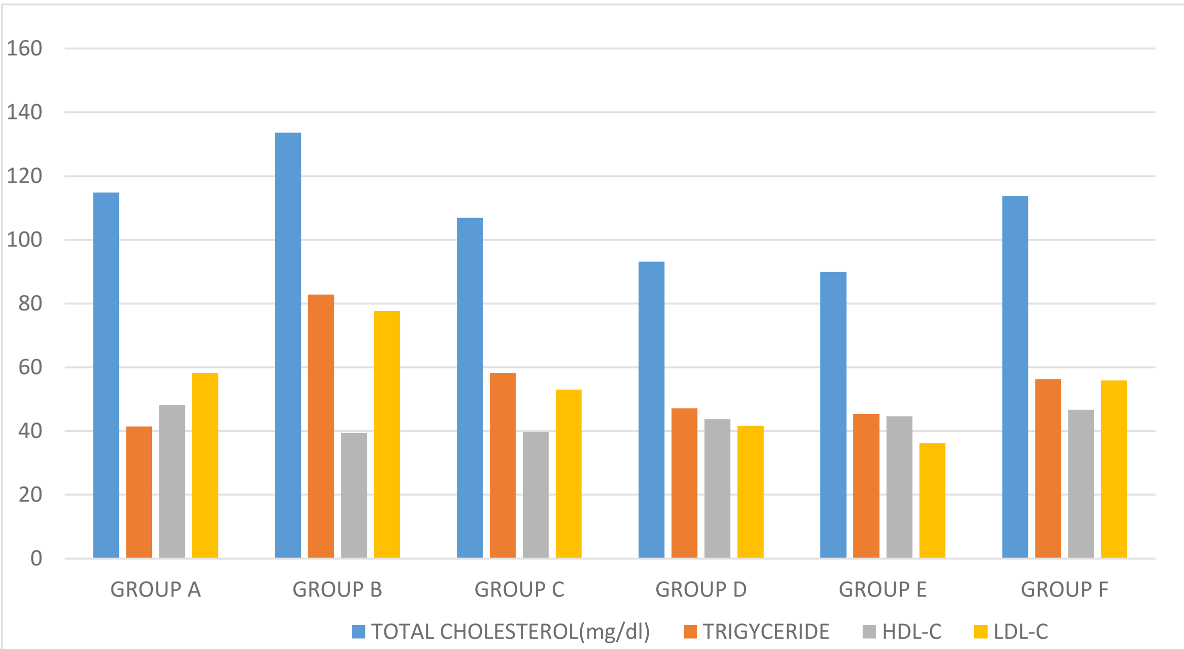

Figure 4: The mean lipid profile (mg/dl) of the test animals

A = Positive control group fed regular rat feed and water; B = Negative control group fed high-calorie diet; C = Treatment group fed high-calorie diet and C. *olitorius;* D = Treatment group fed high-calorie diet and C. *crepidioides*; E = Treatment group fed high-calorie diet and A. *hybridus;* F = Treatment group fed high-calorie diet and S. *biafrae* least. Haemoglobin concentration ranged from 14.88 – 17.75 g/dl. Positive control had the highest while negative control had the least. The group fed *S. biafrae* (16.25 g/dl) had the highest among the treatment groups. The positive control (4.88 $\times 10^9$/ L) had the least white blood cells followed by the negative control (5.63 $\times 10^9$/ L). The white blood cell of the treatment groups was in the range of 6.25 – 9.0 $\times 10^9$/ L. The platelet count was in the range of 247.25 and 289 $\times 10^9$/ L. The lowest platelet count was found in the negative control while the group fed *S. biafrae* had the highest.

Mean lipid profile

Figure 4 presents the values obtained for total cholesterol (mg/dl), triglycerides (mg/dl), high-density lipoprotein cholesterol (mg/dl) and low-density lipoprotein cholesterol (mg/dl). Total cholesterol (133.65 mg/dl), triglyceride (82.85 mg/dl) and LDL (77.65 mg/dl) were high in the rats fed high calorie diet (negative control B) while HDL (39.43 mg/dl) was low . The group given *A. hybridus* (E) had the lowest cholesterol (89.93 mg/dl), triglyceride (45.38 mg/dl), and LDL (77.65 mg/dl) while HDL of 44.65 mg/dl for the group fed *S. biafrae* (F) was the highest. There was no significant difference ($p<0.05$) among the groups.

Discussion

This study tends to determine the potential of some of the vegetables which are available in the tropics in curtailing the abnormalities associated with the consumption of a high-calorie diet. To achieve this aim, vegetable oil was added to the regular rat feed which resulted in a change in the composition of the feed. The addition of oil to the regular rat feed increased the calorie content and reduced the feed's content of other nutrients and increased the calorie content which corroborates an earlier report that an increase in fat content of food reduces the protein content and other important nutrients (26).

The mean change in weight of rats in the positive control A was higher than that of the other groups. This could partly be attributed to the significant reduction in the protein content of the high-calorie feed (26). This finding supports the report of Li and Freeman (27) who reported a 10 g loss in the weight of rats fed high-fat, low-protein diet. However, this is contrary to the report of Buettner et al. (28) who reported significant weight gain in rats fed lard, olive oil, fish oil, and coconut oil. Apart from reduction in protein content, this observation could also be due to impairment of metabolic processes as a result of possible on-set of liver disease (29). Contrary to the weight gain observed in the other groups, a weight loss was observed in the group fed *C. crepidioides* which could have been a result of the blood sugar-reducing property of the vegetable as reported in an earlier study (16).

Haematological parameters are related to the blood and blood-forming organs (30) and are good indicators of the physiological status of animals (31). The results of the haematological parameters showed that the values obtained were within the normal range (32). *S. biafrae* group had the highest PCV among the treatment groups while *C. crepidioides* caused an appreciable increase in the PCV but the effect of *C. olitorius* and *A. hybridus* was not pronounced. The group fed *A. hybridus* had the lowest PCV level of 43.5 % which strongly supports the earlier reports of Olufemi et al. (33) who observed a reduction in haematological parameters of pigs and Akintoye and Olorede (34) who found that *A. hybridus* did not produce any change in rats' haematological parameters. Haemoglobin concentrations of the treatment groups and negative control B were lower but not significantly to that of positive control A. However, the group fed *C. crepidioides* had the lowest haemoglobin concentration (14.88 g/dl) which was also within the normal range (12-18 g/dl.). This observation corroborates the findings of Filho et al. (35) who reported haemoglobin concentration greater than 12 g/dl for growing and aging rats. Haemoglobin concentration was reduced by the diet high in calorie but *S. biafrae* ameliorate the effect better than the other vegetables. The group fed *S. biafrae* had the highest (16.25 g/dl) among the treatment groups which supports the earlier report of Ajiboye and Ojo (36).

The white blood cell count of the treatment groups was high compared to the control groups. This is contrary to the finding of an earlier study that a habitual diet high in vegetables resulted in a lower white blood cell profile (37). The study was carried out on human subjects and the vegetables were subjected to cooking. However, the High WBC recorded for the treatment groups in this study could be attributed to the presence of microorganisms on the vegetables which were not treated before being administered to the rats.

Platelets are colourless blood cells that assist in blood clotting by forming plugs in blood vessel holes. The body has a lower risk of haemorrhaging when there is a sufficient number of platelets. Vegetables and other foods are reported to help in maintaining a healthy level of platelets (38). The platelet count of all the groups was within the normal range. However, the treatment groups had higher platelet counts and the group fed *S. biafrae* had the highest count of 289.0 $\times 10^9$/ L which is similar to what was observed in alloxan-induced diabetic rats (36).

The uncooked dried vegetables used in this study did not have a significant effect on the haematological properties of the rats which is contrary to the findings of the study on effect/efficacy of cooked vegetables on the haematology parameters (5). However, the findings of this study corroborates the report of Hosen et al. (39) who found that supplementation of *C. olitorius* leaves with arsenic-contaminated rice did not significantly ($p < 0.05$) restored the altered hematological parameters and other serum indices toward the normal values. While favourable effect of *S. biafrae* on some haematological parameters was earlier reported (6).

The lipid profile parameters of the groups were all in the normal range (<200 for total cholesterol, (<150 for triglycerides, >60 mg for HDL and <100mg/dl for LDL) according to Delwatta et al., (32). All the vegetables reduced the total cholesterol level of the rats compared to the control groups though not significantly. *A. hybridus* had the highest total cholesterol-lowering potential (89.9 mg/dl). The total cholesterol (89.9 – 132.7 mg/dl) of the test animals was lower than 186 – 209.13 mg/dl reported for rats fed formulated diets from riped, unripe and artificially riped *Mangifera indica* (Mango) pulp (40).

An elevated levels of triglycerides can be a contributing factor to heart disease and stroke (41) and consumption of high-calorie diet had been reported to be one of the factors responsible for elevated triglycerides (42). All the vegetables reduced the triglyceride level of the rats compared to the negative control B which was given only high-calorie diet. *A. hybridus* had the highest triglyceride-lowering potential because the group that was fed *A. hybridus* had the lowest triglyceride level (45.4 mg/dl) among the test groups. The triglyceride level of all the groups was generally lower than 166.40 – 180.0 mg/dl reported by Iheagwam et al. (40).

However, the high density lipoprotein (HDL) of all the groups was between 39.4 – 48. mg/dl. The positive control had HDL level of 48 mg/dl while the negative control group had the lowest HDL level (39.4 mg/dl). All the treatment groups were observed to have higher HDL levels compared to the negative control group. The best level of HDL is 60 mg/dl but, none of the groups attained this level. HDL is considered too low when it is below 40 mg/dl (43) and low levels of HDL cholesterol increase the risk of heart diseases (44). Consumption of high-calorie diet has been linked with lowered HDL (45) and this may be the reason for the reduced level of HDL recorded in the negative control group. However, the group fed *S. biafrae* had the highest HDL level among the test groups (46.7 mg/dl). This finding supports a report that *S. biafrae* extract significantly decreases the level of serum cholesterol, triacylglycerols, low-density lipoprotein (LDL), but significantly increases High-density lipoprotein (6). Also, in a study on *C. crepidioides* it was found that its extract aids in lowering hyperlipidemia in high-fat diet (7).

The LDL cholesterol is generally considered "bad cholesterol" because a high level of LDL leads to a build-up of cholesterol in the arteries. All the vegetables were observed to reduce insignificantly the LDL level of the rats when compared with the control groups. The control group B had the highest level of LDL cholesterol (77.7 mg/dl) which is attributed to the consumption of high-calorie diet (46). A greater reduction of LDL was observed in the group fed on *A. hybridus (*36.8 mg). The LDL for the groups fed on *A. hybridus and C. crepidioides* was within the range reported as reference for rats at the animal house in Sri Lanka (32) and 49.64 mg/dl reported for serum lipid profile of albino rats in an animal facility in Nigeria (47). The vegetables were effective in preventing the deleterious effect of the diet on the lipid profile. Furthermore, the observations in this study of the positive effect of the vegetables on the lipid profile is in agreement with the report of Huang et al. (48) who found that vegetables contribute to the attenuation of hyperlipidemia in rats fed on high-fat fructose diet.

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