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RESPONSE OF YANKASA RAMS TO VARIOUS INCLUSION LEVELS OF PROPAGATED BITTER LEAF (*Vernonia amygdalina*) ON PERFORMANCE AND RUMEN ECOLOGY

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ABSTRACT

The experiment evaluated the response of Yankasa rams fed graded levels of propagated Bitter Leaf (*Vernonia amygdalina*) on growth performance, nutrient digestibility, rumen ecology, and blood profile. Twenty (20) rams were randomly allocated into four dietary treatments consisting of 0%, 5%, 10%, and 15% inclusion levels of dry bitter leaf. Growth performance, nutrient digestibility trail and rumen fluid were collected and analyzed accordingly. Data obtained were analyzed using statistical analysis system (SAS,2002). Growth performance results showed that T3(15%) had an outstanding ($P<0.05$) performance, especially feed intake, and final weight. Nutrient digestibility results indicated that *V. amygdalina* graded level statistically ($P<0.05$) influenced dry matter (DM) digestibility and neutral detergent fiber (NDF). T3 (10%) had statistically higher values ($P<0.05$) of acid detergent fibre (ADF), crude protein (CP) and nitrogen-free extract (NFE) values compared to control group in this study. *V. amygdalina* graded level had profound significant ($P<0.05$) differences in rumen pH and other rumen ecology parameters. It could be concluded that rams fed 10% (T3) *Vernonia amygdalina* had outstanding growth performance followed by 15% *Vernonia amygdalina* level. Bitter Leaf *Vernonia amygdalina* do not negatively affect nutrient digestibility. However, considerable changes occur in VFA and rumen concentration from 0 to 15% of *Vernonia amgydalina.* Farmers are therefore recommended to include *Vernonia amgydalina* into the ram diet up to 15% for growth performance, nutrient digestibility, and rumen fermentation.

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INTRODUCTION

The scarcity of energy and protein in feedstuffs during the dry season poses a significant challenge to ruminant livestock production in Nigeria. This scarcity leads to a marked decrease in voluntary intake, digestibility, and overall productivity (Babayemi *et al.,* 2009). Additionally, the rising prices of conventional energy ingredients, coupled with increased competition between humans and animals due to a growing population, exacerbate the situation. The high cost of maize and other conventional feed resources in Nigeria has resulted in elevated animal feed prices, prompting the need to explore unconventional feed resources as alternatives or replacements for traditional feed ingredients (Sowande, 2004; Lamidi, 2009). Bitter leaf (*Vernonia amygdalina*) is a multipurpose plant in Nigeria that plays a significant biological role (Saalu *et al.*, 2013). It possesses nutritional and phytochemical properties that offer physiological, biochemical, and morphological benefits. The consumption of vegetables is recognized as essential for a healthy life, due to their antioxidative properties (Saalu *et al.,* 2013; Akunna *et al.,* 2013). Bitter leaf has been observed to thrive in Nigeria even under harsh weather conditions; however, very bitter taste can negatively impact intake (Saalu *et al.,* 2013). This bitterness is attributed to anti-nutritional factors such as alkaloids, saponins, tannins, and flavonoids (Saalu *et al.,* 2013). These compounds significantly affect microbial activity in the rumen, thereby modifying the fermentation process.

MATERIALS AND METHOD.

Experimental site

This experiment was conducted at the Pasture unit of Prof. Lawal Abdu Saulawa Livestock Teaching and Research Farm, Department of Animal Science, Federal University Dutsin-Ma, Katsina State. The farm is situated within the latitude 12°27'18' north and 7°29'29' east and 605 meters above sea level with an annual average rainfall of 700mm in the Sudan savannah ecological zone. (Gaddafi *et al.,* 2019).

Experimental design

A total of twenty (20) Yankasa rams, each weighing approximately 23.5 kg, were assigned to four dietary treatments consisting of inclusion levels of dry bitter leaf (*Vernonia amygdalina*) at 0%, 5%, 10%, and 15%. The rams were distributed into four treatment groups, with five rams in each group, in a Completely Randomized Design (CRD). Each animal was housed in a pen measuring 2 m x 1 m, which was disinfected prior to the commencement of the research.

Data analysis

Data were analyzed using ANOVA (SAS, 2002), and significant differences among the treatment means were determined using Duncan's Multiple Range Test.

Data collection

Growth Performance Determination

The Initial weight at the beginning of the experiments were determined and recorded, Feed Intake (Kg), Weight Gain (Kg), Feed Conversion Ratio, Average Daily Weight Gain (g) and Final Weight (Kg) were also determined.

Determination of Nutrient Digestibility

Two Yankasa rams were selected from each treatment and allocated into metabolic cage for faecal and urine samples collection. Animals were allowed to acclimatize with the cage for seven days, where fecal sample was collected daily and weighed. The urine samples was collected in a sample bottle 50mls which it contains 10% concentrated sulphuric acid to prevent bacterial or microbial activities, and nitrogen escape. The Urinealysis samples was taken to laboratory and analyzed for urine nitrogen, ammonia, billirubvin, Cl2, Na, while faecal sample was taken to determine according to procedure outline by association of analytical chemistry (AOAC, 2002) Dry matter (DM), moisture, ADF, NDF, CF, CP, EE Ash, and Lignin.

Rumen Microflora Detection

The rumen fluid was collected using a stomach tube. The tube was inserted into the rumen and pump to obtain rumen fluid. The fluid was put in sample bottles and the samples were taken to the laboratory for rumen Volatile Fatty Acid (VFA) determination which includes, the samples were divided into two portions. The 1st portion is for total volatile fatty acid (VFA) and the proportions of acetate, propionate, and butyrate. The samples were centrifuged at 3000 x g for 10 min; which the samples were allowed to settle. The decant was titrated with 0.1M of sodium hydroxide (4/1000gml H2O) solution each with 2-3 drops of Phenophtaline (1/100gml ethanol) as the indicator. The second portion of the rumen filtrate was used for microbial count and identification. 2ml of rumen liquor was subjected to microbial count. Protozoa count was obtained by direct observation using a microscope at 10 x magnification (Dehority, 1984). Colony-forming units/ml (CFU/ml) of both bacteria and fungi were observed with the pour plate technique using nutrient agar (NA) and Potato dextrose agar (PDA) respectively. The plates were then incubated for 24 hours at 37ºC. All colonies appearing at the end of the incubation period were counted using a digital illuminated colony counter. Colonies grown on nutrient agar plates were suspected to be either gram-positive or gram-negative thus; all colonies found on each plate were used for gram staining as described by Cheesbrough (2005). Colonies grown on the PDA were further incubated for three days after the first 24 hours to check for morphology and isolation of fungi. Physical characteristics of rumen liquor such as temperature, pH, color, and odour.

RESULT AND DISCUSSION

Proximate Analysis of Experimental Diet

The results on proximate analysis of experimental diet were presented in table 4.6 below. The experimental diet in this study were formulated to meet the dietary recommendation of NRC (2002) of grower rams.

Table 4.6: Proximate Analysis of Experimental Diet

Parameters %	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)
LIG	15.98	16.51	16.54	16.51
DM	92.80	94.00	93.60	93.35
ADF	31.36	32.41	32.56	32.70
NDF	52.49	51.88	53.69	53.74
CP	12.44	12.33	12.47	12.22
OIL	3.76	3.94	3.57	3.98
Ash	7.20	7.32	8.11	8.20
NFE	67.30	66.19	64.37	64.37
Energy (Kcal/Kg)	3,601.99	3,641.95	3,685.58	3,714.69

LIG= Ligning, DM= Dry Matter, ADF= Acid Detergent Fibre, NDF= Neutral Detergent Fibre, CP= Crude Protein, NFE= Nitrogen Free Extract.

Effect of Graded Level of Bitter Leaf (*Vernonia amygdalina)* on Growth Performance of Yankasa Rams

The results on graded levels of bitter leaf *Vernonia amygdalina* on the growth performance of Yankasa Rams are presented in Table 2 below. The Experiment revealed that there were no significant (P>0.05) differences in the initial weight of animals used in this study and this clearly shows that the weight of animals was approximately balanced before the commencement of the experiment and this helped to reduce experimental error and bias. The final weight also showed that there were no significant (P>0.05) differences. However, there were considerable numerical differences between groups in which T3 had the highest final weight of (32.73kg) followed by T4 with (31.93kg), T2 (31.00kg), and T1 (30.37kg). The final weight of animals obtained in this study is higher than the final weight of Yankasa Rams of 22.51 - 23.16kg reported by Garba *et al.* (2023). A significantly (P<0.05) higher weight gain was recorded in T3 with 5.03kg followed by T4 with 3.27kg. This indicates that Bitter leaf (*Vernonia amygdalina*) had a profound effect on weight gain improvement of the animal and this may be attributed as a result of proximate constituent and Adegbola *et al.* (2020) reported that secondary metabolites contained in *Vernonia amygdalina* that are responsible for enhancing feed intake, utilization, and absorption thereby improving weight of the animals.

Table 2 Effect of Graded Level of Bitter Leaf (*Vernonia amygdalina*) on Growth Performance of Yankasa Rams

Parameters	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	SEM
Initial Weight (kg)	29.00	28.67	28.67	27.67	3.42
Final Weight (kg)	30.37	31.00	32.73	31.93	2.729
Weight Gain (kg)	1.37^{b}	2.33^{b}	4.06^{ab}	4.26^{a}	1.130
Average Daily Weight Gain (g)	22.00^{c}	38.00^{b}	67.67^{a}	71.00^{ab}	19.47
Feed Intake (kg)	1.163^{b}	1.453^{a}	1.433^{a}	1.607^{a}	0.0932
Average Daily Feed Intake (kg)	0.01933^{b}	0.02400^{a}	0.02400^{a}	0.02633^{a}	0.001616
Feed Efficiency	1.739^{ab}	1.174^{b}	3.431^{a}	2.039^{ab}	0.732
Feed Conversion Ratio	0.3580^{b}	0.8550^{a}	0.3267^{b}	0.6070^{ab}	0.2018

Effect of Graded Level of Bitter Leaf (*Vernonia amygdalina*) on Yankasa Rams Nutrient Digestibility.

The results on the graded level of *Vernonia amygdalina* on Yankasa rams nutrient digestibility are presented in Table 3 below.

The result showed that Acid Detergent Fibre (ADF), Ash, Crude Fibre (CF), Crude Protein (CP), Ether Extract (EE), and Nitrogen Free extract (NFE) were not Significantly (P>0.05) affected by the influence of *Vernonia amygdalina* graded levels across the treatment however there were considerable differences across the treatment groups. For instance, there were considerable increases in CP digestibility with increased levels of *Vernonia amygdalina* in this study. The increasing crude protein digestibility with increasing levels of *Vernonia amygdalina* in the diet could be due to the presence of condensed tannins in bitter leaves. According to Wada *et al.,* (2016) condensed tannins at low levels bind the dietary protein, thus preventing it from ruminal degradation and thus enhance its availability for enzymatic digestion and utilization by the animal tissue. This observation is consistent with the work of Giri *et al.,* (2000) and Aregbeore (2009) who affirmed that the digestibility of nutrients varies with the nutrient composition of a diet.

The result revealed that *Vernonia amygdalina* graded levels in Yankasa rams greatly (P<0.05) influence Dry Matter Digestibility (DM) and Neutral Detergent Fibre (NDF). The relatively decreasing DM with increasing *Vernonia amygdalina* levels in the diets may be attributed to the increasing content of fibre fractions especially NDF in the diet. Bakshi and Wadhwa, (2004) reported that high NDF and ADF depress DM intake and DM digestibility. Minson, (1990) reported that lignin is bound to all plant cell walls and is a significant limiting factor in their digestion in the rumen. The higher values of ADF, CP and NFE at T3 the most probable explanation for this phenomenon is that diet at T3 graded level might have resulted in high palatability, increased activity of rumen microorganisms for rapid fibre digestion in the rumen, and better utilization of the nutrients by the animals.

Table 3: Effect of Graded Level of Bitter Leaf (*Vernonia amygdalina)* on Yankasa Rams Nutrient Digestibility

Parameters	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	SEM
DM	68.64^{a}	70.59^{b}	74.30^{a}	74.25^{b}	1.042
ADF	12.21	17.62	22.14	9.31	7.53
NDF	20.180^{a}	2.300^{c}	7.705^{b}	5.670^{b}	1.141
NFE	6.830	4.690	11.300	10.100	2.99
CP	28.49	19.82	57.89	56.91	14.55
CF	30.00	35.02	27.96	26.00	7.35
ASH	23.08	28.07	24.41	31.09	5.69
EE	42.28	34.89	32.91	45.34	4.49

ADF= Acid Detergent Fibre, CF= Crude Fibre, CP= Crude Protein, DM= Dry Matter, EE= Ether Extract, NDF= Neutral Detergent Fibre, NFE= Nitrogen Free Extract

Effect of Bitter Leaf (*Vernonia amygdalina)* on Volatile Fatty Acids and Rumen Microbes of Yankasa Rams

The rumen Volatile Fatty Acids and Microbial Counts Result of Yankasa rams are presented in the Table 9 below. The result revealed that the graded level of *Vernonia amygdalina* had a significant (P<0.05) role in changing the rumen liquor pH was a progressive linear increase trend with *Vernonia amygdalina* in which T4 had the highest pH value (6.300) while T1 had the lowest (6.150). pH provides a conducive and enabling environment for rumen microbes to work efficiently. Van Soest (1994) states that the pH range for optimal microbial activity was 6.2 - 7.2 which is in line with the value found in this study. The values recorded in this study fell within the range of 6.00 - 7.20 suitable for the growth and activities of microbes reported by Jallow and Hsia (2011). The Result showed that there were significant (P<0.05) differences in Acetic Acid, Butyric Acid, Propionic Acid, and total volatile fatty acid. The highest acetic acids were recorded in T1 (75.38) which is good for energy but it may reduce the level of testostrone reported by (Wagner *et al.,* 2008), followed by T3 (47.62) while T2 had the lowest acetic acid value (42.84) lower acetic acid can lead to energy deficiency and poor sperm quality reported by (Safari *et al.,* 2018). The graded level of *Vernonia amygdalina* greatly influenced the Butyric Acid concentrations with a linear trend with increased levels of *Vernonia amygdalina*. The Total Volatile Fatty Acids (VFA) recorded in this study are higher than VFA reported by Jokthan *et al.,* (2013), Ngele (2008) and Eleman *et al.,* (2009).

Table 4.9: Effect of Bitter Leaf (*Vernonia amygdalina*) on Volatile Fatty Acids and Rumen Microbes of Yankasa Rams

Parameters	T1(0%)	T2(5%)	T3(10%)	T4(15%)	SEM
Ph	6.150^{b}	6.200^{ab}	6.250^{ab}	6.300^{a}	0.0500
AA (mmol/l)	75.38^{a}	42.84^{a}	47.62^{b}	45.16^{b}	2.103
BA (mmol/l)	26.89^{c}	32.05^{b}	35.65^{a}	35.23^{a}	0.549
PA (mmol/l)	43.28^{b}	51.21^{ab}	54.45^{a}	49.98^{ab}	3.38
Bacteria (x10^{3}cfu)	7.465^{b}	7.685^{b}	7.795^{a}	8.615^{a}	0.2675
Fungi (x10^{3}cfu)	1.720	1.775	1.510	1.735	0.2212
Protozoa (x10^{3}cfu)	1.700^{a}	1.490^{b}	1.570^{b}	1.550^{b}	0.0332
NH3(nmol/l)	20.68	20.20	20.19	20.17	0.368
VFA(mg/l)	145.9^{d}	147.8^{c}	156.4^{b}	165.3^{a}	0.498

AA= Acetic Acid, Butyric Acid, PA= Proponoic Acid, NH3= Ammonia, VFA= Volatile Fatty Acid

CONCLUSION

This Experiment could be concluded that rams fed 10% (T3) *Vernonia amygdalina* had outstanding growth performance followed by 15% *Vernonia amygdalina* graded levels. Bitter Leaf *Vernonia amygdalina* graded levels do not negatively affect nutrient digestibility. However, considerable changes occur in VFA and rumen microbes' concentration from 0 to 15% *Vernonia amgydalina.*

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