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Biotechnological Effect of Fermented Mung Beans (*Vigna Radiata*) Flour and Its Protein Quality in Wistar Rats

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Abstract

Fermentation with inclusion of organisms has been shown to improve the nutritional quality of food. This work was designed to investigate the effect of fermented mung beans (*Vigina radiata*) flour and protein quality in Wistar rats. The seed of *V. radiata* was sorted, washed, sun-dried, and later aseptically pulverized. The flour was then divided into seven portions of 500 g each and put in sterile containers. The six portions of flour were subjected to natural and induced fermentation for 4 days, using naturally occurring bacteria singly as a starter culture at 28 ±2°C, while the unfermented portion served as a control. The product of fermentation was used to determine the nutritional quality of the unfermented and fermented mung beans flour by studying the growth weight of visceral organs and body weight of rats and nitrogen retention of animals in various tissues of the internal organs, faeces and urine and protein quality of experimental animals fed. The growth weight of visceral organs and body weight of rats in the kidney (g) ranged from 17.52 ±0.62 to 23.07 ±0.78, liver (g) from 85.77 ±0.30 to 117.52 ±0.50 while muscle (g) ranged from 68.99 ±0.10 to 134.56 ±0.47. Nitrogen retention of animals fed with unfermented and fermented mung beans flour in kidney (g) 13.45 ±0.02 to 50.34 ±0.01, liver (g) 12.44 ±0.01 to 50.38 ±0.02, muscle (g) 12.89 ±0.01 to 50.55 ±0.01, faeces (g) 0.36 ±0.01 to 0.89 ±0.01 while urine 0.35 ±0.01 to 0.84 ±0.02. The protein quality of experimental animals fed in Biological Value (BV) ranged from 0.00 to 70.67 ±1.15, Net Protein Utilization (NPU) 0.00 to 76.33 ±1.53, Protein Efficiency Ratio (PER) 0.00 to 2.27 ±0.15, Net Protein Ratio (NPR) 0.00 to 3.47 ±0.11 while gained/loss ranged from -10.59 ±0.01 to 30.88 ±5.76. The fermentation increase quality of nutrient present in mung beans and fermented mung beans flour may promote the weight of internal organs of Wistar rats.

Keywords: Mung beans flour, fermentation, protein quality
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Introduction

Cowpea is the legume of choice for most Nigerian women mainly because of the relatively short cooking time, swelling capacity, tastes, and colour. Mung bean seed compares favourably with cowpea, however, it is not a legume of choice because of the long cooking time and low digestibility due to high fiber content (Odedeji et al., 2017).

There are thousands of known legume species, however, less than 20 are used extensively as food. Those in common use include peanuts, soya beans, broad beans, bambara groundnuts, peas, lentils, pigeon peas, chickpeas, mung beans, kidney beans, and cowpeas. Most of these have also been extensively studied. Examples of other nutritious but less commonly used legumes include Marama bean, and jackbean (Lawal, 2021; Christenhusz, and Byng, 2016).

Cowpeas contain approximately 25 percent protein (Annor et al., 2010). Additionally, legumes are exceptional energy sources of

complicated carbs because a few scientists have discovered (Hu, 2003; Legumes, 2003). Gallaher and (Jacobs, 2004) to become useful for aerobic illnesses as well as diabetic issues, quite possibly because of substantial quantities of water-soluble fibre as well as a top awareness of phenolics. (Enujiugha, 2010)

There are three subgroups of *Vigna radiata*: One of them developed (*Vigna radiata* subsp. Radiata as well as 2 crazy (Vigna radiata subsp. Sublobata and Vigna radiata subsp. Glabra (Vivid kinds have a tendency to be prostrate, while grown kinds tend to be more erect. Grown styles are golden or green usually, along with regards to the dynamics of a covering or maybe feel, glossy or dark (Godwin and Lambrides, 2006).

Legumes are better than grains as a source of micronutrients (Welch et al., 2000; Broughton et al., 2003) primarily because legumes have a higher initial mineral content, and secondly because many grains are polished before eating.

The use of fermentation in the processing of some grains and legumes in supplementary meals results in reduction anti-nutrients and an increase in product safety. Fermentation technology is being used to create a wide range of foods at home, on small, medium, and large commercial scales across the world (Lawal, 2021). Fermentation generates unique tastes, changes the textural/rheological characteristics, and enhances them. According to Lawal et al. (2009), fermentation improves the digestibility and nutritional content of milk.

Persistent toxicity may be the improvement of negative effects because of long-range contact with another stressor or a toxicant. It can occur as immediate deadly endpoints, but much more normally describes sub lethal endpoints like reduced development, decreased reproduction, or maybe behavioral alterations like influenced shifting efficiency (Di Toro et al. 2001). The target on this analysis is determining the expansion mass of visceral organs, nitrogen retention as well as protein-rich quality of mung beans flour given Wistar rats.

Materials and Methods

Sample Collections

Vigna radiata seeds used for this research were purchased from Irepodun market, Ekinrinade, Ijumu Local Government Area, Kogi State, *Saccharomyces cerevisiae* was collected from the international brewery, Ilesha, Osun State, Nigeria.

Preparation of Sample Vigna radiata Seed

The *Vigna radiata* seedswere sorted, washed, and sun-dried. They were pulverized using a blender. Pulverized *V. radiata* seed flourwas divided into portions of 500 g each and put in plastic containers.

Fermentation of Vigna radiata Flour

The containers with the pulverized *V. radiata* seed flour were divided into seven portions. Two (2) fermentation methods were employed; traditional and induced culture fermentation using plastic containers. A suspension of mung beans flour was prepared by adding 1500 ml of distilled water to one of the 500 g portions of mung beans flour in a clean plastic fermentor and covered for 4 days at room temperature to ferment naturally with indigenous microflora. A suspension inoculated with *S. cerevisiae* obtainedfrom the international brewery, Ilesha, Osun State, Nigeria served as positive control while an uninoculated sterile suspension served as a negative control. All the fermented mung beans flour was kept in airtight containers over a silica gel in desiccators and dried using an electric oven at 50 ^{o}C for 18 hours and the dried samples were used for further analysis (Lawal, 2021).

Growth Performance of Wistar Rats Fed with Unfermented, Fermented Mung Beans Flour and Commercial feed

Forty-eight (48) weaned albino rats were obtained from the College of Health Science Animal Breeding Centre, Obafemi Awolowo University, Ile-Ife, Nigeria. Albino rats were weighed and randomly distributed into metabolic cells. Weight and age range from 45.00 g to 47.43 g and ages 4-6 weeks respectively. Albino rats were housed in digestive cells fixed with a feeding plate and a small plastic bottle for feeding *ad libitum*. The animals were then re-weighed and grouped randomly into eight of six rats per group. The groups, one to eight (1 – 8) weights were not significantly different at the beginning of the study. Groups (1 - 8) were placed on experimental diets for 28 days. They were provided with the observed amount of each experimental diet that delivered libitum in the feeding dish and water through a plastic bottle

attached to the cage. The daily feeding with the unfermented and fermented mung beans flour was carefully recorded and the weight was noted. The weight of each experimental animal was determined every three days using a weighing scale. The live weight of the rats and some organs (kidney, liver, and muscle) were monitored after sacrifice (Ibironke, 2014).

Weight of visceral organs and bodyweight of Wistar rats

Bodyweight (g) = weight of visceral organs and body weight (g)
Weight of the internal organs

Bioassay calculations
The food efficiency ratio (FER), = Gain in body weight (g)
Food intake (g)
Protein efficiency ratio (PER) = Weight gain of test animal (g)
Protein consumed by the test animal (g)
Net protein retention (NPR) = Weight gain of test animal (g)+Average Weight Loss of Animal
Protein consumed by the test animal (g)

Protein retention efficiency (PRE) =NPR×16
The feed conversion ratio was determined by= Feed Consumed (g)

Gain in body weight (g)

Websites protein-rich utilization, or maybe NPU, could be the ratio of amino acids changed to protein-rich foods towards the ratio of amino acids furnished. This particular figure would be to a few degrees affected by the digestibility of crucial amino acids within the body but is clearly affected through the level of restriction of amino acids in meals. It is utilized as a degree of protein-rich quality for man food requirements. NPU values can selected through zero to one (or maybe hundred), having an importance of one (or maybe hundred) indicating the usage of hundred % nutritional nitrogen as a protein, along with an importance of zero indicating that absolutely no availability of nitrogen is changed into protein rich food.

Certain foodstuffs, such as eggs or milk, rate as 1 on an NPU chart.

Experimentally, this value can be determined by evaluatingdietary protein intake and then measuring nitrogenexcretion. One Formula for NPU is

NPU = ((0.16 × (24 hour protein intake in grams)) - ((24 hour urinary urea nitrogen) + 2) - (0.1 × (ideal body weight in kilograms) / (0.16 × (24 hour protein intake in grams))

Ethical Consideration of the Study

This study was approved by the Joint Ethical Review Committee of the Obafemi Awolowo University, Ile- Ife, Osun State, Nigeria, and Kwara State University Research Council Malete, Kwara State, Nigeria.

Statistical Analysis of Data Obtained in the Study
The experiments were conducted in a completely randomized design with three replicates. All data were first subjected to analysis of variance (ANOVA), after checking their normality and later to LSD and Turkey test to determine significant differences among the significant treatments at $p<0.05$. Statistical analyses were carried out using IBM SPSS 24.0 (SPSS Science, Chicago, IL, USA).

Results

Weight of Visceral Organs and Body Weight of Experimental Animals Fed with Unfermented, Fermented Mung Beans Flours and Commercial Feed

The weight of visceral organs and body weight of experimental animals fed with unfermented, fermented mung beans flour and commercial feed are shown in Table 1. The weight of kidneys of experimental animals fed with fermented mung beans flours ranged from 17.52 ±0.62 to 23.07 ±0.78 g, while the weight of kidneys and experimental animals fed with unfermented mung beans flour was 19.80 ±0.29 g. Liver weight of experimental animals fed with fermented mung beans flours and commercial feed (85.77 ±0.30 and 116.65 ±0.27 g) respectively. These values were lower than the value recorded for the liver of visceral organ of experimental animals fed with unfermented mung beans flour (117.52 ±0.50 g). The muscle visceral organ and body weight of experimental animals fed with fermented mung beans flours ranged from 112.02 ±0.16 to 134.56 ±0.47 g which were higher compared with the muscle weight of

experimental animals fed with unfermented mung beans flour (68.99 ±0.10 g).

The Nitrogen Retention of Experimental Animals in various Tissues of the Internal Organs, Feaces, and Urine Fed with Fermented, Unfermented Mung Beans Flour and Commercial Feed

The nitrogen retention in various tissues of the internal organs, feaces, and urine of experimental animals fed with fermented, unfermented mung beans flour and commercial feed is shown in Table 2. The nitrogen retention in the kidney of animals fed with fermented mung beans flours (20.36 ±0.01 to 50.34 ±0.01) were higher than nitrogen retention in the kidney of animals fed with unfermented mung beans flour (13.45 ±0.02). Nitrogen retention in the liver of animals fed with unfermented mung beans flour (12.44 ±0.01) was lower than the nitrogen retention in the liver of animals fed with unfermented mung beans flour and commercial feed (14.53 ±0.02 to 80.26 ±0.02). The nitrogen retention in the muscle of animals fed with unfermented, fermented mung beans flours and commercial feed were as follows; (12.89 ±0.01, 32.54 ±0.02, 50.55 ±0.01, 48.44 ±0.01, 14.88 ±0.02, 36.49 ±0.01, 20.36 ±0.02 and 80.31 ±0.01) respectively. Nitrogen retention in faeces of animals fed with fermented mung beans flours ranged from 0.36 ±0.01 to 0.89 ±0.01, while the nitrogen retention in faeces of animals fed with unfermented mung beans flour was 0.56 ±0.06. The nitrogen retention in urine of animals fed with fermented mung beans flours ranged from 0.35 ±0.01 to 0.84 ±0.02, and nitrogen retention in urine of animals fed with unfermented mung beans flour was 0.57 ±0.01.

Table 1: Weight of Visceral Organs and Body Weight of Animals Fed with Unfermented, Fermented Mung Beans and Commercial Feed in Grams

Groups	UFMF	FMCI	FMLS	FMST	FMBO	FMLM	FMSC	CF
Kidney (g)	19.80±0.29[a]	18.82±0.51[b]	20.93±0.33[c]	20.85±0.27[d]	21.71±0.62[e]	23.07±0.78[f]	17.52±0.62[e]	17.07±0.16[g]
Liver (g)	117.52±0.50[a]	100.99±0.10[b]	107.70±0.64[c]	104.99±0.11[d]	109.59±0.11[d]	116.65±0.27[e]	85.77±0.30[f]	85.77±0.38[g]
Muscle (g)	68.99±0.10[a]	114.60±0.29[b]	134.56±0.47[c]	126.52±0.60[d]	101.31±0.33[e]	112.02±0.16[f]	113.69±0.28[g]	113.59±0.34[h]

Values are expressed as mean ± standard deviation. Data having different superscripts across the row are significantly different ($p < 0.05$).

Key: Sample UFMF-Rats fed unfermented mung beans flour
FMCI- Rats fed fermented mung beans flour by chance inoculation
FMLS- Rats fed fermented mung beans flour with *Lysinibacillussphaericus* starter
FMST- Rats fed fermented mung beans flour with *Streptococcus thermophilus* starter
FMBO- Rats fed fermented mung beans flour with *Brevundomonas olei* starter
FMLM- Rats fed fermented mung beans flour with *Lysinibacillus mangiferihumi* starter
FMSC-Rats fed fermented mung beans flour with *Saccharomyces cerevisiae* starter
CF- Rats fed Commercial feed

Protein Quality of Experimental Animals Fed with Unfermented, Fermented Mung Beans Flour and Commercial Feed

The protein quality of experimental animals fed with unfermented, fermented mung beans flour and commercial feed is shown in Table 3. The Biological Value (BV) of protein quality of the animals fed with fermented mung beans flour 22.00 ±1.00 to 70.00 ±1.00 were lower than the BV of protein quality of the animal fed with commercial feed (76.67 ±2.08) while BV of protein quality of the animal fed with unfermented recorded zero. Net Protein Utilization (NPU) of protein quality of the animals fed with unfermented mung beans flour recorded zero and (NPU) of protein quality of the animals fed with fermented mung beans flours ranged from 23.33 ±0.58 to 76.33 ±1.53 while NPU of protein quality of the animals fed with commercial feed was 73.33 ±1.53.

The Protein Efficiency Ratio (PER) of the protein quality of the animals fed with fermented mung beans flours ranged from 0.60 ±0.10 to 2.27 ±0.15, while the PER of protein quality of the animals fed with commercial feed had a higher value of 3.11 ±0.02 and protein quality of the animals fed with unfermented mung beans flour was zero. The Net Protein Ratio (NPR) of protein quality of the animals fed with commercial feed had a higher value (3.80 ±0.10) than the NPR of protein quality of animals fed with fermented mung beans flours (1.70 ±0.17 to 3.47 ±0.11) while protein quality of the animals fed with unfermented mung beans flour was zero. The gain/loss of protein quality of animals fed with fermented mung beans flours (6.33 ±0.04 to 30.88 ±5.76) were higher than the gain/loss of protein quality of animals fed with unfermented mung beans flour (-10.59 ±0.01).

Table 2: The Nitrogen Retention of Animals in various Tissues of the Internal Organs, Faeces, and Urine Fed with Unfermented, Fermented Mung Beans Flour and Commercial Feed

Groups	UFMF	FMCI	FMLS	FMST	FMBO	FMLM	FMSC	CF
Kidney (g)	13.45±0.02^{a}	32.36±0.02^{a}	50.34±0.01^{b}	48.39±0.04^{c}	15.43±0.02^{a}	34.54±0.01^{b}	20.36±0.01^{b}	80.34±0.02^{a}
Liver (g)	12.44±0.01^{a}	32.58±0.01^{a}	50.38±0.02^{b}	48.33±0.01^{a}	14.53±0.02^{b}	36.36±0.02^{b}	20.56±0.04^{c}	80.26±0.02^{b}
Muscle (g)	12.89±0.01^{a}	32.54±0.02^{b}	50.55±0.01^{a}	48.44±0.01^{a}	14.88±0.02^{b}	36.49±0.01^{a}	20.36±0.02^{b}	80.31±0.01^{a}
Faeces	0.56±0.06^{a}	0.55±0.11^{b}	0.44±0.02^{c}	0.38±0.01^{d}	0.54±0.02^{c}	0.36±0.01^{d}	0.89±0.01^{d}	0.85±0.01^{d}
Urine	0.57±0.01^{a}	0.43±0.02^{b}	0.45±0.02^{b}	0.36±0.01^{a}	0.58±0.01^{a}	0.35±0.01^{a}	0.84±0.02^{b}	0.84±0.02^{b}

Values are expressed as mean ± standard deviation. Data having different superscripts across the row are significantly different ($p < 0.05$).

Key: Sample UFMF-Rats fed unfermented mung beans flour
FMCI- Rats fed fermented mung beans flour by chance inoculation
FMLS- Rats fed fermented mung beans flour with *Lysinibacillussphaericus* starter
FMST- Rats fed fermented mung beans flour with *Streptococcus thermophilus* starter
FMBO- Rats fed fermented mung beans flour with *Brevundomonas olei* starter
FMLM- Rats fed fermented mung beans flour with *Lysinibacillus mangiferihumi* starter
FMSC-Rats fed fermented mung beans flour with *Saccharomyces cerevisiae* starter
CF- Rats fed Commercial feed

Table 3: Bioassay of the Experimental Animal Fed with Unfermented, Fermented Mung Beans and Commercial Feed

Groups	UFMF	FMCI	FMLS	FMST	FMBO	FMLM	FMSC	CF
BV	0.00	59.00±1.00[a]	70.00±1.00[b]	70.67±1.15[c]	22.00±1.00[d]	36.67±1.53[e]	28.00±2.00[f]	76.67±2.08[g]
NPU	0.00	60.67±1.53[a]	76.33±1.53 [b]	74.00±1.00[c]	23.33±0.58[d]	39.33±1.53[f]	31.67±6.43[g]	73.33±1.53[h]
PER	0.00	1.30±0.10 [a]	2.27±0.15 [b]	2.00±0.10 [a]	0.60±0.01 [c]	1.47±0.11[d]	1.30±0.10 [a]	3.11±0.02 [e]
NPR	0.00	2.27±0.06 [a]	3.47±0.11 [b]	3.23±0.15[c]	1.70±0.17[d]	2.30±0.10[e]	2.27±0.06 [f]	3.80±0.10 [e]
Gained/loss	-10.59±0.01 [a]	13.71±0.02 [b]	30.88±5.76 [c]	21.05±0.03 [d]	6.33±0.04 [e]	14.13±0.02[f]	13.33±0.58[g]	31.90±0.03[d]

Values are expressed as mean ± standard deviation. Data having different superscripts across the row are significantly different ($p < 0.05$).

Key: Sample UFMF- Rats fed unfermented mung beans flour

FMCI- Rast fed fermented mung beans flour by chance inoculation

FMLS- Rats fed fermented mung beans flour with *Lysinibacillussphaericus* starter

FMST- Rats fed fermented mung beans flour with *Streptococcus thermophilus* starter

FMBO- Rats fed fermented mung beans flour with *Brevundomonas olei* starter

FMLM- Rats fed fermented mung beans flour with *Lysinibacillus mangiferihumi* starter

FMSC-Rats fed fermented mung beans flour with *Saccharomyces cerevisiae* starter

CF- Rats fed Commercial feed

Discussion

The weight of visceral organs and body weight of animals showed that the values obtained for animals fed with unfermented mung beans flour, fermented mung beans flour with chance inoculation, *Saccharomyces cerevisae* and commercial feed the kidney were lower than those of animals fed with mung beans flour fermented with *Lysinibacillus sphaericus*, *Streptococcus thermophilus*, *Brevundomonas olei* and *Lysinibacillus mangiferihumi*. The livers of animals fed with unfermented mung beans flour, fermented mung beans flour with chance inoculation, *Lysinibacillus sphaericus*, *Streptococcus thermophilus*, *Brevundomonas olei* and *Lysinibacillus mangiferihumi* were higher than that of the animals fed with mung beans flour fermented with *Saccharomyces cerevisae* and commercial feed. The muscle of animals fed with unfermented mung beans flour was, however; lower than the animals fed with fermented mung beans flour with chance inoculation, *Lysinibacillus sphaericus*, *Streptococcus thermophilus*, *Brevundomonas olei,* and mung bean flour fermented with *Lysinibacillus mangiferihumi*, *Saccharomyces cerevisae,* and commercial feed. It is contrary to the work of Ajani *et al*. (2014) and Ewuola *et al*. (2015).

The nitrogen retention of creatures given with Mung Bean flour fermented with commercial feed and *Lysinibacillus sphaericus* displayed the top values within the nitrogen retention wearing different tissue cells of inner organs. The greatest nitrogen retention was captured for creatures fed business feed, and then creatures given with Mung beans flour fermented with *Lysinibacillus sphaericus*. This might be because of the higher protein-rich proportion of fermented mung beans flours, and it is akin to Ewuola *et al* (2015) article as well as Ibironke *et al* (2016). The animal's tissue stores enough nitrogen that could promote growth and carry out other daily activities, however animals fed with unfermented mung beans flour was lower in nitrogen retention compared to the animals fed with fermented mung beans flour, this may be because of a lack of quality protein in animals fed with unfermented mung beans flour (Oloyede *et al*., 2011).

The protein quality of animals fed with unfermented mung beans flour, fermented mung beans flour of chance inoculation, fermented mung beans flour inoculated with *Lysinibacillus sphaericus*, fermented mung beans flour inoculated with *Streptococcus thermophilus*, fermented mung beans flour inoculated with *Brevundomonas olei*, fermented mung beans flour inoculated with *Lysinibacillus mangiferihumi* and fermented mung beans flour inoculated with *Saccharomyces cerevisiae* of Biological Value (BV), Net Protein Utilization (NPU), Protein Efficiency Ratio (PER), and Net Protein Retention (NPR) respectively. The protein quality results of the animals fed with unfermented mung beans flour, fermented mung beans flour of chance inoculation, fermented mung beans flour inoculated with *Lysinibacillus sphaericus*, fermented mung beans flour inoculated with *Streptococcus thermophilus*, fermented mung beans flour inoculated with *Brevundomonas olei*, fermented mung beans flour inoculated with *Lysinibacillus mangiferihumi* and fermented mung beans flour inoculated with *Saccharomyces cerevisiae* obtained in this study corroborated the results earlier reported by Adegunwa *et al.* (2012).

Conclusions

The research work established the effect of fermentation and nutrient content of mung bean (*Vigna radiata*) seed flour. The changes in body weight and internal organs of rats were observed during the feeding and after the sacrifice of experimental animals. The animals fed with fermented mung beans flour retained high nitrogen retention and protein quality.

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