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HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF WEST AFRICAN DWARF RAMS FED ENSILED ELEPHANT GRASS AND GMELINA ARBOREA LEAVES

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Abstract: In a study to evaluate the haematological parameters and serum indices of rams fed with ensiled Elephant grass and *Gmelina arborea* leaves, 16 WAD rams were randomly assigned to four dietary treatments for 4 weeks. Each treatment was replicated thrice in a Completely Randomized Design (CRD). The data obtained were subjected to analysis of variance (ANOVA) using procedure of SAS (2002); where analysis indicated significant difference, the significant means were compared using the Least Significant Difference method. Water and feed were given ad-libitum. Major parameters measured included: Red Blood Cell (RBC), Packed Cell Volume (PCV), White blood Cell (WBC), Hemoglobin, lymphocytes, neutrophils, monocytes, Total Protein (TP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), albumin, glucose, urea, creatinine, cholesterol, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL). The four dietary treatments were 80% Elephant grass+ 20% cassava peels+0% Gamhar (T1), 60% Elephant grass+ 20% cassava peels+20% Gamhar (T2), 20% Elephant grass+ 20% cassava peels+60% Gamhar (T3), and 0% Elephant grass+ 20% cassava peels+80% Gamhar (T4). Except for red blood cell (RBC), white blood cell (WBC), monocytes, creatinine, urea and LDL, all the haematological and biochemical parameters measured were significantly ($p<0.05$) different across the dietary treatments. It was observed that the ensiled diets offered to the rams did not have deleterious effect on the haematological and serum biochemical indices as the values registered across the dietary treatments falls within the normal ranges. It was concluded that all four test diets were suitable for dry season ram feeding and that *Gmelina arborea* could be included in ram diets up to 80% without any harmful effect on their haematological and serum biochemical profile.

Keywords: Haematology, Serum, Indices, Dietary treatment, West African Dwarf rams

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1. Introduction

Small ruminants such as rams play important role in the livestock subsector of the Nigerian agricultural economy (Lakpini et al., 2002). The West African dwarf rams are well adapted to the environment i.e. West African humid zone and trypanotolerant.

Decline in nutritive value of vegetation resulting from senescence combine to make it difficult for livestock to meet their nutritional requirement during the dry season. Such a situation has long been recognized to result in cyclic body weight gain in the rainy season and weight loss in the dry season (Annor et al., 2007). To break this cycle, animal nutritionists have recommended feed supplementation. However, the use of staple cereal, grains as supplements leads to competition between humans and animals and increases the cost of feed supplementation, making supplementation unprofitable or unsustainable, especially in poor communities. The need, therefore, exists to find reliable and sustainable sources for feed supplementation with the view to helping to improve the profitability of livestock production during periods of inadequate and/or poor-quality herbage supply.

According to Ranjhan, 2001 crop residues (straws and stovers) and agro-industrial by-products will remain important sources of feed for livestock production.

Madubuike and Ekenyem (2006) reported that haematological and serum chemistry assay in livestock could indicate the physiological response of livestock to their nutrition. Esonu et al. (2001) had earlier come to the same conclusion that haematological constituents reflect the physiological responsiveness of the animal to its internal and external environment.

Blood is an important index of physiological and pathological changes in an organism (Mitruka and Rawnshey, 1977). The primary function of blood is to transport oxygen from respiratory organs to body cells (Duke, 1975) distributing nutrients and enzymes to cells and carrying away waste products thereby maintaining homeostasis of the internal environment (Bentrick, 1974). The various functions of blood are carried out by the individual and collective actions of its constituents-

the haematological and biochemical components (Akinmutimi, 2004).

Haematological tests have been widely used for the diagnosis of various diseases and nutritional status of animal. The information gained from the blood parameters would substantiate the physical examination and together with medical history provide excellent basis for medical judgment (Schalm et al., 1975).

In general, blood examination is performed for several reasons as a screening procedure to assess general health (Jain, 1993). Glucose, cholesterol, calcium, total protein, alkaline phosphates, uric acid, sodium, potassium, chloride levels are diagnostic values for diabetes mellitus, liver disease, hyperparathyroidism, chronic hepatopathy and liver disease, gout, kidney disease, chronic diarrhea and dehydration respectively. It had been reported that biochemical changes because of toxins have effects on haematological parameters (Karnish, 2003).

A quantifiable variation was reported in blood parameters due to management, feeding level, age, sex, health status, method of blood collection, haematological techniques used, diurnal and seasonal variations, ambient temperature, and physiological status (excrement, muscular exercise, time of sampling, water balance and transportation. (Schalm et al., 1975)

Nutrition, breed, sex, age, reproductive status, environmental factors, stress, and transportation are known to affect haematological and biochemical indices and thought to play major roles in the differences in haematological and biochemical parameters.

These differences have further underlined the need to establish appropriate physiological baseline values for various breeds of livestock in Nigeria, which could help in the realistic evaluation of the management practices, nutrition, and diagnosis of their health condition.

The objective of this work was, therefore, to evaluate the nutritional value of four diets formulated from locally available feedstuffs in Ejigbo district of Osun as feed for rams. The test diets were ensiled mixture of elephant grass and cassava peels with different levels of *Gmelina arborea* leaves. West African dwarf rams were used for the dietary testing and the data collected and evaluated included haematological and serum biochemical indices.

2. Materials and Methods

2.1. Experimental Location

The experiment was carried out at the Research site of Osun State University, Ejigbo campus, Osun State, Nigeria, located on the longitude $7^0 54' 0'' N$ $4^0 18' 54'' E$ and latitude $7.90000^0 N$ $4.31500^0 E$ and at an altitude 426M above sea level (EN.Wikipedia.org/wiki/ejigbo, 2011). Ejigbo is in the middle of 35km to Northeast of Iwo, 30km from Ogbomoso in the North and about 24km to East. The mean annual rainfall in Ejigbo is about 52.35mm and there is deviation from the mean value from year to year.

2.2. Experimental Materials

The materials used for the study are 16 juvenile West African Dwarf rams, jute bags, roll of nylon, roll of robe, plastic bowls and drums, cotton wool, methylated spirit, ice block, sand, Ethylene Diamine Tetra acetic Acid bottles.

2.3. Experimental Animals and Their Management

Sixteen West African dwarf rams of $1^{1/2}$ years were purchased from the rural settlers with an average live weight of 20-30kg. The animals were housed intensively in well-ventilated pens, in an open-sided house with corrugated aluminum roofing sheet and a concrete floor, which was washed, disinfected with Izal and covered with bedding material (wood shavings) before the arrival of the animals. The rams were given prophylactic treatments which consist of intramuscular application of oxytetracycline at the dosage of 1ml/10kg body weight of the animal. Fresh water was supplied ad-libitum.

Before the commencement of the experiment, the animals were left for a week to acclimatize to the new environment; the experimental units were treated against ectoparasites with 0.5ml/10kg body weight of Ivermectin.

2.4. Experimental Diet

Three test ingredients used are *Gmelina* leaves, Elephant grass and cassava peels; they were collected at their early stage in the morning and evening. The test ingredients were ensiled for 21 days. The ensiled mixture of Elephant grass, *Gmelina* leaves and Cassava peel was done at the following ratio T1 (80:0:20%), T2 (60:20:20%), T3 (20:60:20%), T4 (0:80:20%). Experimental units were subjected to the diets without concentrate feeding using a complete randomized design

2.5. Experimental Procedure

10mls of blood samples were collected from each of the animals via jugular vein puncture using syringes. 5mls of the collected blood samples was put into plastic tubes containing the anti-coagulant Ethylene Diamine Tetra Acetic Acid (EDTA) for the determination of haematological parameters i.e., the analysis for packed cell volume (PCV), hemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), lymphocytes, neutrophils, and monocytes. The remaining 5mls of the blood samples was put into anti-coagulant free plastic tubes and allowed to coagulate at room temperature for subsequent biochemical analysis: serum protein, serum glucose, serum albumin, creatinine, urea, serum alanine transaminase (ALT) and serum aspartate transaminase (AST). The blood cholesterol levels were also analyzed including the HDL (High Density Lipoprotein) and the Low-Density Lipoprotein (LDL).

2.6. Data Collection and Analysis

Blood samples were taken from the rams before feeding via the jugular vein puncture between 07:00 and 09:30 h local time at the last day of each experimental period for haematological and blood biochemical assays. The blood samples were taken to the laboratory soon after collection in a sample holder placed in an ice chest. Two different test tubes were used to harvest blood from each of the rams. A plain test tube was used to collect blood to

obtain serum for the determination of blood glucose, total protein, albumin, urea, creatinine, AST, and ALT. The other test tube, which contained Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant, was used to analyze for Hemoglobin (Hb) concentration, Packed Cell Volume (PCV), White Blood Cell (WBC) count, RBC, lymphocyte, neutrophil, and monocytes.

2.7. Chemical Analysis

The packed cell volume percentages were measured for each blood sample in fresh ethylene diamine tetra acetic acid (EDTA) anticoagulant samples within 24hours of collection using the micro-hematocrit method. Hemoglobin concentration was also measured in fresh EDTA anticoagulant samples using the Sahl's (acid hematin) method. RBC was measured in fresh EDTA with the aid of Neubaur counting chamber (hemocytometer). Blood smears were used for total WBC counts. Differential relative and absolute counts were classified as lymphocytes, neutrophils, and monocytes.

Plasma glucose was measured using the enzymatic glucose oxidase method (Bauer et. al. 1974). Total serum protein was measured in serum for individual animal using the biuret method. Serum alanine transaminase and serum aspartate transaminase was analyzed spectrophotometrically by using commercially available diagnostic kits (Randovl Test Kits). Serum creatinine was determined using the principle of Jaffe reaction.

2.8. Statistical Analysis

Resulting haematological and biochemical data obtained from the samples was laid out as Completely Randomized Design and analyzed with one-way Analysis of Variance (ANOVA) using procedure of SAS (2002). The significant means were compared using the least significant different (LSD) method.

3. Results and Discussion

Haematological parameters of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves is presented Table 1. The reference ranges of values were reported by Oyeyemi and Ajani (2014) for West Africa Dwarf (WAD) rams of 18–24 months, which weighed 20–25kg and stated to be within normal range. The observed haematological values show that except for Packed Cell Volume, Hemoglobin, lymphocyte, and monocytes where the mean values between the four diets significantly ($P<0.05$) differs, although the white blood cells count is not significant, the means on the same rows differs. All the other haematological parameters did not. Mean packed cell volume is highly significant $P\leq0.001$ and has the highest mean value in T4 and least in T1. However, these values were within the range of 21-35% reported for WAD goats by Daramola et al. (2005). The implication of this observed PCV values, going by the reports of Dargie and Allonby (1975), is that only the rams on T4 diet could probably have the high tendency for a return of PCV to normal value following an infection through compensatory accelerated production. This is because only the rams on this diet had values above the 32% PCV documented to be normal for circulatory system in sheep.

Table 1. Haematological parameters of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves

Parameters	Reference values	T1	T2	T3	T4	SEM
RBC (10^6/mm^3)	9-15	9.83	9.62	9.94	10.30	1.47
PCV (%)	27-45	28.91	30.05	31.92^b	32.46^a	3.92***
WBC (10^6/mm^3)	4-12	6.88^b	7.53^a	7.02^a	7.75^a	0.80
Hb (g/dl)	9-15	8.79^c	10.01^b	10.69^b	11.71^a	3.63**
Lymphocytes (%)	40-75	53.63^c	54.05^c	55.86^b	57.01^a	4.90*
Neutrophils (%)	10-50	35.76^c	37.58^b	37.51^b	38.41^a	4.56**
Monocytes (%)	7-9	2.07	2.04	2.02	2.05	0.49

^{a,b,c} Means on the same row with different superscript are significantly ($P<0.05$) different. *$P\leq0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. SEM= standard error of mean, RBC= red blood cell, PCV= packed cell volume, WBC= white blood cell, Hb= haemoglobin.

The hemoglobin concentration in the blood of the studied rams showed a similar pattern of variation as with PCV. Nevertheless, the hemoglobin range in this study fell within the range of 7-15g/dL reported by Daramola et al. (2005). However, higher in T4 than the value of 11.40g/dL reported for Red Sokoto goats (Tambuwal et al. 2002) and in cattle fed different levels of extracted rice bran (Singh et al., 2002). With the relatively higher Hb concentration observed in this study, the dietary treatments generally seem to be capable of supporting high oxygen carrying capacity blood in rams.

The values obtained in this study for lymphocytes and neutrophils fell within the broad range of 47-82% and 51.6% reported by Daramola et al. (2005) and Tambuwal et al. (2002) and 36.4% for lymphocytes and 17-52% neutrophils reported by the same authors respectively. These values are suggestive of a well-developed immune system in the WAD rams with such number of immune cells to proffer good health (Daramola et al., 2005). The result also implies that an increase in lymphocytes is associated with a decrease in neutrophils and vice versa (Lazzaro 2001).

White Blood Cell count obtained in this study at the end of the experiment though not significant increases across the dietary treatment row compared favorably with values within the range of 6.8-20.1×10^6/mm^3 reported by Daramola et al. 2005. WAD rams seem to possess a protective system providing a rapid and potent defense

against any infectious agent, and this is probably the physiological basis for the adaptation of these species in their ecological zone (Daramola et al., 2005).

Serum biochemical indices of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves is presented Table 2. Serum biochemistry is a generalized medium of assessing the health status of animals. Aside from the values of urea and creatinine differences between the measured biochemical parameters were not significantly (P≥0.05) different between the diets. ALT and glucose values were highly significantly (P≤0.001) different between the dietary treatments. Serum proteins are important in osmotic regulation, immunity, and transport of several substances in the animal body (Jain, 1986). However, in this experiment, the dietary treatments differ more significantly in terms of their Total Protein levels in the serum of the rams. Besides, the significant difference (P≤0.01) between the diets may be related to the serum protein and to the amount of calories contained in the diet but to the availability of protein.

Urea and creatinine levels did not differ significantly between the diets in this study. This study reports high serum urea values across the diets. This may probably have been due to persistent hypoglycemia; catabolic activity is increased for gluconeogenesis, thus resulting in higher serum urea levels. Enzymes are protein catalysts present mostly in living cells and are constantly and rapidly degraded although, renewed by new synthesis. Normal enzyme level in serum reflects a balance between synthesis and their release, because of the different physiological processes in the body.

Transaminase enzymes are those mostly responsible for the synthesis of non-essential amino acids through the process known as transamination.

Serum levels of AST are significantly high under and morbid conditions involving injuries to large numbers of metabolically active cells. However, the result of this study suggests a contrary situation in this regard thus indicating the potential of the studied plant leaves in the feeding of rams. The monitored activities of transaminases enzymes did not vary widely between the diets. The relatively close mean values observed for transaminases could be an indication that the test diets did not differ in their effects on enzyme secretion mechanism.

Table 2. Serum biochemical indices of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves

Parameters	T1	T2	T3	T4	SEM
TP (g/l)	6.32^{b}	6.38^{b}	7.04^{a}	7.22^{a}	1.52**
AST (UI/I)	77.37^{c}	79.48^{b}	81.62^{b}	83.92^{a}	5.30**
ALT (UI/I)	27.94^{b}	28.16^{b}	29.20^{a}	29.42^{a}	2.49***
Albumin (g/l)	2.65^{b}	2.59b	3.22^{a}	3.29^{a}	0.11**
Creatinine (mg/dl)	1.24	1.03	1.24	1.24	0.05
Glucose (mg/dl)	59.01^{a}	61.36^{a}	54.99^{b}	52.81^{b}	7.92***
Urea (mg/dl)	8.93	9.65	10.54	10.42	2.30

^{a,b,c} Means on the same row with different superscript are significantly (P<0.05) different. *P≤0.05, **P ≤ 0.01, ***P ≤ 0.001. SEM= standard error of mean, TP= total protein, AST= aspartate aminotransferase, ALT= alanine aminotransferase.

Cholesterol level of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves is presented Table 3. The resulting cholesterol values shows that the cholesterol and high-density lipoprotein (HDL) mean values between the dietary treatments significantly differ (P<0.05) while the low-density lipoprotein (LDL) values did not. Mean HDL was highest in diet 4 and lowest in diet 1, however, the implication of the observed values by reports is that there is evidence for a protective effect for dietary fiber against atherosclerosis; a disease of the heart through an increase in the low-density lipoprotein and colon cancer probably through an increased rate of passage of feed residues through the gastro-intestinal tract.

Though, the increase in plasma concentration of HDL cholesterol may be because of polyphenols, which are involved in the regulation of lipid and glucose metabolism. According to some authors, this activates the PPAR-α receptor, with an increased stimulation effect in the liver of the expression of key proteins involved in the metabolism of HDL. It was reported that cholesterol concentration is influenced by the degree of stress.

Table 3. Cholesterol level of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves

Parameter	T1	T2	T3	T4	SEM
Cholesterol (mg/dL)	63.06^{b}	64.33^{b}	68.88^{a}	69.93^{a}	4.41***
HDL (mg/dL)	50.58^{c}	54.13^{b}	54.50^{ab}	55.43	2.56***
LDL (mg/dL)	8.13	7.35	7.73	7.04	2.59

^{a,b,c} Means on the same row with different superscript are significantly (P<0.05) different. *P≤0.05, **P ≤ 0.01, ***P ≤ 0.001. SEM= standard error of mean, HDL= high density lipoprotein, LDL= low density lipoprotein.

4. Conclusion

All the haematological and biochemical parameters of WAD rams from this study fell within the normal range of values. It can be concluded that the ensiled Elephant grass and *Gmelina arborea* leaves can be used as dry season feed for WAD rams without any negative effect on the health status of the animals. More studies should be carried on the ensiled Elephant grass with *Gmelina arborea* using other ruminant animals such as goat and cattle.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	O.A.	V.A.
C	50	50
D	50	50
S		100
DCP	70	30
DAI	40	60
L	90	10
W	100	
CR	20	80
SR	60	40
PM	80	20
FA	100	

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declare no conflicts of interest. The funders had no role in the design of the study, in the collection, analyses, interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Ethical Consideration

The experimental procedures used in the present study were reviewed and validated by the local Animal Care and Ethics Committee of Osun State University (protocol code: 2021/15 and date: January 27, 2021).

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