



Nutritional Evaluation of whole Kenaf (*Hibiscus cannabinus* L.) seed meal in Rats

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Abstract: The study reported herein evaluates with rats the nutritional quality of raw and roasted kenaf seed as sources of nitrogen. Some safety aspects regarding possible feed use were also investigated. Wistar rats were fed four purified diets viz: a basal diet (nitrogen-free), a reference diet that contains casein and two diets made of kenaf seeds. Dietary treatments significantly influenced performance, biological indices and blood parameters ($P < 0.05$). The performance of rats on kenaf based diets was inferior to that of rats on casein diets. The levels of serum glucose were significantly elevated for rats on kenaf based diets compared to rats on casein diet. However, the levels of serum total protein, globulin and albumin/globulin ratio were significantly elevated for rats on the casein diet compared to rats on kenaf based diets. Among the kenaf test proteins, Protein Efficiency Ratio (PER) values of 1.87 and 1.65; Net Protein Ratio (NPR) values of 0.56 and 0.50; Biological Value (BV) of 65.04 and 63.05 and Net Protein Utilization (NPU) values of 44.23 and 42.56 were obtained respectively for a raw kenaf seed meal (RKM) and roasted kenaf seed meal (HKM). There was no significant difference in results obtained for both RKM and HKM.

1. Introduction

Scarcity and high costs of conventional ingredients such as groundnut cake, soya beans, fish meal and maize has led to a renewed interest in the use of non-conventional cheap and easily available ingredients in livestock feeding. Kenaf (*Hibiscus cannabinus* L.) was formerly, primarily used as a fiber crop and secondarily as a livestock feed (1). Its leaves have an acidic flower and are used for the production of local soups. Kenaf is extensively cultivated in tropical Africa, Asia, Central America and the Caribbean for the jute-like fiber. Kenaf seed has a crude protein content of 30.88%, organic matter content of 95.15% and ether extract content of 18.55% on a dry matter basis. The fatty acid content on dry matter basis includes palmitic acid (33.21%), stearic acid (50.02%), oleic acid (31.26%) and linoleic acid (30.51%) (2).

It is customary during the introduction of novel feed resources to assess the nutritive value of such material. Protein utilization is a good indication of proper feed utilization for growth, especially their

protein quality in order to ascertain their competence in meeting recommended allowance (3).

The purpose of this study was to evaluate with rats the nutritional quality of kenaf seeds.

2. Materials and Methods

2.1 Collection and preparation of kenaf seed meal

The kenaf seeds were collected from the Institute of Agriculture Research and Training, Ibadan. The seeds were thoroughly cleaned to remove all foreign materials. After cleaning, part of the seed was roasted for about 10-15 minutes, by a flat pan over an open fire by constant stirring until a golden brownish colour was obtained. It was then allowed cool. The processed kenaf seeds were then ground and then added to the mixed feed.

2.2 Experimental diet formulation

Four experimental diets were prepared (Table 1). The protein sources used for this experiment were the kenaf seed meal viz: raw kenaf meal (RKM), roasted

The experiment was carried out at the college of animal health and production technology, moor plantation, ibadan, nigeria.

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kenaf meal (HKM) and casein (control). The protein content of each of the samples was determined (4).

Preparation of the diets was done by incorporating each protein source separately at the expense of cornstarch to give 10% protein in each. All other ingredients were added in fixed amounts. The feed was offered to the animals in mash form.

2.3 Experimental animals and management

Twenty-four growing albino rats of Wistar strain (weighing 66.7 and 68.3g and 27-33 days old) were obtained from the Department of Veterinary Pharmacology, University of Ibadan, Ibadan, Nigeria. No preference was made for gender since in rats it has been reported that it has no effect on protein utilization (5). The rats were housed individually per unit in modified wire screen metabolic cages with facilities for the supply of fresh clean water and feed as well as urine and faecal collection. The rats were assigned into 4 groups of six rats in each. Diet 1 was the basal diet, diet 2 served as the reference, diet 3 and 4 contained the kenaf seed meal.

The feeding trial lasted for 21 days. Feed and water were provided *ad libitum*. Weight changes were measured weekly while the feed consumption record was taken daily. During the last 7 days, urine and faeces were collected daily from each replicate separately. The faecal samples were oven dried at 65°C, milled and kept in airtight samples bottles and stored in deep freezer until analyzed. The urine was collected in screw-capped plastic bottles containing a drop of toluene and stored in the deep freezer until time analysis. The concentration of nitrogen in urine and faeces was determined by

Kjeldahl method (4). The data obtained were used to calculate the Protein efficiency ratio (PER), Biological value (BV), Net protein retention (NPR), Protein intake (PI) and Net Protein utilization (6).

Feeding period, three rats per treatment were randomly selected, bled through the heart using hypodermic needle and syringe, the blood samples were pooled into carefully labeled sample bottles. The blood samples collected in EDTA were used for the determination of haematological parameters such as packed cell volume (PCV), haemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), blood constant such as mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and Mean cell volume (MCV) and leucocytes differential counts, such as lymphocytes, monocytes, basophils and eosinophils. The remaining blood sample was centrifuged and the serum was dispensed into a clean tube for analysis. The cell counts were carried out by the use of haemocytometer, while Hb, PCV and serum chemistry indices such as serum glucose, total protein, serum albumin, serum globulins, uric acid, serum cholesterol and creatinine were determined using standard methods (7), while the serum enzymes; Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits.

Proximate composition of the feed, faeces, and urine were carried out according to (4). The tannin content of the test ingredient was carried out using vanillin-HCL method modified by (8), oxalate was analyzed as outlined by (9), while the method of (10) was adopted for the estimation of phytates.

Table 1. Composition of rat experimental diets.

Ingredients %	EXPERIMENTAL DIETS			
	Non-protein group 1	Reference (Casein) 2	Raw kenaf Meal 3	Roasted kenaf Meal 4
Corn starch	82.00	70.52	49.75	49.75
Casein	-	11.48	-	-
Kenaf seed	-	-	32.25	32.25
Cellulose (non-nutritive)	5.00	5.00	5.00	5.00
Bone meal	2.00	2.00	2.00	2.00
Oyster shell	0.50	0.50	0.50	0.50
Vitamin/ mineral premix*	0.50	0.50	0.50	0.50
Sucrose	2.50	2.50	2.50	2.50
Glucose	2.30	2.30	2.30	2.30
Salt (sodium chloride)	0.20	0.20	0.20	0.20
Palm oil	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein %	0.00	10.00	10.00	10.00
Determined analysis				
Crude protein %	1.78	10.66	11.54	11.28
Ether extract %	0.35	0.46	0.39	0.38
Ash %	11.96	12.28	12.33	12.45
Crude fibre %	4.62	4.75	5.58	5.42
Moisture content %	10.24	10.42	10.36	10.75

*Vitamin-mineral premix (2.5kg/1000kg): Vitamin A (11,000,000 IU), Vitamin D3 (3,000,000 IU), Vitamin E (30,000 IU), Vitamin K (2.3gm), Vitamin B1 (1.7gm), Riboflavin (5.2gm), Pyridoxine (3.7gm), Vitamin B12 (18mg), Biotin (76mg), Niacin (37gm), Pantothenic acid (9.2gm), Folic acid (0.9gm), Manganese (85gm), Zinc (50gm), Iron (25gm), Copper (6gm), Iodine (1gm), Selenium (120gm), Cobalt (220mg), Antioxidant (126gm), Choline chloride (180gm).

Table 2. Average chemical composition (g/100gDM) of test ingredients.

Nutrients	Raw kenaf meal (RKM)	Roasted kenaf meal (HKM)
Moisture	10.34	9.52
Crude Protein	31.28	31.80
Crude fibre	11.65	13.23
Ether extract	18.73	18.33
Ash	7.23	10.22
Nitrogen-free extract	31.11	26.42
Tannin (%)	0.078	0.045
Oxalate (mg/g)	0.124	0.029
Phytic acid (mg/g)	0.026	0.013

2.4 Carcass evaluation

At the end of the feeding trial, one rat per replicate were selected, weighed, decapitated, bled and eviscerated to obtain empty carcass weight. The weights of the liver, lungs, kidneys, spleen, gastrointestinal tract (GIT) and heart were taken and related to their body weights.

2.5 Statistical analysis

Data generated on performance, digestibility, haematological, serum biochemical indices and carcass yield were subjected to one-way analysis of variance as described for a completely randomized design (CRD) and significant treatment mean were separated using Duncan's multiple range test (11) using (12) package.

3. Results and Discussion

3.1 Performance of rats fed experimental diets

The performance data obtained from rats fed the experimental diets are as presented in Table 3. For all parameters measured, the reference diet with casein as protein source was the most superior. There were significant differences ($P < 0.05$) in the values obtained for weight gain, feed intake and feed: gain ratio.

Although there was no significant difference in the final weight obtained for the kenaf based diets, the highest weight gain was recorded by the rats fed RKM. The same trend was also observed for feed: gain ratio. The reference diet elicited about 100% increase in the rate of body weight gain over both raw and roasted kenaf seed meal. Feed intake were significantly influenced by dietary treatment ($P < 0.05$), with the same trend as average daily weight gain. Rats fed on the casein-based reference diet consumed significantly ($P < 0.05$) more feed than rats on various kenaf based diets. The diet was also most efficient in terms of feed: gain.

3.2 Evaluation of protein quality

Table (4) shows the result of protein quality evaluation of raw and roasted kenaf meal using rats. The biological indices obtained for rats fed the casein reference diet were significantly ($P < 0.05$) higher than the corresponding values for the kenaf test diets. The net protein ratio (NPR) was similar for both RKM and HKM but significantly lower than the reference diet. The same trend was observed for the biological value (BV) and net protein utilization (NPU). While there were no significant ($P > 0.05$) difference in the values obtained for protein efficiency ratio (PER).

Table 3. Performance characteristics of rats fed the experimental diets (g).

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	SEM
	NPG	Reference	RKM	HKM	
Initial weight (g)	68.33	68.33	68.33	66.67	2.88
Final weight (g)	54.83 ^c	120.69 ^a	96.33 ^b	94.67 ^b	6.69
Weight gain (g)	-13.50 ^c	52.33 ^a	28.00 ^b	28.00 ^b	8.61
Feed intake (g/rat/21days)	129.17 ^b	241.50 ^a	154.08 ^b	189.50 ^b	38.41
Feed/Gain	-14.80 ^b	4.77 ^a	5.57 ^a	6.45 ^a	0.27

*abc: Mean on the same row with difference superscripts differ significantly ($P < 0.05$).

NPG = Non-protein group, RKM = Raw kenaf seed meal, HKM = Roasted kenaf seed meal.

Table 4. Biological evaluation of protein quality of whole kenaf meal using rats.

Parameter	Diet 1	Diet 2	Diet 3	SEM
	Reference	RKM	HKM	
Protein intake (g)	1.15	0.80	0.99	0.20
Protein efficiency ratio (PER)	2.15	1.87	1.65	0.15
Net protein ratio (NPR)	1.37 ^a	0.56 ^b	0.50 ^b	0.27
Biological value (BV)	80.38 ^a	65.04 ^b	63.05 ^b	1.25
Net protein utilization (NPU)	70.23 ^a	44.23 ^b	42.56 ^b	2.56

*abc: Mean on the same row with difference superscripts differ significantly ($P < 0.05$).

NPG = Non-protein group, RKM = Raw kenaf seed meal, HKM = Roasted kenaf seed meal.

Table 5. Organ weight and gut characteristics of rats fed the experimental diets (% live weight)**

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	SEM
	NPG	Reference	RKM	HKM	
Lung	0.90	0.71	0.89	0.87	0.12
Heart	0.59	0.45	0.80	0.75	0.48
Liver	6.35 ^a	4.27 ^b	6.78 ^a	7.10 ^a	0.89
GIT	16.94 ^a	9.43 ^c	13.52 ^b	16.79 ^a	1.48
Spleen	0.51	0.53	0.42	0.38	0.27
Kidney	0.90	0.77	0.89	1.01	0.13

abc: Mean on the same row with difference superscripts differ significantly (P<0.05).

*NPG = Non-protein group, RKM = Raw kenaf seed meal, HKM = Roasted kenaf seed meal, GIT** = Gastrointestinal tract, **wet matter basis.

3.3 Effect of treatment on relative organ weights of rats

Among the weight of organs studied, significant (P<0.05) differences were recorded in values obtained for liver and gastrointestinal tract as presented in Table 5. Rats fed HKM had the highest (7.10) liver weight, while the least (4.27) was recorded for rats fed the reference diet. Except for the rats on the reference diet, relative organ enlargement was observed in value obtained for lung, heart, liver, GIT and kidney in all rats fed the test ingredients.

3.4 Effect of treatment on rat blood composition

The effect of test ingredients on haematology and serum biochemistry is as presented in Tables 6 and 7.

Packed cell volume (PCV), haemoglobin concentration (Hb), Red blood cell (RBC), mean cell volume (MCV), Basophil, Monocyte and Eosinophil for rats on the reference diet had the highest values (P<0.05), with the same set of rats having the least values for mean cell haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH). There were no significant differences (P>0.05) in values obtained for PCV, Hb, and RBC in rats fed raw and roasted kenaf meal while significant differences were observed for MCHC, MCV and lymphocyte for the test ingredients. While all other haematological parameters were not significantly (P>0.05) influenced by dietary treatments.

Table 6. Haematological parameters of rats fed the experimental diets.

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	SEM
	NPG	Reference	RKM	HKM	
Packed cell volume (%)	20.67 ^b	39.67 ^a	22.67 ^{ab}	22.67 ^{ab}	6.99
Hb (g/dl)	7.10	13.30	7.80	7.67	2.36
White blood cell ($\times 10^3$ /mm ³)	15.07	13.65	14.38	13.45	4.56
Red blood cell ($\times 10^6$ /mm ³)	3.19 ^b	6.32 ^a	3.64 ^b	3.77 ^b	1.15
MCHC (%)	34.34 ^a	33.53 ^b	34.39 ^a	33.91 ^c	0.40
MCH (μ g)	30.47	21.05	21.32	20.53	7.16
Mean cell volume (μ^3)	647.91 ^a	627.68 ^b	619.90 ^{ab}	605.43 ^c	19.73
Neutrophils (%)	47.00	51.33	45.33	38.68	7.86
Lymphocyte (%)	47.00 ^{ab}	41.00 ^b	51.33 ^{ab}	60.00 ^a	8.93
Basophils (%)	2.00	0.67	0.00	0.00	1.15
Monocytes (%)	3.33	4.00	2.00	1.33	2.16
Eosinophils (%)	0.67	2.33	1.33	0.00	1.32

*abc: Mean on the same row with difference superscripts differ significantly (P<0.05).

NPG = Non-protein group, RKM = Raw kenaf seed meal, HKM = Roasted kenaf seed meal.

Table 7. Serum biochemistry of rats fed the experimental diets.

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	SEM
	NPG	Reference	RKM	HKM	
Glucose (mg/dl)	20.20 ^c	27.20 ^b	36.67 ^{ab}	53.00 ^a	7.65
Total protein (g/L)	35.05 ^c	74.07 ^a	65.37 ^{ab}	51.20 ^b	9.57
Albumin (g/L)	16.50	40.13	35.17	33.87	5.88
Globulin (g/L)	18.55 ^{ab}	33.93 ^a	30.17 ^a	17.33 ^b	5.82
Albumin/Globulin	1.46 ^{ab}	1.20 ^b	1.23 ^b	1.99 ^a	0.32
Uric acid (mg/dl)	8.05	8.10	8.13	7.57	2.53
Cholesterol (mg/dl)	121.00	114.27	96.80	123.97	19.32
Creatinine (mg/dl)	0.95	1.10	1.00	0.90	0.16
ALT (IU/L)	252.90	230.93	147.97	212.57	70.85
AST (IU/L)	38.30	33.93	27.50	29.99	0.32

abc: Mean on the same row with difference superscripts differ significantly (P<0.05).

*NPG = Non-protein group, RKM = Raw kenaf seed meal, HKM = Roasted kenaf seed meal, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase

Among the serum biochemical indices measured, serum glucose, serum albumin, albumin: globulin ratio and serum total protein were significantly ($P < 0.05$) influenced by dietary treatments. Highest values (53.00mg/dl) for glucose were recorded by rats fed HKM, while rats fed the non-protein group had the least (20.20mg/dl) value. For globulin, highest globulin values (33.93g/l) were recorded for rats on reference diet, while rats on HKM had the least value of (17.33g/l). There were no significant ($P > 0.05$) differences in value obtained for serum albumin, uric acid, serum cholesterol, serum creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

4. Discussion

The biological evaluation of the raw and roasted kenaf meal samples showed that although approximately the same protein level was offered (10.00%) in the diets, there were no variation in the performance of the rats among the samples. The weight gains of rats on kenaf based diets were significantly lower than the reference diet. The reduction in weight gain is thought to be associated with the reduction in feed intake of rats on the kenaf based diets and protein quality.

Increase in feed intake will normally result in increased body weight gain (13). The reduced feed intake in this study may also be an aversion for the kenaf based diets based on reducing the palatability arising from odour. The casein diet was also more efficient when measured in terms of feed: gain ratio (14). Reported higher weight gain for rats on animal protein sources compared to rats fed plant protein.

The value obtained for the protein efficiency ratio (PER), net protein ratio (NPR) and biological value (BV) were related to the protein intake. (6) Observed that the BV of a protein is based on the quantity of such protein that is retained, which can provide for growth and health maintenance. The reduction in the BV of protein from the kenaf based diets is thus an indication of the poor quality of the protein compared to that from casein. (15) Explained that PER and NPR are indicators of protein quality based on weight gain and protein intake. Rats fed the kenaf based diets had a lower value PER and NPR compared to the reference diet. This result showed the inability of the rats to properly utilize the kenaf based diets. The lower nutritional value exhibited by kenaf based diets as compared with casein may also be attributed to the high ash content which may have caused an imbalance in the levels of amino acids in the diets as reported by (16, 3).

Protein sourced from kenaf based diets significantly reduced the packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell counts (RBC), serum total protein (TP), serum albumin, serum globulin, alanine aminotransferase, aspartate

aminotransferase, and neutrophil while resulting in an increase in mean cell volume (MCV), mean corpuscular haemoglobin (MCH), serum glucose and albumin: globulin ratio. Since haematocrit and haemoglobin are known to be positively correlated with protein quality and protein level (3), the reduced value of PCV of rats on the kenaf based diets compared to those on reference diet is thought to be indicative of the poor quality of the protein supplied. According to (6), total protein value is an indication of the protein reserve in the animal. In this study, the significant decrease in serum total protein of rats on kenaf based diets is a reflection of the inadequacy of the dietary protein. The significantly lower serum uric acid value recorded in the casein diet (8.05mg/dl) compared to (8.10mg/dl) and (8.13mg/dl) for RKM and HKM respectively signified no observable muscular wastage brought by the inadequacy of protein (17). Explained that in a diet that is deficient in essential amino acids, the amino acid present will be deaminated and hence result in the excretion of urea.

In serum enzymology, the concentration of the enzyme is used in the diagnosis of heart, liver and kidney damage which gives valuable information as regards their state of damage (18). The observed enzyme activity below the physiological range for rats is an indication that the animals did not suffer any heart, liver and kidney damage, as expected in the serum concentration of AST which is an indication to measure the state of heart and liver function in animals (19).

5. Conclusion

It can be concluded that although the protein quality of WKSM is inferior to that of casein, it still holds a promise as a feed resource in livestock nutrition.

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