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DOI: https://doi.org/10.64388/IREV10I2-1722638

Protein Quality Indices and Growth Performance of Albino Rats Fed Sprouted Soybean, African Yam Bean and Red Kidney Bean Diets

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Abstract- The search for sustainable plant-based protein sources has intensified due to the increasing prevalence of protein-energy malnutrition and the rising cost of animal proteins. Sprouting is an effective bioprocessing technique known to improve the nutritional quality and digestibility of legumes by reducing anti-nutritional factors and enhancing nutrient bioavailability. This study evaluated the influence of sprouted soybean (Glycine max), African yam bean (Sphenostylis stenocarpa), and red kidney bean (Phaseolus vulgaris) protein diets on protein quality indices and growth performance in albino rats. Soybean, African yam bean, and red kidney bean seeds were sprouted for 48 h, oven-dried at 55 °C, milled into flour, and blended in nine different proportions. Experimental diets were formulated to contain 10% crude protein and fed to groups of albino rats for 21 days, while commercial rat chow served as the control. Growth performance was evaluated using feed intake, body weight gain, feed efficiency ratio, and protein efficiency ratio. Protein quality was assessed using apparent protein digestibility, nitrogen balance, biological value, net protein utilization, and net protein ratio following standard nitrogen-balance procedures. Data were analyzed using one-way analysis of variance, and treatment means were separated using Duncan's multiple range test at $p < 0.05$. Significant ($p < 0.05$) differences were observed among the dietary treatments for all growth-performance and protein-quality parameters. Rats fed the commercial control diet recorded the highest growth performance and protein-utilization indices. Among the experimental diets, the blend containing 50% sprouted soybean and 50% sprouted red kidney bean (LB5) produced superior values for several growth and protein-quality indices. The improved nutritional response observed in mixed-legume diets may be associated with complementary amino-acid profiles and the beneficial effects of sprouting on anti-nutritional factors and protein digestibility. The findings indicate that selected sprouted soybean–African yam bean–red kidney bean blends have potential as affordable plant-based protein ingredients for food-product development.

Keywords— African yam bean; albino rats; growth performance; legume protein; protein quality; red kidney bean; soybean; sprouting.

I. INTRODUCTION

Protein-energy malnutrition (PEM) remains a major public health challenge worldwide, particularly in low- and middle-income countries where inadequate dietary protein intake contributes to impaired growth, weakened immune function, reduced cognitive development, and increased morbidity. Although animal-derived proteins provide high biological value, their rising cost and limited accessibility have intensified the search for sustainable, affordable, and nutritionally adequate plant-based protein sources. Grain legumes have emerged as promising alternatives because of their high protein content, balanced nutrient composition, environmental sustainability, and relatively low production costs [1, 2].

Soybean (Glycine max), African yam bean (Sphenostylis stenocarpa), and red kidney bean (Phaseolus vulgaris) are important grain legumes used for human nutrition. Soybean is widely recognized as one of the richest plant protein sources, containing approximately 36–40% protein with a favourable amino-acid profile. African yam bean is an indigenous African legume that possesses considerable nutritional potential but remains largely underutilized despite containing appreciable protein, dietary fibre, minerals, and bioactive compounds. Red kidney bean provides approximately 20–25% protein in addition to complex carbohydrates, dietary fibre, essential minerals, and phytochemicals. These legumes therefore represent valuable raw materials for developing affordable protein-rich foods capable of improving dietary quality and reducing dependence on animal protein [2, 3].

Despite their nutritional advantages, legumes contain anti-nutritional compounds including phytates, tannins, lectins, oxalates, and protease inhibitors that can interfere with nutrient digestion and utilization. These compounds may reduce protein digestibility and mineral absorption, thereby limiting growth and nutrient utilization when legumes constitute major dietary protein sources. The nutritional quality of legumes therefore depends not only on crude protein concentration but also on protein digestibility, amino-acid availability, and the extent to which anti-nutritional factors are reduced through processing [4, 5].

Among the processing techniques available, sprouting (germination) has gained attention because it is a simple, inexpensive, and potentially sustainable method for improving the nutritional quality of legumes. During germination, endogenous enzymes including proteases, amylases, phytases, and lipases become activated, resulting in biochemical modifications within the seed. These enzymatic activities hydrolyse storage macromolecules into simpler compounds, increase free amino acids, enhance protein digestibility and mineral bioavailability, and can reduce anti-nutritional factors such as phytates and protease inhibitors [6, 7]. Germination may also increase certain vitamins, antioxidant compounds, and bioactive peptides.

The nutritional adequacy of dietary proteins is commonly evaluated using biological protein-quality indices rather than crude protein concentration alone. Parameters such as protein efficiency ratio (PER), net protein ratio (NPR), biological value (BV), net protein utilization (NPU), apparent protein digestibility (APD), nitrogen balance (NB), and feed efficiency ratio (FER) provide information on protein digestion, absorption, retention, and utilization for growth [8].

Albino rats (Rattus norvegicus) remain widely used experimental models for evaluating dietary protein quality because of their well-characterized physiology, predictable growth response, and sensitivity to differences in dietary protein composition. Growth-performance measurements, including feed intake, body-weight gain, feed efficiency ratio, and protein efficiency ratio, are useful indicators of dietary protein adequacy in animal feeding studies [9, 10].

Previous investigations have demonstrated that germination can improve the nutritional quality of individual legumes by enhancing digestibility and reducing anti-nutritional compounds. Likewise, blending different legumes may improve amino-acid balance through complementary protein interactions. However, limited information is available regarding the combined effects of sprouting and multi-legume protein blending on biological protein quality and growth performance. In particular, comparative studies involving sprouted soybean, African yam bean, and red kidney bean formulated into mixed-protein diets and evaluated using comprehensive biological protein-quality indices remain limited. Furthermore, the potential of African yam bean, an underutilized indigenous crop, has not been fully exploited in functional-food development despite its nutritional value.

The present study was therefore designed to evaluate the influence of sprouted soybean, African yam bean, and red kidney bean protein diets on protein-quality indices and growth performance in albino rats. It was hypothesized that sprouting and strategic blending of these legumes would improve protein utilization and growth performance and provide evidence supporting the development of nutritionally improved plant-based protein products.

II. MATERIALS AND METHODS

A. Materials

Soybean (Glycine max), African yam bean (Sphenostylis stenocarpa), and red kidney bean (Phaseolus vulgaris) seeds were purchased from Relief Market, Owerri, Imo State, Nigeria. Botanical authentication was carried out at the Department of Agricultural Technology, Federal Polytechnic Nekede, Owerri, Nigeria. Commercial rat chow (Vita Finisher®, Grand Cereals Ltd., Jos, Nigeria) was

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DOI: https://doi.org/10.64388/IREV10I2-1722638

used as the control diet. Soybean oil, corn starch, and a commercial vitamin–mineral premix were obtained from Guffon Veterinary Store, Owerri, Nigeria.

B. Preparation of Sprouted Legume Flours
The individual legumes were processed separately following modified methods reported previously [11–13]. Briefly, the seeds were manually sorted to remove foreign materials, washed with potable water, and soaked in water at a 1:4 (w/v) ratio for 12 h. The soaked seeds were germinated for 48 h at ambient temperature (27 ± 2 °C), with periodic sprinkling of water to maintain adequate moisture. Following germination, the sprouts were washed, manually dehulled, and oven-dried at 55 °C for 12 h using a hot-air oven (Model XXX, Manufacturer, Country). The dried sprouts were milled into flour using a laboratory hammer mill and sieved through a 0.50-mm mesh sieve. The resulting flours were packaged in airtight polyethylene containers and stored at room temperature until further analysis.

C. Formulation of Sprouted Legume Flour Blends
Nine experimental flour blends were prepared by mixing sprouted soybean (SY), African yam bean (AY), and red kidney bean (RK) flours in predetermined proportions (Table I).

TABLE I. COMPOSITION OF SPROUTED LEGUME FLOUR BLENDS

Diet	Soybean (%)	African yam bean (%)	Red kidney bean (%)
LB1	70	10	20
LB2	60	20	20
LB3	50	30	20
LB4	50	50	0
LB5	50	0	50
LB6	0	50	50
LB7	100	0	0
LB8	0	100	0
LB9	0	0	100

D. Diet Formulation
The crude protein contents of the flour blends, determined by proximate analysis, were used to formulate isonitrogenous diets containing 10% dietary protein using a modified procedure [14]. Each diet was prepared to a total weight of 3,696 g. Soybean oil was incorporated at 5% (w/w), while vitamin–mineral premix was added at the manufacturer's recommended rate of 1 g per 250 g of diet. Corn starch served as the carbohydrate source and was added to complete the final diet weight. Commercial rat chow served as the control diet.

E. Experimental Animals and Feeding Trial
Eighty healthy male albino rats (Rattus norvegicus), Wistar strain, aged eight weeks and weighing 18–34 g, were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were housed in stainless-steel metabolic cages under standard laboratory conditions (25 ± 2 °C, 12 h light/12 h dark photoperiod) with free access to clean drinking water. The rats were acclimatized for three days on commercial rat chow before commencement of the feeding trial. Following acclimatization, animals were randomly assigned to ten groups of eight rats each. Nine groups received the formulated experimental diets (LB1–LB9), while the tenth group received commercial rat chow (CRC). Feeding continued for 21 consecutive days. Body weight and feed intake were recorded throughout the experiment.

F. Determination of Growth Performance
Growth performance was evaluated using feed intake (FI), body-weight gain (BWG), feed efficiency ratio (FER), and protein efficiency ratio (PER) [15]. Feed intake was determined as the difference between feed offered and feed remaining after 24 h. Body-weight gain was calculated as the difference between final and initial body weights.

FER = Body weight gain / Feed intake
PER = Body weight gain of test animals (g) / Weight of protein consumed (g)

G. Determination of Protein Quality Indices
Protein quality was evaluated using nitrogen-balance techniques. During the feeding trial, urine and fecal samples were collected quantitatively for nitrogen determination using the Kjeldahl method [16]. Protein intake was estimated from feed-consumption records, while nitrogen intake was calculated by multiplying protein intake by 0.16. Apparent protein digestibility (APD), nitrogen balance (NB), net

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protein utilization (NPU), biological value (BV), and net protein ratio (NPR) were determined using established equations for biological evaluation of dietary proteins.

H. Statistical Analysis

The experiment followed a completely randomized design. Data were expressed as mean ± standard deviation. Statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to determine significant differences among treatment means, and Duncan's multiple range test was employed for post hoc mean separation. Statistical significance was accepted at $p < 0.05$.

III. RESULTS AND DISCUSSION

A. Growth Performance of Albino Rats Fed Sprouted Legume-Based Diets

The growth performance of albino rats fed the experimental diets is presented in Table II. Significant differences ($p < 0.05$) were observed among the dietary treatments for feed intake, body-weight gain, feed efficiency ratio, and protein efficiency ratio, indicating that the different sprouted-legume combinations influenced nutrient utilization and growth response.

TABLE II. GROWTH PERFORMANCE OF ALBINO RATS FED SPROUTED LEGUME-BASED DIETS

Group	Feed intake (g)	Body-weight gain (g)	Feed efficiency ratio	Protein efficiency ratio
LB1	341.32	14.89	0.044	1.081
LB2	435.90	33.57	0.077	2.028
LB3	446.70	75.44	0.169	6.104
LB4	445.42	60.96	0.137	4.299
LB5	442.45	92.71	0.210	6.214
LB6	447.52	18.36	0.041	1.576
LB7	444.10	83.17	0.187	4.766
LB8	452.47	26.34	0.058	2.201
LB9	293.63	12.97	0.044	1.503
CRC	457.09	129.84	0.284	9.354

Values are mean ± SD (n = 8). Means within a column with different superscripts differ significantly ($p < 0.05$).

Feed intake ranged from 293.63 g in LB9 to 457.09 g in the control group (CRC). The significantly higher intake observed in CRC suggests superior palatability and nutrient balance compared with the experimental diets. Among the formulated diets, LB3, LB5, LB6, and LB8 showed relatively high feed intake values, whereas LB9 (100% red kidney bean flour) showed the lowest intake.

Body-weight gain varied significantly among treatments, ranging from 12.97 g in LB9 to 129.84 g in CRC. Among the experimental diets, LB5 (50% soybean and 50% red kidney bean) produced the highest body-weight gain (92.71 g), followed by LB7 (100% soybean) and LB3 (50% soybean, 30% African yam bean, and 20% red kidney bean). This pattern suggests that soybean-containing formulations provided a stronger growth response than diets based predominantly on African yam bean or red kidney bean.

Feed efficiency ratio (FER) reflects the efficiency with which consumed feed is converted into body mass. The reported FER was highest in the control group (5.96) and among experimental diets in LB5 (4.38). Lower values were observed in LB1, LB6, and LB9. The differences may reflect variation in amino-acid balance, digestibility, palatability, and residual anti-nutritional factors.

Protein efficiency ratio (PER) is an indicator of the growth-promoting capacity of dietary protein. The reported PER values ranged from 0.47 in LB1 to 5.36 in CRC. Among the experimental diets, Using the stated PER equation and the displayed mean values, LB5 had the highest derived PER (6.213), followed by LB3 (6.104) and LB7 (4.766). The apparent superiority of soybean-containing blends supports the role of soybean as a high-quality plant protein source in composite legume diets.

Overall, the growth-performance results indicate that mixed sprouted-legume diets, particularly LB5 and LB3, supported better growth than diets based predominantly on African yam bean or red kidney bean alone.

B. Protein Quality Indices of Sprouted Legume-Based Diets

The protein-quality indices of the experimental diets are presented in Table III. Significant differences ($p < 0.05$) were observed among dietary treatments for protein intake, apparent protein digestibility, nitrogen balance, net protein utilization, biological value, and net protein ratio.

Protein intake ranged from 8.63 g/rat/21 d in LB9 to 17.45 g/rat/21 d in LB7. The higher value in LB7 is consistent with the relatively high crude-protein concentration of soybean flour. However, protein intake alone does not determine protein quality; digestibility, amino-acid balance, and nitrogen retention are also important.

Apparent protein digestibility ranged from 67.61% in LB1 to 95.01% in the control group. Among the experimental diets, LB5 and LB3 had the highest reported digestibility values (84.20 and 84.11%, respectively), suggesting relatively efficient digestion and absorption of dietary protein. Residual phytates, tannins, protease inhibitors, and other anti-nutritional factors may contribute to lower digestibility in some treatments [4].

Nitrogen balance provides an estimate of dietary nitrogen retained after fecal and urinary losses. Positive values were reported for all treatments, indicating net nitrogen retention. The control group recorded the highest nitrogen balance, while LB9 had the lowest. LB5 and LB3 showed comparatively high nitrogen retention, consistent with their stronger growth responses.

TABLE III. PROTEIN QUALITY INDICES OF DIETS FED TO RAT GROUPS

Group	PI (g/rat/21 d)	N intake (g)	APD (%)	Fecal N (g)	Urinary N (g)	NB (g N)	NPU (%)	BV (%)	NPR
LB1	13.77 ± 0.01f	2.21 ± 0.01f	67.61 ± 0.01j	0.72 ± 0.01b	0.46 ± 0.01f	1.05 ± 0.01g	47.0 ± 0.01h	67.45 ± 0.00j	1.96 ± 0.01j
LB2	16.55 ± 0.01b	2.65 ± 0.01b	71.20 ± 0.01g	0.77 ± 0.01a	0.58 ± 0.01c	1.33 ± 0.01d	50.0 ± 0.01f	67.60 ± 0.01h	2.77 ± 0.01h
LB3	12.36 ± 0.01g	1.99 ± 0.01g	84.11 ± 0.01c	0.32 ± 0.01i	0.51 ± 0.01e	1.17 ± 0.01f	59.0 ± 0.01b	69.74 ± 0.01b	7.09 ± 0.01b
LB4	14.18 ± 0.01d	2.27 ± 0.01d	77.91 ± 0.01e	0.51 ± 0.01f	0.54 ± 0.01d	1.25 ± 0.01e	55.0 ± 0.01d	69.43 ± 0.01e	5.16 ± 0.01e
LB5	14.92 ± 0.01c	2.40 ± 0.01c	84.20 ± 0.01b	0.39 ± 0.01h	0.61 ± 0.01b	1.42 ± 0.01c	59.0 ± 0.01b	69.68 ± 0.00c	7.02 ± 0.01c
LB6	11.65 ± 0.01i	1.86 ± 0.01i	69.91 ± 0.01h	0.57 ± 0.01c	0.40 ± 0.01h	0.92 ± 0.01i	49.0 ± 0.01g	67.50 ± 0.00i	2.61 ± 0.01i
LB7	17.45 ± 0.01a	2.80 ± 0.01a	79.90 ± 0.01d	0.56 ± 0.01d	0.68 ± 0.01a	1.57 ± 0.01b	56.0 ± 0.01c	69.49 ± 0.01d	5.46 ± 0.01d
LB8	11.97 ± 0.01h	1.93 ± 0.01h	72.01 ± 0.01f	0.55 ± 0.01e	0.42 ± 0.01g	0.98 ± 0.01h	51.0 ± 0.01e	67.96 ± 0.00f	3.21 ± 0.01f
LB9	8.63 ± 0.01j	1.38 ± 0.01j	68.11 ± 0.01i	0.45 ± 0.01g	0.28 ± 0.01i	0.66 ± 0.01j	49.0 ± 0.01g	67.82 ± 0.00g	2.90 ± 0.01g
CRC	13.88 ± 0.01e	2.23 ± 0.01e	95.01 ± 0.01a	0.12 ± 0.01j	0.54 ± 0.01d	1.58 ± 0.01a	71.0 ± 0.01a	70.03 ± 0.01a	10.23 ± 0.01a
LSD	0.06	0.003	0.41	0.001	0.01	0.004	0.01	0.04	0.10

Net protein utilization (NPU) and biological value (BV) indicate the proportion of absorbed or consumed nitrogen retained for body maintenance and growth. The control diet recorded the highest

NPU and BV values. Among the experimental diets, LB3 and LB5 showed comparatively high values, suggesting efficient retention of absorbed nitrogen. The observed response may reflect complementary amino-acid profiles among the legumes.

Net protein ratio (NPR) integrates growth response and protein utilization. The control group recorded the highest reported NPR value, while LB3 and LB5 were the strongest experimental formulations. The comparatively lower NPR values in LB1, LB6, and LB9 indicate poorer biological performance under the conditions of the study.

IV. CONCLUSION

The experimental diets supported varying degrees of feed intake, body-weight gain, protein utilization, and nitrogen retention, indicating that legume composition and processing influenced the biological response to dietary protein. Among the experimental diets, LB5 (50:0:50; soybean:African yam bean:red kidney bean) showed the strongest overall growth response and several of the highest protein-quality indices. The findings support further investigation of sprouted soybean–red kidney bean and other selected multi-legume blends as potential plant-protein ingredients for food-product development.

However, the numerical relationships among feed intake, body-weight gain, FER, PER, and protein intake require verification against the original laboratory calculations before the manuscript is submitted. Future studies should also evaluate amino-acid composition, PDCAAS or DIAAS, and consumer acceptability of products developed from optimized blends.

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