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Review Article

Evaluation of Blood Parameters of Rats fed Germinated and Fermented All- Legume Mixed Protein Diets

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Abstract

Young male rats (74g to 127g) were used to evaluate total protein, albumin, globulin, cholesterol, HDL, LDL, serum glucose, triglycerides, PCV, WBC, RBC and haemoglobin. Six groups of six rats were each fed a diet from among the six diets formulated from three legumes: *Mucuna vulgaris* germinated (62h), fermented (62h) and dried at 65^{0}C (MGF 65), *Mucuna vulgaris* dried at 65^{0}C (MVD 65), *Canavalia ensiformis* (jackbean) germinated (87h), fermented (87h) and dried at 57.5^{0}C (JGF 57.5%), *Canavalia ensiformis* (jackbean) dried at 65^{0}C (JBD 65), *Phaseolus vulgaris* (red kidney bean) germinated (48h), fermented (27h) and dried at 57.5^{0}C (RGF 57.5) and *Phaseolus vulgaris* (red kidney bean) dried at 65^{0}C (RKD 65). After a nine day nitrogen balance study, rats were sacrificed and analyzed. Result indicated that serum albumin decreased in rats fed diets RGF 57.5, JGF 57.5 and JBD 65. Total protein, serum glucose, globulin and triglycerides decreased significantly ($p<0.05$) in rats fed all the diets. Cholesterol decreased (3.45 mg/dL, 3.64mg/dL and 3.54mg/dL) in rats fed diets RGF 57.5, RKD 65 and JGF 57.5. HDL increased in rats fed diets RKD 65 and MVD 65. LDL decreased in rats fed diets RGF 57.5 and RKD 65 but increased using diets JGF 57.5, JBD 65, MGF 65 and MVD 65. PCV increased (39.25%, 40.27% and 40.25%) in rats fed diets RGF 57.5, RKD 65 and JBD 65. WBC increased in rats that consumed all the diets. The consumption of the diets appear to have reduced cholesterol, triglycerides, LDL and serum glucose in rats.

Keywords: Blood; Rats; Germination; Fermentation; Legume

Introduction

Most developing countries depend on soy bean (*Arachis hypogea*), cow pea (*Vigna sinensis*), common beans (*Phaseolus vulgaris*) among others as a key protein source for food and feed [1].

However, indigenous food crops and edible seeds and other plant foods such as legumes which are widely grown but neglected, underutilized and rarely consumed by urban dwellers are highly rich in protein, fat, minerals and vitamins than most exotic foods [2,3] are available at certain critical periods of the year [4-6]. To meet the growing demand of these plant based protein, the trend has been towards identification and evaluation of the underutilized and lesser known wild legumes for the nutritional advantages [7,8] and as a viable economical alternative protein source [9,10].

Among the various underutilized legumes is *Mucuna vulgaris* which is widely used in Nigeria and other parts of the tropics as food legumes [11]. *Mucuna vulgaris* contains 26.40% of crude protein [8], ash 2.9 – 5.5%, abundant carbohydrate, high fibre and low fat with high concentration of polyunsaturated fatty acids [12]. The carbohydrate and protein values of red kidney beans is high with low fat content. It is a good source of vitamins A, B1, B2, C and minerals such as iron, calcium and phosphorus [13]. These legumes besides being good protein sources, have effectively played key roles in trado-medicine and medicine for different treatment of diseases. Generally, dry beans are widely known for their fibre, minerals and protein contents, however its nutriceutical value is yet to be known in the prevention of chronic diseases [14]. Kidney beans are low in cholesterol lowering fibre. Consequent upon that, the high fibre content also prevents blood sugar levels from rising too rapidly after a meal. This makes red kidney beans a good choice for diabetic individuals, insulin resistance or hypoglycemia [15].

In as much as these underutilized legumes are nutritionally rich, there is need to investigate with rats the efficacy of the formulated diets as they affect the biochemistry of the body system of the test rats.

Materials and Methods

Collection and preparation of the seeds

Canavalia ensiformis (jackbean) and *Phaseolus vulgaris* (red kidney bean) were obtained form a farmer at Uburu, Oru East LGA, Imo State while *Mucuna vulgaris* was purchased at Edem market in Nsukka, Enugu State. Corn starch and vitamin/mineral premix

were purchased at Gufon Veterinary Centre, Police Shopping complex area (Fire Service roundabout), Owerri, Imo State. Soyabean oil (Sonula brand) was purchased at Ekeukwu Market, Owerri, Imo State. Young male albino rats (36) were obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

The three different legume seeds after cleaning and sorting to remove unwanted materials were soaked in water for 6h (jackbean), 12h (red kidney bean) at 25^{0}C. *Mucuna vulgaris* was soaked in water (24h) at 25^{0}C and later stratified in hot water (2-5min) [16] to facilitate germination.

Germination of legumes

The seeds after soaking were put in three (3) different wetted jute bags and allowed to germinate at different time intervals of 12 – 104.0h (Tables 1 and 2). The legume seeds were washed every 12h to avoid the growing of moulds [17]. At the end of germination, the seeds were manually dehulled by severally rubbing the seeds with the palms to remove the hulls.

	Codes				
Variables	-2	-1	0	+1	+2
X_1 (h)	12	24	36	48	60
X_2 (h)	12	27	42	57	72
X_3 (^{0}C)	50	57.5	65	72.5	80
GT (X_1) = Germination Time (GT)					
FT (X_2) = Fermentation Time (FT)					
DT (X_3) = Drying Temperature (DT)					

Table 1: The coded values of the independent variables for *Phaseolus vulgaris* (Red Kidney bean).

	Codes				
Variables	-1.682	-1	0	+1	+1.682
X_1 (h)	19.9552	37	62	87.10	104.045
X_2(h)	19.9552	37	62	87.10	104.045
X_3(^{0}C)	52.3866	57.5	65	72.5	77.6134
GT (X_1) = Germination Time (GT)					
FT (X_2) = Fermentation Time (FT)					
DT (X_3) = Drying Temperature (DT)					

Table 2: The coded values of the independent variables for *Canavalia ensiformis* (Jackbean) and *Mucuna vulgaris*.

Fermentation of the germinated legume seeds

Labeled plastic containers with covers were used for the fermentation. The germinated seeds were put inside deionised water in the containers at the ratio of 1:3 (w/v) grains of the seeds to water. The germinated seeds were allowed to ferment naturally at the time intervals of 12 – 104.0h (Tables 1 and 2). The seeds were dried respectively in a hot oven at 50^{0}C to 80^{0}C for 50min and then ground into a fine powder in a laboratory hammer mill (60mm mesh screen).

Experimental design

Germination, fermentation and drying were used as variables using central composite rotatable design (CCRD) to investigate the response pattern in which twenty (20) combinations were obtained and utilized as experimental runs. The procedure used was as described by Snedecor and Cochran [18]. The three independent variables used were germination time (DT), fermentation time (FT) of 12h to 104.0h respectively and drying temperature (DT) of 50^{0}C to 80^{0}C. Each variable was at 5 levels coded, -1.682, -1,0, +1, +2. The experimental runs were carried out using the legumes viz: *Mucuna vulgaris* germinated (62h), fermented (62h) and dried at 65^{0}C (MGF 65), *Mucuna vulgaris* dried at 65^{0}C (MVD 65), *Canavalia ensiformis* (jackbean) germinated (87h), fermented (87h) and dried at 57.5^{0}C (JGF 57.5), *Canavalia ensiformis* (jackbean) dried at 65^{0}C (JBD 65), *Phaseolus vulgaris* (red kidney bean) germinated (48h), fermented (27h) and dried at 57.5^{0}C (RGF 57.5) and *Phaseolus vulgaris* (red kidney bean) dried at 65^{0}C (RKD 65) (Tables 3, 4 and 5).

The protein, fat, fibre, ash, moisture and carbohydrate contents of *Phaseolus vulgaris* flour dried at 65^{o}C (RKD 65) and used for the study prior to processing treatment were 21.85%, 2.78%, 4.26%, 3.20%, 8.77% and 59.10% respectively.

The protein, fat, fibre, ash, moisture and carbohydrate contents of *Canavala ensiforms* flour dried at 65^{o}C (JBD 65) and used for the study prior to processing treatment were 21.24%, 4.19%, 5.74%, 4.0%, 6.15% and 58.62% respectively.

The protein, fat, fibre, ash, moisture and carbohydrate contents of *Macuna vulgaris* flour dried at 65^{o}C (MVD 65) and used for the study prior to processing treatment were 22.75%, 1.87%, 4.06%, 3.91%, 11.47% and 55.94% respectively.

Formulation and composition of the diets

Six group of diets were formulated using the respective codes of the runs: RKD 65, RGF 57.5, JBD 65, JGF 57.5, MVD 65 and MGF 65 as the names of the diets with the highest protein values of 21.85%, 21.70%, 21.24%, 23.16%, 22.75% and 23.75% respectively (Tables 3,4, and 5). These values were used to calculate the respective percentage proteins at 10% dietary levels. Vitamin/mineral premix, 5% oil and corn starch respectively were added and thoroughly mixed together in a dough mixer (Hoberl 2000, England) for 30min [19] to obtain a fine consistency. The corn starch was added to dilute the protein (Table 6).

Runs	GT(h)	FT(h)	DT(°C)	Protein(%)	Fat(%)	Fibre(%)	Ash(%)	Moisture(%)	Carbohydrate(%)
1	24	27	57.5	20.65	2.75	4.38	3.17	9.24	59.81
2	48	27	57.5	21.70	3.16	4.20	3.14	8.40	61.37
3	24	57	57.5	19.45	2.90	4.21	3.20	9.96	60.28
4	48	57	57.5	20.16	3.02	4.09	3.06	9.24	60.43
5	24	27	72.5	19.70	3.08	4.15	3.17	9.46	60.44
6	48	27	72.5	20.44	2.84	4.24	3.14	8.16	61.18
7	24	57	72.5	19.78	3.05	4.21	3.20	9.40	60.36
8	48	57	72.5	21.31	3.08	3.92	3.18	8.28	60.23
9	12	42	65	21.44	2.76	4.23	2.97	11.21	57.39
10	60	42	65	21.29	2.82	4.17	3.23	10.87	57.62
11	36	12	65	21.03	2.60	4.09	3.13	10.17	58.98
12	36	72	65	21.56	2.86	3.91	3.17	9.80	58.70
13	36	42	50	21.27	2.70	4.11	2.97	11.23	57.72
14	36	42	80	20.44	2.82	4.17	2.92	10.30	59.35
15	36	42	65	21.59	2.91	4.96	3.07	10.80	56.67
16	36	42	65	20.56	2.92	4.13	3.11	9.29	59.99
17	36	42	65	21.01	2.84	4.19	3.09	10.72	58.15
18	36	42	65	21.03	2.96	4.17	3.03	10.23	58.58
19	36	42	65	21.03	2.98	4.17	3.03	10.20	58.59
20	36	42	65	21.00	2.89	4.15	3.02	10.22	58.58

Table 3: The results of response surface analysis of the variation of proximate composition of *Phaseolus vulgaris* flour with germination time, fermentation time and drying temperature

Runs	GT (h)	FT (h)	DT (°C)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Moisture (%)	Carbohydrate (%)
1	37	37	57.5	12.473	3.126	4.744	3.382	6.93	69.345
2	87	37	57.5	23.120	3.606	5.725	3.489	6.51	57.35
3	37	87	57.5	12.488	3.445	5.376	3.405	5.585	69.701
4	87	87	57.5	23.160	2.772	5.907	4.607	6.174	57.184
5	37	37	72.5	12.504	3.279	4.816	3.311	6.512	69.578
6	87	37	72.5	23.13	3.492	5.915	3.684	5.23	58.349
7	37	87	72.5	12.484	3.39	5.37	3.476	5.594	69.686
8	87	87	72.5	23.051	2.446	5.934	4.629	5.49	58.164
9	19.95518	62	65	16.742	3.109	5.322	3.482	6.19	75.155
10	104.0448	62	65	23.087	2.351	6.499	4.714	6.136	58.213
11	62	19.95518	65	20.908	3.125	5.335	3.372	6.905	60.355
12	62	104.0448	65	20.905	2.464	5.958	4.613	5.855	60.205
13	62	62	52.38655	20.876	4.094	5.235	3.294	5.953	60.548
14	62	62	77.61345	20.909	3.721	5.103	3.681	5.051	61.535
15	62	62	65	20.912	4.119	5.125	3.582	6.025	60.237
16	62	62	65	20.909	4.124	5.033	3.618	6.016	60.3
17	62	62	65	20.894	4.111	5.102	3.507	6.174	60.212
18	62	62	65	20.901	4.13	5.111	3.592	6.043	60.223
19	62	62	65	20.897	4.106	5.109	3.601	6.076	60.211
20	62	62	65	20.910	4.127	5.133	3.424	6.064	60.342

Table 4: The results of response surface analysis of the variation of proximate composition of *Canavalia ensiformis* flour with germination time, fermentation time, and drying temperature.

Runs	GT (h)	FT (h)	DT (°C)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Moisture (%)	Carbohydrate (%)
1	37	37	57.5	18.856	1.870	3.309	2.785	10.029	63.152
2	87	37	57.5	20.676	1.455	3.278	3.886	9.739	60.965
3	37	87	57.5	21.398	1.450	3.603	3.816	10.434	59.298
4	87	87	57.5	21.687	1.306	4.290	3.781	9.976	58.960
5	37	37	72.5	19.358	1.896	4.642	2.405	12.984	58.715
6	87	37	72.5	21.396	1.693	3.036	4.050	12.253	57.572
7	37	87	72.5	21.593	1.621	5.659	4.070	13.534	53.523
8	87	87	72.5	21.865	1.622	4.793	4.023	12.826	54.872
9	19.95518	62	65	20.112	1.752	4.209	2.918	11.583	59.426
10	104.0448	62	65	22.209	1.433	4.101	4.282	10.878	57.098
11	62	19.95518	65	20.149	1.749	4.167	2.896	10.472	60.567
12	62	104.0448	65	22.987	1.418	5.642	4.361	12.098	53.494
13	62	62	52.38655	20.777	1.414	2.045	3.352	8.756	63.656
14	62	62	77.61345	20.989	1.790	4.040	3.865	13.508	55.809
15	62	62	65	21.009	1.551	3.209	3.693	10.488	60.050
16	62	62	65	21.061	1.582	3.538	3.688	10.113	60.017
17	62	62	65	21.030	1.486	3.716	3.711	10.512	59.545
18	62	62	65	23.750	1.590	3.663	3.662	10.475	59.561
19	62	62	65	21.026	1.468	3.366	3.611	10.216	60.313
20	62	62	65	21.201	1.495	3.605	3.444	10.518	59.738

Table 5: The results of response surface analysis of the variation of proximate composition of *Mucuna vulgaris* flour with germination time, fermentation time and drying temperature.

Ingredient (g/kg)	RKD 65	RGF 57.5	JBD 65	JGF 57.5	MVD 65	MGF 65
Protein (10%)	659.04	663.50	677.97	621.76	632.97	606.32
	72.00	72.00	72.00	72.00	72.00	72.00
Vitamin/mineral premix	5.76	5.76	5.76	5.76	5.76	5.76
Corn starch	703.20	698.65	684.27	740.40	729.27	755.92
Total	1440	1440	1440	1440	1440	1440

Table 6: Composition of the formulated Diets.

Feeding of the rats/animal studies

Thirty-six (36) male rats were divided into 6 groups of 6 rats per metabolic cage which separates the faeces from the urine. Each of the experimental group of rats was fed a particular diet daily at a time with water ad-libitum. Earlier in the course of acclimatization, the rats were fed the normal chow with water ad-libitum for 3 days.

Collection of blood samples

Prior to the commencement of feeding, blood (5 – 7ml) was drawn and pooled into labeled bottles for baseline blood analysis by piercing the edge of the eyes of the rats with pointed sterilized capillary tubes and the blood preserved in 1 drop of ethylene diamine tetracetate (EDTA) solution. After the 9 – day nitrogen balance study, another set of blood samples (5 – 7ml) was obtained in the same way and also preserved.

Haematological analysis of blood parameters

Total protein

Total protein was determined according to the standard procedures described by AOAC [20].

Albumin

The concentration of albumin, globulin and serum glucose were determined using the commercial kits (Linear chemicals, Barcelonia, Spain).

Triglycerides (Tg)

The Tg reagent was reconstituted according to the instructions of the manual. The tubes were labeled blank, standard, control unknown. One milliliter (1ml) of reagent was pipetted into all the tubes. The tubes were placed in a 37^0C heating block for at least 4min. About 0.1ml of sample was added to the respective tubes and

mixed. All the tubes were incubated for 5min at 37^{0}C. The spectrophotometer was zeroed at 520nm with blank (wavelength range: 500 – 550nm). The absorbance of all the tubes was read and recorded.

Calculations

$$\text{Triglyceride value} = \frac{\text{Absorbance (unknown) x Conc. of Standard of unknown}}{\text{Absorbance (Standard)mg/dL}} \quad (1)$$

High Density Lipoprotein (HDL)

This was determined by the direct immunoinhibition method of Dialab Kit.

Low Density Lipoprotein (LDL)

A formulae by Friedewald., et al. (1972) was used in calculating low density lipoprotein (LDL).

$$\text{LDL (mg/dL)} = \frac{\text{Total cholesterol} - \text{Triglyceride} - \text{HDL}}{(\text{mg/dL}) \quad 5} \quad (2)$$

Cholesterol

This was determined accordingly: The test tubes were labeled blank, standard, control test rat. One milliliter (1.0ml) of reagent was pipetted into all the tubes and pre-warmed at 37^{0}C for at least 2min. About 0.01ml of sample was added to the respective tubes, mixed and returned to 37^{0}C. All the tubes were incubated at 37^{0}C for 10min. The spectrophotometer was zeroed at 520nm. The absorbance of all the tubes was read and recorded.

Haemoglobin (Hb)

Hb was measured by the method described by Cartwright and Lee [21]. Blood samples (0.02ml) from the test rat and 4ml of Drabkin's reagent were mixed. Absorbance readings were taken after 10min at 540nm to measure the Hb content.

Red blood cell (RBC)

The standard method described by Schalm., *et al.* [22] was used to measure red blood cells and white blood cells. RBC was measured by adding anticoagulant (sequesterine) and 4ml of formal citrate to about 0.02ml blood samples and then mixed thoroughly. Diluted blood was mounted in a counting chamber and the RBC counted.

White blood cell (WBC)

WBC was measured by adding sequesterine, 0.38ml diluting fluid (1.5ml glacial acetic acid, 0.5ml malachite green) and 98.0ml of water (H_2O) to 0.02ml blood samples until they were mixed thoroughly. Diluted blood was mounted in counting chamber and the white blood cells counted.

Packed cell volume (PCV)

The microhaematocrit method described by Hereberg., et al. [23] was used to measure PCV. Two capillary tubes were added blood samples (filled between 5.5 and 6cm of the total length). Dry ends of the tubes were sealed and centrifuged for 5min at 12,000 x g. Microhaematocrit reader was used to measure volume occupied by the red cells and expressed as a percentage of the total blood in the tubes.

$$\text{PCV} = \frac{\text{Volume occupied by red cell}}{\text{Total volume of blood in the tube}} \quad (3)$$

Statistical analysis

The data generated from the haematological analysis were subjected to one-way analysis of variance (ANOVA) at 5% significance and the separation of means of the parameters was done using the Duncan's multiple range tests [24].

Results and Discussion

The values of the haematological parameters obtained for rats fed all-legume protein mixed diets is presented in Table 7. The result showed that serum total protein (T. protein) values are evidence of the quantity of protein reserved in the test rats in this study. It can be stated that the significant ($p<0.05$) decrease in serum total protein of rats fed all the different group diets was an indication of the decreased dietary protein available to the rats [25]. Serum albumin in rats fed the group diets RGF 57.5, JGF 57.5 and JBD 65 decreased from 3.90g/dL to 3.57g/dL, 4.52g/dL to 4.00g/dL and 4.06g/dL to 4.00g/dL while those fed group diets RKD 65, MGF 65 and MVD 65 increased from 3.70g/dL, 3.92g/dL and 4.00g/dL to 3.80g/dL, 4.00g/dL and 4.04g/dL respectively. The normal levels of albumin in human serum ranged from 4.4g/dL to 5.3g/dL [26]. Therefore, all the group diets which produced albumin level below normal value were not nutritionally adequate to maintain normal circulating level of albumin. Low albumin suggests poor clotting ability of the blood and hence poor prevention of haemorrhage [27].

Triglycerides and serum globulins decreased significantly ($p<0.05$) in rats fed all the group diets. Albumin and globulin can be explained in terms of quality and quantity of protein supplemented in the diets [28,29]. High levels of triglycerides and total cholesterol often accompany diabetic condition [30]. The cholesterol levels in rats fed group diets RGF 57.5, RKD 65 and JGF 57.5 decreased in concentration as indicated in the values 3.45g/dL, 3.64g/dL and 3.84g/dL (Table 2). The levels of High density lipoproteins (HDL) in rats fed group diets RGF 57.5, JGF 57.5, JBD 65 and MGF 65 decreased (0.97mg/dL, 1.12mg/dL, 1.07mg/dL and 0.60mg/dL) but increased in diets RKD 65 and MGD 65. Increase in the levels of HDL is a positive occurrence because it plays a defensive role of picking/mopping up fragments of cholesterol and turning them to the liver in a reverse cholesterol transport for excretion as bile salts or used as precursors for vitamin D and many sterol hormones [31].

The concentration of Low Density Lipoproteins (LDL) decreased as shown in the values of 1.67mg/dL and 2.12mg/dL for group diets RGF 57.5 and RKD 65 fed to the rats. The reduction of the serum cholesterol and LDL is very important. This is due to the fact that increased risk of association of high levels of these lipids especially lipoproteins (LDL) with cardiovascular disease is very dangerous [32]. However, the preponderance of LDL in the cell wall of arteries (blood vessels) as a result of oxidation or enzymatic degradation of cholesterol and other lipids deposited, precipitate the formation and accumulation of plaques and as a result, these narrow the arteries [33,34] with serious pressure on the blood. Following this is a short supply of blood to the tissues, with its starvation of oxygen in the tissues and nutrients leading to tissues death [35].

Parameter	RGF 57.5	RKD 65	JGF 57.5	JBD 65	MGF 65	MVD 65	LSD
Total Protein (g/dL) Initial value	$5.06^{b} \pm 0.229$	$5.92^{a} \pm 0.086$	$5.84^{a} \pm 0.86$	$5.64^{ab} \pm 0.201$	$5.52^{ab} \pm 0.092$	$5.54^{ab} \pm 0.154$	0.729
Final value	$3.77^{a} \pm 0.165$	$4.86^{a} \pm 0.189$	$5.08^{a} \pm 0.355$	$4.00^{a} \pm 0.579$	$4.58^{a} \pm 0.146$	$4.72^{a} \pm 0.139$	1.341
Albumin (g/dl) Initial value	$3.90^{a} \pm 0.465$	$3.70^{a} \pm 0.429$	$4.52^{a} \pm 0.282$	$4.06^{a} \pm 0.129$	$3.92^{a} \pm 0.193$	$4.00^{a} \pm 0.095$	1.314
Final value	$3.75^{a} \pm 0.254$	$3.80^{a} \pm 0.141$	$4.00^{a} \pm 0.291$	$4.00^{a} \pm 0.141$	$4.00^{a} \pm 0.071$	$4.04^{a} \pm 0.075$	0.798
Globulin (g/dl) Initial value	$1.16^{a} \pm 0.515$	$2.22^{a} \pm 0.376$	$1.32^{a} \pm 0.287$	$1.58^{a} \pm 0.294$	$1.60^{a} \pm 0.167$	$1.54^{a} \pm 0.143$	1.410
Final value	$0.68^{a} \pm 0.108$	$0.98^{a} \pm 0.376$	$1.08^{a} \pm 0.328$	$1.20^{a} \pm 0.383$	$0.58^{a} \pm 0.109$	$0.88^{a} \pm 0.084$	1.110
Cholesterol (mg/dl) Initial value	$3.82^{a} \pm 0.196$	$3.70^{a} \pm 0.109$	$3.86^{a} \pm 0.140$	$3.66^{a} \pm 0.216$	$3.60^{a} \pm 0.179$	$3.68^{a} \pm 0.294$	0.881
Final value	$3.45^{b} \pm 0.092$	$3.64^{b} \pm 0.147$	$3.84^{ab} \pm 0.163$	$3.77^{ab} \pm 0.201$	$4.62^{a} \pm 0.294$	$3.96^{ab} \pm 0.204$	0.846
HDL (mg/dl) Initial value	$1.12^{ab} \pm 0.174$	$0.58^{b} \pm 0.080$	$1.20^{ab} \pm 0.109$	$1.28^{a} \pm 0.139$	$1.30^{a} \pm 0.195$	$0.86^{ab} \pm 0.169$	0.655
Final value	$0.97^{a} \pm 0.066$	$0.64^{a} \pm 0.093$	$1.12^{a} \pm 0.218$	$1.07^{a} \pm 0.086$	$0.60^{a} \pm 0.071$	$0.94^{a} \pm 0.186$	0.584
LDL (mg/dl) Initial value	$2.12^{a} \pm 0.188$	$2.24^{a} \pm 0.189$	$1.88^{a} \pm 0.092$	$1.70^{a} \pm 0.161$	$1.42^{a} \pm 0.340$	$1.98^{a} \pm 0.369$	1.066
Final value	$1.67^{a} \pm 0.196$	$2.12^{b} \pm 0.325$	$2.26^{b} \pm 0.163$	$2.17^{b} \pm 0.174$	$3.34^{a} \pm 0.268$	$2.36^{b} \pm 0.133$	0.961
Triglycerides (mg/dl) Initial value	$1.34^{b} \pm 0.040$	$1.38^{b} \pm 0.058$	$1.70^{a} \pm 0.071$	$1.54^{ab} \pm 0.068$	$1.66^{a} \pm 0.68$	$1.54^{ab} \pm 0.040$	0.257
Final value	$1.05^{a} \pm 0.50$	$1.14^{a} \pm 0.103$	$0.94^{a} \pm 0.051$	$0.95^{a} \pm 0.067$	$1.18^{a} \pm 0.171$	$1.20^{a} \pm 0122$	0.453

Table 7: Haematological characteristics of rats fed all- legume mixed protein diets.

Mean ± SEM of 3 replications
Means with different superscript within the same row differ significantly ($p < 0.05$)
RGF 57.5 = Red Kidney bean germinated for 48h, fermented for 27h and dried at 57.5°C
RKD 65 = Red kidney bean dried at 65^{0}C
JGF 57.5 = Jackbean germinated for 87h, fermented for 87h and dried at (57.5^{0}C)
JBD 65 = Jackbean dried at 65^{0}C
MGF 65 = *Mucuna vulgaris* germinated for 62h. Fermented for 62h and dried at 65^{0}C
MVD 65 = *Mucuna vulgaris* dried at 65^{0}C
HDL – High density lipoprotein, LDL = low density lipoprotein.

The values of the PCV increased (39.25%, 40.27% and 40.25% in rats fed diets RGF, 57.5, RKG 65 and JBD 65. The Packed Cell Volume (PCV) levels of the rats fed group diets RGF 57.5, RKD 65, JGF 57.5, JBD 65, MGF 65 and MVD 65 were all above human cut-off levels before and after the feeding (Table 8). The PCV levels together with the haemoglobin level confirm the fact that the group diets were adequate to prevent anemic conditions over the period studied. The recommended human PCV level ranged from 32% in children whose ages are not above 4 years to 40% in men above 15 years [23]. The white blood cells (WBC) counts obtained as a result of the rat feeding although increased, were below the normal range of WBC values which is from 5 to 9 x 10^{9}/L. The decrease in count might be due to starvation and debilitating conditions such as nutritional disorders.

Parameter	RGF 57.5	RKD 65	JGF 57.5	JBD 65	MGF 65	MVD 65	LSD
PCV (%) Initial value	$38.20^{d} \pm 0.346$	$37.07^{e} \pm 0.15$	$40.20^{b} \pm 0.346$	$39.43^{c} \pm 0.115$	$41.27^{a} \pm 0.238$	$39.27^{c} \pm 0.462$	0.243
Final value	$39.25^{c} \pm 1.09$	$40.27^{b} \pm 0.238$	$39.00^{c} \pm 1.00$	$40.25^{b} \pm 0.433$	$41.00^{a} \pm 1.00$	$39.27^{c} \pm 0.115$	0.618
WBC (x 10^3 /L) Initial value	$6.07^{a} \pm 0.058$	$5.087^{b} \pm 0.075$	$5.17^{b} \pm 0.144$	$6.20^{a} \pm 0.173$	$4.37^{c} \pm 0.323$	$3.36^{d} \pm 0.312$	0.170
Final value	$5.17^{d} \pm 0.144$	$8.00^{a} \pm 2.00$	$7.25^{b} \pm 0.439$	$7.07^{b} \pm 0.058$	$6.11^{c} \pm 0.92$	$7.01^{b} \pm 0.011$	0.685
RBC (x $10^2/L^3$) Initial value	$2.09^{b} \pm 0.075$	$2.16^{b} \pm 0.271$	$2.29^{ab} \pm 0.248$	$2.07^{b} \pm 0.063$	$2.48^{a} \pm 0.416$	$2.47^{a} \pm 0.404$	0.231
Final value	$3.22^{b} \pm 0.190$	$3.13^{b} \pm 0.109$	$2.27^{c} \pm 0.331$	$3.47^{a} \pm 0.411$	$3.13^{b} \pm 0.115$	$3.37^{ab} \pm 0.37$	0.207
Hb (g/dl) Initial value	$12.13^{c} \pm 0.115$	$10.25^{d} \pm 0.427$	$12.13^{c} \pm 0.115$	$14.21^{b} \pm 0.185$	$15.09^{a} \pm 0.081$	$14.06^{b} \pm 0.104$	0.170
Final value	$15.20^{a} \pm 0.173$	$11.11^{d} \pm 0.092$	$13.09^{b} \pm 0.075$	$13.07^{b} \pm 0.063$	$11.06^{d} \pm 0.104$	$11.27^{c} \pm 0.231$	0.112
Serum glucose (mg/dl) Initial value	$104.00^{c} \pm 0.069$	$108.27^{a} \pm 0.231$	$106.27^{b} \pm 0.231$	$79.27^{f} \pm 0.231$	$97.20^{e} \pm 0.173$	$100.07^{d} \pm 0.058$	0.148
Final value	$61.50^{e} \pm 0.433$	$105.07^{a} \pm 0.058$	$88.13^{b} \pm 0.115$	$81.17^{c} \pm 0.144$	$81.20^{c} \pm 0.173$	$80.27^{d} \pm 0.231$	0.185

Table 8: Packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC), Haemoglobin in (Hb) and serum glucose of rats fed all-legume mixed protein diet.

Mean ± SEM of 3 replications

Means with different superscript within the same row differ significantly ($p < 0.05$)

RGF 57.5 = Red Kidney bean germinated for 48h, fermented for 27h and dried at 57.5°C

RKD 65 = Red kidney bean dried at 65^{0}C

JGF 57.5 – Jackbean germinated for 87h, fermented for 87h and dried at (57.5^{0}C)

JBD 65 = Jackbean dried at 65^{0}C

MGF 65 = *Mucuna vulgaris* germinated for 62h. Fermented for 62h and dried at 65^{0}C

MVD 65 = *Mucuna vulgaris* dried at 65^{0}C

PCV = Packed cell volume, WBC = white blood cell, RBC = Red blood Cell, Hb = Haemoglobin.

The RBC counts in rats fed all the group diets increased but the group diets RGF, 57.5, RKD 65 and MGF 65 as well as JBD 65 and MVD 65 compared well ($p>0.05$) in RBC counts at the end feeding. The Hb levels in rats fed the group diets were above the standard levels or cut-off levels for human and particularly for children. However, people whose level of Hb are below the recommended range from 11g/dL for infants and pregnant women to 13g/dL in adult men taking cognizance of the age, sex or other physiological factors are regarded as anemic [23]. The serum glucose levels significantly ($p<0.05$) decreased in rats fed all the group diets with the exception of diet JBD 65 (Table 8). The decrease in glucose is an indication of hypoglycaemic effect in which oxidative stress is reduced by active antidiabetic ingredients in the group diets. Consequent upon this, pancreatic secretion of insulin is stimulated, thereby enhancing effective glucose transport or key enzymes of the glycolytic pathway involved in glucose utilization [30]. In other words, the hypoglycaemic effect in the serum as shown by the group diets which were significant ($p<0.05$) was important as reduction in glucose level was the best approach to manage diabetes.

Conclusion

It can be concluded that diets formulated from the combined effect of germination and fermentation can be suggested to be able to reduce the levels of cholesterol, triglycerides and serum glucose due to the presence of some active ingredients in the diets.

Bibliography

1. Chel-Guerrero L., *et al.* "Functional properties of flours and protein isolates from Phaseolus lunatus and Canavalia ensiformis seeds". *Journal of Agriculture Food Chemistry* 50.3 (2002): 584-591.

2. Okafor JC and Okolo HC. "Potentialities of some indigenous fruit trees of Nigeria". Paper presented at 5th Annual Conference of Forestry Association of Nigeria (1974): 17-22.

3. Okigbo BN. "Neglected plants of horticultural and nutritional importance in traditional farming systems of tropical Africa. 4th African symposium on horticultural crops". *Acta Horticultural* 53 (1975): 131-150.

4. Martinelli LA., *et al*. "Stable carbon and nitrogen isotopic composition of bulk aerosol particles in a C4 Plant Landscape of South East Brazil". *Atmospheric Environment* 36.14 (2002): 2427-2432.

5. Mbofung CMF., *et al*. "Functional properties of cowpea-soydry red beans composite flour paste and sensorial characteristics of akara (deep fat fried food): effect of whipping conditions, pH, temperature and salt concentration". *Journal of Food Engineering* 54.3 (2002): 207-214.

6. Dahiru D., *et al*. "Antidiarrhoael activity of Ziziphus mauritiana root extract in rodents". *African Journal Biotechnology* 5.10 (2006): 221-225.

7. Bhagya B., *et al*. "Nutritional qualities and in Vitro Starch digestibility of ripened Canavalia carthartica beans of coastal sand dunes of Southern India". *Electronic Journal of Environmental, Agricultural and Food Chemistry* 5 (2006): 21-28.

8. Sridhar KR and Seena S. "Nutritional and antinutritional significance of four unconventional legumes of the genus Canavalia. A comparative study". *Food Chemistry* 99 (2006): 267-288.

9. Famurewa JAV and Raji AO. "Parameters affecting milling qualities of underfatted soybeans (Glycine max I. Merill) (i) selected thermal treatment". *International Journal of Food Engineering* 1 (2005): 1-9.

10. Janardhanan K., *et al*. "Biodiversity in India underexploited/ tribal pulses". In: Improvement strategies for leguminosae Biotechnology. Jaiwal, P. K. and Singh, R. P. (Eds). Kluwer Academic Publishers, Britain (2003): 353-405.

11. Pugalenthic M., *et al*. "Alternative Food/Feed Perspectives of an underutilized legumes". *Plant Foods for Human Nutrition* 60.4 (2005): 201-218.

12. Vadivel Y and Janardhanan K. "Nutritional and antinutritional composition of velvet bean. An underutilized Foodlegumes in South India". *International Journal of Food Science and Nutrition* 51.4 (2000): 279-287.

13. Kay DE. "Hyecinta bean food legumes". Tropical Product Institute Crop and Product. Digest A 3 London 16 (1979): 184-186.

14. Dinelli G., *et al*. "Content of flavonol in Italian bean (Phaseolus vulgaris L.) ecotypes". Food Chem., 99.1 (2006): 101-114.

15. Oomah BD., *et al*. "Phendics and antioxidative activities in narrow-leafed lupins (Lupinus angustifolius L.)". *Plant Food Human Nutrition* 61.2 (2006): 91-97.

16. Jackson JK. "Manual of forestation. Nepal Kathmanchi. Nepal UK Forestry Research Project, Forest Research Division". *Department of Forestry and Plant Research* (1989): 402.

17. Bau HB., *et al*. "Effects of germination on chemical composition, biochemical constituents and antinutritional factors of soybean (Glycine max) seeds". *Journal of the Science of Food and Agriculture* 73.1 (1997): 1-9.

18. Snedecor GW and Cochran WG. "Statistical Methods. 7th ed., Iowa State University Press, Ames, 1A (1980): 215.

19. Okaka JC and Potter NN. "Physio-chemical and functional properties of cowpea powders processed to reduce beany flavour". *Journal of Food Science* 44 (1979): 1233-1240.

20. AOAC. "The official methods of analysis". Association of Official Analytical Chemists, (17th edition) Washington DC. (2005).

21. Cartwright GE and Lee GR. "The anemia of chronic disorders". *British Journal of Haematology* 21.2 (1971): 147-52.

22. Schalm OW., *et al*. "Veterinary hematology". 3rd, Lea and Febiger, Philadelphia (1975).

23. Hereberg Galan P and Dhur A. "Essential mineral and trace element. nutritive methodology: Methods for assessing iron deficiency. In: Fidanza, p. (ed). Nutritional Status Assessment. London: Chapman and Hall, (1991): 374-85.

24. Steel RGD and Torrie HT. "Principles and Procedure of Statistics, A Biometric Approach 2nd edition McGraw-Hill International Auckland. (1981).

25. Odetola OM and Erurbetine D. "Nutritional Evaluation of Whole Kenaf (Hibicus cannabinus L.) Seed Meal in Rats". *Journal of Advanced Laboratory Research in Biology* 3.3 (2012): 152-157.

26. Bender AK. "Dictionary of nutrition and food technology, 2nd edition. London: Butterworth and Co. Ltd. (1965).

27. Robert KM., *et al*. "Mayers Biochemistry, 25th edition. McGraw-Hill, New York (2000): 763-765.

28. Esonu BO., *et al*. "and blood chemistry of weaner pigs fed raw Mucuna bean seed meal". 4 (2001): 45-49.

29. Iheukwumere FC. "Effects of feeding Microdesmis puberula on the haematology and serum biochemistry of weaner pigs". *Journal of the Science of Agriculture, Food and Environment* 8 (2008): 44-48.

30. Nwachukwu N and Nwite IN. "Effect of diet formulated with two local spices on serum and hepatocyte of wistar rats". *Nigerian Journal of Nutri. Sciences*. 30.2 (2009): 84-89.

31. Steinberg D and Witztum JL. "Lipoproteins and artherogenesis. Current Concepts". *The Journal of the American Medical Association* 264.23 (1990): 3047-3052.

32. Davis MJ. "The pathophysiology of acute coronary heart disease". *Paul Duddley white lectures* 94.8 (2000): 2012-2020.

33. Keys A., *et al*. "Serum Cholesterol response to changes in the diet. Particular saturated fatty acids in the diets". *Metabolism* 14.7 (1965): 776-787.

34. Itam EH., *et al*. "Effect of coconut (Cocus nucifera) and palm kernel oil supplemented diets on serum lipid profile of albino wistar rats". *Nigerian Journal of Nutritional Sciences* 31.1 (2010): 30-34.

35. Hegsted DM. "Dietary fatty acids, serum cholesterol and coronary heart disease. In: Nelson, G. J. (Ed). Health effects of dietary fatty acids". Champaign, IL: American Oil Chemist Society, (1981). 50-68.

Volume 3 Issue 7 July 2019