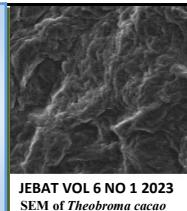


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SEM of *Theobroma cacao*

# Proximate, Nutrient Composition and Glycemic Index of Pearl Millet-Acha-Moringa Blend

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## ABSTRACT

Globally, grains can be regarded as the daily source of diets for many populations. They constitute cereals, pseudocereals and legume and are majorly the seeds of plants. They are made up of a wide variety of macronutrients that are vital to human diet, those nutrients are not limited to carbohydrates, but also proteins lipids and micronutrients such as vitamins and minerals. Grains can be consumed as a source of dietary fibre and bioactives. This research is aimed at formulating a diet using whole grains and cray fish and also to determine the nutrients composition of the diet and its Glycemic Index. A diet was formulated using whole Pearl millet, whole Hungary rice, moringa leaves and cray fish. The Glycemic Index of the diet and the nutrients composition of the formulated diet such as % Fat, % Ash, % Moisture, %Fiber and Vitamin E were determined. Iron and magnesium ions concentration was determined using Atomic Absorption Spectrophotometry while calcium ion was determined using Flame Photometry. The result showed that the blend contains 6.31% moisture, 1.24% ash, 1.50% fat, 12.06% protein, 3.22% fibre, 75.66% carbohydrate, 50.22 mg Vitamin E, 1.37 mg Magnesium (Mg), and the Glycemic Index was 67.7%.

## INTRODUCTION

Mankind has been utilizing plants like grains since ancient times for many purposes, specifically, these include the use of plants as food for nutritional benefits and in some respects as medicine for the treatment of many disease conditions. Plants are considered to be useful in all cultures of the globe and have been dependent upon them to encourage and restore human health [1]. They form a vital component of conventional medicine (CM) and their use for the maintenance of health and well-being is a familiar practice in all African societies. They can be used as remedies for the prevention, treatment, and management of many disease conditions. Grains can be regarded as the daily source of diets for many populations. They constitute cereals, pseudocereals and legumes and are mostly the seeds of plants. They are made up of a wide variety of macronutrients that are vital to the human diet, those nutrients are not limited to carbohydrates, but also proteins lipids and micronutrients such as vitamins and minerals. Grains can be consumed as a source of dietary fibre and bioactives. Grains are the basis of everyday diets for many populations globally. Particularly whole grains, which are of importance for

the production of high-value food crops with enhanced health and nutritional benefits [2]. In quite a lot of countries, nutritional guidelines recommend the addition of a greater amount of wholegrains in the diet for promoting good health. The recently discovered role of whole grains could be attributed to the presence of prebiotics which is useful for gut microbiota which is beneficial to the host's comfort [3]. Utilization of the grain as whole grain makes it a superb source of dietary fibre and is linked with nutraceutical benefits and can be used in managing obesity and diseases, such as diabetes. It is believed that Acha may have nutraceutical properties and it contains resistant starch as well as bioactive peptides which help in preventing and managing prediabetes and type 2 diabetes [4].

Acha (*Digitaria exilis*), popularly known as fonio or hungry rice is a cereal grain belonging to the family *gramineae*. It is developed and cultivated in Guinea and the dry Savanna zone of Mali and Upper Volta with at least 800mm rainfall. Acha is easily utilized by the body, conventionally, it is suggested as food for children, aged individual, diabetic patients, and also can be used to cure abdominal mess and lastly may be utilized in the

management of weight reduction. *Acha* is suitable for consumption by gluten-intolerant people because it is a grain that is gluten-free, consumption of unprocessed acha makes it a good source of dietary fibre and its nutraceutical content makes it beneficial to obese and diabetic individuals [5].

Pearl millet (*Pennisetum glaucum*) is a straight grass that has a summer annual cycle of between 75 and 120 days which depends largely on the environmental circumstances. Generally, it is of rapid growth and reaches an average height of 1.5 to 3 m. The plant develops squashed cylindrical panicles that are 2 to 3 cm wide and 15 to 60 cm long and can produce between 500 and 2000 seeds per panicle [6]. They are called “nutri-cereals” and this is because of the adequate content of protein, fibre, mineral, and fatty acids in it, it is taught to possess antioxidant properties. Also, they can serve as a substitute food for celiac and gluten sensitive people [7]. In addition, the chemical composition of millet grains can support a range of health benefits such as reduction of oxidative stress among others [8]. Pearl millet grains have quite a lot of useful properties, due to their high phytochemical compound's fatty acid composition as well as high fibre content [7].

The leaves of *Moringa oleifera* are consumed as vegetables or as a source of food ingredients because the leaves are rich in vitamins, antioxidants and macronutrients which may improve the nutritional features of humans [9]. The therapeutic use of *M. oleifera* leaves is taught to be a result of quercetin-3-glycoside, rutin, kaempferol and glycosides which are polyphenols that can reduce blood glucose concentration and lipids concentration after ingestion [10]. The Glycemic Index (GI) is a useful parameter of food quality that compares the hyperglycemic effect of a tested food with pure glucose (or of another defined standard food). The GI is a measure of the food's capacity to raise Blood glucose concentration after a meal.

The GI is defined as the relation of the incremental area under the blood glucose response curve (IAUC) of a tested food containing 50 g of digestible carbohydrates and the incremental area under the blood glucose response curve of the standard food, i.e. 50 g of pure glucose (IAUCS). Carbohydrates that break down rapidly during digestion have a high GI because their Blood glucose response is fast and high. Carbohydrates that break down slowly have a low GI [11]. The purpose of this study is to develop a meal plan that includes whole grains and crayfish, and to analyze its nutrient composition and Glycemic Index.

## MATERIALS AND METHODS

### Experimental Animals

Three albino rats weighing between 100- 120 g were used in this study, the animals were obtained from the Animal House of the Department of Biological Sciences, Bayero University Kano. All experimental procedures were approved by the Animal Ethics Committee of the Department of Health Sciences, Bayero University Kano. The environment was maintained with a 12-hour light/ dark cycle. The animals were fed with vital feed with free access to water and they were left to acclimatize for two weeks before the beginning of the experiment. The Fasting blood glucose of the rats was analysed and they were found to be clinically normal and non-diabetic.

### Food Sample Collection and Identification

The ingredients used in the formulation of the diet (Millet, Hungary Rice, Moringa Leaves and Crayfish) were purchased from Dawanau Market, Kano State. The ingredients were cleaned to remove dust and particles and the moringa leaves were dried (shade drying). Three of the ingredients (Millet, Hungary rice and Moringa) were taken to the Department of Plant Biology, Bayero University Kano, for identification. The voucher numbers were BUKHAN 660 for (Hungary rice), BUKHAN 11 for Moringa oleifera leaves and BUKHAN 249 for Pearl millet.

### Preparation of the Formulated Diet

A dietician was consulted before the formulation of the diet and the ratio of the ingredients used for the formulation of the diet was:

Ingredients	%
Composition (/100g)	
Hungry Rice ( <i>Digitaria exilis</i> ),	60%
Pearl Millet ( <i>Pennisetum glaucum</i> )	30%
Moringa Leaves ( <i>Moringa oleifera</i> )	7%
Crayfish	3%

All the ingredients were measured into a mixing bowl and mixed thoroughly.

### Ethical Approval

Ethical approval was obtained from the Ethics sub-committee of the Animal Ethics Committee of the Department of Health Sciences, Bayero University Kano, to conduct the research at the animal house of the Department of Biological Sciences BUK with the approval number (BUK/CHS/REC/108)

### Proximate and Nutrient Composition Analysis

The method of proximate composition determination partitioned nutrients in the diet into 6 components: moisture, ash, protein, lipids/fats, fibre and carbohydrate.

### Determination of Protein (Microkjeldahl Method)

The test is based on the fact that a sample containing proteins is digested with concentrated  $H_2SO_4$  in the presence of a Kjeldahl catalyst. The material was digested with standard alkali, and the ammonia produced was steam-distilled into a standard HCl solution. Titration of the acid indicates the quantity of ammonia ( $NH_3$ ) that has been distilled and may therefore be used to assess the nitrogen content of crude protein. In a 100 mL Kjeldahl flask, 2.0 g of the food sample was weighed precisely, and anti-bumping granules were applied.  $CuSO_4$  and  $K_2SO_4$ , in a ratio of 8:1, acted as the mixed catalyst, to which 15 mL of concentrated sulfuric acid was added.

The flask was positioned on a Kjeldahl digestion rack and heated until a transparent solution was formed. At the end of the digestion, the flask was cooled, and the sample was transferred quantitatively to a 100 mL volumetric flask and made up to the mark with distilled water. Approximately ten millilitres of the digest were pipetted into a Markham semi-micro nitrogen steel tube, 10 mL of 40% NaOH solution was then added carefully. The sample was then steam distilled, producing ammonia into a 100 mL conical flask containing 10 mL of 4% boric acid and a drop of methyl blue indicator was added until the colour changed from pink to green. About 30 mL of sample volume was then collected.

The content of the conical flask was then titrated with 0.1 M HCl. A colour change shows the end point from green to pink and the volume (v) of the acid for each distillate was distinguished. The percentage of nitrogen per sample was calculated from the expression below:

$$\% \text{Nitrogen} = \frac{M \times v \times 14 \times 100 \times 100}{\text{Weight of the sample} \times 1000 \times 10}$$

In this,

M = Molarity of HCl

V= volume of HCl used

14 = Atomic weight of nitrogen.

100 = Total volume of digest.

100 = % conversion.

10 = Volume of the digest taken.

1000 = Conversion to litre.

The protein content was calculated using the relationship below:

$$\% \text{ Protein} = 6.25 \times \% \text{ Nitrogen}$$

#### Fat Content Determination (Method of AOAC 1990)

The principle is based on the concept that non-polar sample components are rapidly removed into the organic solvent. A well-cleaned round-bottom flask containing anti-bumping grains was utilized. In a flask equipped with a Soxhlet extraction device, approximately 210 cm<sup>3</sup> of petroleum ether (60 - 80°C) was added. The material was put into a thimble that had been previously attached to the Soxhlet extraction apparatus. Cold water has been distributed. The heating mantle was activated, and the heating rate was adjusted so that the solvent refluxes at a constant pace. The extraction lasted 8 h. The sample was detached and dried to a constant weight in an oven, it was later cooled in a desiccator and reweighed and the percentage crude fat content was determined from the relationship below;

$$\% \text{ fat} = \frac{\text{Weight of fat extracted}}{\text{Weight of dry sample}} \times 100$$

From the above expression, the weight of fat extracted was the loss in weight of the sample after extraction, drying in an oven and lastly cooling in a desiccator.

#### Ash Content Determination (AOAC 1990)

The test is based on the weight loss that occurs after burning the sample in a muffle furnace at a temperature of about 600 °C; this causes the organic materials to totally burn without influencing the ash elements. For this test, crucibles were utilized; they were preheated in a muffle furnace at approximately 550 °C. Each crucible was weighed after being cooled in a desiccator. In each crucible, 1 g of the sample was weighed. The crucibles and their contents were placed in the muffle furnace at a temperature of 550 °C for five h. The contents of the crucible were weighed and documented. The percentage of ash was determined using the following expression.

$$\frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100$$

#### Moisture Content Determination (AOAC, 1990)

As indicated in the technique, the premise is based on measuring the weight loss caused by drying at a temperature of around 105 °C. After being properly washed and dried in an oven at around 105 °C, the glasses were chilled and weighed. In the watch glasses, precisely 2 g of the sample was weighed. The watch glasses and their contents were dried to a consistent weight in a 105 °C oven with air circulation. The watch glasses and their

contents were refrigerated and reweighed using desiccators. The following expression was used to compute the relative humidity:

$$\% \text{ moisture} = \frac{\text{Loss of weight on drying(g)}}{\text{Initial sample weight}} \times 100$$

#### Crude Fiber Determination (AOAC 1990)

Approximately 2 grams of ground sample was placed in a round bottom flask. 100 mL of 0.25 M H<sub>2</sub>SO<sub>4</sub> was later added, and the mixture was boiled under reflux for 30 min. The insoluble matter was washed several times with hot water until all the acids were removed (C1). It was then transferred into a flask containing 100 mL of 0.25 M NaOH solution. The mixture was boiled again under reflux for 30 min and filtered under suction. The insoluble residue was washed with hot water until all the base was removed (C2). It was then turned to ash in a furnace at 550 °C for 2 h. The furnace was then put off and allowed to cool down. The sample was then removed and cooled in a desiccator and weighed (C3). The crude fibre content was then calculated as loss of weight in ashing. The weight of the original sample was used as W.

#### Determination of Carbohydrate

The carbohydrate content of the sample was obtained by difference thus;

$$\% \text{ Carbohydrate} = 100 - (\% \text{ ash} + \% \text{ crude fibre} + \% \text{ crude fat} + \% \text{ moisture} + \% \text{ crude protein})$$

#### Determination of Energy Value

Percent calories in the formulated diet were calculated from crude protein, fat content, and carbohydrate content values obtained in the experiment. Firstly, crude protein and carbohydrate content values were each multiplied by four while that of crude fat was multiplied by nine and all the values obtained were added. The calculation was as follows;

$$\text{Energy (Kcal/100g)} = \{[\% \text{ C.P} \times 4] + [\% \text{ carbohydrate} \times 4] + [\% \text{ C.F} \times 9]\}$$

Where:

C.P, Crude Protein,

C.F, Crude Fat

#### Determination of Vitamin E (AOAC, 1990).

A gram (1 g) of the sample was weighed, which was then macerated with 20 mL of n-hexane in a test tube for 10 min and centrifuged for another 10 min. The solution was filtered; 3mls of the filtrate were transferred into a dry test tube in duplicates and evaporated to dryness in a boiling water bath. After this, 2 mL of 0.5 N alcoholic potassium hydroxide was introduced and boiled for 30 min in a water bath. Later, 3mls of n-hexane was added followed by vigorous shaking. The n-hexane mentioned earlier was transferred into another set of test tubes and evaporated to dryness. Exactly 2 mL of ethanol was added to the residue. In another container, 1 mL of 0.2% ferric chloride in ethanol was added. Then 1 mL of 0.5% α<sup>1</sup> α<sup>1</sup>-dipyridyl in ethanol was added, followed by 1 mL of ethanol to make it up to 5mls. The solution was mixed, and absorbance of the samples was taken against the blank test tube in duplicate at 520 nm. A standard solution of vitamin E of 100 ppm was also prepared using the above procedure. Accurately 3 mL of calcium working reagent was added and absorbance at 512 nm was taken against the blank.

#### Determination of Calcium (Ca) Using Flame Photometer

When atoms of alkali metals are heated sufficiently to a high temperature, they absorb energy from the heat source and become easily excited. The electrons move from a lower energy level to a higher energy level. The electrons are forced to return to their

original state to maintain stability as they cool to the original state. Therefore, the atoms will emit some radiant energy equivalent in magnitude to the amount of energy absorbed during excitation. The wavelength of the emitted energy has a direct relationship with the electronic transition that occurred. As every element has its peculiar electronic configuration, the wavelength of light emitted is unique for such a particular element. About 1.0 g of each sample is first digested with 20 mL of an acid mixture (650 mL conc.  $\text{HNO}_3$ ; 80 mL perchloric acid; 20 mL conc.  $\text{H}_2\text{SO}_4$ ) in the mixture ratio of 3:1. The samples were taken into the digesting flask and heated until the sample is completely digested. Then it was filtered and made to 100 mL with deionized water. A blank solution was also prepared using a mixture of the acid for the analysis. Calcium, Potassium and Sodium were investigated using Flame Photometer, respectively.

#### Iron and Magnesium Determination Using Atomic Absorption Spectrophotometer

Atomic Absorption Spectroscopy is a much more accurate and sensitive method of analyzing wide ranges of metals. In the most common AAS implementation, an aqueous sample is atomized in a hot flame generated by a highly combustive gas mixture (air-acetylene) at a temperature of 2300 °C. This breaks the metal compounds in the sample into free metal atoms or free radicals. Radiation emanating from the cathode lamp made to pass through the free atoms will be absorbed by the atoms. The lamp produces radiation of an appropriate wavelength while passing through the flame. A photo-detector read-out system measures the absorbed energy, and the extent of this absorption is a measure of the metal concentration in the solution.

The concentration of the sample is usually determined from the standard calibration curve. Sample preparation: By design, the analyte sample has aspirated only as a liquid into the AAS instrument's sample compartment that uses the burner system. Many materials whose samples are to be analyzed, such as tissues, plants, soil, and other minerals, are not liquid. Thus, there is a need for pre-treatment of samples. This is achieved through:

- i. Digestion: This involves heating 1g of the sample with *aqua regia* (a mixture of  $\text{HNO}_3$  and  $\text{HCl}$  in a ratio 1:3) in a fume cupboard until the color of the acid changes pale yellow from the yellow-orange fuming liquid. Allow to cool, filter and makeup to 100 mL with deionized water
- ii. Ashing: This involves heating 1g of the sample in a muffle furnace at about 700-800 °C for 4-6 h (make sure all traces of carbon are completely burnt). Allow to cool, add drops of concentrated  $\text{HCl}$  to dissolve, filter and make up to 100 mL with deionized water.

Exactly 1.0 g of each sample is first digested with 20 mL of an acid mixture (650 mL conc.  $\text{HNO}_3$ ; 80 mL perchloric acid; 20 mL conc.  $\text{H}_2\text{SO}_4$ ) in the mixture ratio of 3:1. The samples were taken into the digesting flask and heated until the sample is completely digested. Then it was filtered and made to 100 mL with deionized water. A blank solution was also prepared using a mixture of the acid for the analysis. Copper, magnesium and iron were investigated using BUCK 205 Atomic Absorption Spectrophotometer.

#### Glycemic Index Determination

Glycemic Index (GI) was determined according to the guideline of Wolever *et al.* [11]. The sample was prepared in the morning using the combination index of the ingredients shown earlier. The fasting blood glucose level (FBG) assessment of the rats commenced at 08:00hrs in the morning after an overnight fast of 12 h. 50 g of the formulated diet was measured and given to the rats; the diet was served with distilled water. Blood sample of the rats were collected with an interval of 15 min for 2 h. The same procedure was carried out the next day using the same set of rats, but they were given standard glucose [12]

#### Blood Analysis

The blood samples from the rats were analyzed using an Accuchek glucometer to determine the FBG levels and the values recorded in mmol/L.

#### Glycemic Index (GI) Calculation

The Glycemic Index (GI) was calculated geometrically using the method described by FAO (1998) as the incremental area under the blood glucose response curve of a 50 g carbohydrate portion of the test food (IAUC) expressed as a percent of the same amount of the carbohydrate from a standard food (IAUCS) taken by the same subject. Glucose-D was used as a reference food. The area under the curve is calculated as the incremental area under the blood glucose response curve.

The GI for the formulated diet was calculated from the formula:

$$\text{GI} = \frac{\text{IAUC}}{\text{IAUCS}} \times 100$$

Here,

GI= Glycemic Index

IAUC= Incremental area under the curve of test food

IAUCS= Incremental area under the curve of standard (glucose-D).

#### Statistical analysis

All values were recorded in triplicates Data analysis was carried and values were reported as mean  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

#### Proximate and some Nutrients Composition of the Blend

**Table 1** shows the result of the proximate compositions of the formulated diet. The results revealed that the percentage of moisture content in the sample was  $6.31 \pm 0.50\%$ , and moisture content of  $5.94 \pm 0.03\%$  was reported for a formulated diet made of Pearl millet and Hungry rice alone [15], the increase seen in the moisture content in the present study could be attributed to the addition of Moringa leaves and crayfish to the formulation. The moisture content of food is a good parameter for food spoilage and acceptability. The ash content was found to be  $1.24 \pm 0.05\%$ . Ash content is a measure of the total amount of minerals present within a sample [13], and hence high ash content indicates that the sample contains a substantial amount of minerals. The fat content was found to be  $1.50 \pm 0.50\%$ , the low value of crude fat found indicates that the formulated diet does not contain much fat as a source of energy, but rather, it could be important in maintaining cell integrity [14].

**Table 1.** Proximate and some nutrient composition of the formulated diet.

Composition	Mean $\pm$ S.D
Moisture Content (%)	6.31 $\pm$ 0.50
Ash Content (%)	1.24 $\pm$ 0.05
Fat Content (%)	1.50 $\pm$ 0.50
Crude Protein (%)	12.06 $\pm$ 0.32
Crude Fibre (%)	3.22 $\pm$ 0.79
Carbohydrate (%)	75.66 $\pm$ 0.40
Energy Value (Kcal/g)	364.2 $\pm$ 1.72
Vit. E, Tocopherol (mg)	50.52 $\pm$ 0.52
Magnesium, Mg (mg)	1.37 $\pm$ 0.12
Calcium, Ca (mg)	60.88 $\pm$ 0.21
Iron, Fe (mg)	18.53 $\pm$ 0.25

Note: All values are expressed in triplicate as mean  $\pm$  S.D, where mg is milligram, mcg is a microgram, Kcal/g is kilo calorie per gram.

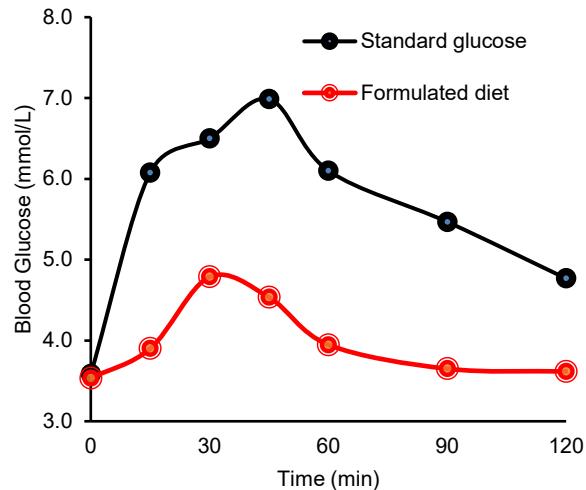
The crude protein content was found to be 12.06 $\pm$ 0.32%, the value was higher than the reported value (5.1% to 11%) obtained for a diet made from combination of pearl millet and hungry rice [15]. The increase seen in the percentage of crude protein in the present study could be majorly contributed by the addition of crayfish to the formulation, research carried out by Ahmad *et al.* [21] indicated that crayfish contains higher concentration of protein needed for body growth and repairs of damaged cells. The crude fiber content of the blend was found to be 3.22 $\pm$ 0.79%, the value obtained was higher than the 2.28 $\pm$ 0.01% obtained by Ojokoh *et al.* [15]. The increase seen in the fibre content in the present study could be attributed to moringa leaves addition to the formulation which contains soluble fiber that enhances decrease in glucose levels when consumed [17].

Diet formulated from whole grains and vegetable (moringa leaves) is a rich source of insoluble dietary fiber, mainly cellulose and insoluble hemicellulose, high-fiber diet may reduce the risk of cardiovascular diseases, colonic cancers and diabetes [18]. The carbohydrate content of the blend was found to be 75.66 $\pm$ 0.40%, carbohydrates are the major components in cereals and contain the main energy sources used by the human body and other organisms [19]. Generally, carbohydrates add to the bulk of the blend, and they play a pivotal role as they provide energy to the body.

The energy value of the blend was found to be 364.2 $\pm$ 1.72%, this shows that the formulated diet is a rich source of energy. The vitamin E and minerals content of the formulated diet was presented in **Table 1**. The vitamin E content of the formulated diet was 50.52 $\pm$ 0.52 mg, vitamin E benefits the body by acting as antioxidants and also protects vitamin A and C and essential fatty acids from destruction. The magnesium content was 1.37 $\pm$ 0.12 mg, magnesium is very important to the wellbeing of the body, because it is required by many enzymes that uses Adenosine Triphosphate and contribute to DNA and RNA synthesis during cell proliferation. The calcium content of the formulated diet was 60.80 $\pm$ 0.21 mg, calcium is the sole essential nutrients for diabetics, because it helps in the development of strong bones. The iron content of the formulated diet was 18.53 $\pm$ 0.25 mg, iron is important in the synthesis of hemoglobin and myoglobin.

#### Glycemic Index of the Blend

The Glycemic Index (GI) of the blend was calculated geometrically from the mean incremental blood glucose response for test food and standard in fig. 1, the incremental area under the curve was determined for both the test diet and the standard from the figure and the Glycemic Index (GI) was found to be 67.6% (moderate Glycemic Index).



**Fig. 1.** Incremental blood glucose responses after ingestion of standard glucose and the test diet.

The Glycemic Index of the formulated diet was found to be 67.7%, this is higher than the reported value obtained for a diet made from unaltered cereals [20], the increase seen could be attributed to the partial grinding undergone by the millet before the formulation of the diet and this was done to increase the surface area of the grain and thereby making it easier for chewing to the rats. Dea *et al.* [20] concluded that changing the physical form of food (e.g., grinding) can have an effect and thereby makes the Glycemic Index of the food high. It was found in similar research that changing the physical form of complex carbohydrates (e.g., grinding of rice) changes the postprandial glucose and insulin responses to it [21]. GI is a useful concept because it measures how rapidly the carbohydrates are absorbed and result in blood glucose and insulin elevations, Glycemic Index is a measure of the rise in blood sugar caused by the intake of a measured quantity of a particular food and is not related to whether the carbohydrate is simple or complex [22]. A moderate Glycemic Index diet is digested and absorbed moderately and hence does not cause a significant increase in blood sugar [23].

#### CONCLUSION

This study has shown that a diet made from millet, acha, moringa and crayfish contained essential nutrients, vitamins, proteins, and minerals and may have a potential for use in the management of diseases because the Glycemic Index of the diet is moderate and hence cannot results in a significant increase in blood glucose level after ingestion.

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