Serum Biochemistry Profile, Haematological Indices and Body Weight Gain of Albino Rats Fed Cookies Produced from Wheat (*Triticum spp*), Supplemented with Soyabeans (*Glycine Max*) and Pro-Vitamin a Cassava (*Manihot Utilisima Crants*) Flour Blends

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Abstract- In this study, the serum biochemical, haematological indices and body weight gain of albino rats fed cookies produced from, supplemented with soya beans and pro-vitamin A cassava flour blends were determined, A preliminary work was carried out to ascertain the optimal acceptable level of soybeans using 0 to 20% (w/w) addition to wheat flour cookies production. Soybeans yielded the most acceptable cookies at 10% (w/w). Consequently in the main study, the level of soybean was maintained at 10% (w/w) while wheat and pro-vitamin A cassava were varied in the ratios of 100:0:0, 80:10:10, 70:10:20 and 60:10:30 giving a total of four samples. The cookies produced by standard methods were fed to 20 albino rats for thirty (30) weeks and were characterized for serum biochemistry profile, haematological indices and bodyweight gain using standard analytical methods.

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GJMR-L Classification: NLMC Code: QV 180

SERUMBIOCHEMISTRYPROFILEHAEMATOLOGICALINDICESANDBODYWEIGHTGAINOFALBINORATSFEDCOOKIESPRODUCEDFROMWHEATTRITICUMSPPSUPPLEMENTEDWITHSOYABEANSGLYCINEMAXANDPROVITAMINACASSAVAMANIHOTUTILISIMACRANTSFLOURBLENS

Strictly as per the compliance and regulations of:

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I. INTRODUCTION

Cookies are nutritive snacks produced from unpalatable dough that is transformed into appetizing product through the application of heat in an oven (Anozie, 2014). Cookies are traditionally made from soft wheat and are nutritious and convenience foods with long shelf life. Soft wheat flour has been the major ingredient used in the production of cookies and other pastry products, but they can also be made with non-wheat flours such as sorghum, maize, pearl millet, acha grain, Soybean etc. (Okpala, 2013). In Nigeria, reliance on wheat flour in the bakery industries has over the years restricted the use of other cereals and tuber crops available for domestic use (Racheal and Margaret, 2016).

In recent years, government has through intensive collaboration with research institutes encouraged the use of composite flours in the production of cookies and related food products such as bread. The adoption of these locally produced flours in the bakery industry increased the utilization of indigenous crops cultivated in Nigeria and also reduces the cost of bakery products (Ayo and Gaffa, 2002).Wheat grains are relatively low in total protein and vitamins (Vitamin A), generally low in lysine and certain other amino acids, which could be supplemented by the use of soybeans and Pro-vitamin A cassava in cookies production (Agu, 2007). The most obvious result of such blending is that the mixture is higher in protein than the cereal component alone. Soybeans usually improve the quality of cereal protein by supplementing them with limiting amino acids such as lysine and sometimes tryptophan and threonine (Chinma, 2012). Soybean *(Glycine max)* a grain legume, is one of the richest and cheapest sources of plants protein that can be used to improve the diet of millions of people especially the poor and low income earners in developing countries because it produces the greatest amount of protein used as food by man (Lui, 2004). It is also known to be a good source of the trace elements copper, zinc and manganese (Ampofo, 2009). It is the only source that contains all essential amino acids such as Lysine, tryptophan etc. Protein content of soybean is about two times that of other pulses, four times that of wheat, six times that of rice grain (Akubor, 2005). Pro-vitamin A Cassava (*Manihotesculenta Crantz*) is currently been used as an aid in reducing the prevalence of dietary

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Vitamin A deficiency (VAD) due to its high content of B-carotene (Sogo *et al.,* 2016) Haematology refers to the study of the numbers and morphology of the cellular elements of the blood, and this results is used in diagnosing and monitoring of disease (Merck, 2012). Haematological indices generally provide information on inflammation, various infections of the visceral organs and the presence of stress factors (Jurick *et al.,* 2007). Haematological Indices i. e the packed cell volume (PCV) is an index of toxicity factor in the blood and it suggests presence of toxic factors in the food eaten which has adverse effect on blood formation (makinde, 2016). RBC serves as a carrier of haemaglobin, the haemoglobin in turn react with oxygen in the blood during respiration to form oxy-haemaglobin (Johnson and Morris, 1996). White blood cells (WBC) performs a defensive actions in the body. These indices are necessary to be able to conclude that the cookies produced does not have adverse effect on the rat being fed. Serum biochemistry refers to the chemical analysis of the blood serum (Dessouky, 1992). nvestigation of blood serum constituents can provide valuable benefits and indication about the general health of animals and humans (Dessouky, 1992). Observation of a deviation of certain blood parameters from their normal limits could be an indication for diagnosis or differential diagnosis of a diseased condition (Dessouky, 1992). Information on the effect of these selected food products on the serum biochemical profile and haematological indices of albino rats is limited hence the impetus of this study.

II. Materials and Methods

a) Procurement of Materials

Wheat flour and soyabean seeds were purchased from wurukum market in Makurdi, Benue State. Pro-Vitamin a Cassava (TMS 1368) tubers (*Manihotutilisimacrantz*) was purchased from BERNADA in Makurdi, Benue State.
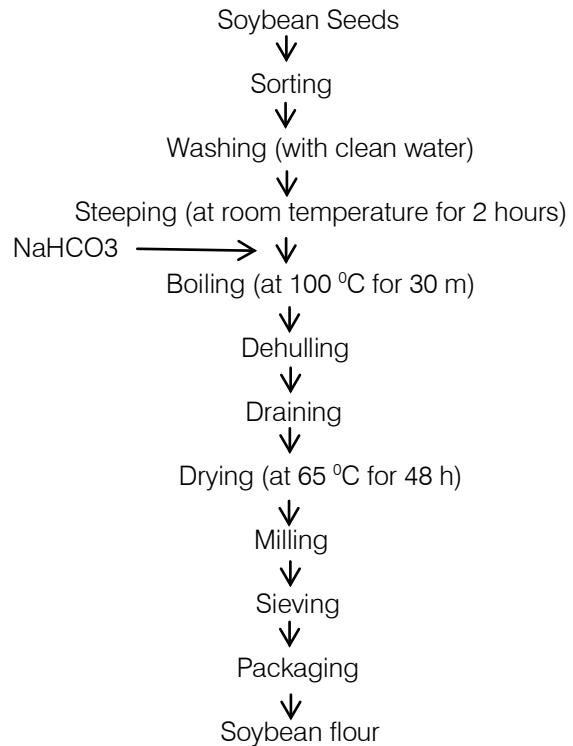
b) Reagent

Chemical used such as Sodium bicarbonate to facilitate removal of beany off-flavor and dehulling of soy bean seeds is of analytical grade.

c) Sample Preparation

Fresh pro-vitamin A cassava roots were peeled with knife, grated and pressed using a laboratory scale hydraulic press (Fisher, USA Hydro DL, 2017) and oven dried at 60°C for 4 h, they were then ground into flour using laboratory grinder (M/S Sujata: New Delhi India) and sieved through a 0.5mm size mesh and were packaged in Low density polyethylene bags prior to analyses (Ubbor *et al.,* 2009). Soy bean flour was prepared as shown in Figure 1. The flour blend formulation is captured in Table 1. The flour blends were conditioned to 25% moisture content, mixed manually with other ingredients for cookies production and baked following the methods of Igbabul *et al.,* 2013). The recipe for the production of cookies from flour blends of wheat, soybeans and pro-vitamin A cassava is captured in Table 2.

Soybean Seeds
↓
Sorting
↓
Washing (with clean water)
↓
Steeping (at room temperature for 2 hours)
NaHCO3 ⟶ ↓
Boiling (at 100 °C for 30 m)
↓
Dehulling
↓
Draining
↓
Drying (at 65 °C for 48 h)
↓
Milling
↓
Sieving
↓
Packaging
↓
Soybean flour

Source: (Modified Ndife et al., 2014). NaHCO3: Sodium metabisulphite

Figure 1: Flow chart for the production of soybeans flour

d) Feeding Trial

A total of twenty (20) mixed breed, albino rats of both sex were obtained from a reputable disease free albino rat farm and used for ten (10) week experiment. The cages, feeders and drinkers were properly cleaned and disinfected using izal, seven days before the arrival of the rats. The rats were reared in four (4) cages made of wood/wire netting measuring 60 cm x 40cm x 40 cm having wire mesh flour, raised 60 cm above the floor. Rats in cage 1, 2, 3 and 4 were fed with cookies A, B, C and D respectively. The rats were allowed to acclimatize to the new environment for seven days, after which 3 rats were randomly selected from each cage and allocated to each treatment group, live weight differences between treatment groups was minimized. Individual rats were given the experimental diet (cookies) and fresh water. Standard rat husbandry practices including medication, recommended sanitary measures and other health practices were strictly observed throughout the experimental period.

III. Analytical Methods

Haematological indices and serum biochemistry profile was done using the method as

described by Adekojo *et al*. (2014). While weight gain was done following the method as described by Mmoreole *et al.* (2011)

a) Statistical Analysis

Data Obtained was subjected to Analysis of Variance (ANOVA) followed by Duncan Multiple Range test to compare treatment means; differences was considered significant at 95% (P≤0.05) (SPSS V21 software).

IV. RESULT AND DISCUSSION

Haematological parameters generally provide information on inflammation, necrosis, various infections of visceral organs and the presence of stress factor (Jurcik *et al.,* 2007). Serum biochemical investigations have been explored extensively to distinguish normal state from from stress and disease conditions in animals (Awosanya *et al.,* 2000). The haematological indices and serum biochemical indices of rat fed cookies produced from wheat, soybeans and pro-vitamin A cassava composite flour is presented in Table 3, while Table 4 presented the weight gained by Albino Rat fed with cookies produced from wheat, soybeans and pro-vitamin A cassava composite flour. Total protein ranged from 5.22 to 6.55 g/dL with sample Dhaving the highest value while the control had the least. The high serum total protein is an indication of nutritional adequacy since high serum They were no significant difference (p≤0.05) in albumin and White Blood Cell (WBC) content with increased addition of soybeans flour in rat fed cookies. WBC performs a defensive action in the body, its values were within the normal range reported by Hewitt *et al.,* (1986), therefore, the feeding of rats with the experimental diet did not affect the ability of the rats to fight infections and distribute antibodies for immune response. Glucose, ASTU/L, ALTU/L, PCV and RBC increased while cholesterol decreased with increasing quantity of soybeans flour in rats fed cookies, PCV is an index of toxicity factor in the blood and it suggest a presence of a toxic factor which has adverse effect on blood formation (Makinde, 2007), the PCV values in this study ranged from 32.0 to 41.0 % which was within the reference range (33-50%) reported by Medi-rat (2007) for healthy rats. This is an indication that the cookies fed were not toxic to the health status of the rats. Serum glucose values range of 69.50 to 74.33 mg/dL observed in this study was in line with standard reference range of 75 to 150 mg/dL recommended by RAR (2011), which implies that the experimental diets met the requirements for growth and development. Normal cholesterol level (10-80 mg/dL) reported by Karen (2002) was in line with the cholesterol values (85.10-85.50 mg/dL). Possibilities of anorexia, diabetes, liver dysfunction and mal-absorbtion of fat which are symptoms of abnormal glucose and cholesterol levels in blood (Bush, 1991) were ruled out. These results were similar to the study by Ogu *et al. (*2006) for quality evaluation of *Pannicum maximum* and *Leucaenaleucocephala* leaves in rabbit feeding.

Table 1: Flour blends formulation

	SAMPLES			
	A (Control)	B	C	D
Wheat flour	100	80	70	60
Pro-vitamin A cassava flour	0	10	20	30
Soybeans flour	0	10	10	10

Table 2: Recipe for preparation of cookies from wheat, soybeans and pro-vit a cassava flour blends

Ingredient	Quantity(g)
Flour	100
Salt	1.0
Sugar	20
Baking fat	7.5
Skimmed milk	80
Water	76-80 (mL)

Source: Igbabul et al., 2013

Table 3: Hematological indices and Serum Biochemical Indices of Rat Fed with Cookies Produced from Wheat, soybeans and Cassava Composite Flour

	SAMPLES			
	A (Control)	B	C	D
Wheat flour	100	80	70	60
Pro-vitamin A cassava flour	0	10	20	30

Soybeans flour	0	10	10	10
Total protein (g/dL)	5.20[b]±0.1	6.55[a]±0.0	6.56[a]±1.1	6.55[a]±2.3
Albumin (g/dL)	4.15[a]±1.0	4.16[a]±1.2	4.13[a]±2.3	4.14[a]±1.3
Glucose (mg/dL)	69.50[c]±1.	73.79[b]±1.1	73.87[b]±1.6	74.33[a]±1.4
Cholesterol (mg/dL)	85.10[c]±1.1	88.89[b]±3.1	86.82[a]±2.1	85.80[a]±2.2
ASTU/L	28.33[c]±1.1	28.65[c]±3.1	32.48[b]±2.4	38.19[a]±2.6
ALTU/L	21.83[b]±1.1	24.53[a]±3.1	25.48[a]±2.1	25.80[a]±2.2
PCV (%)	32.0[d]±1.1	35.25[c]±3.1	37.00[b]±2.1	41.0[a]±2.2
RBC (10[12/L])	6.70[c]±1.1	7.89[b]±3.1	6.82[a]±2.1	6.88[a]±2.2
WBC (10[9/L]	4.45[a]±1.1	4.49[a]±3.1	4.32[a]±2.1	4.30[a]±2.2

Values are Mean± Standard deviation of triplicate determination. Mean values with common superscript letter(s) along each row are not significantly (P>0.05) different

Key: PCV= Packed Cell Volume
RBC= Red Blood Cell
WBC= White Blood Cell

Table 4: Weight Gained by Albino Rat Fed with Cookies Produced from Wheat, Soybeans and Pro-Vitamin A Cassava Composite Flour

	SAMPLES			
	A (Control)	B	C	D
Wheat flour	100	80	70	60
Parameter Pro-vitamin A … cassava flour	0	10	20	30
Soybeans flour	0	10	10	10

Initial Weight (g)	120.34[a]±0.2	121.40[a]±0.1	120.20[a]±0.3	123.32[a]±0.2
Final Weight (g)	370.21[b]±0.1	385.45[a]±0.1	388.22[a]±0.1	391.33[a]±0.1
Total Weight Gain (g)	249.89[b]±0.1	264.05[a]±0.1	268.02[a]±0.1	268.01[a]±0.1
Feed Conversion Ratio	3.20[a]±0.1	3.27[a]±0.1	3.26[a]±0.1	3.30[a]±0.1

Values are Mean± Standard deviation of triplicate determination. Mean values with common superscript letter(s) along each row are not significantly (P>0.05) different

V. CONCLUSION

In conclusion, response of the rabbits to the test diet (Cookies) revealed that weight was affected positively. The high conversion ratios are indications that adequate nutrients available for growth and maintenance. The value of haematology and serum biochemistry were comparable with normal reference values, an indication of nutritional adequacy and safety of the experimental diet (cookies)

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