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Effects of Fermented Mung Bean IRU on Experimental Animals and its Toxicology Implications

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Abstract: The impact of fermentation on the nutritional and vitamin content of fermented mung bean 'iru' as a protein condiment is being investigated. The mung bean was processed into mung bean 'iru' using the local method of producing 'iru' from African locust beans. The mung bean underwent sorting, washing, and boiling for 1 hour. Following the hour-long boiling process, the seeds were dehulled to eliminate the seed coat, and subsequently, they were cleaned. After draining the water and spreading the seed on a sack bag to cool, the mixture was wrapped in enough Musa sapientum banana leaves and placed inside a cleaned plastic container to ferment for five days at room temperature in a warmed environment. For chemical analysis and albino rat feeding, mung bean "iru" was dried in an electric oven set to 50°C for eighteen hours. The mineral and vitamin analysis of fermented mung bean iru was carried out using standard analytical methods. Standard procedures were used for the feeding of experimental animals. The results showed that mineral and vitamin parameters were as follows: Na 48.10, K 18.36, Mg 15.60, Ca 23.00, and Fe 24.20 mg/g, while vitamin A 481.97 and B 0.38 units/g. The albino rats fed fermented mung bean iru were in line with commercial feed in growth response. The histoarchitecture of the liver had no negative effect in rats fed fermented mung bean iru. The fermented mung bean iru shows that it has haematinics properties.

Keywords: Nutrient, Haematology, Histology

Introduction

Numerous biological activity found in mung beans help to prevent sickness in people. The usage of mung beans in Asian cuisine has long been thought to be a healthful ingredient that boosts vitality and delays aging (Lawal, 2021). After germination, the mung bean becomes increasingly richer in metabolites and activity. The beans are utilized as functional food supplements in conjunction with human diets based on cereal because they are an excellent source of protein and amino acids, particularly lysine (Van-Horn and Ernst, 2011). It is also a very high source of copper, manganese, vitamin K, riboflavin, folate, vitamin C, and dietary fiber. Additionally, according to Lawal and Awe (2020), it is a good source of magnesium, iron, potassium, phosphorus, thiamin, niacin, vitamin B6, and pantothenic acid. It is exceptionally low in saturated fat and sodium, and it has very little cholesterol. Lowering blood cholesterol can be aided by soluble fiber. The key components of mung beans' antioxidative, anti-inflammatory, antibacterial, and anticancer properties are believed to be their high quantities of amino acids, proteins, polyphenols, and oligosaccharides. These components also help to regulate lipid metabolism (Tang et al., 2014).

In addition to its well-known detoxifying properties, mung beans (*Vignaradiata*) are used to relieve heat stroke, improve mental clarity, and lessen summertime swelling. It was found to be helpful in controlling the growth of vigna sprouts, which are popular in western and Indian countries and consumed as a fresh salad vegetable. When consumed as food, they provide a good supply of bioactive phytochemicals together with well-balanced nutrients such dietary fiber and protein (Edwards et al., 2006).

Carbs can be transformed into alcohol, carbon dioxide, or other acids through the metabolic process of fermentation. Through the anaerobic process of fermentation, glucose is converted to energy without the need for oxygen. Yeast cells undergo fermentation in order to produce alcohol and energy from the conversion of sugar. In fermentation, bacteria also play a role by converting carbohydrates to lactic acid (Lawal et al., 2023).

The dietary requirements of laboratory rats are better understood than those of other laboratory animals; yet, depending on the parameters used, there may be a substantial variance in the requirements that are projected (Baker, 2016). For example, the amounts of nutrients required to enhance functional metrics like enzyme synthesis or to maintain tissue concentrations may be different from those needed to support optimal development in young rats. Moreover, age, reproductive activity, and developmental stage all affect nutritional needs.

The demands of different inbred and outbred strains, as well as those of males and females, differ significantly. Although the dietary requirements outlined in this chapter are averages, they might not always be adequate. Further research is required to identify the causes of the variation in nutritional demand (Baker, 2016). Preparing mung beans, or "iru," testing for minerals and vitamins, and evaluating the growth performance of Wistar albino rats are the goals of this study project.

Materials and Methods

Purchases and processing of mung bean sample collections were made shortly after at Irepodun Market in Ekinrinade, (Ijumu Local Government Area), Kogi State, Nigeria.

Mung beans are processed to create "Iru."

The procedure outlined by Lawal et al. (2023) was the model for the indigenous "iru" method of processing mung beans. Mung beans weighing two hundred and fifty grams (250 g) were sorted, cleaned, and then cooked for one hour. After the seed was cooked for an hour, it was dehulled to remove the seed coat and then rinsed. After draining the water and letting it cool on a sack bag, it was wrapped in banana leaves (*Musa sapientum*), placed inside a clean plastic container, and let to ferment for five days at room temperature. The mung bean "iru" was utilized for chemical analysis and feeding of experimental animals after being dried for eighteen hours at 50°C in an electric oven.

Mung beans



Boiled for one hour



Allowed to cooled



Dehulled



Boil for another one hour



Spread on a sack bag to cooled



Wrapped with enough banana leaves



Pack in a cleaned container, fermented for five days in a warmed place



Iru

Figure 1: A flow chart for the production processes of Mung beans 'Iru'

Mineral Analysis of Mung Beans, Iru

While P was evaluated using a UV-visible spectrophotometer after the ammonium vanadate-molybdate complex was made at 436 nm using the established protocols of Ojokoh et al. (2012), an atomic absorption spectrophotometer (AAS) was used to determine the nutritionally essential elements Na, Ca, Mg, and K.

Determination of Vitamin Content of Mung Beans, Iru

The following was determined about the vitamin content of the chickpea flour: The Association of Vitamin Chemists' methodologies were used to determine the vitamins present in the fresh mung beans (AOAC, 2016). The Ojokoh et al. (2012) spectrophotometer method was utilized to assess the levels of vitamins A and B.

Animal Experimentation Procedure

The Ibrinke (2017) and Ibrinke (2014) methodology was used. We acquired twenty Wistar albino rats from ObafemiAwolowo University's pharmacy faculty in Ile-Ife, Osun State, Nigeria. The white albino rats were between three and six weeks old, weighing between sixty and seventy grams. The test animals were divided into two groups—typically with one animal per group—and weighed at random before being kept in a metabolic cage. For seven days, they were given animal chow to help them get used to their new surroundings. For a duration of 28 days, the experimental animals were fed the experimental meals. Ad libitum food and water were given to the experimental animal. The experimental animals' daily feed intake was monitored throughout the trial, and every three days, their weights were measured. Seven days afterwards the conclusion of the experiment, the urine and feces of each group of experimental animals were collected independently. Before analysis, each group's urine was preserved in a container with 6NHCl. On the other hand, the excrement was dried for 12 hours at 60°C in an oven, cooled, weighed, and then sealed in polythene bags. The animals were weighed, sedated, and slaughtered after the 28 day period. Samples of tissue were taken, including those from the kidney, liver, and plantaris muscles. The microKjeldahl method was used to measure the nitrogen levels in the urine and feces (AOAC, 2010; DasS, 2011). The animal's organs, which included the liver, kidney, and heart, were promptly preserved in 10% formyl saline in preparation for more research on nitrogen retention (Ibrinke, 2024).

Haematological Analysis

A heart puncture was used to get the blood sample, which was then placed into a test tube with an anticoagulant (EDTA). The blood was submitted to the lab so that an automated counter using an Abbott Cell-Dyn 1700 automatic analyzer could process it right away. The blood was put in a cabinet inside the analyzer after being thoroughly mixed but not shook. The device contains apertures for the analysis of various components in the blood, flow cells, and photometers. The components of the cell count identify the different types and numbers of blood cells. The following variables were recorded: WBC(10^3 /UL), LYM(%), MON(%), GRAN(%), LYM($\#^3$ /UL), MON($\#^3$ /UL), GRAN($\#^3$ /UL), RBC(6^6 /UL), HCT(%), MCV(fl), MCH(pg), MCHC(g/dl), RDW(-SDfl), RDW(-CV%), PLT(10^3 /UL), MPV(fl), PDW(%), PCT(%), and P.LCR(%). It printed the results (Lawal et al., 2021).

Bioassay Calculations

This method was used to compute the food efficiency ratio (FER), protein efficiency ratio (PER), net protein retention (NPR), and protein retention efficiency (PRE):

$$\text{The food Efficiency Ratio} = \frac{\text{Gain in body weight(g)}}{\text{Food intake (g)}}$$

$$\text{Protein Efficiency Ratio} = \frac{\text{Weight gain of test animal (g)}}{\text{Protein consumed by the test animal (g)}}$$

$$\text{Net Protein Retention} = \frac{\text{Weight gain of test animal (g)} + \text{Average weight loss of animal}}{\text{Protein consumed by the test animal}}$$

Using method of Ibrinke (2024).

Histopathology of the Liver

Tissue was fixed, dehydrated, cleared, infiltrated, embedded, sectioned, and stained with haematoxylin and eosin (Awe et al., 2013). The slides were then mounted with Canada balsam as the mountant, and viewed under a Leitz microscope, with photomicrographs obtained and examined.

The Study's Ethical Considerations

The Joint Ethical Review Committee of ObafemiAwolowo University, Ile-Ife, Osun State, Nigeria, and the University of Ilesa, Ilesa, Osun State, Nigeria, Research Council gave their approval for this work.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was used in SPSS 17 for Windows to analyze the data. Significant differences at $p < 0.05$ were found using Duncan's Multiple Range Test (DMRT).

Results

Mineral Composition of Mung Beans Iru

The mineral results of the fermented mung bean Iru were as follows in Table 1: sodium 48.10, potassium 18.36, magnesium 15.60, calcium 23.00, and iron 24.20 mg/g.

Table 1: Mineral analysis of mung beansiru (mg/g)

Parameters	Values
Sodium (Na)	48.10 \pm 0.00
Potassium (K)	18.36 \pm 0.00
Magnesium (Mg)	15.60 \pm 0.00
Calcium (Ca)	23.00 \pm 0.00
Iron (Fe)	24.20 \pm 0.01

The data is displayed as the average \pm standard deviation. A row of separate superscripts indicates a significant difference in the data ($p < 0.05$).

Vitamin analysis of Mung Beans Iru

The results of vitamin A and vitamin B shown in Table 2 were 481.97 and 0.38 (unit/g).

Table 2: Vitamin Analysis of Mung Beans Iru (unit/g)

Parameters	Values
Vitamin A	481.97 \pm 0.00
Vitamin B	0.38 \pm 0.00

The data is displayed as the average \pm standard deviation. A row of separate superscripts indicates a significant difference in the data ($p < 0.05$).

The Growth Response of the Experimental Animals during the Feeding Period

The growth responses of the experimental rats given a commercial diet and fermented mung beans (Iru) are displayed in Figure 1. The test animals fared well

overall. The animals fed fermented mung beans (Iru) gained weight more favourably than those fed commercial diet.

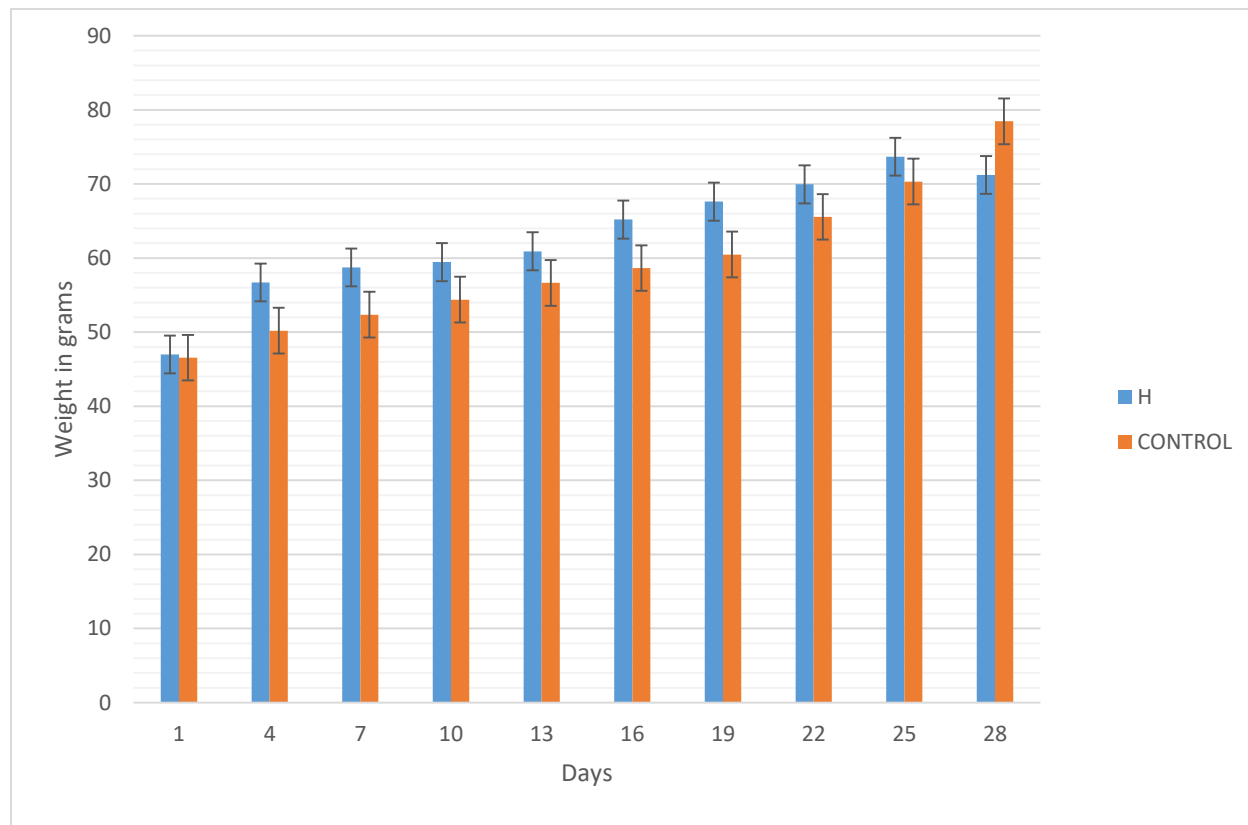


Figure 1: Growth response histogram for groups of albino rat fed with fermented mung bean iru for 28 days

Key: H- Fermented mung bean iru
Control- Commercial feed

Haematological Study of White blood cell Parameters of Albino Rats Fed with Fermented Mung Beans Iru and Commercial Feed

Figure 2: Haematological analysis of white blood cell parameters in albino rats fed commercial diet and fermented mung beans (Iru). The haematological analysis of WBC ($10^3/\mu\text{l}$) in experimental animals fed commercial feed and fermented mung beans Iru. The fermented mung bean Iru had higher value compared with commercial feed. The LYM (%) of fermented mung beans Iru was higher than the commercial feed/ MON (%) of fermented mung beans Iru and commercial feed were almost the same the value. The GRAN (%) of fermented mung beans Iru was lower compared with the value obtained for commercial feed. LYM# $\times 10^3/\mu\text{L}$ of fermented mung beans Iru had higher value compared with commercial feed value. The MON# $\times 10^3/\mu\text{L}$ of fermented mung beans Iru and commercial were the same while

the GRAN #3/μL of fermented mung beans Iru compared favourably with commercial feed.

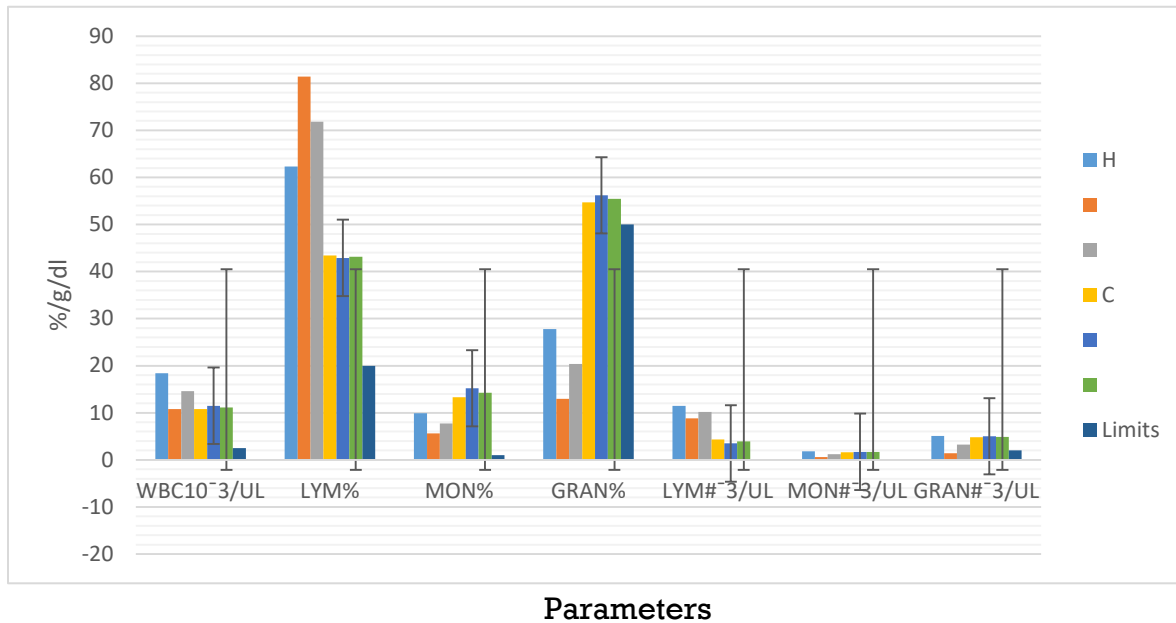


Figure 2: Haematological study White blood cell parameters of experimental albino rats fed with fermented mung bean iru

Key: H- Fermented mung bean iru

Control- Commercial feed

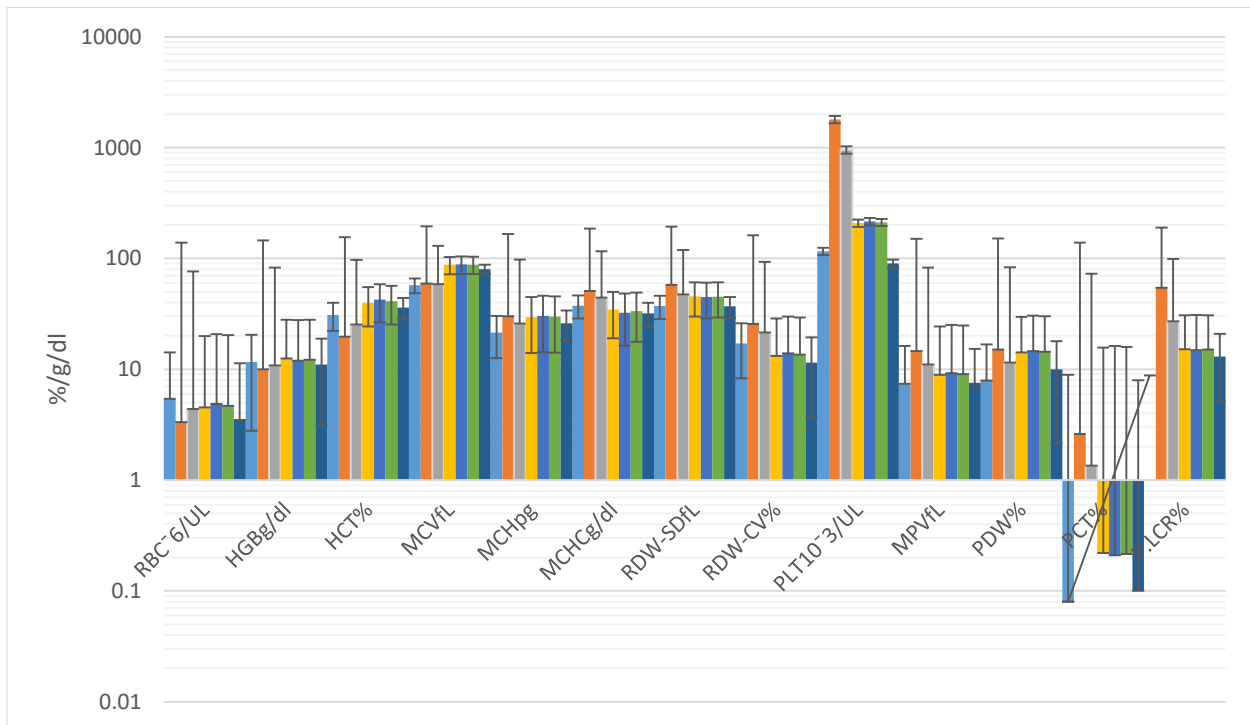
WBC= White blood cell, LYM= Lymphocyte, MON= Monocyte, GRAN= Granulocytes

Haematological Study of Red Blood Cell Parameters of Experimental Albino Rats Fed with Fermented Mung Beans, Iru, and Commercial Feed

Figure 3 depicts a haematological analysis of the red blood cell characteristics of experimental albino rats given commercial chow and fermented mung beans (Iru). Red blood cell 6μL from experimental animals fed commercial feed and fermented mung beans (Iru) ranged between 5.4 and 5.0 mm, within the acceptable ranges for males (4.3–5.9 million/mm) and females (3.5–5.5 million/mm). The experimental animals fed both commercial feed and fermented mung bean Iru showed the same HGBg/dl values (12.6 and 12.2 g/dL), in line with the limit range of 12–16 g/dL. When compared to commercial feed, the HCT (%) of experimental animals fed fermented mung bean Iru was lower (30.9 and 42.7%).

The MCH (g/dl) results of experimental animals fed commercial feed and animals fed fermented mung bean iru were identical. When compared to experimental

animals fed commercial feed, the MCH (g/dl) of the animals fed fermented mung bean Iru was higher. The RDW, SDF, and RDWCV% of test animals given commercial diet and fermented mung bean Iru fell into the same range. $PLT10^{-3}/\mu L$ of the test animals given commercial diet and fermented mung bean fell within the same range. The experimental animals fed both commercial feed and fermented mung bean Iru exhibited greater values of MPV (fL) and PDW (%). When compared to the value obtained for experimental animal feed using commercial feed, the PLT (%) of animal feed including fermented mung beans (IRU) was greater. When compared to animals fed commercial feed, the PLCR (%) of experimental animals fed fermented mung bean was lower.



Parameters

Figure 3: Haematological study red blood cell parameters of experimental albino rats fed with fermented mung bean iru

Key: H- Fermented mung bean iru, Control- Commercial feed, RBC = Red blood cell, HGB= Hemoglobin, HCT= Hematocrit, MCV= Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, RDW=Red blood cell distribution width, PLT= Platelet, MPV= Mean platelet volume, PDW= Platelet distribution width, PCT=, P-LCR= Platelet large cell ratio

Weight of the Internal Organs of Experimental Animals in Grams

The internal organ weight of the experimental animals expressed in grams. Table 3 shows the values obtained for the liver (H 3.43 ± 0.01 and C 3.81 ± 0.01), kidney (H 0.66 ± 0.02 and C 0.68 ± 0.01), muscle (H 0.55 ± 0.01), and kidney (C 0.56 ± 0.01) of the two experimental animals fed with fermented mung bean Iru and commercial feed.

Table 3: Weight of the Internal Organ of the experimental Animal in gram

Dietary	Liver(g)	Kidney(g)	Muscle(g)
H	3.43 ± 0.01	0.66 ± 0.02	0.55 ± 0.01
CONTROL	3.81 ± 0.01	0.68 ± 0.01	0.56 ± 0.01

The data is displayed as the average \pm standard deviation. A row of separate superscripts indicates a significant difference in the data ($p < 0.05$).

Key: H- Fermented mung bean iru

Control- Commercial feed

The Internal Organs' Diverse Tissues' Retention of Nitrogen

The nitrogen retention in various tissues shown in Table 4 revealed that experimental animals fed fermented mung bean Iru had lower values, while the animals fed commercial feed had higher values. The waste product of the experimental animals fed with commercial feed had higher values compared with animals fed with fermented mung bean Iru.

Table 4: The Nitrogen Retention in Various Tissues of the Internal Organ of Animals Fed With Fermented Mung Beans Iru

Dietary	Kidney mg/g	Liver mg/g	Muscle mg/g	Fecal mg/g	Urine mg/g
H	50.35 ± 0.01	50.38 ± 0.01	50.55 ± 0.01	0.45 ± 0.01	0.46 ± 0.01
CONTROL	80.35 ± 0.01	80.25 ± 0.01	80.30 ± 0.01	0.86 ± 0.01	0.84 ± 0.01

The data is displayed as the average \pm standard deviation. A row of separate superscripts indicates a significant difference in the data ($p < 0.05$).

Key: H- Fermented mung bean iru

Control- Commercial feed

Protein Quality

The result of the protein quality of experimental animals fed with fermented mung bean Iru and commercial feed as presented in Table 5 showed that animals fed with commercial feed had higher values of the following parameters; BV%, NPV%, PER, NPR, and gained compared with experimental animals fed with commercial feed.

Table 5: Protein Quality of animals fed with fermented mung beans iru

Dietary	BV %	NPU%	PER	NPR	Gained
H	71.00 ±1.00	75.00 ±1.00	2.50 ±0.10	3.47 ±0.11	24.24 ±0.01
CONTROL	76.33 ±1.53	75.00 ±1.00	3.10 ±0.01	3.80 ±0.10	31.89 ±0.01

The data is displayed as the average ± standard deviation. A row of separate superscripts indicates a significant difference in the data (p <0.05).

Key: H- Fermented mung bean iru

Control- Commercial feed

Histological Analysis

Histological research revealed that Plate 1 is a representation of the higher micrographs of the experimental animal liver sections stained with hematoxylin and eosin. While the experimental animals fed fermented mung bean iru are close to control, the animals fed commercial food exhibit normal liver histoarchitecture with characteristic polyhedral hepatocytes, round euchromatic nuclei with prominent nucleoli, radially apparent sinusoids, and a prominent central vein.

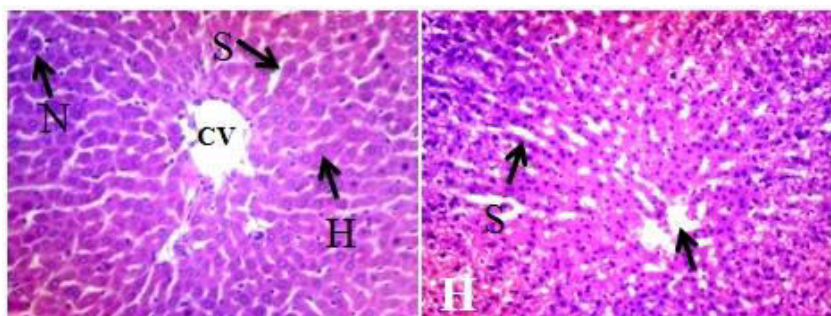


Plate 1: Photomicrographic Sections of Liver of Albino Rats Fed with Fermented Mung Beans Iru and Commercial Feed Subjected to H & E Stain

Observe: [CT]: normal liver histoarchitecture with characteristic polyhedral hepatocytes (H), round euchromatic nuclei with prominent nucleoli (N), radially apparent sinusoids (S), and prominent central vein (CV); while [H] is close to control (CT). Magnification 400x

Discussion

The mineral values for sodium in fermented mung bean iru were lower than those obtained from supplemental meals derived from maize, soybean, and pigeon pea (Aderonke et al. 2014). While the potassium content of fermented mung bean iru was lower than that

reported by Aderonke et al. (2014), it was lower than that of basil leaf (Ibironke et al., 2017b).

The fermented mung bean iru contained less calcium than bitter leaves (Ibironke et al., 2017b). Calcium-rich meals are recommended for promoting bone and heart health. The iron content of fermented mung bean iru was higher than that of basil and bitter leaves (Ibironke et al., 2017b). According to the study, beans can help vegetarians meet their iron requirements.

The fermented mung bean iru had a higher vitamin A content, which is consistent with Lawal (2021), who studies the influence of microbial fermentation on the nutritional quality of mung beans (*Vignaradiata*) and its toxicological implications in Wistar rats. While fermented mung bean iru has a lower vitamin B content than breakfast meals made from animal polypeptides, crayfish, and maize (Ibironke and Adepeju, 2016), it aids in the development of healthy red blood cells.

The quality and quantity of protein in the fermented mung bean iru, as well as the flavour, had a significant impact on weight gain. The results from fermented mung bean iru and commercial feed were consistent with Ibironke et al. (2016) but contradicted Lawal et al. (2022).

The haematology investigations of white blood cells (10-24) and LYM% of albino rats fed fermented mung bean iru and commercial feed yielded higher results than the experimental animals fed fermented mung bean inoculated with *Lysinibacillusphaericus* (Lawal, 2021). The MON% and GRAN#-3UL of fermented mung bean iru and commercial feed were consistent with findings Lawal (2021).

The study found that a haematology investigation of the RBC and HGB counts in the blood of animals fed fermented mung bean iru and commercial feed aligned with the findings of Ibironke and Owotomo (2019). Fermented mung bean iru can be utilized to produce blood for anaemia patients, which complements the research results of Ibironke et al. (2017a).

The HCT and MCV counts in the blood of animals fed fermented mung bean iru and commercial feed were comparable to those reported by Ibironke and Owotomo (2019). The MCH and MCHC counts in the blood of animals fed fermented mung bean iru were greater than in the study by Faleye et al. (2018). The RDW, PLT, MPU (FL), PDW, PCT, and P.LCR were all greater than what was discovered of Ibironke et al. (2017a), who conducted an in vivo and haematological research of several selected vegetable condiments.

The weight of various organs of experimental albino rats in grams, including the liver, kidney, and muscle. Animals fed fermented mung bean iru had somewhat lower weights in the liver, kidney, and muscle than those fed commercial feed. The results of animals fed fermented mung bean iru and commercial feed were consistent with those described by Ewuola et al. (2015).

The protein quality of albino rats given fermented mung bean iru versus commercial diet. The protein quality results correlated with the work of Adegunwa et al. (2012) and were greater than the fermented mung bean inoculated with *Saccharomyces cerevisiae* (Lawal et al., 2022). The animals fed fermented mung bean iru and commercial diet have normal liver architecture traits, which is in agreement with the findings of Awe and Olayinka (2011).

Conclusion

The study discovered that fermented mung bean 'iru' had haematinic characteristics and could be utilized to treat anaemia. The study discovered that fermented mung bean 'iru' has haematinic characteristics and can be utilized to treat anaemia.

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