



Haematological and Serum Biochemical Indices of Weaner Rabbits fed Varying Levels of Dried *Gmelina arborea* Leaf Meal

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Authors' contributions

This work was carried out in collaboration between all authors. Author PCJ designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author DOU managed the literature searches, analyses of the study performed the spectroscopy analysis and author OEK managed the experimental process and author ED identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IBRR/2016/27947

Editor(s):

(1) Dharmesh Chandra Sharma, Incharge Blood Component & Aphaeresis Unit, G. R. Medical College, Gwalior, India.

Reviewers:

(1) Ahmed NF Neamat –Allah, Zagazig University, Egypt.

(2) Agu Kingsley Chukwunonso, University of Benin, Nigeria.

Complete Peer review History: <http://www.sciencedomain.org/review-history/15673>

Original Research Article

Received 27th June 2016
Accepted 12th July 2016
Published 5th August 2016

ABSTRACT

Aims: Rabbit production fulfils important socio-economic functions and has the ability to alleviate the animal protein inadequacy. In Nigeria and most other developing countries, inadequate nutrition undermines rabbit in expressing their full potential. Therefore, a 49-day study was carried out to determine the effect of varying levels *Gmelina arborea* leaf meal (GALM) on haematology and serum biochemistry of weaner rabbits.

Study Design: Completely randomized design (CRD).

Place and Duration of Study: At the Rabbit unit of the Teaching and Research farm of Federal College of Agriculture, Ishiagu, Ivo Local Government Area of Ebonyi state Nigeria between June and August, 2015.

Methodology: 80 weaner rabbits were used for this study. Four diets were formulated such that

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diets T₁, T₂, T₃ and T₄ contain MOLM at 0%, 10%, 20% and 30%, respectively. Other ingredients remained constant for the four diets. The diets were offered to the rabbits which were randomly divided into four experimental groups of twenty animals each, with five rabbits constituting a replicate. Blood samples were drawn from each animal per treatment on the last day of the trial and evaluated for haematological and serum biochemical profile and data obtained analysed statistically.

Results: Results showed that the haematological parameters differed ($P < 0.05$) significantly among the treatment groups. Packed cell volume, red blood cell and haemoglobin ranged between 35.31 – 41.37%, 9.55-12.62 g/dl and 4.39-7.10x10⁶ /ul for T₁ and T₄ respectively and were improved ($p < 0.05$) by GALM supplementation at 30% inclusion level. White blood cell (4.90-10.52x10⁶ /dl) count of weaner rabbits in treatment groups was significantly ($p < 0.05$) higher and better than the control. There were significant ($p < 0.05$) difference in total proteins, urea, creatinine levels, and cholesterol for the treatment groups. The proteins (61.07-55.99 g/l), albumin (38.21-32.23 g/l), urea (35.40–30.96 mg/dl), and cholesterol (46.47-11.58 mg/dl) values tended to decrease with increasing levels of GALM for T₁ and T₄ respectively. Cholesterol was significantly ($P < 0.05$) reduced at 30% inclusion.

Conclusion: These results showed that inclusion *Gmelina arborea* leaf meal had a beneficial effect on health status of weaner rabbits and therefore 30% was recommended for optimum rabbit production.

Keywords: Gmelina arborea; leaf meal; rabbits; haematology; serum biochemistry; alternative feed stuff; medicinal plants.

1. INTRODUCTION

The growing population has informed the need to increase livestock production to satisfy the requirement for animal protein. The requirement for animal protein can be achieved by the production of animals with high prolificacy and short generation interval. Among the livestock of interest is rabbits, a caprophagous herbivore whose production before now has been low [1]. Rabbits play significant roles in solving the animal protein inadequacy, due to their high prolificacy, high growth rate, short generation interval, limited vital space, early maturity, ample nutritional spectrum, remarkable capacity to convert roughages into meat, easy management, good meat quality and low capital intensive.

Intensive approach to rabbit production would however entail the use of alternative nutritionally viable sources other than the conventional ones to enhance the production of meat at affordable price. Such alternative plant sources are currently under investigation; they are being evaluated for nutritiveness, availability, acceptability and affordability. It is in light of the above that *Gmelina arborea* leaf meal, relatively unexploited forage, is being assayed for its feed value.

Gmelina arborea is an evergreen perennial fast growing medicinal tree, of which are used as laxative and anthelmintic, improve appetite,

useful in hallucination, piles, abdominal pains, burning sensations, fevers, and urinary discharge [2]. The leaves are unconventional materials that can be explored for the production of feedstuff. The leaf is one of the tree leaves considered as an important source of nutrient for ruminants and non- ruminants, especially in those areas with a pronounced dry season. Previous records by [3] and [4] on the nutrient profile of *Gmelina arborea* leaves have shown that the leaf meals contained 18.00-20.05% crude protein, 14.40-15.05% crude fibre, ash 4.55±0.49%, fat 0.79±0.02% and metabolizable energy values of 1368 Kcal Kg-1. The leaves, flower, roots and bark are used in medicine. They are useful in blood diseases [2]; thus the use of *Gmelina arborea* in the present study fits in the strategy of increasing immunity and health status of rabbits that is reflections on improving physiological and productive performance.

Generally, both the haematological and biochemical components of blood are influenced by the quantity and quality of feed [5]. Haematological and serum biochemical studies are widely used for the diagnosis of animal diseases as well as investigation of blood damage and feed toxicity and protein quality. They are relevant since blood constitutes change in relation to the physiological conditions of the animals. Blood profile studies are important because the blood is the major transport system of the body and evaluations of the blood profile

usually furnish vital information on the body's response to injury of all forms, feed quality and toxicity. Therefore, this experiment was designed to determine the haematological and serum biochemical indices of rabbits fed dietary levels of *Gmelina arborea* leaf meal.

2. MATERIALS AND METHODS

2.1 Location of Experiment

The research work was carried out at the Rabbit Unit of Federal College of Agriculture, Ishiagu, Ivo Local Government Area of Ebonyi state Nigeria. The College is situated at latitude 5.56°N and longitude 7.31°E, with an average rainfall of 1653 mm and a prevailing temperature condition of 28.50°C and relative humidity of about 80%.

2.2 Sources and Processing of Experimental Material

Fresh leaves of *Gmelina arborea* were harvested within the College environment and air dried for some days to a moisture content of about 10%. The dried leaves were processed and milled and used in the formulation of the experimental diets.

2.3 Management of Experimental Animals

Eighty weaner rabbits weighing averagely 831.25 (g) were randomly divided into four experimental groups of twenty animals each, with five rabbits constituting a replicate. The four treatment groups were assigned the four experimental diets in a Completely Randomized Design. Each rabbit received an assigned diet for 49 days. Each animal was housed in a standard hutch of 120 cm by 150 cm and raised 120 cm from the ground floor. The animals were provided with

feeders and drinkers. Each animal was vaccinated against prevalence under current diseases and were quarantined for 21 days before the commencement of the experiment. They were also dewormed and given accaricides bath prior to the experiment.

2.4 Experimental Diets

Four experimental diets were formulated and designated as T₁, T₂, T₃ and T₄ to contain *Gmelina arborea* leaf meal (GALM) at 0%, 10%, 20% and 30% respectively. Treatment one (T₁) did not contain the test ingredient, thereby serving as the control as presented in Table 1.

2.5 Haematological and Serum Biochemical Studies

Five ml of blood samples were drawn from each animal on the last day of the study. The rabbits were bled through the ear marginal vein. The samples were separated into two lots and used for biochemical and haematological determinations [6]. An initial 2.5 ml was collected from each sample in labelled sterile universal bottle containing 1.0 mg/ml ethyldiamine tetracetic acid (EDTA) and used for haematological analysis. Another 2.5 ml was collected over anti-coagulant free bottle. The blood was allowed to clot at room temperature and serum separated by centrifuging within three hours of collection. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000+ HbA1c analyzer, respectively. Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentrations (MCHC) were calculated.

Table 1. Composition of the experimental diets

Ingredients	Dietary levels (%)			
	T ₁	T ₂	T ₃	T ₄
Maize	43.00	40.00	38.00	35.00
Wheat	22.00	20.00	16.00	12.00
Fish Meal	3.00	3.00	3.00	3.00
Soybean	27.00	22.00	18.00	15.00
<i>Gmelina arborea</i> leaf meal	0.00	10.00	20.00	30.00
Bone meal	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00
Premix	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Total	100	100	100	100

2.6 Proximate Analysis

All feeds and experimental materials were analyzed for proximate compositions according to [7].

2.7 Statistical Analysis

The results were analyzed using the Package for Social Sciences Window 17.0. One -way analysis of variance (ANOVA) was employed to determine the means and standard error. Treatment means were compared using Duncan's new multiple range test.

3. RESULTS AND DISCUSSION

3.1 Proximate Compositions

The proximate compositions of the test ingredient (*Gmelina arborea* leaf meal) and experimental diets are presented in Table 2. Proximate analysis of *Gmelina arborea* leaf meal and experimental diets revealed the presence of dry matter, crude protein, crude fibre, ether extract, ash and nitrogen free extract. The result of the present study compared favourably with the findings of [3] for the same leaf meal. The crude protein value (19.35%) reported in the present study is comparable to 20.05% reported by [4] for the same leaf meal. [8] reported lower value for dry matter (52.75%), crude fibre (11.00%), ether extract (0.50%) and a comparable ash (9.15%) and crude protein content of 19.26%. The differences on the proximate compositions of the leaf meal could be as a result of different climatic conditions, time and age at which the leaves were harvested and processing methods used.

Dry matter (DM) content of *Gmelina arborea* leaf meal containing diets (T₂, T₃, and T₄) compared well with the control diet (T₁). The dry matter content of the experiment diets tend to decrease with increasing levels of the test ingredient (*Gmelina arborea* leaf meal). However, the dry matter values reported in the present study is higher than the range (85.39–89.96%) reported by Ogunsipe *et al.* (2014) for rabbits. The crude protein values also tend to increase with increasing values of GALM among the treatment groups. The crude protein range of values (18.09–18.79%) for the present study is higher than the range (17.12–17.43%) reported by [9], but favourably compared with the range of (18.45–19.50%) reported by [3] for rabbits. The crude fibre contents of the experimental diets did not show any consistent trend. The crude fibre

content of the diet met the crude fibre requirement of 14–18% on dry matter basis as stated by [10]. The ether extract and ash range of values are in agreement with the range of values (4.94–5.73% and 4.82–5.27%) reported by Nkwocha *et al.* [3] for ether extract and ash respectively for the rabbits. The nitrogen free extract (NFE) in this study tended to decrease with increasing levels of GALM, but however, comparable with the control diet. The Metabolisable energy (ME) content of *Gmelina arborea* leaf meal containing diets (T₂, T₃, and T₄) compared with the control diet (T₁), but however were decreasing with increasing levels of the test ingredient.

3.2 Haematological Indices

The haematological profile of rabbit fed dietary levels of *Gmelina arborea* leaf meal is presented in Table 3. There were significant differences (P<0.05) among the treatment groups for all the haematological parameters. Similarly all the haematological parameters examined except packed cell volume tend to increase with increasing levels of GALM. However, packed cell volume (PCV) did not show any particular trend, but recorded the highest value for T₄ animals. The Packed Cell Volume (PCV) values (32.41–41.37%) obtained in this study fell within the normal range of 33.00–50.00% reported by [11] for rabbits. T₄ had the highest value of 41.37 while T₂ had the lowest value of 32.41%. The normal range of PCV observed in this study suggests absence of toxic factor like haemagglutamin and anemia which causes reduced oxygen carrying-capacity of blood, dizziness, leg cramps and rapid heartbeat. PCV values obtained for all treatment groups were within normal range for rabbits which is an indication that the treatment diets were nourishing and nontoxic and influenced adequate blood supply.

The haemoglobin value of T₁ differed (P<0.05) significantly from the treatments containing GALM. The haemoglobin (Hb) concentration compared favourably with the values of 9.70–13.70 g/dl reported by [12] for rabbits fed grasshopper meal. Animals given T₄ diet had the highest Hb concentration of 12.62 g/dl, and fell within the normal range of 9.4–17.4 reported by [13] for healthy rabbits. PCV and Hb being are very strong indicators of nutritional status of animals. The high level of Hb of the treatment diets may imply that the dietary proteins were of high quality.

Table 2. Proximate composition of experimental diets and *Gmelina arborea* leaf meal (% dry matter basis)

Diets	T ₁	T ₂	T ₃	T ₄	GALM
Dry matter	93.29	92.36	91.98	90.95	89.56
Crude fibre	16.23	16.30	16.30	16.86	19.35
Crude protein	18.09	18.17	18.45	18.79	17.56
Ether extract	5.31	5.64	5.36	5.96	2.36
Ash	3.94	3.46	3.75	3.49	8.24
Nitrogen free extract	49.72	48.79	48.12	45.85	42.05
Metabolisable energy (Kcal/kg)	2824.70	2823.00	2785.55	2771.45	2286.95

GALM = *Gmelina arborea* leaf meal

The red blood corpuscles (RBCs) count showed significant differences ($P < 0.05$) among treatments. The values were within the range of 3.07 to $7.50 \times 10^6 / \text{mm}^3$ reported by [13]. Increased RBC values are associated with high quality dietary protein and with disease free animals. These observations were related to the composition of the diet and the health status of the rabbits since no rabbit died during the experiment.

The mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were all significant ($P < 0.05$). The values are relative to the normal range of $60 - 73$ fl, 16.23 pg and $26-34\%$ for MCV, MCH and MCHC respectively [14]. MCV range of ($78.97 - 88.97$ fl) was in tandem with the values reported by [15] for MCV (63.4 ± 0.7 fl) and higher than ($54.71-68.34$ fl) reported by [16] for rabbits fed millet offal based diet. Similarly, the values $28.34 - 33.51\%$ observed for MCHC in this study relatively compared well with $31.00-31.70$ reported by [16]. [17] explained that the MCV and MCH values could reflect anaemic condition and the capacity of the bone marrow to produce RBC of normal size and metabolic capacity. However MCHC is very significant in the diagnosis of anaemia. The normal MCV, MCHC and MCH recorded in this study for the rabbits gave a clear

indication of the absence of anaemia among the experimental groups.

The white blood cell count (WBC) ranged from $3.80-9.70 \times 10^6 / \text{dl}$, the values were within the range of $5-13 \times 10^9 / \text{l}$ reported by [11] for healthy young rabbits. These results indicated that the animals were healthy because decrease in number of WBC below the normal range (leukocytopenia) is an indication of allergic conditions and certain parasitism, while elevated values (leukocytosis) indicate the existence of a recent infection. This perhaps highlights the ethno-veterinary properties of *Gmelina arborea* as reported by [18].

3.3 Serum Biochemistry

Table 4 shows the blood biochemistry of weaner rabbits fed dietary levels of *Gmelina arborea* leaf meal. The total proteins (g/l) values obtained differed significantly ($P < 0.05$) with T₁ having the highest value and T₄ having the lowest value. Blood albumin (g/l) concentrations, differed ($p < 0.05$) significantly among the treatment groups. However, globulins (g/l) was not affect ($p > 0.05$) by the treatment diets, but tended to increase with increasing levels of the test ingredient. Blood urea concentrations (mg/dl), differed ($P < 0.05$) among all the groups and however decreases with increasing levels

Table 3. Haematological profile of weaner rabbit fed dietary levels of *Gmelina arborea* leaf meal

Parameter	T ₁	T ₂	T ₃	T ₄	SEM
Packed cell volume (%)	35.31 ^c	32.41 ^d	37.96 ^b	41.37 ^a	1.25
Haemoglobin (g/dl)	9.55 ^b	11.06 ^a	11.31 ^a	12.62 ^a	0.48
Red blood cell ($\times 10^6 / \text{ul}$)	4.39 ^d	5.36 ^c	6.51 ^b	7.10 ^a	0.40
MCHC (%)	28.34 ^d	30.45 ^c	32.60 ^b	33.51 ^a	0.76
Mean corpuscular haemoglobin (pg)	19.21 ^c	21.51 ^b	22.37 ^b	23.41 ^a	0.60
Mean corpuscular volume (fl)	78.97 ^c	84.01 ^c	86.00 ^b	88.97 ^a	1.39
White blood cell ($\times 10^6 / \text{dl}$)	4.90 ^c	5.69 ^c	7.95 ^b	10.52 ^a	0.83

^{a, b, c} means in the row with different superscripts are significantly different ($p < 0.05$)

MCHC = Mean corpuscular haemoglobin concentration

SEM = Standard error of mean

Table 4. Serum biochemistry of rabbit fed graded levels of *Gmelina arborea* leaf meal

Parameter	T ₁	T ₂	T ₃	T ₄	SEM
Total proteins (g/l)	61.07 ^a	58.70 ^b	57.52 ^b	55.99 ^c	1.38
Albumin (g/l)	38.21 ^a	35.72 ^b	34.46 ^b	32.23 ^c	1.00
Globulins (g/l)	22.86	22.98	23.06	23.76	0.31
Urea (mg/dl)	35.40 ^a	33.61 ^b	31.57 ^c	30.96 ^c	0.68
Creatinine (mg/dl)	0.85 ^b	0.91 ^b	0.94 ^b	1.17 ^a	0.05
Cholesterol (mg/dl)	46.47 ^a	20.39 ^b	15.99 ^c	11.58 ^d	5.13

^{a, b, c, d} means in the same row bearing different superscripts are significantly ($P < 0.05$)
SEM=Standard error of mean

Gmelina arborea leaf meal. T₁ differed significantly from T₂, T₃ and T₄, while T₃ and T₄ were similar ($p > 0.05$) for blood urea. The creatinine level tend to increase with increasing levels of the test ingredient with T₄ showing significant ($p < 0.05$) with T₁, T₂ and T₃. The cholesterol values of this study showed significant ($p < 0.05$) difference among the treatment groups.

The range of value (55.99–61.07 g/l) for total proteins obtained in this present value compared with the values (69.7±2.6-70.5±1.4 g/l) for rabbits as reported by [15]. However, the values fell within the normal range of (5–8 g/dl) reported by [19] reported for healthy rabbits. The result suggests that there were no muscle wastage in the rabbits and that the animals did not survive at the expense of body reserves since protein synthesis is related to the amount of dietary protein. This entails better utilization of the dietary proteins by the animals within each treatment thereby facilitating total protein availability.

The range of values (28.23-38.21 g/l) for albumin obtained in this present study fell within the normal range reported of (2.5-4.5 g/dl) reported by [14] which is an indication of proper functioning of the liver in the rabbits. However, the values reported in this study favourably compared well with 2.81-3.91 (g/dl) reported by [12] for growing rabbits fed grasshopper meal as a substitute for fish meal. The globulins values showed no ($P > 0.05$) difference, but was highest numerically for T₄ and lowest for T₁, and fell within the normal range (1.5-3.3 g/dl) reported by [11] which is indicative of high immunity and good resistance to disease in the experimental animals. This also highlights the ethno-veterinary properties of *Gmelina arborea* leaves as reported by [18].

Blood urea concentrations (mg/dl), differed ($P < 0.05$) among all the groups and fell within the range of 31.37 - 38.00 mg/dl reported by [20] for

weaner rabbits fed different processed *Laptadenia hastata* leaves. The low and non-significant blood urea observed in T₃ and T₄ is an indication that the amino acids of *Gmelina arborea* are balanced since high blood urea levels are associated with poor protein quality [21] or excess tissue catabolism associated with protein deficiency. The result of this study is in agreement with the findings of [21] who observed that amino acid imbalance would results in an increase in blood urea concentration. This perhaps highlights the high quality protein of *Gmelina arborea* as reported by [22].

Creatinine level of the experimental rabbits followed a particular trend, with the rabbits receiving the treatments exhibiting significant ($P < 0.05$) higher values than those in the control group. Similar results were obtained by [23] in their experiment with *Gmelina arborea* on swine. [24] associated lower serum creatinine level with increased risk of type 2 diabetes. The increased level of serum creatinine of the animals receiving the test ingredient relative to those on the control group could suggest the anti-diabetic property of *Gmelina arborea* leaves [2]. This is corroborated by the significant ($P < 0.05$) decrease in serum cholesterol of the test animals. Since cholesterol levels were within the normal range, possibilities of anorexia, diabetes, liver dysfunction and mal-absorption of fat, which are the symptoms of abnormal cholesterol levels in the blood are ruled out.

4. CONCLUSION

This study showed that *Gmelina arborea* could stimulate erythropoiesis and influence metabolism. Therefore, its use is recommended in the improvement of haematological and serum biochemical indices of rabbits. Similarly, from physiological point, blood profiles of rabbits in all treatment groups are within normal range for rabbit; an indication that the test ingredient enhanced feed quality and inadvertently the

nutritional and health status of experimental animals.

CONSENT

It is not applicable.

ETHICS APPROVAL

This paper followed all the guidelines for the care and use of laboratory animal model of the Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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