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A Study on the Chemical and Phytochemical Composition of *Gongronema Latifolia* (Utasi) Leaf Meal, Using Performance and Organ Histopathology of Grower Rabbits as Indices of Its Nutritional Significance

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Abstract

Studies were carried out to determine the chemical composition of the leaf meal of *Gongronema latifolia* (Utasi), using organ histopathology of mixed breed grower rabbits as an in-vivo confirmatory reflector of its nutritional significance after a feeding trial. The proximate analysis of the leaf meal yielded 8.04%; 14.25%, 60.39%, 6.26%, 2.84% and 2.84% of moisture; crude protein, NFE, ash, ether extract and crude fibre respectively on dry matter bases and 2903.41Kcal/kg. In the mineral analysis, minerals detected were: Calcium (10.80mg/ 100g), Magnesium (45.00mg/ 100g), Potassium (486.00mg/ 100g), Sodium (3.86mg/ 100g), and Phosphorus (395.30mg/ 100g). The phytochemical screening of the leaf meal yielded 1.03, 0.37, 0.47, and 0.55g/100g of Alkaloids, Flavonoids, Saponins and Tannin, respectively. Other phytochemicals found were Phenols (0.17mg/100g), Phytates (0.12mg/100g), and cyanogenic glycosides (7.07mg/100g). Analysis of the leaf meal protein detected 17 (seventeen) amino acids, comprising of both essential and non-essential amino acids, almost in the ratio of 1:1. In the feeding trial with rabbits, 4 experimental diets were formulated such that diet 1 (control) contained 0% *Gongronema latifolia* leaf meal (GLLM) while diets 2, 3 and 4 contained 10%, 20% and 30% GLLM, respectively. Each diet was fed to a group of 9 grower rabbits for 49 days. All performance parameters (average initial body weight, average final body weight, average body weight gain, average daily feed intake, average daily body weight gain, and feed-to-gain ratio), were not significantly ($P<0.05$) affected by dietary treatments. There were no lesions of pathologic significance in the tissues (liver, kidney and pancreas) examined. The results of the in-vivo and in vivo investigations suggest that GLLM is not toxic, and is sufficiently nutritious to support rabbit production.

Keywords: *Gongronema Latifolia, Grower Rabbits, In-Vitro, In-Vivo, Leaf Meal, Organ Histopathology*

1.0 INTRODUCTION

The use of leaf meal of plants as feed ingredients, as an alternative to conventional feed sources is a novel area of research in animal nutrition. The competition for food between man and his livestock has been a major concern to nutritionists. Conventional feed ingredients high in energy which are used in compounding diets for livestock (e.g. maize, millet, sorghum, etc.) are expensive. The current pressure on these feedstuffs resulting in high cost of feed and livestock products calls for the need to explore the nutritional and feeding value of some unconventional feedstuffs that are cheaper and readily available. Utilization of leaf meal such as *Glyricidia sepium* (1, 2), cassava plant (3), wild sun flower (4), mimosa leaf meal (5), *Calapogonium mucunoides* (6), etc as sources of protein and/or energy in livestock nutrition are well documented (7). A number of workers including (8, 9) have shown that various alternative feedstuffs have been fed to poultry and rabbits with remarkable results. Some of these alternative sources including the leaf meals of some tropical legumes and browse plants, rich in nutrients like vitamins, minerals and oxycarotenoids have been reported (10, 11, 12, 13, 14, 15).

Gongronema latifolia (utazi) is a wild tropical creeping plant with lush deep green vegetation. Where it grows in a swampy area or inland valley, the vegetation is perennial, otherwise, it is deciduous (16). *Gongronema latifolia* is abundantly available in Cross River State, Nigeria. It can readily be found in its southern part, up to areas beyond the central region of the state. The location of which lies along longitudes 8^{o} and 9^{o} East, and latitudes 6^{o} and 7^{o} North of the equator, with a warm weather and an ambient temperature range of 21 – 30^{o}C. It experiences an annual rainfall of 500 – 1070mm (17). The forest areas of Akamkpa, Biase, Ugep, Mkpani, and Obubra among others are rich reservoirs of the plant.

So far, studies on *G. latifolia* have been basically *in vitro* investigations. It is worthy of note that the nutritional and pharmacological principles of the plants oftentimes behave differently in the animal's alimentary environment from what obtains in *in vitro* studies. This is because the factor(s) under investigation may be inactivated in the *in vivo* environment, or they may form complexes which limit or inhibit their bio-availability. Sometimes the factor(s) may be flushed out of the system so rapidly that they have no time to act. At other times active ingredients in the plant materials can be poisonous or toxic at certain concentrations or administration regimen. This is why an *in-vivo* investigation of GLLM in feeding trial with growing rabbits also became necessary alongside the in-vitro study.

Rabbits are unique animals, which serve as a flexible financial reserve for rural population and as well play other socio-cultural roles in the customs and traditions of many Nigerian societies. The prolific nature of rabbits, coupled with its short gestation period makes it the animal of choice for multiplication, and a short way of increasing animal protein intake (18). The acute shortage of animal protein in the diets of Nigerians demands that efforts should be directed to animals which are prolific and have short gestation period such as pig, poultry and rabbits. However, the increased price of pork and chicken constitute a hindrance to their consumption. Moreover, pigs and poultry have the disadvantage of competing directly with man for available feed ingredients, but rabbits can be raised on high fibre feedstuffs not utilized by man (19). The rabbit appears to be the most sustainable means of producing high quality animal protein for the expanding populations of the lesser developed countries like Nigeria. The rabbit is small-bodied (2.5 – 5.4Kg) animal, and has a short generation interval (6 months). Other desirable attributes of this animal include rapid growth rate, genetic diversity, large litter size, ability to utilize forage and agricultural by-products;

and adaptation over a wide range of ecological environments. Rabbits do not compete with humans for grains as strongly as chickens (20, 21). Unlike bigger animals such as cattle, rabbits can be tended by women, children or old men, as they do not need force to be restrained (22). The small body of a rabbit provides a small carcass that can be consumed by a family in one meal, thus eliminating the need for meat storage and refrigeration. In addition, it is affordable and its management requirements are low-cost. Rabbit production can provide the impoverished urban population and the resource-poor rural dwellers the opportunity to meet part of their total protein needs, as well as earn additional income (22).

Histopathology is a branch of pathology concerned with the study of disease in a tissue section. It constitutes histological procedures aimed to provide good quality tissue sections that can be used for a light microscopic evaluation of animal (or human) tissue changes in either spontaneous or induced diseases (23). Histopathological assay, like hematological and serum biochemical analysis provides information on the health status of animals. After a feeding trial with graded dietary levels of *G. latifolia*, histopathological examination of internal organs of the experimental rabbits will complement the *in-vitro* evaluation in the determination of the nutritional status of GLLM.

There is paucity of information on the nutritional, phytochemical and toxicological properties of *G. latifolia* leaf meal. Research work on GLLM leaf meal have largely been in-vitro, hence the synergy of in-vitro and in-vivo investigation has become necessary in order to generate more authentic data on its nutritional properties. It would therefore seem necessary to carry out a study to determine its nutritional significance as feed ingredient in rabbit diets, using histopathology of internal organs of the experimental animals as a reflector of the same.

2.0METHODOLOGY

Location of Study

The research was carried out in the Teaching and Research Farm of the School of Agriculture and Agricultural Technology (SAAT), of the Federal University of Technology (FUT), Owerri, Nigeria. The histopathological aspect of the study was carried out at the laboratory of the Department of Anatomy, College of Medicine, University of Calabar, Nigeria, while the amino acid assay and phytochemical analysis were done at the Department of Biochemistry laboratory, University of Jos, Nigeria, and the central laboratory of the National Root Crops Research Institute (N. R. C. R. I.), Umudike, respectively.

Preparation of *Gongronema latifolia* Leaf meal (GLLM)

Fresh leaves of *G. latifolia* were harvested from the forest area of Ovonum in Obubra Local Government Area of Cross River State, Nigeria. Harvested leaves were dried under shade to prevent inactivation of its chemical constituents by direct sunlight. The drying process which was done in the months of December and January was carried out for 5 – 7 days until the leaves became crispy to touch. The dried leaves were milled to particle sizes that would pass through a 2mm sieve, using a hammer mill. The *G. latifolia* leaf meal (GLLM) so prepared was stored in air-tight plastic containers under cool dry conditions prior to use.

Determination of Proximate and Metabolisable Energy of GLLM

Samples of the leaf meal were subjected to proximate analysis using the methods described by [24]) to determine the following proximate fractions:

Moisture content, Percentage dry matter (DM), Crude protein (CP) as a percentage of DM, Crude fibre (CF), as % DM, Ether extract (EE), as % DM, Ash, as a Percentage of dry matter (DM), Nitrogen free extractives (NFE), as % DM .

The metabolisable energy (ME) of the leaf meal was calculated, using the methods of (25), using data from proximate analysis.

Mineral Analysis

The following minerals were determined using the standard procedures (AOAC, 2006 [24]):

Calcium (Ca), Magnesium (mg), Potassium (k), Phosphorus (P), and Sodium (Na)

Amino Acid Analysis

The analysis of GLLM protein to determine its constituent amino acids was carried out in the Department of Biochemistry laboratory, University of Jos, Nigeria, using methods described by (26). Fresh samples of GLLM were subjected to acid hydrolysis using 6N HCl. Thereafter the hydrolysate was recovered by removing the acid through evaporation at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0), stored in plastic bottles and kept in a freezer. Between 5 – 10 micro-liters of the hydrolysate was loaded into a TSM amino acid analyzer (Technicon Sequential Multi-sample Amino acid analyzer (TSM), Technicon Instruments Corporation, New York). The TSM analyzer separated and analyzed free acidic, neutral and basic amino acids of the hydrolysate, at the end of which it produced a chromagram which represented corresponding values of the different amino acids.

Phytochemical Screening of GLLM

The phytochemical screening of *G. latifolia* leaf meal was carried out at the Central laboratory of the National Root Crops Research Institute (N.R.C.R.I.), Umudike. Factors screened included alkaloids, flavonoids, tannins, saponins, cyanogenic glycosides and phenols.

Alkaloid was determined using the alkaline precipitation method of (27); The ethyl acetate precipitation method of (28) was used in the determination of flavonoids; Follins-Dennis spectrophotometric method described by (29) was used in the determination of Tannins;The content of saponin in the sample was determined by double solvent extraction gravimetric method of (27); Whilst The Follins-Dennis method described by (29) was used to determine phenol content of GLLM.

Feeding Trial with Rabbits

This experiment was carried out in the School of Agriculture and Agricultural Technology (SAAT) Teaching and Research Farm, Federal University of Technology, Owerri.

Experimental Diets

Four grower rabbit experimental diets were formulated to contain *C. latifolia* leaf meal at 0.00 (control), 10.00, 20.00 and 30.00 per cent inclusion levels, for treatments 1, 2, 3, and 4 respectively. The experimental diets composition is shown in table 1.

Experimental Rabbits and Design

A total of thirty-six (36) crossbred rabbits (hybrid) of Dutch and Chinchilla) aged between seven and nine weeks were used for the study. The animals were procured from the Nigerian Veterinary Research Institute (NVRI), Vom-Jos, Nigeria

After seven days of stabilization with conventional concentrate diet, they were distributed into four treatment groups of nine rabbits each, on weight equalization basis and randomly assigned to the four experimental diets in a completely randomized design (CRD). Each treatment was sub-divided into three replicates of three rabbits each. Animals in each replicate were housed in a seperate wooden cage equiped with wire mesh floors. The animals were fed for fourty-nine (49) days. During this period, animals in all the treatments were offered feed and water *ad libitum*, and all necessary prophylactic medications were also carried out.

Data Collection

The rabbits were weighed at the beginning of the trial and thereafter on a weekly basis. Daily feed intake per treatment was measured. This was determined by the difference between the weight of feed offered and that of the left-over the next morning. On the whole, data collected included: initial body weight, weekly body weight, final body weight and mortality; total feed intake, weekly body weight gain, total body weight gain and feed conversion ratio (g feed/g gain) were calculated at the end of the trial.

Table 1: Ingredient and Chemical Composition of Experimental Diets

Ingredients	GLLM	Dietary	Levels	(%)
	0	10	20	30
White maize	52.00	47.00	42.00	37.00
Soybean meal	10.00	10.00	10.00	10.00
GLLM	-	10.00	20.00	30.00
Wheat offal	20.00	15.00	10.00	5.00
Palm kernel cake	10.00	10.00	10.00	10.00
Fish meal	2.00	2.00	2.00	2.00
Blood meal	2.00	2.00	2.00	2.00
Bone meal	3.50	3.50	3.50	3.50
Common salt	0.25	0.25	0.25	0.25
Vit./mineral premix**	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated chemical composition				
Crude protein	18.86	19.42	19.88	17.88
Crude fibre	4.63	4.39	4.15	3.91
Ether extract	3.84	3.75	3.66	3.57
Ash	3.37	3.61	3.85	4.09
Calcium	1.49	1.48	1.48	1.48
Phosphorus	0.77	0.79	0.81	0.83
L-methionine	0.30	0.37	0.42	0.49
L-lysine	0.88	1.28	1.56	1.87
ME (kcal/kg)	2765.98	2781.12	2816.26	2841.40

** To provide the following per kilogram of diet: Vit.A, 10,000 iu; Vit.D3, 2000 iu; Vit.E, 5 iu; Vit.K, 2mg; Riboflavin, 4.20mg; Vit.B_{12}, 0.0mg; panthotenic acid, 5mg; Nicotinic acid, 20mg; folic acid, 0.5mg; choline, 3mg; Mg, 56mg; Fe, 20mg; Cu, 10mg; Zn, 50mg; Co, 125mg.

Harvest of Internal Organ

At the end of the feeding trial, three rabbits per treatment group were randomly selected for harvest of internal organs. The animals were starved of feed only for twenty-four hours. Thereafter they were slaughtered and thoroughly bled. The carcasses were cleaned, dissected and eviscerated, after which the kidney, liver and pancreas were harvested. Thereafter the organ samples were quickly transferred to formalin solution in preparation for histopathological study.

Histopathological Study of Rabbits

This study was executed at the Department of Anatomy Laboratory, College of Medical Sciences, University of Calabar, Nigeria

After organ weight evaluation, internal organs used for the procedure (liver, kidney and pancreas) were cut and fixed in Bouin's fixative for 24 hours, then hydrated, cleared and infiltrated in molten paraffin. Thereafter, the tissues were embedded in pure paraffin wax and sectioned at 5 - 6 micron in a microtome, after which they were stained with haematoxilin and eosin (H and E) and subsequently examined by light microscopy for histopatological changes.

Statistical Analyses

Data on feed intake, body weight gain, feed conversion ratio, hematology, serum biochemistry, and organ weights evaluation were subjected to one-way analysis of variance (ANOVA) as outlined by (30). Where ANOVA detected significant treatment effects, means were separated using the Duncan's New Multiple Range Test (DNMRT) as outlined by (31).

3.0 FINDINGS

The Proximate/mineral composition of GLLM, its amino acid profile and phytochemical composition is shown in Tables 2, 3, and 4, respectively.

Table 2: Proximate/Mineral Composition of GLLM

Nutrients	Concentration (%)	SE (±)
Moisture	8.04	0.19
Dry matter	91.96	0.01
CP (%DM)	14.25	0.01
EE (%DM)	2.84	0.02
Ash (%DM)	6.26	0.01
Crude fibre (%DM)	2.84	0.02
NFE (%DM)	60.39	0.01
ME (Kcal/kg)	2903.41	0.99
Calcium (mg/100g)	10.80	0.12
Mg (mg/100g)	45.00	1.20
K (mg/100g)	486.00	0.58
Na (mg/100g)	3.86	0.01
P (mg/100g)	395.30	0.15

Values are means of triplicate determinations

Source: Ukorebi and Akpet (2019)

Table 3: Amino Acid Profile of GLLM

Amino acids (g/100g)	Concentration
Lysine	3.97
Histidine	2.19
Arginine	4.42
Aspartic acid	7.75
Threonine	3.02
Serine	2.41
Glutamic acid	9.27
Proline	2.97
Glycine	3.60
Alanine	4.09
Cystine	0.86
Valine	4.01
Methionine	0.86
Isoleucine	3.39
Leucine	6.70
Tyrosine	2.90
Trytophan	ND
Phenylalanine	3.97

ND: Not detected

Source: Ukorebi (2021)

Table 4: Phytochemical Composition of GLLM

Compound	Concentration
Alkaloids (%)	1.03
Flavonoids (%)	0.37
Saponins (%)	0.47
Tannin(%)	0.55
Phenols (mg/100g)	0.17
Phytates (mg/100g)	0.12
Cyanogenic glycosides (mg/100g)	7.07

Source: Ukorebi (2021)

For the proximate fractions, the moisture content was 8.04%. The percentage composition of other proximate fractions on dry matter basis was crude protein, 14.25; ether extract 2.84 and ash, 6.26. Others were crude fibre, 2.84 and nitrogen free extractives, 60.39.

The five minerals analysed for yielded the following results (mg/100g): calcium 10.8 magnesium, 45; potassium, 486; sodium, 3.86 and phosphorus, 395mg/100g.

The result of analysis of GLLM protein to determine its amino acid profile is showed that the concentrations of lysine, histidine, arginine and aspartic acid (g/100g protein) were 3.97, 2.19, 4.42 and 7.75 respectively. Others were threonine, 3.02; serine, 2.41; glutamic acid, 9.27; proline, 2.97; glycine, 3.60 and alanine, 4.09. Cystine concentration was 0.86, valine, 4.01 methionine,

0.86 while yields of 3.39, 6.70, 2.90 and 3.97 were recorded for isoleucine, leucine, tyrosine and phenylalnine, respectively.

From the quantitative phytochemical analysis of GLLM, the percentage composition of alkaloid, flavonoid, saponin and tannin were 1.03, 0.37, 0.47, and 0.55%, respectively. Others were phenol, 0.17; phytate, 0.12 and cyanogenic glycoside, 7.07 (all in mg/100g).

Results of In-Vivo Study (Feeding Trial)

The calculated nutrient composition of the treatment diets is shown in Table 1.

The metabolizable energy values of the diets increased with increasing levels of GLLM. The control diet which contained 0% GLLM had the lowest energy level of 2765.98kcal/kg, whilst treated diets with 10.0, 20.0 and 30.0 percent levels of GLLM were 2781.12, 2816.26 and 2841.40kcal/kg, respectively. On the other hand, the diet with the highest crude protein content (19.88%) was the treatment diet containing 20.0% GLLM. This was followed by the T_2 (10.0% GLLM) which had 19.42% crude protein. The control treatment and T_4 (30% GLLM) contained 18.86% and 17.73% crude protein, respectively.

The effects of the different dietary levels of GLLM on the performance of grower rabbits is summarized in Table 5. The initial average body weights of the rabbits assigned to the different treatments were 716.7, 765.6, 777.8 and 788.9 grams for 0% (control), 10.0% (T_2), 20.0% (T_3) and 30.0% (T_4), respectively.

There were no significant differences ($P>0.05$) among the treatment groups in respect of initial average body weights.

Table 5: Effects of Different Dietary Levels of GLLM on Growth Performance of Grower Rabbits

Parameters	Dietary Level of GLLM (%)				
	T_1 (0)	**T_2 (10)**	**T_3 (20)**	**T_4 (30)**	**SEM**
Av. Initial body wt. (g)	716.7	755.6	777.8	788.9	21.16
Av. Final body wt. (g)	1542.2	1422.2	1328.8	1300.0	73.39
Av. Body wt. gain (g)	825.5	666.6	551.0	511.1	82.51
Av. Daily feed intake (g)	71.9	74.9	73.3	70.8	1.65
Av. Daily body wt. gain (g)	16.8	13.6	11.2	10.4	1.47
Feed conversion Ratio	4.27	5.51	6.54	6.81	0.49

Average Final Body Weights

The average final body weights of the rabbits after 49 days of feeding trial were 1.54, 1.42, 1.33 and 1.3kg, respectively for T_1 (control), T_2. (10% GLLM), T_3 (20% GLLM) and T_4 (30% GLLM). There were no significant differences ($p>0.05$) among the treatment means.

Average Body Weight Gain

Data on average body weight gain of the rabbits were 0.83kg (T_1), 0.67kg (T_2), 0.55kg (T_3), and 0.51kg (T_4). The weight gains were not affected ($p>0.05$) by the treatments.

Average Daily Feed Intake

The average daily feed intake of the rabbits were 71.9gm (T_1), 74.9gm (T_2), 73.3gm (T_3) and 70.8gm (T_4). There were no significant differences ($p>0.05$) among the treatments.

Average Daily Body weight Gain

The average daily body weight gains of the groups were, 16.8, 13.6, 11.2 and 10.4 grams, respectively for T_1, T_2, T_3 and T_4. There were no significant differences ($p>0.05$) among the groups.

Feed Conversion Ratio (FCR)

The feed conversion ratio were 4.27 (T_1), 5.51 (T_2), 6.54 (T_3), and 6.81 (T_4). There were no significance differences ($P>0.05$) among the treatment.

Organ Histopathological Studies (Rabbits)

The effects of graded levels of GLLM in the diets of grower rabbits on the histological integrity of livers, kidneys and pancreas tissues of the experimental rabbits are shown in table 6

The photomicrograph of liver tissues from all treatment groups presented a liver lobule with its central vein at the center. The hexagonal shaped hepatocytes are shown radiating from the central vein. The hepatocytes had the normal open faced nuclei. Some of the hepatocytes had more than one nucleus. The radiating patterns of the hepatocytes were well outlined.

The kidney tissues showed venal cortex with rounded glomeruli. The endothelial cells were intact. The glomerular capsules were outlined and their nuclei prominent. The distal and proximal convoluted tubules were also differentiated with the nuclei well stained.

Pancreas tissues examined in T_1 animals showed aciner tissues in a background of congested and oedematous matrix with focal areas of hemorrhage, and the islet cells were not well stained. Tissues of T_4 animals showed poorly stained cytoplasm and nucleus, but no inflammatory cells were seen. However, tissues from T_2 and T_3 showed no degenerative changes.

Table 6: Effects of Graded Dietary Levels of GLLM on Histopathological Structures of the Kidneys, Livers and Pancreas of Rabbits

Parameters	Dietary	Levels	Of	GLLM (%)
	0	10	20	30
Pancreatic necrosis (Pancreas)	-	-	-	-
Edematous cytoplasm (pancreas)	+++	-	-	++
Intercellular reaction (Kidney)	-	-	-	-
Hepatic necrosis (Liver)	-	+	+	-
Fatty change (Liver)	+	-	-	-
Portal fibrosis (Liver)	-	+	-	-

(+) Relative presence of Histopathological lesions.

(-) Absence of Histopathological Lesions

Discussion

Proximate/mineral Composition of GLLM

The proximate composition of GLLM (Table 2) showed that crude protein and nitrogen free extractive (NFE) values were 14.25 and 60.39 percent of dry matter respectively. These values are quite reasonable for a leaf meal. The Cp value compares favorably with those of some multipurpose tree leaves like *Enterolobiam cyclocarpum*, which contains 14.45% cp; and *Pterocarpus santalonoides* (15.32% cp). However, when compared to *Gliricidia sepium* (19.26%) and *Leucaena leucocephala* (26.27%) reported by (32), it tends to rank low. The Crude fibre

content (CF) of 2.84% is amazingly low for a leaf meal, far less than those of other plants leaves investigated and compares favourably with those of cereal grains like maize, guinea corn, wheat, wheat, and barley used in livestock feeding (33). This properly recommends GLLM above its counterparts for monogastric feeding programmes (34). Its metabolisable energy (ME) value of 2903.41 Kcal/kg compares favourably with those of conventional energy concentrates such as maize, guinea corn, millet, wheat, etc., while its ash content are comparable to those of most leaf meals investigated.

Of the five minerals assayed (mg/100g), Sodium (3.86) and Calcium (10.8) were the least abundant, while Phosphorus (395.3) and Potassium (486.0) were the highest in concentration. The value of Potassium in G. latifolia, tends to agree with the reports of (35) which noted that Phosphorus and Calcium are always found together in the body (within the blood, teeth and bones), as well as in animal products like milk and in poultry products like egg, as well as egg shell. The ratio of Calcium to Phosphorus in the body is of significant importance for certain physiological processes. In this study, the Ca/P ratio of 0.027 in GLLM is less than the recommended dietary ratio of 0.05, this implies that GLLM Calcium concentration may not be sufficient for normal Calcium/Phosphorus metabolism in the body unless its use in feeding programmes is carried out with Calcium supplementation (34). According to (36), low Ca/P ratio in the diet facilitates calcinations in the small intestine. Indeed, apart from its role in the skeletal and associated structures, Calcium is also important in blood clotting, muscle contraction and in certain enzymatic processes. The ratio of Sodium to Potassium (Na/K ratio) measured in mg/100g in GLLM was found to be 0.008. The ratio of Sodium to Potassium (Na/K ratio) in the body is of human health care concern because it is a factor in the control of high blood pressure. Accordingly in this respect, a ratio of less than one (1.00) has been recommended (36). It would therefore seem that the consumption of G. latifolia could be beneficial to humans in alleviating the malaise in question.

Phytochemical Composition of GLLM

The quantitative phytochemical analysis of GLLM for anti-nutritional factors presented in table 4.0 showed that alkaloid had the highest concentration of 1.03%, followed by tannins, saponins and flavonoids. Among the compounds measured in mg/100g, cyanogenic glycoside had the highest value (7.07mg/100g), followed by phenols and phytates.

Out of the several thousands of alkaloids known, two forms (aristolochic acid and pyrrolizidine), are of particular nutritional concern because of their toxicity (37). Aristolochic acids are nephrotoxic, carcinogenic and mutagenic (38), whereas pyrrolizidine is often implicated in hepatotoxicity (39). These compounds also cause damage to lungs and kidney (40, 41). The result of this study, however, suggest that the alkaloids contained in GLLM are none of the toxic forms described above, as no pathological effects characterizing them were observed in the histological intergrity of the internal organs of the experimental animals.

Tannins are reputed for their capacity to bind dietary proteins thereby reducing the nutritive value of feeds in that regard. (42) reported that the serum biochemical studies of weaner rabbits fed graded dietary levels of GLLM showed a consistently high serum protein, when compared to the control animals. This result suggests that the 0.55% content of tannins in GLLM is a tolerable level or that the bulk of the compound (tannins) is the condensed form which is less toxic than the hydolysable ones.

Flavonoids are classified into several groups that perform functions of pigmentation and defense in plants. Reports by (43) indicated that such groups include anthocyanins that attract insects, flavones and flavonols that protect the plant from excessive ultra violet radiation and iso-flavonoids that provide antifungal and antibacterial defenses to the plant. This indicates that flavonoids may impart antibacterial properties on GLLM.

The content of phytate in GLLM refers. Phytates usually form insoluble salts with calcium, this condition is likely to adversely affect the absorption of dietary calcium, and/or utilization of the element in the animal body. Phytate can also affect digestibility by chelating with calcium or by binding with substrate or proteolytic enzymes (44). However, the performance of experimental rabbits and histopathological characteristics of their internal organs did not reflect these adverse effects on the animals.

The concentration of saponins in GLLM was 0.47%. Saponins are steroids or tri-terpenoid glycosides which are characterized by their bitter or astringent taste, foaming properties, and hemolytic effects on red blood cells. Saponins have been shown to possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic and permeabilization of the intestine) effects (45, 46). The adverse effects saponins on experimental rabbits could not be detected within the scope of the experiment.

Another important phytochemical detected in GLLM was cyanogenic glycoside, with a value of 7.07mg/100g. This concentration is higher than those recorded for some legume seeds, as reported by (47). The toxicity of cycanogenic glycosides and their derivatives is dependent on the release of hydrogen cyanide (HCN) on hydrolysis. Cyanide toxicity can occur in animals including humans at a dose between 0.5 and 3.5mg HCN per Kilogram body weight (48). The deleterious effects exhibited by a lethal dose of HCN is characterized by its rapid reaction with serum metal ions of iron and copper, leading to a series of reactions to form cyanohaemoglobin which is not an oxygen carrier. Large doses of cyanogenic glycosides would therefore cause death, by inhibition of cell respiration. However the cyanogenic glycoside value of GLLM (7.07mg/100g) recorded in the study, did not seem to elicit toxic effects on experimental rabbits at dietary inclusion levels of GLLM reaching 30%. This inference is made from the performance characteristics of the experimental animals as well as from the histopathological investigation of their internal organs.

According to (42) up to 30% dietary inclusion of GLLM in the diets of weaner rabbits elicited no deleterious effects on hematological and serum biochemical characteristics of the animals. This suggests that the phytochemical factors in GLLM are not such as could cause health hazards in humans and livestock.

Amino Acid Analysis

The analysis of GLLM protein detected 17 amino acids, made up of both essential and non-essential amino acids, almost in the ratio of 1:1. The most abundant amino acids were glutamic acid and aspartic acid. These were followed by leucine, arginine, alanine and valine respectively. Apart from arginine, other amino acids in this second group rank with those of oil seeds such as peanuts, soybean, buffalo gourd and colocynthis in value (49). The concentration of aspartic acid was comparable to those of the oil seeds earlier mentioned. The values of the sulphur containing amino acids, methionine and cystein were generally low. This trend is common to most leaf meal proteins already studied.

On the other hand, the value of lysine compared with those of the oil seeds except for Soybean (50). This implies that GLLM protein could be of a better quality than cereal proteins which are generally deficient in lysine. The significance of the amino acid profile of a protein cannot be over emphasized, as protein quality and its utilization in the animal's physiological environment depends much on it, especially in monogastric nutrition. According to Ukorebi [52], the values of Leucine (6.70g/100g protein), and Phenylalanine (3.97g/100g protein) in GLLM protein are higher than the reference values of 4.20g/100g protein and 2.80g/100g protein, respectively reported by (51); lysine value (3.97g/100g protein), and the value of valine (4.01g/100g protein) compare favourably to the reference values of 4.20g/100g protein and 4.20g/100g protein, respectively as published by (51). The researcher however posited that GLLM methionine and threonine values of 0.86g/100g protein and 3.02g/100g protein, respectively are far below the reference standards of 2.2g/100g protein and 17.5g/100g protein, respectively as indicated by (54). He then concluded that GLLM protein cannot be used as a sole protein source in diets but needed methionine and threonine supplementation, since they are essential amino acids.

Performance of Experimental Rabbits

The performance characteristics of rabbits fed graded dietary levels of GLLM refers.

Body Weight Gain

There were no significant differences (P>0.05) among treatment groups for average final body weights, average body weight gain and average daily body weight gain. However there was a general dose-related depression in growth of the rabbits induced by dietary levels of GLLM. The mean daily weight gain range of between 10.4 to 16.8g obtained in this study is similar to 10.1g reported by (53) when groundnut leaves were fed to rabbits; 11.15g reported by (54) in a feeding trial with angora rabbits involving brewer's dried grain; and the result of 10.2 to 12.8g obtained by (55) in a study on the evaluation of the feed value of sugar cane scrapping meal for weaner rabbits. Also comparable to daily growth rates pattern of rabbits in this experiment is the result obtained by (56) which reported a mean daily growth rate of 9.06 to 10.31g per rabbit in a study which compared the performance of young growing rabbits fed diets containing cracked and cooked Jackbean soaked in water prior to cooking, but lower than 17.65 to 18.80g recorded by (57) on cassava leaf and peel meals.

Average Daily Feed Intake

There were no significant differences (P>0.05) among the treatment groups for average daily feed intake. However, apart from T_2 (10.0% GLLM) which recorded the highest daily feed intake, the feed consumption trend indicated a general decline with increasing levels of GLLM. This result compares favorably with the work of (58) which reported a non-significant (p>0.05) difference among treatments in the daily feed intake of weaner rabbits fed graded dietary levels of wild sunflower (*Tithonia diversifolia* Hemsb A.Gray) leaf-blood meal mixture.

The lower feed intake of the diets containing higher GLLM inclusion levels might be attributable to the combined effects of low palatability, higher energy contents and perhaps the antinutritional factors (ANFS) content of the diets. The consumption of feed by animals is a function of its palatability, energy, fibre and ANFS contents among others. It is important to note that *G.latifolia* leaves have a strong bitter taste which could discourage its consumption by animals. It will also be recalled that the calculated analysis of the treatment diets for the experiment indicated that their energy values increased linearly with the level of GLLM, while the fibre content followed the

opposite trend. It is well known that rabbits have preference for diets with reasonable fibre content than those very low in fibre. This might account for their higher consumption of the control diet which was more palatable and richer in fibre than the test diets.

Feed Conversion Ratio

There were no significant differences ($p>0.05$) in feed to gain ratios among the treatment groups. The range of values (4.27 – 6.81) obtained in this study is comparable to 4.63 - 7.09 reported by (59) on the growth performance of growing rabbits fed graded levels of garlic (*Allium sativum*), but higher than 3.45 - 5.12 reported by (60) on utilization of different levels of gliricidia leaf meal by growing rabbits. It is also higher than 4.43 - 5.25 reported by (61) on evaluation of growth performance, blood mineral profile and carcass characteristics of weaner rabbits fed rice milling waste, but superior to the value of 11.27 - 16.35 reported by (62) on performance of young rabbits fed two grass and two legume species supplemented with compounded diet. This shows that the test ingredient (GLLM) can compete with some non-conventional feed ingredients used in rabbits feeding.

Histopathological Observations

Results of the histopathological studies conducted were represented by presence or absence of lesions on the organs investigated. Lesions are described as structural or functional abnormalities in cells, tissues or organs which is an indication of static presentation of a dynamic process (63). When many cells in an organ or tissue undergo degeneration or necrosis, a visible lesion is produced which in turn interferes with homeostasis.

Table 4.9 shows observed histopathological state of the livers, kidneys and pancreas tissues of the grower rabbits fed graded levels of GLLM in their diets.

There were no lesions of pathologic significance in liver tissues of experimental animals. The increase in the number of nuclei in the hepatocytes showed that the cells were not necrotic but rather normally dividing. It would therefore seem that GLLM is not hepatotoxic.

Kidney tissues of the experimental animals showed no degenerative changes in all the treatments. The clearance between the glomerular capsule and the glomerulus indicated the presence of a functional urinary space. In addition, the non-distortion of the entire tissue cyto-architecture is indicative of a normal renal integrity. Pancreas tissues of T_2 (10% GLLM) and T_3 (20% GLLM) experimental animals showed no degenerative changes.

However, poor staining of tissues and edematous cytoplasm were observed in T_1 (0% GLLM) and T_4 (30% GLLM). This condition might not be attributable to toxic factors in GLLM as the diet of T_1 animals did not contain the test material. The abnormalities could be traceable to poor histopathological processing of tissues prior to staining and microscopic examination.

4.0 CONCLUSIONS AND APPLICATIONS

1. Dietary inclusion of GLLM up to 30% showed no significant performance impairment of grower rabbits, this suggests that it is nutritionally beneficial in raising grower rabbits at that level.
2. The analysis of GLLM protein detected 17 amino acids, made up of both essential and non-essential amino acids, almost in the ratio of 1:1. This suggests GLLM protein is good quality protein that could be harnessed in livestock feed production.

3. Up to 30% dietary level of CLLM did not significantly affect the histological integrity of the livers, kidneys and pancreas of grower rabbits, indicating that its phytochemicals are not lethal to the animals.
4. In general, the findings of the experiment suggests that GLLM contains phytochemicals and nutrients that can be of beneficial nutritional significance in rabbit production.

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