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Effects of Gliricidia-Based Diet on Heamatology, Serum Biochemistry and Blood Morphology of Weaned Rabbits

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Abstract:

The search for alternative feed resources for farm animals as a way of reducing production costs is the way out to solve competition between human and animals while putting the health of the animal into consideration. A total of 24 cross breed unsexed weaner rabbits of 6 weeks was divided into three dietary treatments (Treatment 1; control, Treatment 2; 25 % inclusion level of gliricidia leaf meal (GLM) and Treatment 3; 50% GLM). Each treatment had 8 rabbits with 4 replicate of two rabbits each in a completely randomized design. Blood samples was collected into labeled Ethylene-deamine-tetra-acetic acid (EDTA) treated tubes for haematological analysis, also into tubes without anticoagulant for serum biochemical evaluation. Data were analysed using descriptive statistics and ANOVA at α0.05. There were no significant differences in all the haematological indices analysed. Rabbits on 50% gliricidia-based diet were significantly higher in neutrophils but least neutrophils was obtained in rabbit fed control diet. Lymphocytes was higher in rabbits fed the control diet while rabbits on 50% gliricidia-based diet had the least lymphocytes counts. Similar trend was observed for the monocytes and eosinophils count. Meanwhile, basophils count was higher in rabbits fed 25% gliricidia-based diet while least count was obtained in rabbits fed 50% gliricidia-based diet. Both the total protein and cholesterol was significantly higher in rabbits fed control diet, while least values were obtained in rabbits fed 25% and 50% gliricidia-based diet respectively. Triglyceride (mmol/L) levels in rabbits fed gliricidia-based diet decreases as the GLM inclusion levels increases. The same trend was observed for high density lipoprotein. The low density lipoprotein was higher in the rabbits fed with the control diet, but decrease in those fed with 25% gliricidia-based diet. Gliricidia leaf meal can be fed to rabbits at 50% level of inclusion without any detrimental effect on haematology and serum biochemistry of the rabbits. The total cholesterol triglyceride and low density lipoprotein were reduced while the physiological and oxidative status of the rabbits was improved.

Keywords:
Gliricidia-Based Diet, Heamatology, Serum Biochemistry, Blood Morphology and Weaned Rabbit

1. Introduction

The search for alternative feed resources for farm animals as a way of reducing production costs, and making livestock products more readily available to the populace in the tropics is far from over. A situation in which the average Nigerian still consumes only about 7.4g of animal protein per day [1], which is still far below the recommended animal protein level of 35g required by an average adult human for proper health, is undesirable. It is known that some tree legumes in the tropics, notably *Gliricidia sepium*, have attracted attention for their ability to provide large quantities of high quality forages all year round as well as their ability to maintain a sustainable environment and soil fertility through nitrogen fixation [2]. The use of these legumes as livestock feeds has the potential of significantly bringing down the cost of commercial livestock feeds, which are very high in Nigeria, primarily because the conventional ingredients used to supply the protein components in these feeds also serve as food for humans, and also raw materials in industries. However, studies by [3] have shown that the use of some legumes as protein sources could produce some undesirable physiological and biological alterations when such legumes are fed in their raw state to monogastric animals, which may manifest in the haematological and serological qualities of the animals. Since these qualities reflect in the physiological responses of these animal's health to their environment and nutrition values as reported by [4]. This study was therefore, carried out to ascertain the effect of feeding varying levels of GLM on the haematological, serological and carcass characteristics of weaned rabbits.

2. Material and Method

2.1. Study Location

The research was carried out at Dagwom farm division of the National Veterinary Research Institute (NVRI) Vom, Jos south Local Government Area of Plateau State Nigeria. Vom lies on longitude 8°45 East and latitude 9°48 North and has an altitude of about 1280m above sea level with an average temperature of between 19 °C to 22 °C (NVRI).

2.2. Experimental Animal

A total of 24 cross breed unsexed weaner rabbits of 6 weeks' old were divided into three dietary treatments of 8 rabbits in 4 replicate of two rabbit each in a completely randomized design.

The initial weight of all the animals in each treatment were taken before the beginning of the experiment. Feed (gliricidia-based diet) and clean drinking water was given ad-libitum. Dewormer was administered to all the rabbits at the beginning of the study. The experimental animal was housed two per hutch.

2.3. Test Ingredients

Gliricidia leaves was harvested from Dagwom farm division of the National Veterinary Research Institute (NVRI), by hand picking, washed with distill water and air dried under shade to prevent the leaves from being denatured until they are crispy to touch. The leaves were thereafter crushed with hammer mill before incorporation in the test diets at 25% and 50% inclusion levels.

2.4. Experimental Diet

The experimental diet is shown in Table 1.

Table 1. *Composition of the experimental diet.*

Ingredients	Control	25% gliricidia-based diet	50% gliricidia-based diet
Maize	38.50	33.00	25.00
Full fat soya	17.00	15.00	10.75
Wheat offals	15.00	5.00	0.00
Rice offals	16.50	14.75	10.00
Palm kernel cake	8.75	3.00	0.00
Gliricidia leaf meal	0.00	25.00	50.00
Gliricidia tigs meal	0.00	0.00	0.00
Bone meal	2.50	2.50	2.50
Lime stone	1.00	1.00	1.00
Salt	0.30	0.30	0.30
Premix	0.25	0.25	0.25
Methionine	0.10	0.10	0.10
Lysine	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated:			
Crude Protein	15.89	16.00	16.07
Metabolizable energy	2591.68	2609.48	2590.55
Crude fibre	8.96	8.82	8.97

2.5. Data Collection Procedure

2.5.1. Blood Sample Collection

Blood samples was collected into labeled Ethylene-deamine-tetra-acetic acid (EDTA) treated tubes for haematological analysis and into tubes without anticoagulant for serum biochemical evaluation. Evaluations was conducted according to the method described by [5].

2.5.2. Hemoglobin Determination

N/10 HC1 was taken into an ordinary pipette and poured in the graduated dilution tube up to 20% mark. The heparinized blood was filled into the hemoglobin pipette up to 0.02 ml and transferred it into the dilution tube. The blood and HCl was stirred in the dilution tube with the stirrer. Distilled water was added until the colour of the dilution and standard tubes matched with each other. The reading was noted which gave hemoglobin as g/dl of blood according to [6].

2.5.3. Red Blood Cell Count

For RBC count, blood with an anticoagulant was used. Blood was drawn into the RBC diluting pipette exactly to the 0.5 mark, using gentle suction on the mouth piece. The lip of the pipette was wiped free of blood before inserting it into the diluting fluid

(Toission Solution). The diluting fluid was drawn up to the mark 101 above the bulb. The tube was rotated in a horizontal position to ensure uniform dispersion of the blood cells in the pipette [6].

RBCs was calculated using the following formula:

RBC (million/mm) = Cells counted x 10x200

2.5.4. Packed Cell Volume (PCV)

Packed cell volume was measured using the heparinized blood in the plain capillary tubes (75 mm x I mm). Tubes was filled approximately 1 cm from the end. Holding it in the flame sealed. Care was taken not to heat the blood. Capillary tubes were fixed in the hematocrit centrifuge machine. Then centrifugation was done at 13000 rpm for 5 minutes [6].

2.5.5. Red Blood Cell Indices

From the values of PCV, Hb and RBC count following useful erythrocyte indices was empirically calculated.

2.5.6. Mean Corpuscular Volume (MCV)

MCV expresses the average volume of the individual RBC and is calculated from the formula as given by [6] and [7].

MCV = Hematocrit X 10/R.B.C., MCV is expressed in femtoliter.

2.5.7. Mean Corpuscular Hemoglobin (MCH)

MCH is the amount of hemoglobin by weight in average Red blood cell count and is calculated using the formula given by [6] and [7].

MCH = Hemoglobin X 10/R.B.C. It is expressed in picogram.

2.5.8. Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC is the concentration of hemoglobin in the average red blood cells or the ratio of weight of hemoglobin to the volume in which it is contained and is calculated from the formula given [6] and [7].

MCHC = Hemoglobin X 100/ Hematocrit

2.5.9. White Blood Cell Differential Count

The white blood cell differential count was carried out and measured; White cell count ($x10^9$/L), Neutrophils ($x10^9$/L), Lymphocytes ($x10^9$/L), Monocytes ($x10^9$/L) and Eosinophils ($x10^9$/L L).

2.5.10. Serum Biochemistry Indices

Serum biochemistry indices such as Total protein (g/ 100ml), Albumin (g/ 100ml), Total cholesterol (mg/100ml) was measured.

2.5.11. Experiment design

A completely randomized design was used

2.5.12. Data Analysis

Data obtained was subjected to analyses of variance using SPSS statistical package version 25. Significant differences between treatment means were separated using Duncan's Multiple Range Test.

3. Results and Discussions

3.1. Results

Table 2 shows the effect of gliricidia-based diet on heamatology of weaner rabbit. There were no significant differences in all the haematological indices analysed. The white blood cell differentials are shown in Figure 1. Rabbits on 50% gliricidia-based diet are significantly higher in neutrophils but least neutrophils was obtained in rabbit fed with the control diet. Lymphocytes was higher in rabbits fed the control diet while rabbits on 50% gliricidia-based diet had the least lymphocytes counts. Similar trend was observed for the monocytes and eosinophils count. Meanwhile, basophils count was higher in rabbits fed 25% gliricidia-based diet and least count was obtained in rabbits fed 50% gliricidia-based diet.

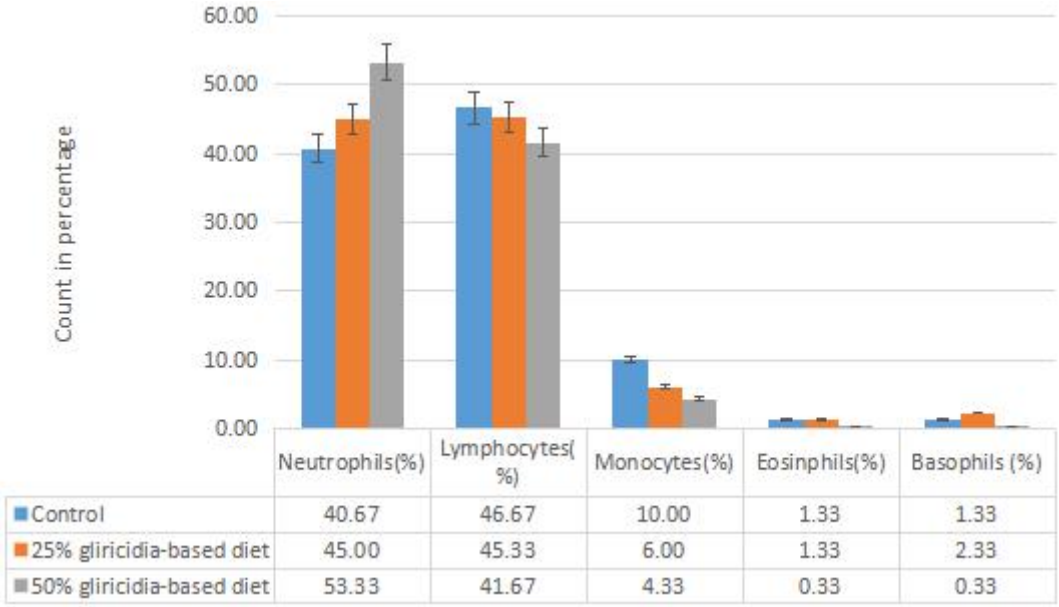

Figure 1. *White blood cell differentials.*

Table 2. *Effect of gliricidia-based diet on heamatology of weaner rabbit.*

Parameters	Control	25% gliricidia-based diet	50% gliricidia-based diet	SEM
Packed Cell Volume (%)	40.00	38.33	37.00	1.32
Red blood cell (x1012/l)	6.66	6.39	6.16	0.22
Haemoglobin (g/dl)	13.33	12.78	12.33	0.43
MCHC (g/dl)	33.33	33.33	33.33	0.15
MCH (pg)	60.02	60.02	60.02	0.34
MCV (fl)	20.01	20.01	20.01	0.11
White blood cell (x109/l)	6.73	8.10	6.30	0.66

[a, b] Means in the same row not sharing superscript are significantly different at P<0.05.

MCV=Mean corpuscular volume MCH=Mean Corpuscular Haemoglobin MCHC=Mean Corpuscular Haemoglobin Concentration

SEM: Standard error of the mean.

The effect of gliricidia-based diet on selected serum biochemistry of weaned rabbit could be seen on Table 3. Both the total protein and cholesterol was significantly higher in rabbits fed control diet with gliricidia leaf meal while least values were obtained in rabbits fed 25% and 50% gliricidia-based diet respectively. Triglyceride (mmol/L) levels in rabbits fed gliricidia-based diet decreases as the gliricidia leaf meal inclusion levels increased as showed in Figure 2. The same trend was observed for high density lipoprotein as shown in Figure 3. The low density lipoprotein was higher in the rabbits fed the control diet, decreases in 25% in rabbits fed gliricidia-based diet.

Table 3. *Effect of gliricidia-based diet on serum biochemistry of weaned rabbit.*

Parameters	Control	25% gliricidia-based diet	50% gliricidia-based diet	SEM
Total protein (g/L)	108.67	64.00	82.33	9.49
Albumin (g/L)	38.00	35.33	30.00	2.46
Total cholesterol (mmol/L)	6.30[a]	4.90[b]	4.37[b]	0.33

a, b Means in the same row not sharing superscript are significantly different at P<0.05. SEM: Standard error of the mean.

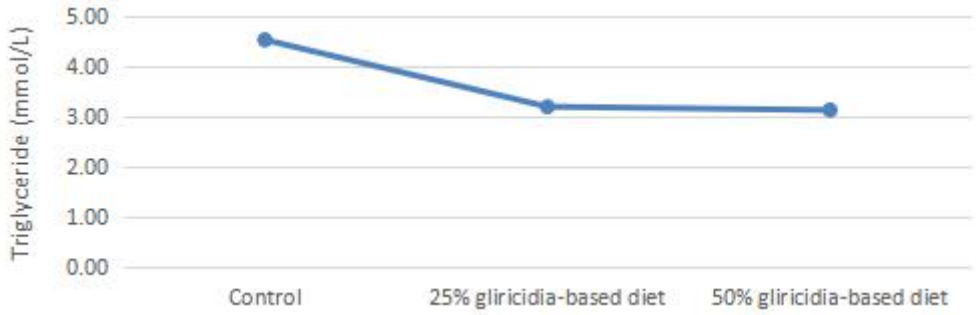

Figure 2. *Triglyceride (mmol/L) levels in rabbits fed gliricidia-based diet.*

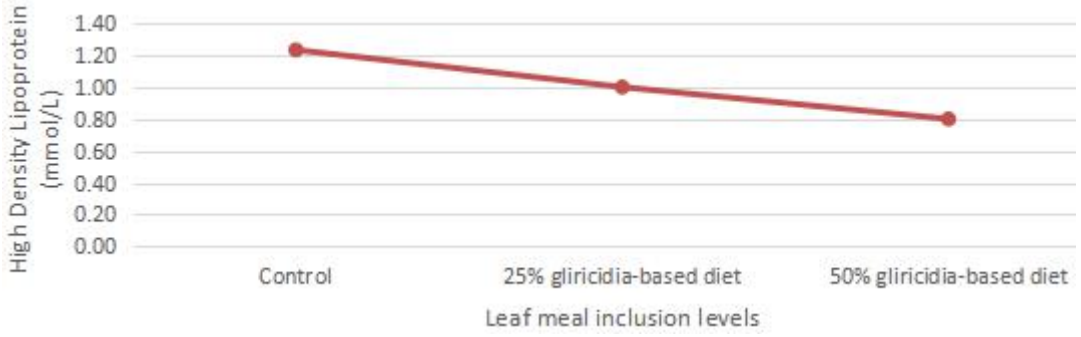

Figure 3. *High Density Lipoprotein (mmol/L) levels in rabbits fed gliricidia-based diet.*

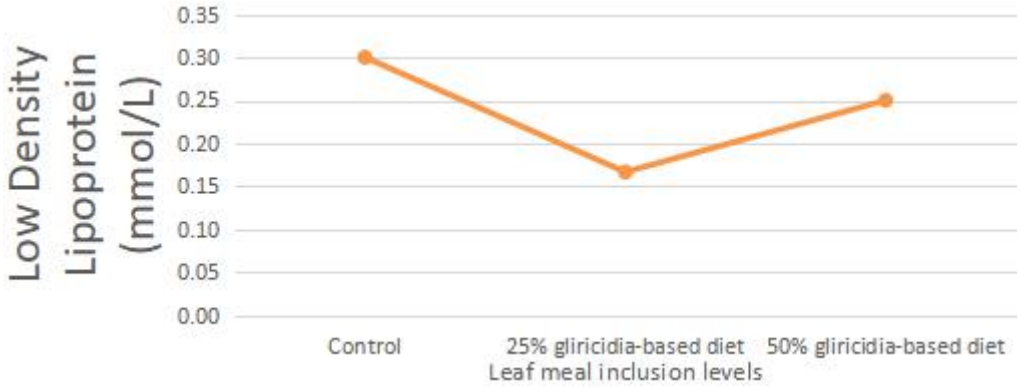

Figure 3. *Low Density Lipoprotein (mmol/L) levels in rabbits fed gliricidia-based diet.*

Table 4. *Effect of gliricidia-based diet on blood morphology of weaner rabbit.*

Parameters	Control	25% gliricidia-based diet	50% gliricidia-based diet
Red blood cell	MA+, HYPO+, MI ++, HYPER +	MI ++. HYPER ++	ANISO +, SPURR ++. BURR +, ELIP +
White blood cell	NTG +	LS+, NTG ++	NTG +
Platelet	N	N	L +

Keys: BURR = BURR CELLS, ELIP= ELIPTOCYTES, HYPER= HYPERCHROMASIA, HYPO= HYPOCHROMASIA, L= LARGE, LS = LEFT SHIFT, MA= MACROCYTES, MI= MICROCYTES, N= NORMAL, NTG = NOT-TOXIC NUETROPHILIC GRANULATIOS and SPURR = SPURR CELLS (ACANTOCYTES).

3.2. Discussions

The physiological status and health of farm animals depends on the nutrition fed to the animal aside genetic and environment influence. According to [8], the values of haematological traits are indicators of the nutritional status of the animals which was earlier reported by [9], [10] and [11]. The present result obtained in this study shows no significant differences among all the haematological indices measured across the dietary groups which also fall within the normal range [12]. Similar results were obtained in the study of [8], who use gliricidia leaf meal and multi-enzyme in rabbits' diet. The result obtained in this study is in agreement with [13] who used varying dietary levels of water spinach (*Ipomoea aquatic)* leaf meal to feed rabbits. In contrary to this result, [9] reported a variation in white blood cells, lymphocytes and platelets in rabbits fed 0% and 10% pawpaw leaf meal inclusive diet. [11] also reported a variation in the haemoglobin concentration, red blood cells and mean cell volume when processed cocoa pod husk meal was fed to rabbits. The results obtained in this study confirms the nutritional adequacy of the diets used and in particular indicating the safety of the treatment applied in supporting the normal haemapoietic process in the experimental rabbits when compared to the rabbits fed control diet without gliricidia leaf meal. This study revealed that blood cells morphology of both control group and rabbits fed with gliricidia leaf meal had some degree of similarities in their blood picture. Rabbits fed with 25% gliricidia-based diet presented with 5-10 red cells showed microcytosis and hyperchromasia per high power field (HPF), however, rabbits fed on 50% gliricidia-based diet presented with red cells of varying sizes and shapes does not show tandem with the control groups. Although the reason for varying blood morphologies could not be determined in this study, [14] had reported that Rabbits transported at 28°C (82.4°F) for 1 to 3 hours would often show varying haematological parameter and picture. Cold stress has been shown to affect RBC [15]. White blood cells of all groups shows some level of Non-Toxic Neutrophilic Granulations (NTG). Higher NTG has been proven to indicate underlining pathologic conditions however, mild NTG could also be related to increased exposure to stress [16]. Thrombocytes appeared normal except for groups fed with 50% gliricidia-based diet, indicating presence of immatured thrombocytes and could result from acute thrombopoiesis.

The total protein was not significantly ($p>0.050$) influenced by dietary treatments. Values obtained was higher than the normal range (6.00 to 8.30) recommended by [17] and [12]. This study is in agreement with [13] who used varying dietary levels of water spinach (*Ipomoea aquatic)* leaf meal to feed rabbits. Albumin was not

significantly (p>0.050) influenced by dietary treatments. Increase in triglyceride level may be a risk factor for atherosclerosis, pancreatitis and liver disease. In this study, it was observed that the levels of triglycerides decreases as the level of gliricidia leaf meal increases. This result is in line with the data obtained by [8] who got a reduction in triglyceride level in rabbits fed 15% gliricidia-based diet compared to the rest test diets. This result obtained could be due to the presence of some bioactive compounds in gliricidia leaf meal which impaired fat absorption and consequent fat depletion. This findings also supported the reduction in cholesterol and Low-Density Lipoprotein level (LDL) in rabbits fed 50% gliricidia-based diet compared to the control diet as this is also of health benefit to the consumers, especially those predisposed to heart disease. The inclusion of gliricidia-based diet in the diets lower the uptake of cholesterol or increased loss or catabolism of cholesterol as stated by [9] which further confirms the health benefits of including gliricidia leaf meal in rabbit's diet. However, the present findings agreed with earlier reports of [10], [11] and [9], who all reported a reduced cholesterol level in rabbits fed *Alchornea cordifolia* leaf meal based diets, processed cocoa pod husk meal based diet and pawpaw leaf meal inclusive diet, respectively. The reduction of cholesterol level in this study may be linked to the presence of saponins, one of the components of gliricidia which exerts inhibitory effects on cholesterol uptake in the gut through intra-lumenal physiochemical interaction as stated by [18].

4. Conclusions

The findings showed that gliricidia leaf meal can be fed to rabbits at 50% level of inclusion without any detrimental effect on haematology and serum biochemistry of rabbits while total cholesterol triglyceride, and low density lipoprotein were reduced also the physiological and oxidative status of the rabbits was improved.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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