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Journal of Human Nutrition & Food Science

Review Article

Nutritional Evaluation of Germinated and Fermented all Legume Mixed Protein Diets in Rats

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Submitted: 28 September 2017
Accepted: 10 November 2017
Published: 13 November 2017
ISSN: 2333-6706

Keywords
• Nutritive quality; Germination; Fermentation; Legume; Protein diets; Rats

Abstract

Young male albino rats (74 -127g) were used to evaluate the nutritional quality of germinated, fermented and dried underutilized legumes: *Canavalia ensiformis* (jackbean), *Mucuna vulgaris* and *Phaseolus vulgaris* (red kidney bean). Six different diets were formulated respectively from the legumes and fed to thirty-six rats, which were divided into six groups of six rats per metabolic cage for 9-day nitrogen balance study in a 12-day study. Three day acclimatization was used during which time the rats were fed the normal rat chow and water ad-libitum. The diets were coded as red kidney bean germinated (48h), fermented (27h) and dried at 57.5°C (RGF 57.5), red kidney bean dried at 65°C (RKD 65), jackbean germinated (87h), fermented (87h) and dried at 57°C (JGF 57.5), jackbean dried at 65°C (JBD 65), *Mucuna vulgaris* germinated (62h), fermented (62h) and dried at 65°C (MGF 65) and *Mucuna vulgaris* dried at 65°C (MVD 65). At the end of the nine (9) day experimental feeding with the diets and water ad libitum, the result indicated that diet MVD 65 was most superior in all the performance and protein quality characteristics determined. This was evidenced in the fact that the highest food intake, digested nitrogen, nitrogen intake, weight gain, nitrogen balance and apparent digestibility (AD) of 12.25g, 0.955g, 1.176g, 43.42g, 0.884g and 81.227% were obtained using diet MVD 65. These values were significantly ($p<0.05$) higher than the values of the other diets. Net protein utilization (NPU) and Biological value (BV) using MGF 65 and RKD 65 diets were high (77.92% and 99.21%) respectively. Absorbed/digested nitrogen and retained nitrogen / nitrogen balance were comparable in values. The result tends to indicate that good quality protein diets can be produced from the mixed legumes.

INTRODUCTION

Grain legumes occupy an important place in the world's traditional food and nutrition economy. Legumes form inexpensive sources of protein, carbohydrates, vitamins, minerals and polyunsaturated fatty [1] in human and animal food. Generally, protein in legume seeds represents about 20% (dry weight) in peas and beans, upto 38 – 40% in soy bean and lupin [2].

Despite the high nutrient content of the raw legumes, their utilization is impaired by long cooking time and by some inherent antinutrients such as trypsin inhibitors, tannins, phytates, hemagglutinins, saponins and flatulence factors [3,4]. There are other toxic factors that reduce the food value (such as protein digestibility) of these plant foods by binding with some digestive enzymes in the body system [5]. Cooking, germination, fermentation, dehulling, autoclaving, etc reduce these factors and at the same time improve the quality of human diet.

Weaning/complimentary foods have been developed from underutilized legumes to increase the nutritional status of these foods [6] and from maize, soybean, and fluted pumpkin blends [7]. It has been shown that legume proteins rich in lysine and tryptophan can complement the protein in cereal grains, rich in methionine and cystine since the chemical and nutritional characteristics of legumes make them natural complements to cereal based diets [8].

In Nigeria and other African and Asian countries, *Canavalia ensiformis* (jackbean) is considered as a non-conventional source of high protein (23.4%), carbohydrate (55%) and some minerals such as magnesium, copper, phosphorus, calcium, zinc and nickel [9,10].

Mucuna vulgaris is regarded as a legume with better potential to meet the increasing protein requirements of human and livestock industries at large, but remains untapped. Mucuna seeds as a rich source of protein supplement (ranging from 27 – 30%) and carbohydrate (42.32 -64.26%) in food and feed has been documented [11,12].

Phaseolus vulgaris (red kidney bean) is a known protein rich legume. The nutritional value per 100kg was indicated as protein (24g), fat (1g), carbohydrate (60g) and energy (1390kj). Dry beans are widely known for their fibre, mineral and protein contents [13].

The main purpose of this study is to evaluate the nutritional quality of germinated and fermented all-legume mixed protein diets using rats (Figure 1).

MATERIALS AND METHODS

Source of materials

Phaseolus vulgaris (red kidney bean) and *Canavalia ensiformis* (jackbean) were obtained from a farmer at Uburu in Oru East LGA, Imo State. *Mucuna vulgaris* was purchased at Edem market in Nsukka, Enugu State. Vitamin/mineral premix and corn starch were purchased at Gufon Veterinary Centre, Police shopping complex (fire service roundabout area), Owerri, Imo State. Oil (Sonuola soya oil brand) was purchased from Ekeukwu Market, Owerri, and Imo State. Albino rats were purchased from the Department of Veterinary Medicine, University of Nigeria, Nsukka, and Enugu State, Nigeria.

Preparation of the legume seeds

Phaseolus vulgaris (red kidney bean) and *Canavalia ensiformis* (jackbean) were cleaned and sorted to remove all foreign materials. After cleaning, they were soaked in distilled water for 12h and 6h respectively at room temperatures of 25°C. *Mucuna vulgaris* was soaked in distilled water for 24h at room temperature of 25°C and then stratified in hot water for 2-5min before being germinated.

Germination and fermentation of legume seeds

The hydrated legume seeds were placed separately in wetted jute bags and germinated at different time intervals of 12 – 104.0h (Tables 1 and 2). The seeds were washed every 12h to prevent mould growth [14]. The germinated seeds were dehulled manually by rubbing the seeds multiple times with the palms to get them ready for fermentation.

The dehulled germinated seeds were later placed in deionised water in ratio of 1:3 (w/v) grams to water in covered labeled plastic containers of the same sizes. The seeds were allowed to ferment at varied time intervals of 12h to 104.0h using natural fermentation process (Tables 1 and 2). The fermented seeds were dried at different temperatures of 50°C to 80°C in a Hot Air Oven (Model AV-160) for 50mins. The dried seeds were respectively ground using Moulinex blender and sieved with 60mm mesh size to separate the fine sample flours from coarse particles. The flours were separately put in labeled plastic containers and stored in the refrigerator.

Experimental design

A central composite rotatable design (CCRD) for three variables germination, fermentation and drying was used to examine the response pattern in which twenty (20) variable combinations were obtained and used as experimental runs. The procedure described by Snedecor and Cochran (1980) was used. The combination runs of jackbean germinated (87h), fermented (87h) and dried at 57.5°C (JGF 57.5), jackbean dried at 65°C (JBD 65), *Mucuna vulgaris* germinated (62h), fermented (62h) and dried at 65°C (MGF 65), *Mucuna vulgaris* dried at 65°C (MVD 65), red kidney bean germinated (48h), fermented (27h) and dried at 57.5°C (RGF 57.5) and red kidney bean dried at 65°C (RKD 65) were used.

Diet formulation

The diets (6) were formulated from processed and unprocessed (raw) seed flours of *Canavalia ensiformis* (jackbean), *Mucuna vulgaris* and *Phaseolus vulgaris* (red kidney bean) respectively. The following combination runs of JGF 57.5, JBD 65, MGF 65, MVD 65, RGF 57.5 and RKD 65, which were used as the codes for the respective diets, were based on the fact that they had the highest protein values of 23.16%, 21.24%, 23.75%, 22.75%, 21.70% and 21.85% (Tables 1-4). Corn starch, vitamin/mineral premix and 5% oil were added to the legume flours and thoroughly mixed differently in a dough mixer (Hobert 2000, England) for 30min (Okaka, 1997) to obtain six all-legume mixed protein diets of fine consistency. The corn starch was added to dilute the protein. The diets provided 10% dietary protein for the entire period of the nitrogen balance study. The diets were each put in labeled plastic containers and stored in a freezer until required for feeding (Table 5,6).

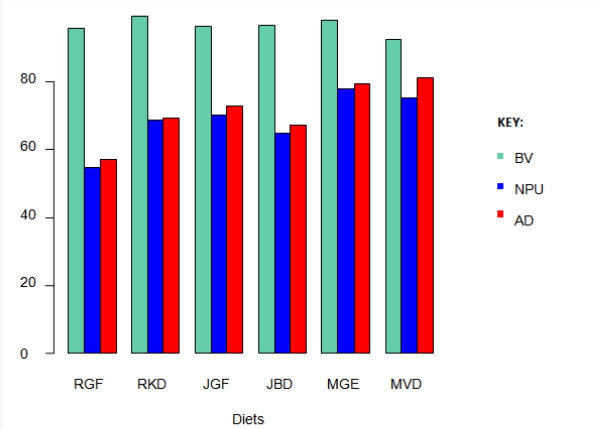

Figure 1 Protein quality of all legume mixed diets fed to rats.

Table 1: The coded values of the independent variables for *Canavalia ensiformis* (Jackbean) and *Mucuna vulgaris.*

		Codes			
Variables	-1.682	-1	0	1	1.682
X_1 (h)	19.9552	37	62	87.1	104.45
X_2(h)	19.9552	37	62	87.1	104.45
X_3(°C)	52.3866	57.5	65	72.5	77.6134

Table 2: The coded values of the independent variables for Phaseolus vulgaris (Red Kidney bean).

			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 (°C)	50	57.5	65	72.5	80

Calculation of the recipe for diet JGF 57.5

To obtain the total grams of diet to be consumed daily by the rats, assume that 20g of diet will be consumed daily by rats in a cage and for 12 days.

∴ 20g X 12 days X 6 rats = 1440g of diet.

1. The value of processed Jackbean (JGF 57.5- that is

Table 3: 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 (^{0}C)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Moisture (%)	CHO (%)
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

The protein, fat, fibre, ash, moisture and carbohydrate contents of *Canavalia ensiformis* flour dried at 65^{0}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.
CHO = Carbohydrate; GT = Germination time; FT = Fermentation time; DT = Drying time

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

Runs	GT (h)	FT (h)	DT (^{0}C)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Moisture (%)	CHO (%)
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

The protein, fat, fibre, ash, moisture and carbohydrate contents of *Mucuna vulgaris* flour dried at 65^{0}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.

CHO = Carbohydrate
GT = Germination time
FT = Fermentation time
DT = Drying time

Table 5: 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 (^{0}C)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Moisture (%)	CHO (%)
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

The protein, fat, fibre, ash, moisture and carbohydrate contents of *Phaseolus vulgaris* flour dried at 65^{0}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.

CHO = Carbohydrate
GT = Germination time
FT = Fermentation time
DT = Drying time

Table 6: Composition of the formulated diets.

Ingredient (g/kg)	JGF 57.5	JBD 65	MGF 65	MVD 65	RGF 57.5	RKD 65
Corn starch	740.48	684.77	755.92	729.27	698.65	703.20
Fat (5%)	72.00	72.00	72.00	72.00	72.00	72.00
Protein (10%)	621.76	677.67	606.32	632.97	663.50	659.04
Vitamin/mineral premix	5.76	5.76	5.76	5.76	5.76	5.76
Total	1440	1440	1440	1440	1440	1440

Jackbean germinated for 87h, fermented for 87h and dried at 57.5°C) (Table 3) used for the diet formulation = 23.16%

The gram of diet JGF 57.5 was obtained based on 10% dietary protein level.

Let x = the gram of Jackbean's protein

Using % protein of processed Jackbean = 10% dietary protein level

100% Unknown gram (x) of jackbean's protein

∴ 23.16 = 10%

100 x

x = 1000 = 43.18g

23.16

If 100g of protein in JGF 57.5 is required for the production of 1440g of the diet

∴ 1440 X 43.18 = 621.76g of the protein of diet JGF 57.5

100 1

2) Let the percentage of fat (Oil) used be 5%

If 100% of the oil was required by 1440g of diet

∴ 5% of the oil will be required by 1440 X 5g

100 1 = 72g of oil

3) Vitamin/Mineral Premix

If 250g of the diet was required by 1g of vitamin/mineral premix

∴ 1440g of diet will require 1440g of vitamin/mineral premix 250 =5.76g of vitamin/mineral premix

4) Corn starch was obtained by subtracting the total values of the ingredients from the total value of the diet per day by the six rats.

∴Corn starch = [1440 – (621.76 + 72 + 5.76)]

= [1440 – 694.336]

= 740.48

Experimental feeding of rats

The 36 rats, 6 per a labeled stainless metabolic cage were divided into 6 groups. Each group of rats was fed a particular formulated diet from the 3 different legumes. The metabolism cage was to separate the urine from the faeces. The rats were weighed prior to access to their respective diets and everyday to evaluate the body weight change. The feeding was daily with water ad-libitum for the entire 9-day nitrogen (N) balance study period. At the end of feeding on the 9th day, the rats were sacrificed and on evacuation of the internal organs, the liver was carefully removed, weighed and dried in a hot air oven drier (70-75°C) for 14 – 16h and the weight taken again. One (1) gram of the liver was mixed with 10ml Tris-HCL buffer solution pH 7.5, crushed with mortar and pestle and the paste filtered for 15 min [14]. The supernatant was stored at room temperature (27°C) for analysis. A 0.05g of carmine red powder was introduced into the diets and fed the rats on the mornings of day 1 as well as day 9. Coloured faeces appeared on day 2 and day 10 and other procedures followed were according to Obizoba (1983) [16].

The experimental rats were fed equal weights (20g) of their respective diets and water ad-libitum. Reminants of diets, urine and faeces were collected daily throughout the entire 9-day period and recorded. The daily food intakes mean weight gains were also recorded and data generated were used to estimate protein quality such as Nitrogen (N) intake and Biological Value (BV), Net Protein Utilization (NPU), Apparent Digestibility (AD) and nitrogen retention.

Analysis of nitrogen

Urinary nitrogen, faecal nitrogen and liver nitrogen were analyzed according to the method of AOAC (2005) [17].

Statistical analysis

The data obtained for the protein quality indices and performance characteristics were analyzed using analysis of variance (ANOVA) at 5% level. The test for significant differences between the means was carried out using the multiple range tests [18].

RESULTS AND DISCUSSION

Performance characteristics

The result from the work on the all-legume protein mixed diets is shown in Table 2. The group diet *Mucuna vulgaris* dried at 65°C (MVD 65) was highly (12.25g) consumed by the rats (Table 7). The diet least (5.039g) consumed was red kidney bean germinated 48h, fermented 27h and dried at 57.5°C (RGF 57.5). Chikwendu and Obizoba (2003) [19] reported that food intake is associated with nitrogen source, palatability, flavor and essential amino acids rather than the level of nitrogen [20] also maintained that growth rate in experimental rats is influenced by the amount of diet consumed. The fact that there were higher food intakes, showed that the food had the necessary nutrients required by the rats for growth or that the nitrogen balance study revealed the food growth – promoting quality of the diets without minding if germination or fermentation treatment was given to the legumes (Table 7).

Food intake from the result showed a varied influence on the weight gain. The rats that consumed more had increased weight gain as the values varied form 10.96 – 43.42g in diets RGF 57.5 to MVD 65 while rats that consumed the least diet also significantly ($p<0.05$) gained the least weight [21,22]. The gain in weight in rats fed the group diets showed that the diets contained good quality amino acids which the rats utilized. Food intake determines the level of nitrogen intake in the body. Increased food intake as a result of feeding the rats with diets MGF 65 and MVD 65 was an indication of increased nitrogen intake and nitrogen balance/ retained nitrogen as reflected in the values of 1.108g and 1.176g and 0.8633g and 0.884g respectively.

The lower digested/absorbed nitrogen of 0.2766g by the rats that consumed diet red kidney bean germinated 48h, fermented 27h and dried at 57.5°C (RGF 57.5) was due to the fact that the nitrogen intake was lower when compared to the other test diets. However, the lower digested nitrogen of RGF 57.5 may be attributed to lower apparent digestibility (AD) value of 57.27% obtained by rats that consumed the group diet. In other words, the lower nitrogen intake influenced absorbed/digested nitrogen which made it to be lower.

The lower (0.2653g, 0.473g and 0.440g) values of retained nitrogen/nitrogen balance of rats that consumed diets RGF 57.5, RKD 65 and JBD 65 were expected since the rats had lower nitrogen intake values than the other diets. However, the retained nitrogen/nitrogen balance and the absorbed nitrogen/digested nitrogen were comparable ($p>0.05$) in values in all the diets fed the rats (Table 7). The rats that consumed diets RGF 57.5 and RKD 65 also had lower (9.57% and 10.24%) liver nitrogen.

The liver weight was highest (1.30g) and significant ($p<0.05$) in rats that feed on diet MGF 65 while the value of the liver weight was least (0.64g) in rats that fed on diet RGF 57.5. The diets JBD 65, MGF 65 and MVD 65 were comparable ($p>0.05$) in values of liver weight. The low liver weight of rats fed diet RGF 57.5 was comparable to the works of Anyika (1988) [23] who compared the supplementary effect of soaked bambara groundnut and sorghum and Obizoba (1985) [24] who also compared the supplementary effect of crayfish and brown bean to rice protein. The low liver weight value might be attributed to low food intake, low weight gain, low nitrogen (N) intake and apparent digestibility (AD), poor nitrogen absorption and utilization.

Protein quality

The measurement of the efficiency of the absorbed nitrogen/ nitrogen balance is known as Biological value (BV) [25]. The

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

Parameters	RGF 57.5	RKD 65	JGF 57.5	JBD 65	MGF 65	MVD 65	LSD
Food intake (g)	5.02ᵉ ± 0.173	7.11ᵈ ± 0.981	8.27ᶜ ± 0.236	7.03ᵈ ± 0.288	11.31ᵇ ± 0.271	12.17ᵃ ± 0.144	0.134
Weight gain (g)	10.62ᶠ ± 0.536	23.20ᵈ ± 0.173	28.48ᶜ ± 0.421	16.28ᵉ ± 0.248	33.32ᵇ ± 0.560	43.14ᵃ ± 0.121	0.314
Faecal Nitrogen (g)	0.2064	0.2112	0.219	0.2220	0.2290	0.2208	-
N intake (g)	0.483ᵈ ± 0.058	0.688ᶜ ± 0.0081	0.808ᵇ ± 0.0035	0.677ᶜ ± 0.045	1.108ᵃ ± 0.0078	1.176ᵃ ± 0.013	0.094
Urinary N (g)	0.0113	0.004	0.022	0.0150	0.0157	0.071	-
Absorbed N/ digested N (g)	0.2766 ± 0.012	0.4768 ± 0.058	0.589 ± 0.0098	0.455 ± 0.008	0.879 ± 0.043	0.955 ± 0.015	0.145
Retained N/N balance (g)	0.2653ᶜ ± 0.072	0.473ᵇᶜ ± 0.052	0.567ᵇ ± 0.058	0.440ᵇᶜ ± 0.017	0.8633ᵃ ± 0.05	0.884ᵃ ± 0.09	0.288
Liver Nitrogen (%)	9.57ᶜ ± 0.172	10.24ᵇᶜ ± 0.471	12.21ᵃ ± 0.513	12.47ᵃ ± 0.135	12.72ᵃ ± 0.134	11.33ᵇᶜ ± 0.246	1.394
Liver weight (dry) (g)	0.64ᵇ ± 0.040	0.90 ± 0.084	1.16ᵃᵇ ± 0.150	1.20ᵃ ± 0.164	1.30ᵃ ± 0.084	1.20ᵃ ± 0.173	0.550

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°C
JGF 57.5 – Jackbean germinated for 87h, fermented for 87h and dried at 57.5°C
JBD 65 = Jackbean dried at 65°C
MGF 65 = *Mucuna vulgaris* germinated for 62h. Fermented for 62h and dried at 65°C
MVD 65 = *Mucuna vulgaris* dried at 65°C

biological value (BV) of 92.56% - 99.21% for the rats was higher than the recommended value of 75% for children [26]. The higher BV was affected by higher food intake and higher nitrogen intake. The BV of a protein is related to the amount of the protein that is retained. This quantity accounts for growth and the healthy maintenance of the animal [27]. The decreased urinary excretion even reduced Net Protein Utilization (NPU) as well as AD and Nitrogen balance of rats that consumed diet RGF 57.5. The discussion was further depicted in Fig 1. The highest (92.21%) BV was in diet RKD65 and the lowest (92.56%) in diet MVD65. Diet MGF65 had the highest (77.92%) NPU value while diet RGF 57.5 had the lowest (54.93%) value. The highest (81.22%) AD value was in diet MVD65 and the lowest (57.27%) in diet RGF57.5. Biological value (BV) is always greater than NPU as indicated by the rats that consumed the group diets. The higher values of food intake, nitrogen intake, weight gain, nitrogen balance, apparent digestibility, NPU and biological value were significantly ($p<0.05$) influenced by the dietary treatments (protein intake) given to the rats during the 9-day nitrogen balance study. A protein material is regarded as nutritionally good in quality when its biological value (BV) is high (70 – 100%) [28,29].

REFERENCES

1. Chung H, Liu Q, Peter P, Ming Z, Richey Y. *In vitro* starch digestibility, expected glycemic index and some physiochemical properties of starch and flour from common bean (*Phaseolus vulgaris L.*) varieties grown in Canada. Food Res Int. 2008; 111: 316 -321.

2. Duranti M. Grain Legume proteins and nutraceutical properties. Fitoterapia. 2006; 77: 67 - 82.

3. Osho SM. Soybean processing for house use in food crops production, utilization and nutrition. BN Mbah, DO Nnanyelugo (eds). Dotam Publ Ltd Ibadan. 1989; 68-78.

4. Fadahunsi IF, Sanni AI. Chemical and Biochemical changes in Bambara nut (*Voandzeia subterranean L.*) flours during fermentation to tempeh. Elect J Environ Agric Food Chem. 2010; 9: 275-283.

5. Agbede JO, Aletor VA. Comparative evaluation of weaning foods from Gliricidia and Leucaena leaf protein concentrates and some commercial brands in Nigeria. J Sci Food Agric. 2003; 84: 21- 30.

6. Ali-Asma M, EL Fadil, Babikar E, EL Tinay Abdullahi H. Development of weaning food from sorghum supplemented with legumes and oil seeds. Food Nutri Bull. 2006; 27: 26 -33.

7. Agu HO, Aluyah E. Production and chemical analysis of weaning food from maize, soybean and fluted pumpkin seed flour. Nigeria Food J. 2004; 22: 171- 177.

8. Altschul A M. New Protein Foods. Academic Press 1st Edn. 1974; 20-23.

9. Bamiro FO, Tairu AO, Oderinde RA. The nutritive values of Nigerian Jackbeans (*Canavalia ensiformis*). La rivisita Heliana Delle Sastance citrasse. 1994; LXXI: 421- 424.

10. Liener IE. Implications of antinutritional components in soybean foods. Crit Rev Food Sci Nutr. 1994; 34: 31- 67.

11. Siddhuraju P, Becker K. Preliminary evaluation of Mucuna seed meal (*Mucuna pruriens var utilis*) in common carp (*Cyprinus carpio L.*): An assessment by growth performance and feed utilization. Aquaculture. 2001; 196: 105-123.

12. Bressani R. Factors influencing nutritive value in food grain legumes: Mucuna in comparison to other grain legumes. In: Flores, editors. Mucuna as a Food and Feed: Current uses and the way forward. Proc. International Workshop held April 26- 29, 2000. Tegucigalpa, Houndras. 2002; 164- 188.

13. Dinelli G, Bonetti A, Minelli M, Marotti I, Catizone P, Mazanti A. Content of flavonols in Italian bean (*Phaseolous vulgaris L*) ecotypes. Food Chem. 2006; 99: 101-114.

14. Bau HB, Villanne G, Nicolos JP, Mejean L. Effects of germination on chemical composition, biochemical constituents and antinutritional factors of soybean (*Glycine max)* seeds. J Sci Food Agri. 1997; 73: 1-9.

15. Nwachukwu N, Nwite IN. Effect of diet formulated with two local spices on serum and hepatocyte of Wister rats. Nigeria J Nutri Sci. 2009; 30: 84-89.

16. Obizoba IC. Growth and Nitrogen utilization of rats fed pigeon pea-rice mixtures. Nigeria J Nutri Sci. 1983; 4: 35- 40.

17. AOAC. Official methods of Analysis. Association of Official Analytical Chemists. 17th Edn. Washington DC. 2005.

18. Steel RGD, Torrie HT. Principles and Procedures of Statistics. A Biometric Approach. 2nd edn, International Auckland; McGraw-Hill. 1981.

19. Chikwendu NJ, Obizoba IC. Nutritional evaluation of protein quality of ground bean (Kershingiella geocarpa) based diets. Nigeria J Nutri Sci. 2003; 24: 64- 68.

20. Akeredolu AA, Addo AA, Akeredolu OA. Clinical evaluation of Pearl Millet Conophor Weaning Mix as supplementary food for Nigerian children. Brazilian Arch Biol Technol. 2005; 48: 531- 536.

21. Ugwu FM, Ekwu FC, Okoye IC. Protein quality indices and Food intake pattern of parboiled and roasted breadfruit-corn diets. J Sci Agri Food Tech Envir. 2002; 2: 97- 100.

22. Oke DB, Oke MO, Adeyemi OA. Prediction of cowpea seed protein quality through total sulphur determination. In contributory role of animal production in national development (AO Fanimo and JA Olanite editors. Proc 7th Annual Conf of Animal Science of Nigeria, 16th-19th Sept. University of Agriculture, Abeokuta, Nigeria. 2002.

23. Anyika JU. The nutritive value of legume proteins supplemented with sorghum or crayfish. Unpublished B.Sc Project. Department of Home Science and Nutrition, University of Nigeria, Nsukka, Nigeria. 1988.

24. Obizoba IC. Evaluation of the protein quality of rice supplemented with beans or crayfish. Plant food for Human nutri. 1985; 35: 43- 49.

25. Hackler LR. Methods of measuring protein quality: A review of bioassay procedures. Cer Chem. 1977; 54: 984 - 995.

26. PAG. Protein Advisory Group guideline. No. 8 Protein-rich mixtures for use as weaning foods. New York. FAO/WHO/UNICEF. 1971.

27. Pallet PL, Yeong VR. Nutritional evaluation of protein foods. The United Nation University, Tokyo. 1980.

28. Ijarotimi SO, Keshinro OO. Determination of nutrient composition and protein quality of potential complementary foods formulated from the combination of fermented popcorn, African locust bean and bambara groundnut see flour. Pol J Food Nutri Sci. 2013; 63: 155-166.

29. Okaka, JC, Potter NN. Physio-chemical and functional properties of cowpea powders processed to reduce beany flavour. J Food Sci. 1979; 44: 1233- 1240.

Cite this article

Okorie SU, Okaka JC (2017) Nutritional Evaluation of Germinated and Fermented all Legume Mixed Protein Diets in Rats. J Hum Nutr Food Sci 5(3): 11157.