Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.64, 2018

Hematological Indices and Growth Performance Studies of Albino Rats Fed Bioprocessed and Non-Bioprocessed Bambara Groundnut (Vigna Subterranean (L) Verdc.)

Chude, Charles[1*] Okoyeuzu, Chigozie[2] Amadi, Elechi Regina[3]
1. Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria
2.Department of Food Science and Technology, University of Nigeria, Nsukka Nigeria
3.Federal Polytechnic, Nekede, Owerri, Imo State, Nigeria

Abstract
The hematologic indices and growth performance of albino rats that was fed bioprocessed and non-bioprocessed bambara groundnut was studied to ascertain its suitability for use as food and feed. Among the 3 groups of albino rats used for the study; group A was fed diet 1 (control), while group B and C was fed the experimental diets 2 and 3 (non-bioprocessed and bioprocessed bambara groundnut) respectively. When hematological indices obtained from all the groups were compared, only group 2 had a significant ($p < 0.05$) increasing effect on the red blood cell (RBC), packed cell volume (PVC) and hemoglobin (Hb) counts; however, the counts obtained for all the groups where within hematologic reference range for rats, thus could not indicate or give specific information about infections, toxicity, allergy, immune-suppression or poisoning. When values for feed intake, weight gain, protein efficiency ratio, biological value and net protein unit were compared, rats fed basal had the highest feed consumption (286.10g); while feed intake varied for rats fed non-bioprocessed and bioprocessed samples with values of 209.50 and 191.30g respectively. The study observed that weight gain of rats was proportional to feed intake but when results for protein efficiency ratio, biological value and net protein unit were compared; the highest PER value was obtained for diet 1 followed by diet 3 and the BV values for the test groups fed diets 2 and 3 were comparable while diet 2 showed a much higher values for NPU. However, dietary nitrogen (DN) was used as an index of diet quality to ascertain nitrogen intake, fecal and urinary nitrogen, digested and retained nitrogen of the albino rats.
Keywords: Bioprocess, Bambara Groundnut, Hematology, Growth Performance

INTRODUCTION

Bambara groundnut (*Vigna subterranean* (L) *Verdc.*), sometimes called round beans are widespread most commonly in West and Central Africa and have various names. Its cultivation remains one of the crops most neglected by research and development. Yet empirical evidence and fragmentary research results suggest that it is a crop with great potentials (National Research Council, 2006); and excellent nutritive value. This legume is an important source of dietary protein, particularly when intake from animal sources is low or not available (Gupta *et al.*, 2005). When compared to other food legumes, bambara groundnut is rich in iron and the protein contains high lysine and methionine (Iqbal *et al.*, 2006). However, lack of adequate processing techniques to overcome the hard-to-cook effect has limited its utilization and hence reduced its production.

Thus, despite the full potentials of this bambara groundnut, their use in food and feed is still limited by the presence of several antinutritional factors (ANFs). These include tannins (Reddy *et al.*, 1994), phytic acid, trypsin inhibitors and flatulence causing oligosaccharides (Gupta *et al*., 2005). Among all the antinutritional components, phytic acid is one of prime concern for human nutrition and health management (Iqbal *et al*., 2006). Regardless of this negative side of bambara groundnut, research must strive to find ways to harness the potentials of this crop to solve food security issues since food security could only be solved through effective utilization of indigenous crops. The purpose of this research is to study and compare the benefits of bioprocessing of bambara groundnut on hematological indices and growth performance of albino rats; thus improve its utilization in food and fed applications.

MATERIALS AND METHODS

Collection and Preparation of Samples: Bambara groundnut was purchased from Ogbete Main Market, Enugu State of Nigeria. The bamabara groundnuts were carefully cleaned and freed of all extraneous materials as well as damaged nuts prior to use. The nuts was washed twice with ordinary water, rinsed with distilled water, and cooked to softness as a pretreatment measure and to eliminate existing microflora. Pure cultures of freeze dried *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] preserved in a dormant state by drying a heavy suspension of cells in sterile bovine serum was obtained from Agricultural Research Services Culture Collection, Bacterial Foodborne Pathogens and Mycology Research Unit; National Center for Agricultural Utilization Research of the United States Department of Agriculture, Peoria Illinois USA. The

freeze dried cells was brought to active state by growing in 25 ml sterile M.R.S. broth, and incubated in CO_2 enriched jars for 24 h and centrifuged at 3600-x g for 15 min. The recovered cells were rinsed using 10 ml sterile distilled water and spine twice at 3600-x g for 15 min. After this, a 9 ml suspension of the cells was made using sterile distilled water. The suspensions was serially diluted and plated out on plate count agar using the pour plate method. After 24 h incubation period in CO_2 enriched jars, the colonies on each plate of dilution factor was counted and the plate with approximately 10^6 cfu/ ml was noted and used at every inoculation of the fermentation process.

Fermentation: Twenty (20) kg of bambara groundnuts were cooked to softness and was rinsed with distilled water and poured into a basin. Ten (10) ml inoculum suspension of *Lactobacillus plantarum* [NRRL B-4306] and *Lactobacillus fermentum* [NRRL B-1932] containing approximately 10^6 cfu/ml was then inoculated aseptically into the 10kg of bambara groundnuts used for this study and 15 liters of distilled water added. The basin was covered completely and allowed to stand on the laboratory bench for three days at room temperature for the nuts to ferment. The fermentation was carried out without stirring, in accordance with the usual household practice. Thus, the uninoculated 10 kg of the nuts were used as control. The uninoculated (non-bioprocessed) sample was drained of water and the nuts spread on a tray and dried in a cabinet dryer at 60^{o}C for 14 h; hence, the same process was repeated for the inoculated sample after the fermentation period. To obtain the whole bambara groundnut flour; the samples were finely milled using commercial attrition grinder and sieved 3 times using a laboratory test sieve (Sethi Standard Test Sieve 100 BSS) to obtain the flour. The flour was stored in an airtight nylon bags at 4°C until it was used for experiments.

Animal Feeding Experiment: The bioassay was carried out using 30 male albino rats (50 - 60g) which was sourced commercially and used to conduct a 37-day study, which included a 30-day growth and a 7-day nitrogen balance period. The rats were fed rodent laboratory chow during a 3-day adjustment period and was randomly divided into 3 groups of 10 rats each and housed in metabolic cages fitted with steel funnels and perforated discs to facilitate separate collection of feces and urine. Rats that show symptoms of ill health were excluded from the experiment. One group of rats was placed on a standard basal commercial diet (control). The basal diet contains protein 20%, corn oil 8%, salt mixture 5%, vitamin/trace minerals premix 1%, Non nutritive fiber (cellulose) 1%, fish meal 3%, bone meal 3% and maize meal 60%. The basal diet was prepared and sold commercially by F. Hoffman – La Roche and company Ag. Switzerland based on formulation for laboratory animals. The remaining two groups were placed on diets of bioprocessed and non-bioprocessed bambara groundnut flour used as complete feed.

Feeding regime: The albino rats were placed on the diets and water given *ad libitum* throughout the feeding period. Weighed foods were placed in small porcelain mortars and about 5 ml of water added and mixed to minimize spillage and scattering. Spilled food with the fecal contaminants was collected daily and dried. The dry spilt food was combined with the dry unconsumed food for the determination of total amount of food actually consumed by the rats in each of the groups. The proximate daily food consumption was determined by weight difference between the served, unconsumed and spilt foods. Oral health of all rats was screened for abnormalities and documented by a licensed veterinarian. Thus, the weight of the rats was monitored during the feeding period (every 3 days).

Collection and treatment of urine and feces: Urine and feces were collected twice daily. Collected samples for various groups was emptied into sterile containers for urine and fecal samples and weighed to the nearest 1g using a digital scale. After initial emptying and before washing, the floor was duly checked to account for any residual fecal content. Samples was frozen at -20°C within 12 h of collection and stored until analyzed for nitrogen.

Sacrifice of rats: After the feeding period, the rats were weighed and their physical conditions such as fur, appearance and agility noted and recorded prior to sacrificing them. The rats were put to sleep by placing them in a sealed container containing diethyl ether. Incision was made in the abdomen extended to the thorax. Blood was collected directly from the heart with a syringe and needle into a sample bottle containing ethylene diamine-tetra acetic (EDTA).

Determination of Hematological Indices

Red blood cell count: This was determined using the method described by Henry (1979). Blood (0.02 ml) was pipetted into a clean test tube and 4 ml of the red blood cell diluting fluid was added to make a 1:200 dilution of the sample. Diluted blood sample was loaded into a Neubauer counting chamber and all red blood cells in the five groups of 16 small squares in the central area of the Neubauer chamber were counted using a light microscope at 40x objective. The number of cells counted for each sample was multiplied by 10,000 to obtain red blood cell count per microlitre of blood.

Packed cell volume (PCV): The packed cell volume (PCV) was determined using the conventional method of filling capillary tubes with blood and centrifuging to settle the erythrocytes. A micro capillary tube was nearly filled with the blood sample and sealed at one end with flame. It was then centrifuged at 10,000 rpm for 5 minutes using a micro haematocrit centrifuge. After this, the PCV was read using a haematocrit reader.

White blood cell count: This was determined using the method described by Henry (1979). Blood (0.02 ml) was pipetted into a small test tube containing 0.38 ml of white blood cell diluting fluid to make for 1:20 dilution of the blood sample. The diluted sample was loaded on to the Neubauer counting chamber and all cells on the four corners square was counted using a light microscope at 10x objective. The number of cells counted for each blood was multiplied by 50 to obtain the total white blood cell count per microlitre of blood.

Hemoglobin estimation: This was determined using the cyanomethaemoglobin method described by Kachmar (1970). Drabkin's haemoglobin reagent (5 ml) was added to a clean test tube. Then, 0.02 ml of the blood sample was added to the reagent and mixed properly. The mixture was allowed to react for 20 minutes before reading the absorbance at 540 nm against a reagent blank on a spectrophotometer. Standards was also prepared as above and read at 540 nm. The concentration of haemoglobin of the blood sample was obtained by multiplying the absorbance of the sample with a calibration factor derived from the absorbance and concentration of the standard.

Urine and Fecal Analysis

For nitrogen balance studies, a method as described by Anshu (2000) was used to collect and preserve the urine and fecal samples. Carmine red, a biological marker was added to the diets to mark the beginning and the end of fecal collections and was fed to the rats at the mornings of days 30 and 37. Colored feces appeared beginning on days 31 and 38. Colored feces appearing on day 31 were included in the pooled fecal sample while those appearing on day 38 (7 days) were discarded, however, all the uncolored feces excreted on the other days were collected. The individual fecal collections were dried in an air oven at approximately 60^0C to a constant weight. The dried feces were weighed, ground into fine power and stored in a refrigerator until used for fecal nitrogen determination. Urine was collected from 7.00am of day 30 through the morning of day 37 (7 days). One ml of 0.1N hydrochloric acid was added as a preservative to the urine samples to prevent the loss of ammonia. They were stored in a refrigerator until analyzed for urinary N. The nitrogen content of the feed sources, diets, feces and urine were analyzed using the micro-Kjeldahl procedure (AOAC, 1990).

Growth Performance Studies

The protein qualities of the raw and fermented bambara groundnut feed as well as the basal diet was evaluated based on their ability to promote growth and nitrogen retention in the albino rats. The data collected during the growth study and N-balance periods were used in calculating the feed intake, body weight gain and protein efficiency ratio (PER) of the diets. The urinary and fecal N and N intakes were used for calculating apparent N digestibility, N retention, net protein utilization (NPU %) and biological value (BV %) according to the formula given below:

Nitrogen digestibility (digested nitrogen) = N intake (I) - fecal nitrogen (F)
Nitrogen retention (N- balance) = Digested nitrogen – Urinary nitrogen (U)
Biological value (BV %) = N-retention / Digested nitrogen X 100
Net protein utilization (NPU %) = N-retention / N-intake X 100.
The protein efficiency ratio (PER) was determined by the formula of Osborne *et al.* (1990).

$$\text{PER} = \frac{Weight\ gain}{\text{Pr}otein\ consumed}$$

Statistical Analysis: Data generated from this study were represented as mean ± standard deviation and statistically analyzed using one-way analysis of variance and mean separation was done by Duncan's new multiple range and Paired T-tests at 95 % level.

RESULTS

The hematological indices of albino rats was studied to ascertain the effect of bioprocess on feed diets of bambara groundnut and the results presented in Table 1. However, among the 3 groups of albino rats; group A was fed diet 1 (control), while group B and C was fed the test diets 2 and 3 (non-bioprocessed and bioprocessed bambara groundnut) respectively. When indices obtained from all the groups are compared, only group B had a significant ($p < 0.05$) increasing effect on red blood cell (RBC), packed cell volume (PVC) and hemoglobin (Hb) counts. The sample from group C also had a significant ($p < 0.05$) increasing effect on white blood cell (WBC).

The crude protein value for the control diet was 87.28%, raw bambara groundnut was 20.8%, while bioprocessed bambara groundnut was 19.70%. Thus, table 2 presents the values for food intake, weight gain, PER, BV and NPU of the rats fed the control and test diets. The rats fed basal (diet 1) had the highest feed consumption (286.10g); while the feed intake varied significantly for rats fed diet 2 (Non-bioprocessed Sample) and diet 3 (Bioprocessed Sample) with values of 209.50 and 191.30g respectively.

The study observed that weight gain of rats was proportional to feed intake. The rats fed diet 1 with relatively high feed consumption had significantly higher weight gain compared to the respective weight gains of the test groups. However, there was a significant ($p < 0.05$) difference in the weight gain of rats fed diets 2 and 3 with group C showing higher weight despite lower fed consumption.

The BV values for the test groups fed diets 2 and 3 were comparable. However, they were significantly ($p < 0.05$) higher relative to that of the control group. The test group fed diet 2 showed a much higher values for NPU and these were comparable to that of the control diet.

Table 3 presents the values for nitrogen intake, fecal and urinary nitrogen, digested and retained nitrogen of the rats fed basal, raw and fermented bambara groundnut diets. The control group that consumed diet 1 had a higher mean N Intake as compared to other groups of rats followed by test group fed diet 2; thus, there was no significant ($p < 0.05$) difference between the test groups. Analysis of fecal nitrogen (N) excretion showed variations with the test groups fed diets 2 and 3; and there was no significant ($p < 0.05$) difference between the groups fed diets 1 and 3. The non-bioprocessed bambara groundnut diet induced higher fecal N excretion in rats, thus, the fecal N excretion affected N digestibility. The rats fed diet 1 had higher N intake, excreted less fecal N and had higher digested N value; while the test group fed bioprocessed sample had lowest N intake, excreted less fecal N and had higher digested N value when compared to the test group fed raw sample.

Table 1: Hematological Indices of Albino Rats Fed Diets of Basal, Non-bioprocessed and Bioprocessed Bambara Groundnut

Parameters	Basal	Experimental Diets		Heamatologic Reference Range for Male Rats*
		Non-Bioprocessed	Bioprocessed	
RBC ($x10^6/\mu L$)	8.05±0.2	9.28±0.3	8.84±0.5	7.74 to 9.72
WBC ($x10^3/\mu L$)	5.70±0.1	6.95±0.4	6.25±0.2	5.29 to 10.80
PCV (%)	39±0.4	42±0.1	39±0.1	39 to 48
Hb (g/dL)	15.5±0.2	17.4±0.4	15.8±0.2	14.7 to 18.0

Values are means ± SD (n = 3)

Table 2: Growth Performance Study of Rats Fed Diets of Basal, Non-bioprocessed and Bioprocessed Bambara Groundnut

Parameters	Basal	Experimental Diets	
		Non-Bioprocessed	Bioprocessed
Feed Intake (g)	286.10±0.3	209.50±0.6	191.30±0.1
Weight Gain (g)	48.50±0.5	25.50±0.1	28.10±0.7
PER (g/g)	2.80±0.2	0.9±0.1	1.04±0.1
BV (%)	84.73±0.1	70.49±0.3	93.24±0.1
NPU (%)	79.28±0.8	50.0±0.5	83.13±0.1

Values are means ± SD (n = 3)

Table 3: Nitrogen Utilization of Rats Fed Diets of Basal, Raw and Fermented Bambara Groundnut

Parameters	Basal	Experimental Diets	
		Raw Sample	Fermented Sample
N Intake (g)	1.40±0.2	0.86±0.6	0.83±0.1
Fecal N (g)	0.09±0.1	0.25±0.2	0.09±0.1
Digested N (g)	1.31±0.3	0.61±0.2	0.74±0.2
Urinary N (g)	0.20±0.2	0.18±0.2	0.05±0.1
Retained N (g)	1.11±0.6	0.43±0.5	0.69±0.3

Values are means ± SD (n = 3)

DISCUSSION

Hematological parameters are important indices of the physiological and pathological status for both animals and humans (Adeneye *et al.*, 2006). It can also be used to determine the extent of deleterious effect of foreign compounds, including plant extracts, on the blood of the albino rats (Odeyemi *et al.*, 2009). Besides the significant increase in the counts for White Blood Cell (WBC), Red Blood Cell (RBC), Packed Cell Volume (PVC) and Hemoglobin (Hb) which was observed for group B; when compared to reference counts for laboratory animals, the results however, cannot give specific information about the results obtained for all the 3 groups studied in this experiment. In essence, the results could not indicate or give specific information about infections, toxicity, allergy, immune-suppression or poisoning.

The highest feed consumption observed in rats fed diet 1 could be attributed to the fact that basal is an animal protein and has more organoleptic appeal to the rats and provided a better profile of essential amino acids (EAAS) (Almas and Bender, 1980). The study observed that weight gain of rats was proportional to feed intake.

The rats fed diet 1 with relatively high feed consumption had significantly higher weight gain compared to the respective weight gains of the test groups. However, there was a significant difference in the weight gain of rats fed diets 2 and 3 with group C showing higher weight despite lower fed consumption. This may consequently suggest that bioprocess improved the availability of nutrients. The PER values were influenced by feed consumption and subsequent body weight. The highest PER value was that of the rats fed diet 1; followed by rats fed with diet 3. The decrease in weight gain and PER value of rats fed with diet 2 may be attributed to the presence of anti-nutrients in the diet (Almas and Bender, 1980).

The observed decrease in the mean percent NPU values for diet 3 tend to imply that the heat treatments for cooking and drying might have been severe. Almas and Bender (1980) reported increased nitrogen susceptibility to heat processing in protein foods containing significant amounts of carbohydrate; thus, legumes are considered rich sources of carbohydrates. The study concludes that utilization of the dietary protein was adversely affected by alteration in pattern of EAAS resulting from denaturation of proteins which occurred during processing.

The digested N values for rats fed diets 2 and 3 clearly demonstrate that bioprocess improved N digestibility of bambara groundnut proteins in albino rats (Almas and Bender (1980). The control group that ate diet 1 had comparatively higher urinary N and lower fecal N excretions and this may have been consequent upon the fact that basal is basically a protein diet and they may have suffered a physiological disorder due to nutrition imbalance. The test group fed diet 2 had a higher urinary N when compared to the test group fed diet 3; thus, this may also be consequent upon the fact that their diet contained significant amounts of tannins, which bind with proteins thereby making it less accessible (Almas and Bender (1980). The control group fed diet 1 showed induced high N retention than the test groups fed diets 2 and 3 with diet 2 showing the lowest N retention. Obviously, the basal diet possessed better pattern of EAAS. This promoted N- retention despite the high urinary N losses. Judging from the retention value, heat treatment which was applied to diets 2 and 3 before (cooking) and after (drying) fermentation may have denatured some of the EAAS resulting in less N retention.

CONCLUSION

In most African countries where population is constantly on the increase, food security becomes paramount and there is no better means of ensuring food security than harnessing the potentials of indigenous crops. Considering the qualities of bambara groundnut, it has great opportunities towards food security, sustainability, income generation, product development, dietary diversification and animal feed. It also has the potential to be utilized in many forms as food and fed but anti-nutritional factors hinder these possibilities. This necessitates an investigation of this sort to ascertain the benefits of bioprocess as a treatment measure to reduce the anti-nutritional factors and improve nutrient digestibility, thus promote health and growth. Hence, it becomes imperative to employ the bioprocess of fermentation as a treatment measure to reduce these anti-nutritional components found in bambara groundnut to a safe level. While bambara groundnut is not a toxic substance, the present study observed that albino rats fed fermented bambara groundnut performed better in terms of all the parameters studied and maintained a good range of growth and hematological parameters for the duration of the study, thus indicating more availability of nutrients and removal of anti-nutritional factors.

Acknowledgement: The researchers wishes to acknowledge the Agricultural Research Services Culture Collection; National Center for Agricultural Utilization Research of the United States Department of Agriculture, Peoria Illinois USA; for providing the freeze-dried bacterial strains used for the fermentation.

REFERENCES

Adeneye, A.A., Ajagbonna, O.P and Bello, S.O. (2006) preliminary toxicity and Phytochemical studies of the stem bark aqueous extract of *musanga cecropioides* in rats. *Journal of Ethnopharmacology* 105(3): 373 – 379.

Almas, K and Bender, A. E. (1980), Effects of heat treatment of legumes on available lysine, *Journal of Food. Chemistry* 31: 448-452.

Anshu, V. K., Ameeta, H.S., Kushwah, H.S., Singh and Malik, M.R. (2000). Haemato-biochemical and histological changes in rats upon dietary supplementation of cultivated mushroom. *Indian Journal of Nutrition and Dietetics*, 38:191-198.

AOAC (1990). Official Methods of Analysis, 14th ed. *Association of Official Agricultural Chemists*, Washington, DC.

Charles River Laboratories. (1998). Baseline hematology and clinical chemistry values for Charles River Wister Rats as a function of sex and age. Technical Bulletin, 2.

Gupta, V., Modgil, R and Kalia, M. (2005) Effect of domestic processing on the Invivo protein quality of faba bean (Vicia faba). *Journal of Food Science and Technology* **42**:501-503.

Henry, J.B. (1979). Clinical diagnosis and management by Laboratory methods, *W.B.Saunders and company*, Philadelphia, P.A., 365.

Iqbal, A., Khalil, I.A., Ateeq, N and Khan, S.M. (2006). Nutritional quality of important food legumes. *Food*

Chemistry **97**(2): 331-335.

Kachmar, J. F. (1970). Determination of blood haemoglobin procedure, In: *Tietz New Edition, Fundamental of Clinical Chemistry*, W. B. Saunders Company, Philadephia. 268-269.

National Research Council (2006). Bambara Bean. Lost Crops of Africa: Volume II: Vegetables. Lost Crops of Africa. *National Academies Press*, New York. ISBN 978-0-309-10333-6.

Odeyemi, O.O., Yakubu, M.T., Masika, P.J and Afolayan, A.J. (2009) Toxicological evaluation of the essential oil from *Mentha Longifolia L Suosp. Capensis* Leaves in rats. *Journal of Medicine and Food* 12(3): 669-674.

Osborne, T. B., Mendel, L. B. and Ferry, E. L. (1990). A method of expressing numerically the growth-promoting values of proteins. *Journal of Biological Chemistry*, 37: 223-229.

Reddy, B.S., Ekelund, G., Bohe, M., Engle, A. and Domellöf, L. (1994). Metabolic epidemiology of colon cancer: Dietary pattern and fecal sterol concentrations of three populations. *Nutrition Cancer* **5:** 34-40.