

## **Haematology and Serum analysis of West African Dwarf (WAD) rams fed silage combinations of Maize Forage and *Mucuna pruriens* L. foliage**

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**Target audience:** Ruminant producing farmers, Researchers, Academics, Students

### **ABSTRACT**

*Forage is of good quality when it can meet the nutrient requirements of ruminants all year round without deleterious effects on the health status of animals. Ruminants in the tropics are mostly sustained on native pastures, which are not available in the dry season. Scarcity of native pasture during the dry season predisposes flocks to weight loss and high mortality. Ensiling to preserve forage resources such as maize forage and *Mucuna pruriens* can circumvent this problem all year round. However, information on the haematology and serum biochemical status of WAD rams fed silage combinations maize forage and *Mucuna pruriens* foliage has not been adequately documented. Therefore, the haematology and serum biochemical analysis of West African dwarf (WAD) rams fed silage combinations of Maize Forage (MF) and *Mucuna pruriens* foliage (MPF) was assessed. Downy mildew and streak resistant (DMR-SR) yellow variety maize was planted and harvested as MF at six weeks of growth and ensiled with MPF at four levels : 1:0, 3:1, 1:1 and 1:3 for 21 days. The silages were then fed to 20 WAD rams (12.00±0.25 kg) in a completely randomised design for 105 days. Haemoglobin (g/dL), neutrophils (%), glucose, High Density Lipoproteins (HDL, mg/dL) and Low Density Lipoproteins (LDL, mg/dL) of blood (5 mL) collected from the rams before and after 105 days feeding trial were determined using standard procedures. Data obtained were analysed using descriptive statistics and ANOVA at  $\alpha_{0.05}$ . Higher haemoglobin, neutrophils and HDL were recorded for T2 (9.49±0.91, 41.2±3.8%, 54.70±7.20) and least in rams on T4 (6.11±0.20, 35.2±3.1%, 30.50±5.30), respectively. The LDL was highest in rams on T4 (3.25±0.60) and least in rams on T1 (1.20±0.30). Glucose level was significantly higher in T1 (73.00 mg/dL) compared with T2 (63.48 mg/dL), T3 (58.24 mg/dL) and T4 (52.28 mg/dL), respectively. Ensiling maize forage with *Mucuna pruriens* foliage at 3:1 improved performance, enhanced silage preference and increased dressing percentage in West African dwarf rams without any deleterious effect on blood profile.*

**Key words:** *Mucuna pruriens* foliage; Maize forage; Silage combinations; Blood profile; WAD rams

### **Description of problem**

Generally, the feeding pattern of WAD rams is a characteristic of the native husbandry practice whereby they scavenge for food to meet their daily nutrient requirement (1). However, due to scarcity of green fodder plants, particularly in the dry season, attempts have focused on the utilisation of the abundant foliages by ruminant in the eco-zone which tend to be green all-year round. With a large proportion of plants being used for the nourishment of various domestic animals (2), certain forage legumes thus appear as important component in the diets of sheep and goat to meet their nutrient requirement.

*Mucuna pruriens* has been used as food and feed in a large number of countries where it has been introduced. Where *Mucuna pruriens* is consumed by human, it is typically the beans that are consumed, though in some areas immature pods and leaves have also been eaten (3). For animal fed both bean and foliage, its potential as food and feed are affected by two opposing forces. On the positive side, by its relatively good seed and forage yield and beneficial composition of nutrients and minerals, and on the negative side, by its anti-nutritional components (3).

Haematological and biochemical determinations in animals have been well documented by (4) and (5). According to (6) examining blood for their constituents is used to monitor and evaluate diseases prognosis. Thus, information on blood parameters of rams offered silage combination of maize forage and *Mucuna pruriens* foliage as feed have mostly been scanty.

The objective of this study is to provide

information on some haematological, serum biochemical parameters of WAD rams fed silage combinations of maize forage and *Mucuna pruriens* foliage.

### **Materials and methods**

#### **Study location**

The study was carried out at the Teaching and Research Farm of University of Ibadan, Ibadan, Nigeria.

#### **Planting of *Mucuna* and maize**

Two acres of land were used for the planting of *Mucuna* and maize seeds. The land was adequately ploughed twice, harrowed once and fenced before planting to prevent accidental grazing. The plot was divided into 1 acre each for separate planting of maize and *Mucuna* seeds. Seeds of both downy mildew/streak resistance (DMR-SR) yellow variety maize and *Mucuna* were purchased from IITA, Ibadan. *Mucuna pruriens* seeds were planted at 2 seeds per hole two weeks before the planting of DMR-SR yellow variety maize at 2-3 seeds per hole. Weeding was done manually once respectively, for the *Mucuna pruriens* and maize at four weeks after planting during this period. Thinning was done on maize plot to 2 stands at 3 weeks after planting, while re-supplying of seeds was done on *Mucuna pruriens* plot at two weeks after planting.

#### **Harvesting and silage making**

*Mucuna pruriens* foliage was harvested at eight weeks after planting (onset of flowering) while maize was harvested at dough stage as maize forage (including maize stalks, leaves, immature cobs and tassels excluding the roots) at six weeks after planting. Harvested *Mucuna pruriens* foliage and maize forage were

chopped into 2-3 cm pieces (for easy compaction) as recommended by (7). Chopping of forages was done by using chopping machine fabricated for the purpose of silage production located at the dairy unit of Teaching and Research Farm, University of Ibadan, Nigeria (8). Thereafter, the chopped materials were wilted under shade for 24 hours on polyethene sheets spread on concrete floor. That was followed by ensiling the maize forage and *Mucuna pruriens* foliage in different proportions to form five dietary treatments as follow;

- T1 = 1MF: 0MPF
- T2 = 3MF: 1MPF
- T3 = 1MF: 1MPF
- T4 = 1MF: 3MPF

#### **Collection and evaluation of blood samples**

Before and at the end feeding trial which lasted for 105 days, blood samples (5mL) were collected from the jugular vein of each of 20 WAD rams ( $12 \pm 0.25$  Kg) randomly allotted to the four experimental silages at five rams per treatment for haematology and serum biochemical analysis. Blood samples for haematology were collected into sterile vacutainer tubes containing EDTA (Ethylene di amine tetra acetic acid) while that of serum analysis was without EDTA to allow blood clotting and serum was decanted for the analysis. During the collection, care was taken to avoid contamination with hairs, dirt and microorganisms.

#### **Haematology**

The packed cell volume (PCV) and haemoglobin (Hb) were determined using micro haematocrit method and cyanmethaemoglobin method as described by (9). Red Blood Count

(RBC) and white blood count (WBC) were determined using haemocytometer after appropriate dilution.

#### **Serum Biochemistry**

Serum total protein was determined using Biuret method as described by (10) serum urea was determined by urease method and creatinine by Folinwu filtrate method as describe by (11). Also serum glucose was determined by O-Tluioline method using acetic acids (12), while serum cholesterol was determined using appropriate laboratory kits (13; 14). The activity of the enzymes alanine transaminase (ALT) and aspartate transaminase (AST) was measured using the method of (15) and alkaline phosphatase (ALP) was by method of (16).

#### **Experimental design**

The experimental design was completely randomized design (CRD).

#### **Statistical analysis**

All data collected were analysed using analysis of variance (ANOVA) with the procedure of (17). Statistically significant observed means were compared using LSD of the same package at 5 % level of probability.

### **Results and Discussion**

#### **Results**

Table 1 shows the haematological parameters such as packed cell volume (PCV), haemoglobin, Red blood cells (RBC), White blood cell (WBC), neutrophils, lymphocytes and monocytes. There were no significant ( $p > 0.05$ ) differences in the values obtained for PCV, haemoglobin, RBC, WBC, lymphocytes and monocytes of WAD rams across the dietary

treatments. However, there were observed significant ( $P < 0.05$ ) differences in the values obtained for neutrophils of WAD rams across the

dietary treatment. Neutrophils values was significant highest (41.23) in WAD rams on T2 (3 MF: 1 MPF) and lowest (35.28) in WAD rams on T4 (1 MF: 3 MPF).

**Table 1: Haematological parameters of WAD rams fed ensiled maize forage and *Mucuna pruriens* foliage.**

Parameter	Treatment				
	T1	T2	T3	T4	SEM
PCV (%)	29.27	30.02	29.21	29.20	1.03
Haemoglobin (g/dL)	9.87	9.64	9.56	9.11	0.34
RBC ( $10^6$ /mm <sup>3</sup> )	11.87	11.66	11.14	11.02	1.27
WBC ( $10^6$ /mm <sup>3</sup> )	8.85	8.85	8.43	8.02	0.77
Neutrophils (%)	41.01 <sup>a</sup>	41.23 <sup>a</sup>	40.05 <sup>a</sup>	35.28 <sup>b</sup>	3.24
Lymphocytes(%)	56.28	57.03	56.62	56.02	3.46
Monocytes(%)	2.00	2.00	2.00	2.00	0.17

a, b : \_ means along the same row with different superscript differs significantly ( $P < 0.05$ ).PCV- Parked Cell Volume, WBC – White blood cell, RBC – Red blood cell.

T1- 1 MF: 0 MPF

T2- 3 MF: 1 MPF

T3 - 1 MF: 1 MPF

T4 - 1 MF: 3 MPF

MF = Maize Forage

MPF = *Mucuna pruriens* Foliage

Table 2 shows serum biochemical indices of WAD rams fed ensiled maize forage and *Mucuna pruriens* foliage. Urea, Glucose, Total protein, Creatinine and values for liver enzymes such as Aspartate trans aminase (AST), Alanine trans aminase (ALT) and Akaline phosphatase (ALP) were determined. There were no significant ( $p > 0.05$ ) differences in the values of Urea, Total protein, Creatinine, AST, ALT and ALP of WAD rams across the dietary treatments. However, there were significant ( $p < 0.05$ ) differences in the values of glucose of WAD rams across the dietary treatments. Glucose level was significantly highest (73.00 mg/dl) in WAD rams on T1 (1 MF: 0 MPF) and lowest (52.28 mg/dl) in WAD rams on T4 (1 MF: 3 MPF). Generally, the glucose

levels decreased with increased levels of *Mucuna pruriens* in the silage.

Table 3 shows the blood cholesterol fractions of WAD rams fed ensiled maize forage and *Mucuna pruriens* foliage. There were no significant ( $p > 0.05$ ) differences in blood cholesterol level of WAD rams across the dietary treatments. Cholesterol level ranged from 59.81 mg/dl in T1 (1 MF: 0 MPF) to 58.61 mg/dl in the blood level of WAD rams on T4 (1 MF: 3 MPF). There were significant ( $p < 0.05$ ) differences in High Density Lipoproteins in the blood of rams across the dietary treatments. HDL values were significantly higher for rams on T2 (45.70 mg/dl) and T1 (45.50 mg/dl) compared with T3 (40.00 mg/dl) and T4 (30.50 mg/dl) respectively. (LDL) in the

blood of WAD rams across the dietary treatments were generally low. LDL values were 10.20 mg/dl (T1), 10.35 mg/dl (T2), 10.39 mg/dl (T3) and 10.85 mg/dl (T4) respectively. However, no significant difference ( $p>0.05$ ) was observed for LDL values across the dietary treatments.

**Table 2: Serum biochemical indices of WAD rams fed silage combinations of maize forage and *Mucuna pruriens* foliage.**

Parameter	Treatment				SEM
	T1	T2	T3	T4	
Urea (mg/dL)	16.28	16.30	17.42	18.26	3.28
AST (UI/l)	23.22	24.43	24.00	23.78	1.03
ALT (UI/l)	26.21	26.79	26.77	26.69	2.28
Glucose (mg/dL)	73.00 <sup>a</sup>	63.48 <sup>b</sup>	58.24 <sup>c</sup>	52.28 <sup>d</sup>	3.02
ALP (UI/l)	62.01	62.72	62.52	62.13	29.8
Total proteins(g/dL)	6.84	6.98	6.64	6.37	0.06
Creatinine (mg/dL)	0.98	1.07	1.21	1.33	0.04

a, b, c, d: means along the same row with different superscript differs significantly ( $P<0.05$ ).

AST- Aspartate amino transferase, ALT- Alanine amino transferase, ALP- Alkaline phosphatase,

T1 – 1 MF: 0 MPF                      T2 – 3 MF: 1 MPF

T3 – 1 MF: 1 MPF                      T4 – 1 MF: 3 MPF

MF = Maize Forage

MPF = *Mucuna pruriens* foliage

**Table 3: Blood cholesterol fractions of WAD rams fed silage combinations of maize forage and *Mucuna pruriens* foliage.**

Parameter	Treatment				SEM
	T1	T2	T3	T4	
Cholesterol (mg/dL)	59.81	58.70	58.64	58.61	1.03
HDL (mg/dL)	45.50 <sup>a</sup>	45.70 <sup>a</sup>	40.00 <sup>b</sup>	30.50 <sup>c</sup>	1.98
LDL (mg/dL)	10.20	10.35	10.39	10.85	0.04

a, b, c: means along the same row with different superscript differs significantly ( $P<0.05$ ).

HDL- High Density Lipoprotein

LDL- Low Density Lipoprotein

T1- 1 MF: 0 MPF

T2 – 3 MF: 1 MPF

T3 – 1 MF: 1 MPF

T4 – 1 MF: 3 MPF

MF = Maize Forage

MPF = *Mucuna pruriens* foliage

## Discussion

The values obtained for haematology parameters in this study were within the normal physiological range reported for healthy sheep. The PCV obtained in this study were within the range of 28.3-35.0% reported by (18) and of 22.0-37.0% obtained by (19) for normal healthy sheep. The result showed that

the experimental rams were not anaemic.

The haemoglobin was closer to the value ranged of 9.0 - 12.4 g/dL reported by (19) for clinically health sheep. This is an indication that the silages were capable of supporting high oxygen capacity in the experimental rams.

The red blood cell (RBC,  $10^6/\text{mm}^3$ ) values were within the normal range of

8.8 - 12.0 reported by (18) but higher than 2.40 - 4.20 reported by (19). This revealed that, the experimental rams used in this study were not susceptible to anaemia related diseases.

The values for white blood cell (WBCs), lymphocytes and monocytes obtained in this study were within the normal range (1) for clinically healthy sheep. The WBCs in animals possess phagocytic function. The function of WBC is responding to antigen or foreign substance by forming antibodies that circulates in the blood or in the development of cellular immunity (3; 1). Therefore, the higher neutrophil levels recorded as a component of white blood cell in this study indicated that the WAD rams seem to possess protective system, thereby providing a rapid and potent defense against any infection.

**Serum biochemical indices of WAD rams fed ensiled maize forage and *Mucuna pruriens* foliage.**

Serum urea level of WAD rams fed the silages fell with the normal values reported (15.0 - 36.0 mg/dL) by (9) for healthy sheep but lower to the range values (29.7- 40.50 mg/dL) reported by (19) for normal healthy WAD sheep. However, the values obtained in this work fell within the normal values healthy sheep. The result showed the ability of experimental rams to digest and metabolise protein effectively. The serum urea measures the amount of nitrogen contained in the urea. High serum urea levels can indicate kidney dysfunction (19).

Aspartate trans aminase (AST), Alanine amino trans aminase (ALT) and Alkaline phosphates (ALT) values were also within the normal range, which is an

indication of normal function of liver. The glucose is the chief source of energy of all living organisms. The glucose levels obtained in this study were within the normal range of (55.0 - 131.00 mg/dL) reported for normal healthy sheep by (9) but higher than the value (59 - 65 mg/dL) reported for serum glucose levels by (20). The values reported across the treatment shows that, the silages were adequate in supplying energy to the rams. The serum creatinine were within the normal ranged values (0.5- 2.00 mg/dL) reported by (9) for clinical healthy sheep. This indicated that, there was no muscle waste in the rams.

**Serum cholesterol in WAD rams fed silage combinations of maize forage and *Mucuna pruriens* foliage**

The serum cholesterol levels obtained in the present study were within the normal range of 48.00 - 120.00 mg/dL reported by (18) for normal healthy sheep. However, the values obtained in this study were below 96 - 117 mg/dL reported by (20) for normal healthy sheep. Cholesterol is a group of fats vital to cell membranes, nerve fibres and bile salts, and a necessary precursor for the sex hormone. High levels indicate diet high in carbohydrates while low levels indicate diet low in fat, malabsorption, or carbohydrates sensitivity. (21) established that cholesterol level of 180 mg/dL and below is safe and may not result in arteriosclerosis in ruminants. The serum cholesterol contents obtained in this study is considered to be safe. High density lipoprotein (HDL) is a form of cholesterol that is called 'good cholesterol'. HDL contains high density molecular protein than fat and it protect

against heart disease by remove excess cholesterol deposited in the arteries. In this study, the values of HDL are normal for healthy rams. (21) recommended the HDL ranged values of 40 – 50 mg/dL for clinical healthy rams. The Low density lipoprotein (LDL) is said to be 'bad cholesterol' because cholesterol deposited in the arteries with high levels of LDL which causes blockage of the arteries (22; 18). The LDL values in this study were within the range considered save for WAD rams fed on the dietary treatments.

### Conclusion

From the results of this finding, it is concluded that:

1. The heamatology parameters and serum biochemical indices of WAD rams on all the silage treatments were within the normal physiological range recommended for clinical healthy rams
2. However, ensiling maize forage with *Mucuna pruriens* foliage at 3:1 improved performance in West African dwarf rams without any deleterious effect on blood profile

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