Original Article

Impact of Consuming Maize-Cassava- Soybean Flour Blends on the Nutritional Value and Safety of the Diet in Wistar Rats

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ABSTRACT

Background and Objectives: The nutrient contents of maize and cassava composite flour used in the preparation of cakes, pastas and porridges are low in protein with high carbohydrates, leading consumers to several nutritional and health challenges. The study aimed to investigate impact of consuming maize-cassava-soybean flour blends on the nutritional value and safety of the diet in wistar rats**.**

Materials and Methods: The flours were prepared from maize, cassava and soybean at the ratios of 70:25:5, 70:20:10, 70:15:15 and 70:10:20 (maize: cassava: soybean). The proximate composition was determined using standard methods. The flour blends were, thereafter, fed to 15 wistar rats for 21 days using 100 % maize flour, 70:30% (maize-cassava) and 70:15:15% (maize-cassava-soybean). Subsequently, their blood samples were collected and analyzed for blood glucose and biochemical indices.

Results: Protein, fats and ash increased with the addition of soybeans, whereas, moisture, fibre and carbohydrate decreased. The animals were observed to consume more feeds of soybean inclusion, with a significant weight gain and a reduced fasting and post prandial blood glucose. The intake of maize-cassava flour supplemented with 15 % soybean flour by the rats, elevated serum protein, albumin, globulin, cholesterol, high density lipoprotein, sodium, chloride and magnesium. The intake, however, decreased triglycerides, low density lipoprotein as well as calcium, potassium and phosphorus levels. It also caused reduction in the activities of liver enzymes, suggestive of no liver damage and toxicity.

Conclusions: Therefore, the incorporation of soybean to our maize-cassava-based diet can enhance its nutritional value and safe for healthy living.

Keywords: Nutrients, Serum, Protein, Electrolytes, Cholesterol and Enzymes

HIGHLIGHTS

- It has established that incorporation of soybean into maize- cassava flours enhanced its nutrients.
- The study demonstrated that maize-cassava based flour supplemented with soybean supports growth and reduction of blood glucose level
- The consumption of the flour blends elevated serum protein, albumin, globulin, cholesterol, high density lipoprotein, sodium, chloride and magnesium to a normal level
- The intake maize, cassava, soybeans blends, however, reduced triglycerides, low density lipoprotein, calcium, potassium, phosphorus and liver enzymes.

INTRODUCTION

Legumes are reported to be good source of protein, fat, minerals as well as high density lipoprotein that help lowers the levels of total cholesterol, LDL-c and have ability to elevate serum protein and electrolytes to a normal level (1).

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High concentration of low-density lipoprotein cholesterol (LDL-c) is said to be bad as it is a risk factors of cardiovascular disease. Researchers have reported an increase HDL-c level, serum protein and electrolytes of diet supplemented with legumes. An increased level of HDL-c in the blood has been reported to facilitate the removal of cholesterol from the tissue to the liver for breakdown and subsequent excretion thereby reducing the prevalence of cardiovascular diseases (2). It has also been shown that sufficient intake of legume seeds contributes to about 82 % reduction in the possible risks of developing coronary heart disease and liver damage (3).

The incorporation of legumes such as soybeans, cowpea, ground nut and other legumes seeds into cereals, roots and tubers meal has been shown to enhance products of high protein, fat and mineral values and such a formulation can be used to address cases of protein and micronutrients deficiencies (4). Legumes are known to have anti-nutrient factor which can reduce bioavailability of food products and however, can be greatly reduced by soaking, boiling or precooking and other processing operations like dehulling (5).

The National Nutrition and Health Survey (NNHS) (6) report in Nigeria revealed that about 32 %, 19.9 % and 7 % of fewer than five years children were stunted, underweight and wasted, respectively. There is about 30 % and 20 % increase in the rates of stunting and underweight, respectively, among under-five children when compared to the report of 2014. The report of National Food Consumption and Micro-nutrients Survey (NFCMS) (7) of Federal Republic of Nigeria also revealed the prevalence of stunting, wasting, underweight and overweight in children (aged 6-59 months) nationally as 33.3 %, 11.6 %, 25.3 % and 1.5 %, respectively. The report had it that, stunting was highest in the North West zone (47.9 %), wasting was highest in the North East zone (17.2 %) and underweight was highest in the North West zone (35.5 %).

Several efforts have been put forward to combat this menace of malnutrition using native meals such as maize and cassava products, especially in Nigeria. Blending of flours from legumes which are rich in protein, fat and minerals with flour from cereals, roots and tubers which are high in carbohydrates in proper proportion to make a balanced enriched meal can help address malnutrition challenges (8). Studies have been reported on the use of cereal-tuber-legume flour blends in the production of several products targeting at enhancing nutrient proficiency of food product accrued to proportional composition of the composite flour. Kadam *et al.* (9); Igbua *et al.* (10) separately reported high protein, fat and mineral contents in maize-cassava -soybean composite flour. However, a study yet to profile biochemical indices of such blend; this has necessitated this work using wistar rats. Results of such profile shall guide the choice of flour blend of this nature as food products to address some nutritional deficiencies.

MATERIALS AND METHODS

Source of Materials

Maize (SC 649), soybean (TGx 1989-19F) and fresh cassava roots (TME 419) for the study were purchased from local farmers at Tse-Igbum, behind Command Secondary School, Makurdi, Benue State, Nigeria. They were first subjected to cooling, cleaning, sorting and packaging, before been processed into flour.

Preparation of Maize Flour

Maize flour was prepared in accordance with a method as reported by Houssou and Ayemor (11). Maize grains were cleaned, soaked in potable water for 1h., with an occasional change of the water. This was done to reduce the anti-nutrients. The grains were then drained, rinsed, sun dried to a constant weight and milled into powder. The powder obtained was sieved through a muslin cloth (250 µm) into a clean plastic bowl and packaged in an airtight plastic container for use.

Preparation of Cassava Flour

Cassava flour was produced according to the method described by Adekunle *et al.* (12). As outline by the method, cassava roots were peeled manually and cut into slices with a sharp stainless knife, washed and soaked in water for 72 h. to ferment. The fermented cassava roots were packed in a sack and dewatered by pressing with hands, and then molded, sundried, milled, sieved and packaged.

Preparation of Soybean Flour

The procedure for production of soybean flour followed the method as described by Edema *et al.* (13). Soybean was sorted to remove pebbles, stones and other materials, and the seeds were wet cleaned and steeped in water for 2 h. It was drained and boiled for 30 min at 100^{o}C, dehulled manually by rubbing in between clean palms and removed by rinsing with clean water. The dehulled soybeans were sun dried and milled into flour.

Determination of proximate composition

The Association of Official Analytical Chemists (14) was used for the determination of moisture, ash, fat, fibre, protein and carbohydrate.

a) Determination of moisture content: Empty dish and lid were oven dried at 105^{o}C for 3 h and then transferred to a desiccator to cool, weighed thereafter. Three grams of the sample in triplicate were weighed into the dish and spread to the uniformity. The dish with the samples were placed in the oven and dried for 8 h at 105^{o}C, and transferred with partially covered lid to the desiccator to cool. The dish and its dried samples were then reweighed to get the moisture content. The moisture content of each of the samples was calculated using equation (1).

$$\text{Moisture Content (\%)} = \frac{Initial\ weight - Final\ weight}{Weight\ of\ sample} \text{ X } 100 \quad (1)$$

b) Determination of ash content: A crucible and lid were placed in a furnace at 105ºC overnight to ensure that impurities on the surface of the crucible were burned off. They were cooled in the desiccator for 30 min, and were weighed to 3 decimal places. Five (5) grams of the sample in triplicate was weighed into the crucible and heated over low Bunsen flame with lid half covered. When fumes were no longer produced, the crucible and lid were placed in the furnace and heated at 105ºC overnight. During heating, the lid was not covered and was placed after complete heating to prevent loss of fluffy ash. They were then allowed to cool in a desiccator and weighed at room temperature when the samples turned gray. The percentage residue weighed was expressed as shown in equation (2).

$$\text{Ash content (\%)} = \frac{Weight\ of\ ash}{Weight\ of\ sample} x\ 100 \quad (2)$$

c) Determination of fat content: The flask and lid were placed in an incubator at 105ºC overnight to ensure that the weight of the flask was stable. Four (4) grams of the sample in triplicate was weighed and wrapped with a filter paper and placed in an extraction thimble and transferred in to Soxhlet. Petroleum ether (250 mL) was filled into the bottle and taken on the heating mantle. The Soxhlet apparatus was connected and water was turned on to cool them. The heating mantle was then switched on. The samples were then heated for 14 h (heat rate of 150 drop/min). The solvent was evaporated by using the vacuum condenser. The flask was incubated at 80 to 90ºC until the solvent was completely evaporated and the flask completely dried. After drying, the flask was transferred with partially covered lid to the desiccator to cool. The flask and its dried content were reweighed and the difference in weight was expressed as percentage fat content as shown in equation (3).

$$\text{Fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} x\ 100 \quad (3)$$

d) Determination of fibre content: Two (2) grams of the sample in triplicate and 200 mL of 1.25 % H_2SO_4 was heated for 30 min and filtered with a Buchner funnel. The residues were washed with distillated water until they were acid free. About 200 mL of 1.25 % of NaOH was used to boil the residues for 30 min, filtered and washed several times with distilled water until they were alkaline free. They were rinsed once with 10 % HCl and twice with ethanol and finally rinsed with petroleum ether three times. The residues were put in a crucible and dried in a muffle furnace at 550ºC for 90 min to obtain the weight of the ash. Fibre content was expressed as percentage loss weight on ignition as shown in equation (4).

$$\text{Fibre content (\%)} = \frac{Oven\ dried\ weight - Weight\ after\ ahsing}{Weight\ of\ sample} \text{ x}100 \quad (4)$$

e) Determination of protein content: About one (1) gram of the sample in triplicates was placed in digested flask and 5 g Kjedahl catalyst and 200 mL concentrated H_2SO_4 were added. The flasks were placed in inclined position and heated gently until fronting ceased. Brisky was boiled until the solution was clear. The solution was allowed to cool and 60 mL of distilled water was added cautiously. The flask was connected immediately to the digestion bulb on the condenser and with tip of the condenser immersed in standard acid and 5 to 7 drops of mix indicator in the receiver. The flask was rotated to mix the content thoroughly and then heated until all NH_3 was distilled. The receiver was removed and the tip of the condenser was washed and the excess acid standard distilled was titrated with standard NaOH solution.

$$\text{Protein (\%)} = \frac{(A-B)xNx14.007x6.25}{w} \quad (5)$$

where; A is the volume (mL) of 0.2 N HCl used in sample titration, B the volume (mL) of 0.2 N HCl used in blank titration, N the Normality of HCl, W the Weight of the sample, 14.007 atomic weight of nitrogen and 6.25 is the protein nitrogen conversion factor for fish and its by-product.

h) Determination of total carbohydrates: The total carbohydrate content was calculated by difference as:

Total Carbohydrate (%) = 100 – [Moisture % + Protein % + Fat% +Fibre%+ Ash %].

Experimental Animals and Design

A total of 15 male Wistar rats within the age of 4-8 weeks old weighing between 115-207 grams were used for feeding trial. The Animals were procured from the Animal House Unit of the Theosalem Farm North Bank, Makurdi-Nigeria. The animals were housed in a constructed wooden cages in an animal house and fed with growers feed with free access to water.

The animals were acclimatized for 7 days, after which their body weight, serum protein, lipid, electrolyte and liver enzymes were determined and served as initial before feeding experiment and after the experiments. The rats were randomly grouped into three groups; labeled MF, MCF and MCSF of five (5) rats each with average body weight of 147.03, 149.79 and 146.72, respectively, in a well-ventilated cages with free access to food and drinking water at an average temperature between 75ºC to 88ºC. Group MF fed on 100 % maize flour; Group MCF fed on 70 % maize flour-30 % cassava flour and Group MCSF fed on 70 % maize flour- 15 % cassava flour-15 % soybean flour. Each of the experimental rats' groups was served daily with weighted 120 g of maize, cassava and soybean composite flour.

Determination of Feed Intake

Feed intake was monitored every day by weighing back leftover feeds in the buckets and the sum was subtracted from the total weight of the feed allocated to each cage. This was done using digital weighing balance (model; 18189c).

Determination of Body Weight

The body weight of each rat was assessed using digital weighing balance (model; 18189c) once before commencement of the experiment and was monitored in an interval of a day to observe the changes at the end of the experiment.

Blood Sample Collection

After feeding trial period of 21 days, the animals were fasted overnight without access to water. Then the blood sample was collected by ocular puncture from the eyes using capillary tube, and placed in plain tubes that allowed clotting. The ocular puncture method was used to avoid the damage of the heart. The blood samples were centrifuged at 3000 *x g* for 25 min to obtain the sera, stored, thereafter, in a deep freezer (-20°C) and then used for determination of protein, lipid, electrolyte profiles and liver enzymes.

Determination of Fasting and Postprandial Glucose

Fasting Blood Glucose (FBG) was measured after overnight fasting of 12 h. using a glucometer (Acute-check, Germany) and glucose test strips. A 2-hour postprandial glucose (PPG) was measured exactly 2 h. after feeding the wistar rats with the formulated meal. Blood glucose curve was drawn from blood glucose response of animals at time 0, after 30, 60, 90 and 120 min intervals after consumption

Determination of serum Lipid Profile

Serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) were determined using Agape TC, TG and HDL reagents following method described by Ani and Besthshel (15). While Low density lipoprotein (LDL) was calculated by the subtraction of sum of total cholesterol minus HDL-c minus TG and The very Low density lipoprotein (VLD) levels were calculated using the formula TG/5 mg/dL

Determination of Serum Electrolytes

The concentration of the electrolytes, namely calcium (Ca^{2}+), chloride (Cl^{-}), potassium (K^{+}), phosphorus(P^{3+}), Magnesium (Mg^{2+}) and Sodium (Na^{+}) were determined using auto analyzer as described by Tietz (16).

Determination of serum protein and liver enzymes

Serum total protein (TP) and albumin (ALB) were determined using Randox TP and ALB reagents following method described by George (17) while serum globulin was determined by subtracting the albumin fraction from the total protein fraction. The measurement was taken in g/dL while Aspartate Amino transferase (AST), Alanine Amino transferase (ALT) and alkaline phosphatase (ALP) activity were determined using the method described by Rej and Hoder (18).

Data Analyses

All samples were carried out in triplicate and the results were expressed as mean value ± standard deviation. Data were statistically analyzed using SPSS version 23 and the means were calculated at 0.05 level of significance

Ethical consideration

With the ethical approval (MOH/STA/204/VOL.1/238) from Ministry of Health and Human Services, Benue State, Nigeria, the animals were treated in conformity with rules and regulations guiding the use of animals.

RESULTS AND DISCUSSION

The proximate composition of the formulated composite flours is as shown in Table 1.The moisture content of the blends ranged from 11.45 % to 11.91 %. From the table it can be seen that, the moisture content decreased with the addition of soybean flour. The values of the moisture content of the samples (11.45 % to11.91 %) are comparably higher than 6.63% reported by Ikujenlola and Adurotoye (19) for a blend of quality protein maize and cowpea, 8.60 to 9.71 % as recorded by Adeola *et al.* (20) for sorghum, pigeon pea, and soybean flour blends, but within 10.05 to13.03% reported for wheat-soybean-carrot based foods (21). Low moisture content of this study is an indication that the composite flour samples may have prolong shelf life, if properly packaged (22). High moisture content is not a desirable quality of flour because it can influence microbial growth and spoilage of flours (23). In general, the moisture content of this study is however, within the acceptable range of < 14 %, as recommended by USRDA (24).

The protein content of the samples ranged from 6.96 to 19.57 %, showing significant increase (p>0.05) in protein content with the addition of soybeans flour. These values are relatively similar to 8.92 to 20.77% reported in the literature for maize-soy flour ((25). The protein values of the present study is lower than 20.78 to 28.09 % reported by Fusuan *et al.*(26), 22.40 to 25.0 % of Gbadamosi *et al.*(21) and 21.73- 22.63 % as documented by Adeola *et al.*(20) but higher than 1.37 to 13.17% reported by Adeoye *et al.*(8)**.** The protein values of the study as seen in the blends of soybeans are within the recommended value of 10-35 % (24). High level of protein, however, demonstrated the beneficial effect of soybeans supplementation.This can promote growth, increase resistance to infections and repair damage tissues (27).

Table 1. Proximate composition of maize-cassava-soybean composite flour

MF:CF:SF	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohydrate (%)
100:0:0	11.91^{a}±0.01	8.66^{e}±0.02	4.16^{e}±0.06	1.47^{a} ± 0.02	1.05^{e}±0.02	73.41^{b}±0.48
70:30:0	11.81^{b}±0.01	6.96^{f}±0.02	1.71^{f}±0.01	1.08^{c} ± 0.01	0.82^{f}±0.01	78.28^{a}±0.02
70:25:5	11.63^{c}±0.01	12.10^{d}±0.02	4.33^{d}±0.04	1.00^{d} ± 0.01	1.13^{d}±0.01	69.79^{c}±0.010
70:20:10	11.57^{d}±0.02	14.13^{c}±0.02	4.53^{c}±0.03	0.97^{b} ± 0.02	2.56^{c}±0.03	66.24^{d}±0.07
70:15:15	11.53^{d}±0.01	16.75^{b}±0.03	4.73^{b}±0.02	0.93^{be} ± 0.01	2.75^{b}±0.02	63.40^{e}±0.04
70:10:20	11.45^{e}±0.02	19.57^{a}±0.02	5.82^{a}±0.02	0.91^{e} ± 0.02	2.81^{a}±0.02	59.43^{f}±0.08
USRDA	<14	10-35	20-35	10-35	-	45-65

Values are means + standard deviation of triplicate (3) replications
Means within a column with the same superscript are not significantly different (p>0.05). MF=maize flour, CF=cassava flour, SF=soybean flour
USRDA= United States Recommended Dietary Allowance, 2015
Source Igbua *et al*. (10).

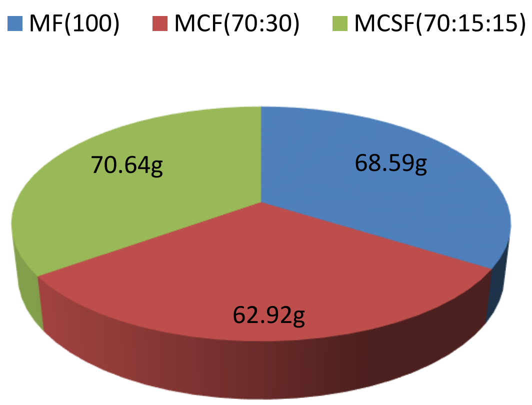
The fat content of the samples ranged from 1.71 to 5.82 %, indicating increase in the level of substitution of soybean flour. Soybean generally is a good source of fat. The fat content of the formulation is higher than 2.57 to 2.94 % reported by Adeola *et al.*(20) for sorghum, pigeon pea, soybean flours, but lower than 4.85 to 7.99 % for maize-soybeans blends as reported by Ikya *et al.*(25).The fat content in this study was found to be lower than 20 to 35 % recommended dietary allowance (RDA). Fats are reported to have health benefit of lowering blood cholesterol level and absorption of water soluble vitamins, however, low fat would be suitable for weight watcher (28). Furthermore, higher fat composition may cause rancidity and encourage contamination of flours.

The crude fibre content of the formulated samples ranged from 0.91 to 1.47 %, which are comparably lower than 1.92 to 2.74 % previously reported for maize-soybeans composite flours by Ikya *et al.*(25) The fibre content decreased with increasing level of soybean substitution. This decreased value could be due to dehulling of the soybean during processing. The result of the fibre was far below the recommended fibre value of 10 to 35 % of USRDA (24). Fibre is needed to assist in digestion and keep gastrointestinal tract healthy and also adds bulk to the stomach and can also keep blood sugar stable and reduces constipation (29). Interestingly, low fibre content of this study may be advantageous, since high dietary fibre may negatively affect nutrients bioavailability, making them insufficient to the body.

The results of the ash contents of the samples shows significant differences (p<0.05) with values ranging from 0.82to 2.81 %. The ash content increased with increasing level of soybean in the blends. The increase in ash content is an indication of high mineral content of soybeans. The ash content in the present study is higher than the 0.99 to 1.39% reported in the literature for the maize-soybeans composite flour (25).

The carbohydrate content of the formulated samples ranged from 59.43 % to 78.28 %, decreasing considerably from 78.28 % maize-cassava flour to 59.45 % in the blends containing 20 % soybeans flour. The decrease in carbohydrate content of the blends with addition of soybean flour is expected; following the trend reported in several studies that substitution of cereals or roots with legumes reduces carbohydrate content. Higher carbohydrate values have earlier been reported in maize-soy flour blends 67.11 % to 84.31 % with a decrease trends in carbohydrate as result of legumes supplementation (25).The lower content of carbohydrate in the composite flours could be due to the soybeans flour that contributed to high proteins and low carbohydrate. The carbohydrate results from sample 70:15:15 (63.40 %) and 70:10:20 (59.45 %) are however, within the acceptable range of 45 to 65 % as recommended by USRDA (24).

Mean feed intake of different experimental groups is shown in Figure 1. The result shows that the wistar rats fed on MCSF (70:15:15) had the highest mean feed intake (70.64 g) while those fed 70:30 % maize-cassava diets had the lowest feed intake of 62.92 g compared significantly to the group of rats fed 100 % maize flour diet (68.59 g). High consumption of feeds by rats from MCSF (70:15:15) could be due to the pleasant flavor, aroma, palatability and adequate nutrients of soybean while low intake of the group containing 30 % of cassava substitution could be attributed to the unpleasant aroma of the fermented cassava as well as an imbalance of nutrients in cassava flour diet. Oibiokpa *et al.* (30) demonstrated that high levels of anti-nutritional factor, low protein and unpalatability affected food intake of rats.



MF=Maize flour, MCF= Maize-Cassava Flour, MCSF= Maize-Cassava-Soybean Flour
Figure 1. Mean feed intake of wistar rats fed maize-cassava soybean flour blends
Source: Igbua *et al.* (31).

The result of mean body weight gain of the experimental rats is shown in Figure 2.The weight gain of this present study is directly or indirectly influenced by the dietary protein, fat and feed intake of the wistar rats. The mean weight gain of the experimental rats fed 100 % maize flour, 70: 30 % maize-cassava flours and 70:15: 15 % maize- cassava-soybean flour blends were 17.03 g, 2.56 g and 37.62 g, respectively. The body weight gain in the present study is comparably higher than 1.31 to 8.09 g reported by Adeyibi *et al.* (32). The body weight gained by rats fed 15 % soy flour substitution was significantly higher than weight gained by rats from other groups, while those fed 30 % cassava substitution had the least weight gain (2.56 g). The weight gain witnessed in this study agreed with the previous report of Adeyibi *et al.* (32) who recorded a positive weight gain from a blend of cereals and legumes.

The lower weight gain observed in rats fed 30 % cassava flour substitution is expected because cassava flour has low protein and fat which makes the diet inadequate to support growth of the animals (33). The lower weight gained may also be due to the fermentation of cassava during processing that resulted to poor feed intake due to unpleasant aroma which in turn affects the weight gain. The lower weight gain observed in this study is desirable for those who are suffering from diabetes, overweight and weight watchers. Protein is required for building and maintenance of muscle which in turn contribute to weight gain (27). However, these attributes of protein and fat were significantly lacking in the cassava-based composite diet.

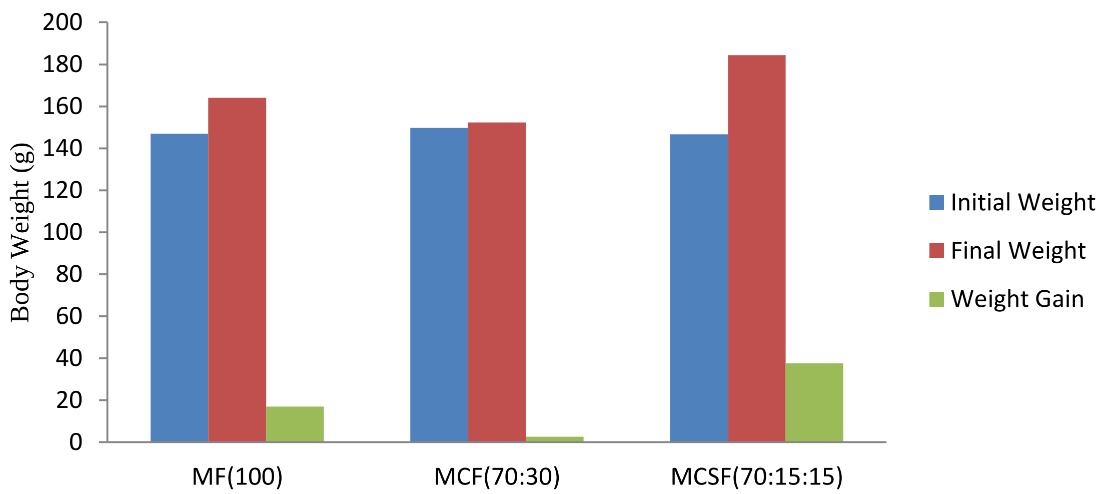
The higher weight gain observed in the rats fed 15 % soybean addition suggests high amount of protein and fat in soybeans that enhanced the growth of the rats (33). It is clearly understood that, the consistent weight gain by rats fed maize-cassava-soybean diet was due to high nutrient content of soybean diet as well as high feed intake by the wistar rats. This weight performance exhibited by rats in the present study is in line with earlier report of Bintu *et al.* (34).

Fasting and postprandial blood glucose response of rats after fed experimental diet is presented in Figure 3 and 4, respectively. Carbohydrates based foods are ingested and absorbed from the gastrointestinal tracts which are subsequently transported through the portal veins to the liver and to the other parts of the body (35). This carbohydrate released as glucose functionally provides cells, tissues and organs with energy and then stored as glycogen in the liver and muscles (35). The improper carbohydrate metabolism result to diabetes mellitus, which is caused by lack of or resistance to insulin leading to hypoglycemia or hyperglycemia. The fasting blood glucose levels of the experimental animals were 61.40 mg/dL (MF), 51.80 mg/dL (MCF) and 39.4 mg/dL (MCSF), respectively. The fasting blood glucose of the present study was lower than 65 to 125 mg/dl reported by Adeoye *et al.* (36). Fasting blood glucose is an indication of overall glucose balances in the blood. The group of rats fed on the sample containing 15 % soybeans supplementation had lower fasting blood glucose (39.40 mg/dL). This lower value could be attributed to the addition of soybean to maize-cassava flour in the blends which has a low glycemic index, high protein as well as calcium and magnesium (23). The low levels (hypoglycemia) indicate liver disease, insulin overdose; severe bacterial infection which resulted to dizziness, sweating and blurred vision, while higher concentration (hyperglycemia) is an indication of risk to diabetes, pancreatitis (36). However, the fasting blood glucose of the study is within the normal range of 50 to 135 mg/dL except those that fed MCSF and may be desirable for people suffering from diabetes mellitus.

The incremental area under glucose verse intake time is an indication of the blood glucose rise potential. After feeding the respective diets, postprandial blood glucose level rose to the peak from 51.80 to 134 mg/dL (MCF) and 39.40 to114 mg/dL (MCSF) compared to the response of the control(MF) from 61.40 to 130 mg/dL) in the first 0 min. of blood glucose determination. Thereafter, the blood glucose level of all the experimental groups decreased slowly to 30 min. and sharply to 60 min. of intake and started increasing slowly up to 120 min.

The decreased postprandial blood glucose levels of the present study were lower than the 200 mg/dL reported by Akinjayeju *et al.* (35). The decrease in the glucose levels is in agreement with Oluwajuyitan and Ijarotimi (37) who reported that plantain flour-based product manages diabetes, reduces the blood glucose level and lower starch digestion rate in the body. The group of rats that fed maize-cassava flour had the highest blood glucose response (134 mg/dL) while those that fed maize-cassava-soybeans had the least (114 mg/dL) blood glucose response. The higher level of postprandial blood glucose in maize-cassava based diet indicates a high absorption and metabolizing rate of blood glucose which may be a risk for diabetes (35). Famakin *et al.* (38) reported lower glycemic index and blood glucose level of rats fed composite flour when compared to synthetic antidiabetic drugs. The lower glucose levels observed in rats fed soybean-based diet is attributed to the fact that protein based diet are not easily digested, which however increase the fullness of the stomach thereby delaying the onset of hunger and sugar absorption (39). This lower postprandial blood glucose level may also be due to the inhibition of glucose, digestion of starch and absorption process of alpha- glucosidase as reported by Inoue *et al.* (40).

It is interestingly to know that, the postprandial blood glucose response of all the experimental groups were within the normal range hence does not have any negative effect for whoever may consume the formulated diet.



MF=Maize flour, MCF= Maize-Cassava Flour, MCSF= Maize-Cassava-Soybean Flour

Figure 2. Initial and Final mean body weight of experimental rats fed maize-cassava-soybean flour blends
Source: Igbua *et al*. (31).

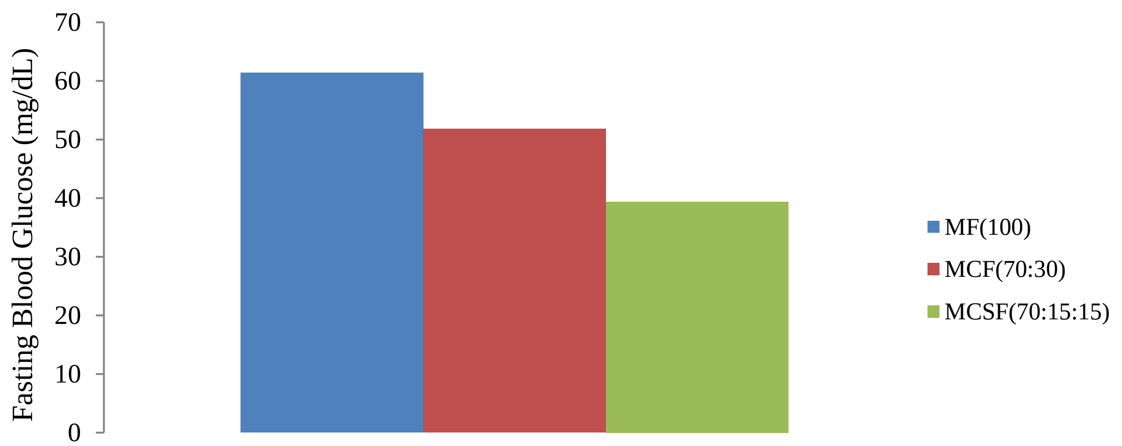


Figure 3. Effect of maize-cassava-soybean flour on the fasting blood glucose level of wistar Rats

Source: Igbua *et al*. (31).

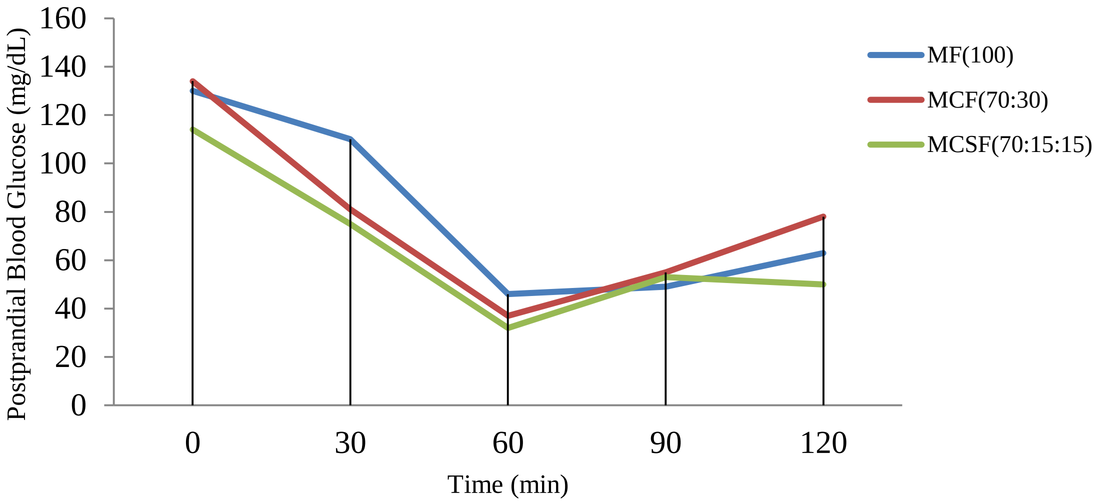


Figure 4. Postprandial blood glucose levels of wistar rats feeding maize-cassava-soybean diet
Source: Igbua *et al*. (31).

The serum protein profile of rats fed maize-cassava-soybean flour blends is shown in **Table 2**. Serum proteins indicate the state of body cells, tissues and organs as well as metabolism of the consumed diet by animals (41). High value of serum proteins shows a better protein quality in the feeds of animals and it level reduces if the animal is severely malnourished (41). In this study, the serum total protein increased from 6.20 to 6.48 g/dL (MCF) and 6.25 to 6.80 g/dL (MCSF) relative to the increased level of 6.14 to 6.61g/dL for those fed the control diet (MF). The group of wistar rats fed the MCF formulation had the least (6.48 g/dL) levels of total protein while those fed MCSF had the highest (6.80 g/dL). The low levels of serum total protein observed in the group (MCF) of the wistar rats might be attributed to high anti-nutritional content of MCF blends or animal starvation. The high levels of serum total protein observed in the group fed MCSF is in agreement with Ardo *et al*. (42). Serum total protein is responsible for the transportation of hormones, lipids, ions, and assists in immune function (43). High levels indicate dehydration, inflammation, chronic infection and certain cancers. Low levels indicate intestinal absorption problems, liver disease, severe burns and losses through the kidneys (44). The serum total proteins in all groups were at the normal range of 6.20 to 7.30 g/dL, indicating that the kidney and the liver were free from any related diseases of low or high total protein.

Serum albumin indicates the amount and type of protein in the blood as well as nutrition and health status and also used as an indicator of protein status (41). It carries various substances through the blood and is important in maintaining blood pressure within the vessels. High levels indicate dehydration while low levels indicate chronic inflammation, liver disease, kidney disease, starvation and blood loss (41). The serum albumin content of the rats as observed in the study increased from 4.25 to 4.41 g/dL (MCF) and 4.28 to 4.53 g/dL (MCSF) when compared to 4.27 to 4.49 g/dL of control (MF) group. The increased level of albumin in the present study is in agreement with Eteng *et al.* and Egbung *et al*. (1).

Similarly, the intake of the flour blends significantly increased the serum globulin levels from 1.95 to 2.09 g/dL (MCF) and 1.97 to 2.27 g/dL (MCSF) when compared to 1.87 to 2.12 g/dL of the control group (MF). The wistar rats fed on all the experimental diets (MF, MCF and MCSF) were at the normal range for total protein, albumin, and globulin, indicating that the body organs such as the kidney and the liver were free from diseases related to low or high protein.

The levels of total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) levels of rats are presented in Table 3**.** The result revealed that the formulated diet (maize-cassava-soybean) has a significant impact on the lipid profile as it increases total cholesterol level and high density Lipoprotein cholesterol (HDL-C). However, it termed to decreased triglyceride, low density lipoprotein (LDL) and Very low density lipoprotein.

Serum total cholesterol level increased from 78.78 to 90.31 mg/dL (MCF) and 77.92 to 86.33 mg/dL (MCSF) when compared to the control of 100% maize flour (83.9 to 92.51 mg/dL). Total cholesterol level exhibits significant ($p<0.05$) increase among the samples. Lower serum total cholesterol was observed in the flour blends of MCSF (86.33 mg/dL) when compared to MCF (90.31 mg/dL) and control group MF (92.51 mg/dL) after the feeding period.

This result was comparably higher than (45.24 mg/dL) reported in rats fed rice-based composite flour cookies (2). The low total cholesterol of Groups MCF, MCSF and control-MF are desirable since they fall within the acceptable range of 37-95 mg/dL. Cholesterol is important in the synthesis of certain hormones, but high levels are undesirable, as it is considered a risk factor of cardiovascular disease; however, low levels indicate liver disease, starvation, kidney disease, pancreatitis, diabetes and hypothyroidism (46). Therefore, being within the acceptable range shows the desirability of the flour blend.

Table 2. Serum protein profile of rats fed maize-cassava-soybean flour blends

Serum	MF(100)	MCF(70:30)	MCSF(70:15:15)	Normal Range (45)
TP(g/dL)	($6.14^c \pm 0.22$) $6.61^b \pm 0.14$	($6.20^b \pm 0.95$) $6.48^c \pm 0.34$	($6.25^a \pm 0.61$) $6.80^a \pm 0.44$	5.2-7.10
ALB(g/dL)	($4.27^a \pm 0.12$) $4.49^b \pm 0.36$	($4.25^b \pm 0.50$) $4.41^c \pm 0.22$	($4.28^a \pm 0.13$) $4.53^a \pm 0.35$	3.40-4.80
GBL(g/dL)	($1.87^b \pm 0.19$) $2.12^b \pm 0.26$	($1.95^a \pm 0.71$) $2.09^b \pm 0.19$	($1.97^a \pm 0.38$) $2.27^a \pm 0.11$	1.50-2.50

Values are expresses as mean ± standard deviation, n = 5. Means within a row with the same superscript are not significantly different (p>0.05) .Values in the parenthesis are initial values before feeding experiments. TP= Total Protein, ALB= Albumen, GBL= Globulin.

Table 3. Impact of maize-cassava-soybean flour blends intake on serum lipid profile of rats

Serum	MF(100)	MCF(70:30)	MCSF(70:15:15)	Normal Range(45)
TC(mg/dL)	($83.91^a \pm 3.98$) $92.51^a \pm 8.24$	($78.78^b \pm 15.41$) $90.31^b \pm 12.53$	($77.92^c \pm 7.02$) $86.33^c \pm 2.55$	37.00-95.00
TG(mg/dL)	($75.06^b \pm 14.74$) $76.17^a \pm 10.13$	($59.35^c \pm 16.29$) $69.31^b \pm 9.95$	($82.15^a \pm 13.00$) $61.12^c \pm 11.50$	32.06-78.24
HDL(mg/dL)	($41.76^a \pm 0.63$) $50.66^c \pm 8.48$	($40.23^b \pm 1.14$) $55.30^a \pm 10.20$	($37.90^c \pm 3.11$) $55.47^a \pm 1.00$	36.78-56.65
LDL(mg/dL)	($26.97^a \pm 2.86$) $26.78^a \pm 13.29$	($26.40^a \pm 15.17$) $21.46^b \pm 6.58$	($23.88^b \pm 2.68$) $19.16^c \pm 2.84$	15.58-35.09
VLDL(mg/dL)	($14.89^b \pm 2.99$) $15.08^a \pm 1.98$	($12.18^c \pm 3.35$) $13.73^b \pm 2.11$	($16.25^a \pm 2.66$) $12.18^c \pm 2.37$	6.40-16.50

Values are expresses as mean ± Standard deviation, n = 5. Means within a row with the same superscript were not significantly different (p<0.05). Values in the parenthesis are initial values before feeding experiments. TC= Total Cholesterol, TG= Triglycerides, LDL= Low Density Lipoprotein, HDL= High Density Lipoprotein, VLDL= Very Low Density Lipoprotein. MF=Maize flour, MCF= Maize-Cassava Flour, MCSF= Maize-Cassava-Soybean Flour

A significant increase ($p<0.05$) in serum triglyceride (TG) level of Group MCF fed with 70% maize: 30% cassava flour (59.35 to 69.69.31mg/dL) was observed, with a decrease in MCSF value (82.15 to 61.12 mg/dL) when compared with control MF (75.06 to 76.17 mg/dL). The level of Group MCSF is favorable and in disagreement with an increased triglyceride value (96.55 to 136.4 mg/dL) of rats fed *Vernonia amygdalina* supplementation on *Vigna subterrenea* (bambara groundnut) pudding as reported by Egbung et *al*.(1). A decrease in triglycerides level of the present study as observed in group MCSF may be linked to consumption of soybeans-based diet. However, the triglyceride values of all the experimental groups were within the normal range of 20-160 mg/dL. Triglycerides are needed for storage of fat and releasing of fatty acids in the body (46). A high level increases the risk for heart disease and metabolic syndrome whereas low levels indicate starvation or malnutrition (46).

In a related development, serum high-density lipoprotein cholesterol level (HDL-c) significantly increased at $p<0.05$ in the groups fed with maize-cassava based flour blends (40.23 to 55.30 mg/dL), maize-cassava-soybean based flour blends (37.90 to 55.47 mg/ dL) with the control group (41.76 to 50.66 mg/dL), when compared with HDL-c level before introducing the trial diet. The HDL-C levels of all the groups are within the normal range of 36.78 to56.65 mg/dL for healthy living. Egbung et *al*. (1) reported an increase in high density lipoprotein levels in rats following consumption of leguminous diet. An elevated level of HDL-c observed in the study is desirable since it can facilitate the removal of cholesterol from the tissue to the liver for breakdown and subsequent excretion. Also it helps carries excess cholesterol back to the liver where it converted into bile acids and excreted in the small intestine (46). In regards to the numerous functions of HDL-c, it is considered as good cholesterol with it high levels decreasing the risk of heart diseases and keep off the LDL-c or "bad" cholesterol from building up in the arteries (47).

On the other hand, Low-density lipoprotein cholesterol level (LDL-c) decreased from 26.40 to 21.46 mg/dL (MCF) and 23.88 to 19.16 mg/dL (MCSF) when compared with control MF (26.97 to 26.78 mg/dL). It was also observed that the rat groups fed maize-cassava flour blends (MCF) had the highest (21.46. mg/dL) LDL-c level while the group of maize-cassava-soybean flour blends (MCSF) had the least (19.16 mg/dL) relative to 26.78 mg/dL of the 100 % maize flour. The decreased LDL-c levels of this study are in agreement of early report of Sada *et al*. (48). The lower content of LDL-c in group MCSF is desirable because, Low-density lipoprotein (LDL) cholesterol is “bad" cholesterol which deposit fat substance on the walls of the arteries. It can also link with white blood cells to form artery-narrowing plaque that restricts the free flow of blood (49). However, the LDL of all the samples is within the accepted range of 15.58-35.09 mg/dL which is said to be desirable and important for diabetic patients or heart diseases as pointed out by Chigbo (50).

The result of very-low-density lipoprotein cholesterol level (VLDL-c), on the hand, was observed to be higher as the control group MF (15.08 mg/dL) as was compared with the group fed maize- cassava flour blend MCF (13.73 mg/dL) and maize-cassava-soybean flour blends (12.18 mg/dL), respectively. A significant decrease was observed in all the groups, with them all within the normal range of 2.00 to 30.00 mg/dL. Like LDL-c, very Low-density lipoprotein (LDL) is also referred as bad cholesterol. It is produced by the liver and released into the bloodstream in order to provide the blood tissues with triglycerides; about half of a VLDL particle is made up of triglycerides. Consequently high levels of VLDL-c narrow the artery walls and restrict blood flow (49).

Results of serum electrolyte levels of rats fed maize-cassava-soybean flour blends are presented in Table 4. The serum calcium level decrease significantly at $p<0.05$ in group MCF (2.57 to 2.33 mmol/L) and MCSF (2.82 to 2.57mmol/L) and when compared with the increased value of the control group MF (2.06 to 2.58 mmol/L). However, group MCSF had higher serum calcium level (2.57 mmol/L) than sample MCF (2.33 mmol/L) after feeding maize-cassava-soybean flour and maize-cassava flour. The higher absorption level of calcium by group MCSF could be attributed to low antinutrients value witnessed in the flour samples incorporated with soybean flours and may be advantageous since calcium is one of extracellular cations in the body that helps in bone formation, muscles contraction, blood clotting and hormonal secretion (51). However, all the groups were within the normal range of 2.43-2.80 mmol/L except group MCF that was below normal range. Serum sodium levels increased significantly ($p<0.05$) in all the groups, ranging from 134.27 to 142.70 mmol/L (MCF) and 136.10 to 142.30 mmol/L (MCSF) relatively to the control group MF (137.21 to 140.65 mmol/L). A normal sodium level is 142-151 mmol/L for normal functioning of body activities. These results show that serum sodium is within the acceptable range in all the study groups, implicitly meaning that the formulations are desirable since they do not affect the sodium uptake by body but rather cause an elevation. Egbung *et al. (1)* reported lower serum sodium levels of 132.67 - 141.33 mmol/L in rats fed diet supplemented with bambaranut as compared to the present study. Sodium is an extracellular fluid that combines with potassium in maintaining normal function of muscle and nerves. It is an important nutrient needed for the control of blood pressure, blood volume, pH and osmotic equilibrium. High level (hypernatremia) indicates dehydration, lack of water, diabetes and excess salt intake, whereas low level indicates starvation, severe diarrhea, vomiting and metabolic acidosis (Hyponatremia) (52).

The serum magnesium level of the rats fed the flour blends ranged from 1.06 to 1.13 mmol/L (MCF) and 1.13 to 1.14 mmol/L (MCSF) comparably to 1.13 to 1.02 mmol/L of sample MF (Control). There was a significant difference ($p<0.05$) between sample MCF and MCSF with an increase respectively. Though there was a decrease in the magnesium level of the control MF. The levels of magnesium in the rats fed the different flour blends were within the normal range of 0.85-1.15mmol/L. Magnesium is an intracellular cation that involves in the ATP metabolism that generate body energy and normal muscles function as well as regulation of blood pressure and control of sugar level. The increased level of magnesium in this study is favorable since low level (Hypomagnesemia) increased renal losses with diuretics (51).

Serum potassium levels of the study decreased significantly $p<0.05$ in group MCF (7.76 to 7.44 mmol/L) and MCSF (6.50 to 6.40 mmol/L) when compared to the control sample MF (8.36 to 6.15 mmol/L). However, higher decrease was observed in sample MCF compared with sample MCSF. The serum potassium level of this study were higher than 3.32-4.82 mmol/L reported in rats fed cereal- based diet (2). Potassium is an intracellular electrolyte responsible for the proper functioning of the nerves and muscles, particularly the heartbeat as well as maintenance of body fluid (52). High levels (hyperkalemia) indicate diabetes, certain toxin ingestions, urinary obstruction, acute kidney failure and severe muscle damage. Low levels (hypokalemia) indicate vomiting and diarrhea, gastrointestinal cancer, insulin overdose, overuse of diuretics and starvation as well as increase irregular heartbeats (52). The potassium value of this study is not desirables since all the experimental groups were out of normal range of between 3.82 - 5.55 mmol/L. However, the potassium levels of these formulate would have reduced to a normal if the animals were placed for a longer time.

Table 4. Effect of maize-cassava-soybean flour blends on serum electrolyte of rats

Serum	MF(100)	MCF(70:30)	MCSF(70:15:15)	Normal Range (45)
Calcium(mmol/L)	(2.06^{c} ±0.54) 2.58^{a} ±0.21	(2.57^{b} ±0.56) 2.33^{b}±0.15	(2.82^{a} ±0.03) 2.57^{a}±0.17	2.43-2.80
Sodium(mmol/L)	(137.21^{a}±1.44) 140.65^{b}±1.96	(134.27^{c}±1.96) 142.70^{a}±0.37	(136.10^{a}±0.98) 142.30^{a}±3.15	142-151
Magnesium(mmol/L)	(1.13^{a} ±0.31) 1.09^{a}±0.12	(1.06^{a}±0.21) 1.13^{a}±0.08	(1.13^{a}±0.15) 1.14^{a}±0.15	0.85-1.15
Potassium(mmol/L)	(8.36^{a}±0.51) 6.15^{b}±0.73	(7.76^{b}±0.56) 7.44^{a}±0.33	(6.50^{c}±0.48) 6.40^{b}±0.19	4.60-6.00
Chloride(mmol/L)	(98.73^{a}±0.81) 102.17^{a}±1.27	(97.37^{b}±1.07) 103.10^{a}±0.67	(96.37^{c}±0.77) 100.83^{b}±1.39	100-106
Phosphorus(mmol/L)	(3.53^{a}±0.22) 2.28^{b}±0.39	(3.41^{a}±0.17) 2.53^{a}±0.32	(2.97^{b}±0.73) 2.34^{b}±0.53	1.62-3.46

Values are expresses as mean ± Standard deviation, n = 5. Means within a row with the same superscript are not significantly different (p>0.05). Values in the parenthesis are initial values before feeding experiment.
MF=Maize flour, MCF= Maize-Cassava Flour, MCSF= Maize-Cassava-Soybean Flour

Chloride functions to maintain the body's fluids as well as balance acid in the blood. High level of chloride in the blood (hyperchloremia) is an indication of dehydration, metabolic acidosis as well as kidney disease. However, Low levels (Hypochloremia) indicate vomiting and metabolic alkalosis (52). The normal range of serum chloride range is 100 - 106 mmol/L. In the study, the control group MF (102.17 mmol/L), maize-cassava flour group MCF (103.10 mmol/L) and maize-cassava-soybeans flour base (100.83) concentration of chloride were within normal range.

The serum chloride contents of this study were comparably lower than 109 mmol/L and 107.33 mmol/L reported in rice-based composite flour cookies and wheat flour cookies (Eteng *et al.*(2).
Furthermore, serum phosphorus levels showed decrease in sample MCF and MCSF as well as the control sample MF. The serum phosphorus ranged from 3.41 to 2.53 mmo/L (MCF) and 2.97 to 2.34 mmol/L (MCSF) comparable to the control group MF (3.53 to 2.28 mmol/L). There was a significant decrease in serum phosphorus of all the experimental groups. Egbung *et al.* (1) reported significant difference in serum phosphorus levels of rats fed with a composite diet supplemented with Bambara nut. The phosphorus levels of this finding were lower than 1.37 to 1.75 mmol/L reported by Egbung *et al.* (1). However, the results fall within the normal range of 1.62 to 3.46 mmol/L.

The physiological status of liver function is determined by the activities of serum enzymes such as Aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase as presentenced in Figure 5, 6 and 7. These enzymes are the indices of measuring functional status of liver .The increase in the levels of theses enzymes indicate a signal for liver damage as well as disease condition of the body (53). In specific the elevated levels of AST indicate cell and tissue damage with specific to the liver and ALT specific to the skeletal muscle (54). On the other hand, an increase in the levels of these enzymes may not be associated with liver organ damage or injury but as a result of active vitamin B6 as co- enzymes in unhealthy rats and low dietary protein can lead to elevated levels of enzymes **(**55). In this study, The serum AST values of the flour blends were 102.06 IU/L (MCF) and 88.45 IU/L (MCSF) relatively to 93.09 IU/L of the control group (MF) whereas the serum ALT levels of the rats fed the flour blends were 33.37 IU/L (MCF) and 31.96 IU/L (MCSF) comparable to the 27.80 IU/L (MF) of the control were within the normal range (56). Significant decreases were witnessed in AST and ALT among group MF, MCF and MCSF from the initial to final values respectively. There was a significant reduction of AST from initial to final values as shown in Figure 5, 6 and 7. The decreased level of AST and ALT of the formulation suggests no liver damage neither leakage of enzymes into the blood stream. The evidence of no liver damage observed in the study shows that the flour blend is safe for consumption as a food. Anaemene 57) reported cereal-based complementary foods fortified with pigeon pea (cajanus cajan) flour have no negative effect on the activity of liver enzymes which is in agreement with the present study. Higher levels of AST and ALT were however observed in the group of rats fed 70% maize to 30 % cassava flours (MCF) which may be due to low protein content as was determined in the flour (10). Charles *et al.* **(**41**)** fed rats with diet supplemented with Pigeon pea (*Cajanus cajan*) seeds he however, opined that the sample with low protein elevated AST and ALT levels of the rats.

Alkaline Phosphatase (ALP) is a biomarker enzyme for testing the ability of plasma membrane, increase in the activities of alkaline phosphatase is an indication that there is damage due to cytotoxic (48).. High concentration of alkaline phosphatase (ALP) is found in bone, kidney, liver and mucosa of the small intestine of animals. However, the leakage or outflow of this enzyme ALP into the blood stream is due to organ injuries (41). Also an increase level of ALP is connected to growth and development of bone and may not necessary cause by liver damage.

The ALP levels of rats fed with the blended flour in the present study was observed to decrease from 690.68 IU/L to 520.65 IU/L (MCF) and 610.73 to 522.35 IU/L (MCSF) relatively to 555.93 to 470.45 IU/L (MF) witnessed in the control sample. The result obtains in this flour blends are within the acceptable range for normal body function with suggestion that the maize-cassava-soybean formulation does not have negative effect to the integrity of tissues and organs of the body.

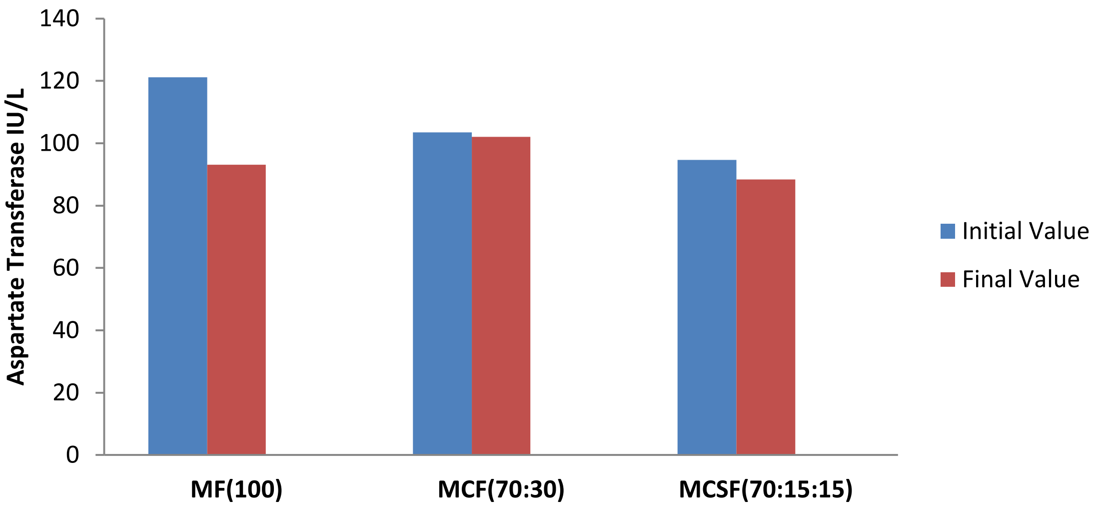


Figure 5. Aspartate aminotransferase activity of rats fed maize-cassava-soybean flour blends.
MF=Maize flour, MCF= Maize-Cassava Flour, MCSF= Maize-Cassava-Soybean Flour.
Initial value: Value before consuming the formulated diet. Final Value: Value after consuming the formulated diets

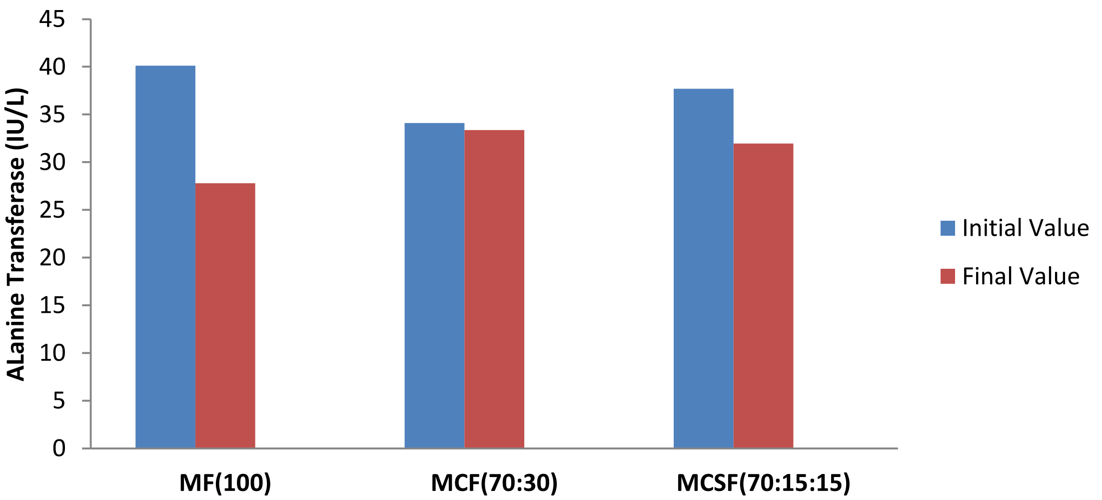


Figure 6. Alanine aminotransferase of rats fed maize-cassava-soybean flour blends
MF=Maize flour, MCF= Maize-Cassava Flour, MCSF= Maize-Cassava-Soybean Flour
Initial value: Value before consuming the formulated diet. Final Value: Value after consuming the formulated diets

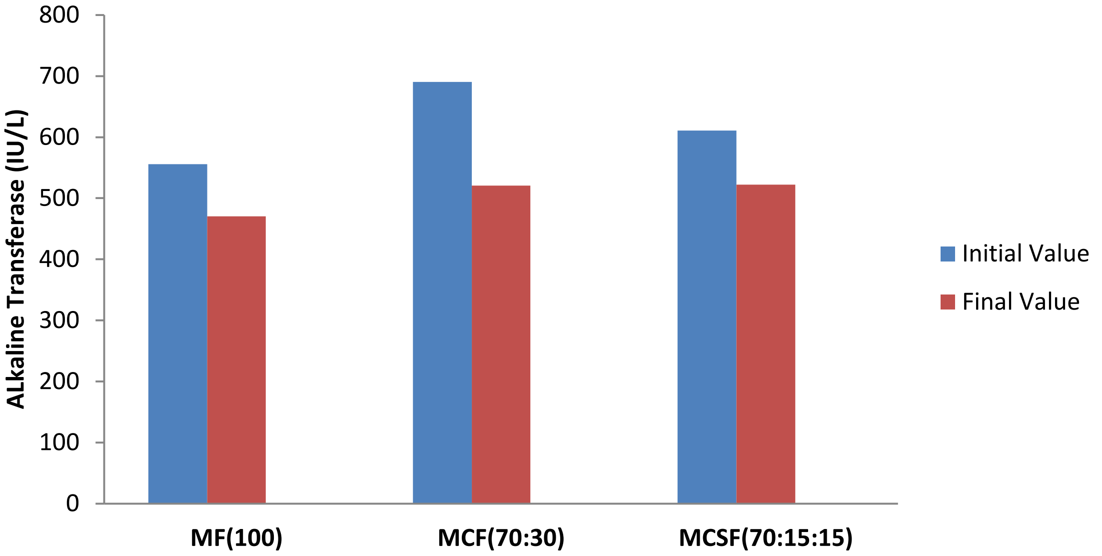


Figure 7. Alkaline Phosphate Transferase activity of rats fed maize-cassava-soybean flour blends
MF=Maize flour, MCF= Maize-Cassava Flour, MCSF= Maize-Cassava-Soybean Flour
Initial value: Value before consuming the formulated diet. Final Value: Value after consuming the formulated diets

Conclusions

The substitution of cassava flour with soybeans flour increased the protein, fat and ash content of maize, cassava and soybean composite blends. The incorporation of soybean flour however, decreased the moisture, fibre and carbohydrate constituents of the blends.

Feed intake by the animals revealed that the wistar rats preferred the 15 % soybean supplemented diet than 30 % cassava diet inclusion. However, all the animals gained weight during the experimental period most, especially group MCSF which suggests that the formulated diet supported the growth of the animals and can lower fasting and postprandial blood glucose level.

The intake of maize-cassava flour supplemented with soybean flour elevated serum total protein, albumin, globulin, cholesterol (TC), high density lipoprotein (HDL), sodium, chloride and magnesium levels in the blood of the tested rats. The intake however, decreased triglycerides, low density lipoprotein (LDL), calcium potassium and phosphorus levels of the blood as well as serum aspartate aminotransferase(AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), indicating that the formulate does not have negative effect to the integrity of tissues and organs of the body.

Therefore, the incorporation of this new source of food to diet does not only enhance its nutritional value, and safe for healthy living but can help fight against metabolic diseases.

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