

Haematological and Serum Biochemical Indices of White Fulani Calves Fed *Rauvolfia vomitoria* Leaves and Brewers' Spent Grain-based Diet

*Uduak D. Etteh and Udoh A. Inyang

Department of Animal Science, University of Uyo, Uyo, Nigeria

*Corresponding Author Email: uddydanni@gmail.com

Abstract

The blood profile of White Fulani calves fed ensiled *Rauvolfia vomitoria* (RV) leaves and Brewers' Spent Grains (BSG) based diet was assessed. Nine (9) Fulani calves, aged between 4 – 8 weeks, were randomly assigned to 3 treatment groups of three animals per replicate, such that there was a control diet (Treatment 1) and two treatment diets (Treatment 2 and Treatment 3). Based on body weight, Treatment 1 (control) was fed 3% concentrate, 0% silage, and 4% forage; Treatment 2 was fed 3% silage and 4% forage; and Treatment 3 was fed 4% silage and 3% forage. The experiment was carried out using a Completely Randomized Design (CRD) and lasted for 56 days. Data were obtained on haematological (white blood cell, red blood cell, packed cell volume, haemoglobin, neutrophils, lymphocytes, platelets, MCH, MCV, MCHC, eosinophils, monocytes and basophils) and serum biochemical indices (urea, creatinine, total protein, ALT, ALP, AST, glucose and total cholesterol) of the calves. The haematological indices of calves fed RV/BSG-based diets were similar to those on the control diet, except for MCHC of calves on T3 (34.08g/dl), which was significantly ($p < 0.05$) higher. The effect on the haematology also showed that feeding the silage at 4% of the calves' body weight can boost the haemoglobin concentration of the red blood cell. The highest values for Alanine Aminotransferase (43.50 iu/L), Aspartate Aminotransferase (83.33 iu/L) and Alkaline Phosphatase (152.34 iu/L) were obtained for the blood of calves fed T3 diet. The serum biochemistry indices revealed that the calves fed with silage at 3% of their body weight yielded the lowest creatinine concentration in the serum, but within the range for healthy cattle. The findings of this study proved that feeding calves with brewers' spent grains and *Rauvolfia vomitoria* did not elicit any adverse effects on the calves and proved that it can be utilized as a feed resource.

Keywords: *Rauvolfia vomitoria*, Brewers' spent grains, Haematological indices, Serum biochemical indices, White fulani calves

1.0 Introduction

The status of ruminants such as cattle, sheep and goats to the socio-economic wellbeing of individuals in emerging nations like Nigeria in terms of nourishment, revenue and

imperceptible profits like savings, assurance against disasters, cultural and traditional resolutions cannot be over-emphasized (Kosgey, 2004). This is because of their ability to convert forages, crops and household residues into meat, fibre, skin and milk. Rangeland degradation and economic instability of developing countries like Nigeria have led to serious challenges in livestock feeding related to the quantitative and qualitative provision of nutrients, which is exacerbated by the continuous increase of feedstuff prices (Mureithi *et al.*, 2016).

White Fulani cattle are Zebu cattle with long legs and long horns, which are reared by the nomadic Bororo people (Kubkomawa, 2017). The cattle originated from Northern and Southern Nigeria and northern Cameroon, from where they migrated to other West African countries and beyond (Glowatzki-Mullis *et al.*, 1995). The breed is noted for its large humps with lyre-shaped horns that are 80–105 cm long (Kubkomawa, 2017) and a white coat colour that is usually interspersed with black dots. The ears of the cattle are erect, and some have red patches on the ears, legs, and sides (Onasanya *et al.*, 2021).

Blood acts as a pathological reflector of the status of the exposed animals to toxicants and other conditions. The assessment of blood therefore provides the opportunity to clinically investigate the presence of metabolites and other constituents in the body of animals, as it plays a vital role in the physiological, nutritional and pathological status of a farm animal. The ability of nutrition to influence serum biochemical indices of animals, for instance, has been reported by Onasanya *et al.* (2015).

Rauvolfia vomitoria (Afzel Apocynaceae), generally known as “poison devil’s pepper”, is a small shrub which grows to 15m high. In Nigeria, it is referred to as “Asofeyeje” in Yoruba, “Akanta” in Igbo, and “Penpe” in Ashanti, Twi, and Wassaw languages (Ajayi and Ojelere, 2014). It has oval or oblong shiny leaves in whorls, with its tuberous roots having a pale brown cork texture. Chukwu *et al.* (2015) had earlier reported the ash, nitrogen, and phosphorus content of *Rauvolfia vomitoria* matured leaves to be 9.18%, 2.61% and 0.135% respectively. However, concerns have been raised about the extracts from both leaves and roots by Eteng *et al.* (2009), who found that ethanol extracts of the roots and leaves significantly raised fluid levels of transaminases and alkaline phosphatases, unfavorably disturbing the biological and experimental catalogues of liver and various meats.

Brewers’ Spent Grain (BSG), on the other hand, is also an excellent source of high-quality protein (25.80 %) and fibre content of 15.80 % on a dry matter basis (Mussatto *et al.*, 2006), while gross energy is 19.70 MJ, it contains various essential and non-essential amino acids, such as methionine, histidine, and proline (Huige, 2006; Mussatto and Roberto, 2006). It is also a source of vitamin H, vitamin B1, and vitamin E, with the latter acting as antioxidants and providing health benefits (Ikram *et al.*, 2017). Additionally, BSG contains minerals like calcium, magnesium, potassium, and manganese (Mussatto, 2009; Meneses *et al.*, 2013). Other compounds found in BSG include tannic acid, essential oils, and lipids, primarily triglycerides and fatty acids (Ikram *et al.*, 2017; Mussatto and Roberto, 2006).

The utilization of BSG in animal nutrition does not only help reduce feeding costs but also taps into an available resource. Its diverse nutritional profile and proven efficacy across various animal diets position BSG as a valuable component in the quest for sustainable and efficient animal nutrition strategies (Aliyu and Bala, 2011). Nevertheless, there is a dearth of information regarding the haematology and serum biochemical indices of calves of White Fulani fed ensiled *Rauvolfia vomitoria* (RV) leaves and BSG; hence, this study was

conducted to determine the effects of introducing this relatively unknown forage into ruminant diets.

2.0 Materials and Methods

The experiment was conducted at the Cattle Unit of the Teaching and Research Farm of the Department of Animal Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria. It has an average annual rainfall range of 2200 mm to 3500 mm. The temperature of the area ranges from 26 °C to 28 °C. Uyo can be found between latitudes 4°31 and 5°30 North and longitude 7°30 and 8°20 East of the Equator. The mean altitude is 38.10 m above sea level, and relative humidity ranges from 71% to 88% annually (University of Uyo Meteorological Station, 2020).

A total of nine (9) White Fulani calves were used for this experiment. The calves were separated into 3 treatment groups of one calf per replicate using a Completely Randomized Design. Before the start of the experiment, the pens were cleaned and disinfected thoroughly with a disinfectant (Izal). The animals were quarantined and acclimatized for 14 days. Feedstuff and water were provided at *ad libitum* throughout the study period which lasted for 60 days.

Medications such as ivermectin and LA antibiotics were administered to prevent internal and external vermin. Adequate record-keeping on all aspects of management practices was ensured. The experimental animals were fed ensiled *Rauvolfia vomitoria* leaves and a brewers' spent grains-based diet. The blood sample for serum biochemical analysis was collected from the calves two days before the end of the feeding trial via the jugular vein using a 10ml syringe. The blood was collected from all 9 calves into appropriate test tubes. The sample for haematological breakdown was composed using ethylene diamine tetra acetic acid decanters to avert coagulation, while those of the blood serum biochemistry were poised in a laboratory test cylinder.

After collection, the test tubes were taken to the laboratory within 30 minutes and were centrifuged to separate the serum from the cellular components of the blood. The serum composition of the different serum parameters was projected using profitable kits and resulting standard procedures prearranged by the producer Randox Laboratories Limited, U.K. Haematological examination of the blood models on the other hand was achieved in a computerized haematology analyzer (BC-2300 model, Mindray Medical Co., China) with the technique carried out as stated by the producer.

Table 2.1 presents the feeding schedule for the calves. Calves on Treatment 1 (control) were fed 3% Concentrate, 0% Silage, and 4% forage, while those on Treatment 2 were offered 3% Silage and 4% forage and those on Treatment 3 were 4% silage and 3% forage for 56 days. All the data obtained were analyzed using a one-way analysis of variance (ANOVA) in a completely randomized design (CRD) with the statistical analysis system package (SAS, 1999). Significant Differences observed were separated with means as prescribed by the Duncan Multiple Range Test (DMRT) at a 5% probability level.

Table 2.1: Ingredients constituents of the experimental diet

Ingredients	Control (0% Silage)	T2 (3% Silage + 4% Forage)	T3 (4% Silage + 3% Forage)
Maize	40	-	-
Soybean meal	20	-	-
Brewer's spent grain + <i>Rauvolfia vomitoria</i> leaves silage	-	Silage with the best characteristic (96.00%)	Silage with the best characteristic + a little high margin (96.00%)
Wheat offal	20	-	-
Palm kernel cake	16	-	-
Bone meal	3.0	3.0	3.0
V/TM premix	0.50	0.50	0.50
Salt	0.50	0.50	0.50
Total	100	100	100

V/TM Premix = vitamin A, D3, E, K, B1, B2, B6, B12, Niacin, Pantothenic acid, Folic acid, Biotin, Choline, Chloride, Manganese, Zinc, iron, Copper, iodine, selenium, cobalt and Antioxidants.

3.0 Results and Discussion

3.1 Haematological Indices of Experimental Calves

The result of the haematological indices of White Fulani calves fed Brewers' spent grain ensiled with *R. vomitoria* is presented in Table 3.1. The outcome shows that the treatment diets did not have any significant effect ($p>0.05$) on all the measured haematological parameters except in the Mean Corpuscular Haemoglobin Count for T₃ (34.08g/dl) which was significantly higher ($p<0.05$) in the calves fed T₃ compared with those on control diet (30.23g/dl) and T₂ (32.23g/dl).

Table 3.1: Haematological indices of White Fulani calves fed BSG ensiled with *R. vomitoria*

PARAMETER	Control	T2	T3	SEM
WBC ($\times 10^3/L$)	13.30	10.83	11.12	2.66
RBC ($\times 10^6/L$)	8.56	7.37	7.26	1.08
PCV (%)	25.17	23.33	29.13	2.46
Hb (g/dl)	11.73	9.99	8.23	1.16
Neutrophils (%)	44.13	48.79	40.63	7.26
Lymphocyte (%)	42.63	35.00	38.00	8.11
Platelet ($\times 10^9/L$)	367.10	323.67	338.98	40.40
MCH (Pg)	13.27	12.00	13.02	0.48
MCV (fl)	42.83	36.70	34.67	2.82
MCHC (g/dl)	30.23 ^b	32.23 ^b	34.08 ^a	1.07
Eosinophil ($\times 10^3/ul$)	2.33	2.00	2.00	0.38
Monocyte ($\times 10^3/ul$)	1.33	1.67	2.00	0.43
Basophil ($\times 10^3/ul$)	0.67	0.67	0.67	0.33

^{ab} Means with different superscripts within a row are significantly different ($P<0.005$), SEM Standard error of the mean. WBC - White Blood Cell count; RBC - Red Blood Cell count; PCV - Packed Cell Volume; Hb - Haemoglobin concentration; MCH - Mean Corpuscular Haemoglobin; MCV - Mean Corpuscular Volume; MCHC - Mean Corpuscular Haemoglobin Concentration, control -0% silage, T2-3% silage + 4% forage, T3 -4% silage + 3% forage.

MCHC measures the average concentration of haemoglobin in the red blood cells and is calculated by dividing the haemoglobin by the hematocrit (Peng *et al.*, 2023). Haemoglobin is an iron-rich protein that carries oxygen to tissues; hence, a raised MCHC value is often associated with good health (Huang and Hu, 2016). In the present study, feeding the calves silage at 4% of their weight raised the MCHC value, but the increase was only significant in the group fed silage at 3% of their body weight. More importantly, all the MCHC values are within the range (32-36g/dl) as reported by RAR (2009). A similar trend was also reported by Busari *et al.* (2022), where the supplementation of BSG improved the haematological and red blood cell haemoglobin of Bunaji bulls. However, this is the first study where a combination of BSG and *Rauvolfia vomitoria* silage was fed to cattle.

Red blood cell indices are blood tests that provide information about the haemoglobin content and size of red blood cells. Abnormal values indicate the presence of anaemia, a condition in which there are too few red blood cells. Anaemia can also be defined based on the cell size (MCV) and amount of Hb (MCH). Red blood cells transport haemoglobin, which, in turn, transports oxygen (Etim *et al.*, 2013). However, from our study, the values obtained were within limits for healthy calves (RAR, 2009). White Blood Cells (WBC), or leucocytes, are cells of the immune system involved in defending the body against both infections, disease and foreign materials (Etim *et al.*, 2013). Etim *et al.* (2013) had reported that elevated white blood cell count could be as a result of infection, immune system disorders, stress among others while increase in leucocytes may be due to anaemia, bone marrow tumour, infectious diseases inflammatory disease, severe physical stress, tissue damage (for example, burns) among others. The values obtained in this study fell within the range for healthy calves as reported by RAR (2009).

3.2 Serum Biochemical Indices of Experimental Calves

Table 3.2 shows the result of the serum biochemical indices of calves fed ensiled BSG and *R. vomitoria*. The result showed that there were significant differences ($p < 0.05$) in creatinine, ALT, AST, and ALP values of the calves. The creatinine values of the calves fed the T₃ diet (0.6umol/L) were highest but were statistically the same ($p > 0.05$) to those fed the control (0.56umol/L), and both were significantly different and higher ($p < 0.05$) than those fed T₂ diets. The calves on Treatment 2 had statistically similar ($p > 0.05$) ALT, AST, and ALP values to those on the control. The animals on Treatment 3 had significantly higher values (43.50iu/L, 83.33iu/L, and 152.34iu/L), respectively.

Table 3.2: Serum Biochemical indices of White Fulani Calves fed ensiled BSG and *R. vomitoria*.

PARAMETER	Control	T2	T3	SEM
Urea (mmol/L)	13.63	14.42	13.91	0.25
Creatinine (umol/L)	0.56 ^a	0.47 ^b	0.60 ^a	0.02
Total Protein (%)	57.00	59.67	59.00	6.84
ALT (iu/L)	20.50 ^b	21.00 ^b	43.50 ^a	6.07
AST (iu/L)	65.50 ^b	59.50 ^b	83.33 ^a	4.87
ALP (iu/L)	118.00 ^b	115.00 ^b	152.34 ^a	6.99
Glucose (iu/L)	53.40	52.80	56.40	1.59
Total Cholesterol(mmol/L)	121.17	116.01	122.46	6.14

^{ab} Means with different superscripts within a row are significantly different ($P < 0.005$); SEM= Standard error of the mean. ALT - Alanine Aminotransferase; AST - Aspartate Aminotransferase; ALP - Alkaline Phosphatase, control -0% silage, T2-3% silage + 4% forage, T3 -4% silage + 3% forage

The serum creatinine as a byproduct of amino acids metabolism can be affected by nutrition and in the present study, feeding BSG and *R. vomitoria* silage at 3% of body weight lowered the creatinine level while feeding 4% of body weight did not, but all the values are within the range reported by Alalade *et al.* (2021) of 0.45-0.65mg/dl.

Creatinine is a nitrogenous organic acid that is generated predominantly in the kidney and liver, and to some extent in the pancreas, using the three amino acids, namely: glycine, arginine, and methionine. This process consumes up to 10% of the daily intake of glycine, 22% of arginine, and 42% of methionine (Kashani *et al.*, 2020). Its abundance is often associated with kidney dysfunction, as this could be an indication of the inability of the kidney to filter out creatinine through urine.

Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) are serum enzymes that are commonly measured in blood tests to assess liver health in cattle. These enzymes are produced by liver cells, and their levels in the blood can indicate the degree of liver damage or dysfunction (Thapa and Walia, 2007). For these liver enzymes, the highest values for ALT (43.5mg/dl), AST (83.33mmol/L) and ALP (152.34iu/L) were recorded by the calves fed T3 diets, and they were significantly higher ($p<0.05$) than those fed the T2 diet and control.

The enzyme values for the calves fed the control diet were generally higher than those fed T2, but there was no significant difference ($P>0.05$) between them. Past studies (Oke *et al.*, 2022; Khan and Line, 2010) provided a range for which the liver enzymes of cattle could be compared. In tandem with their range for ALT (6.9-35u/L; 5.12-9.6iu/L) and AST (60-125mmol/L), the ALT and AST found in this study are hereby reported to be normal, signifying that the effect of the calf with ensiled BSG and *R. vomitoria* at 3% and 4% of body weight is not detrimental. However, ALP values recorded in all the treatments (118.00iu/L, 115.00iu/L, and 152.34iu/L) are higher than the range reported by Oke *et al.* (2022) (90-170iu/L) and Ozegbe *et al.* (2000) (138.51-172.91iu/L) and may be attributed to damage to the liver (RAR, 2009).

4.0 Conclusion

In conclusion, this study attempted to utilize Brewers' Spent Grain and *Rauvolfia vomitoria* by formulating a silage-based diet on different compositions of the two feed sources. The haematology of the calves showed that feeding the silage at 4% of the calves' body weight can boost the haemoglobin concentration of the red blood cells. The serum biochemistry indices also revealed that the calves fed with silage at 3% of their body weight yielded the lowest creatinine concentration in their serum, but within the range for healthy cattle. Generally, the dietary supplementation of calves' diets with silages at 3% and 4% of their body weight did not have any adverse effect on the calves' blood parameters, therefore proving its potential as a veritable feed resource, especially in the dry season.

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