

Rumen Fermentation Characteristics of West African Dwarf Sheep Fed Supplementary Diets Containing Water-Washed Neem (*azadirachta indica* A. Juss) Fruit and Garlic (*Allium sativum*)

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Abstract

An experiment was conducted for 37 days for the purpose of investigating Rumen Fermentation Characteristics of West African Dwarf Sheep Fed supplementary diets containing Water-Washed Neem (*Azadirachta indica* a. Jusso Fruit and garlic (*Allium sativum*). The experimental design used was a completely randomized design and the obtained data were subjected to analysis of variance (ANOVA). 12 West African dwarf Sheep were randomly allotted into 4 treatments of 3 replicates per treatment, these treatments were supplements containing 0%, 5%, 10% neem and 1.25% garlic. Panicum maximum was fed as the basal diet. The basal and supplementary diets were fed at ratio 50:50 and at 3% body weight on dry matter bases. At the end of 37 days feeding trial, rumen liquor from the experimental animals were collected for rumen fermentation characteristics and in vitro gas production. The pH of the rumen liquor had values ranging from 6.30 - 7.00 and there was a significant difference between the 4 treatments. Treatment IV had the highest acetic acid and butyric acid production while treatment II had the lowest. However, there was no significant difference amongst the 4 treatments. Propionic acid was highest for treatment I while treatment II had the lowest value. For the propionic acid, the treatment that had no inclusion of neem and garlic (the control treatment) had the highest mean value for the study. The in vitro production obtained for a standard feed sample incubated in the rumen liquor obtained from the 4 treatments were significantly affected by the inclusion of neem and garlic. It is then concluded that the inclusion of neem at 10% and garlic at 1.25% in the supplementary feed of WAD sheep will significantly reduce methane production and support ruminant production.

Keywords: Water-washed Neem (*Azadirachta indica* A. Juss) Fruit, Garlic (*Allium sativum*), West African Dwarf Sheep

Introduction

The role played by crop residues in tropical livestock nutrition has been stressed by several researchers. Crop residues are low quality roughages that are low in protein, minerals and vitamins they are easily available at low cost and have great potential if properly harnessed. They are key elements in tropical ruminant nutrition (Dominique *et al*, 1991) and can meet the nutritional requirements of livestock for maintenance, growth and reproduction during adverse climatic conditions chemical composition of crop residues can give an idea of their nutritive value (Oyenuga 1968; Golu, 1981). However, the chemical components of crop residues are not always directly related to the response of an animal because it is also a function of the ability of the animal to derive useful nutrients from the ration. Beside digestibility, the voluntary intake of roughage is another essential fact in quality assessment (Minson, 1990). The digestibility nutrients content is determined by *in vitro* experiments or estimated using *in vitro* procedures which are cheaper and more convenient. There is scanty literature report using a combination of chemical constituents and gas released on incubation of feed in an *in vitro* medium containing rumen microbes (Menke and Steingass, 1998) to assess the nutritional quality of tropical crop residues. Inedible residues from industrial processing such as brewers dry grain (BDG), sugarcane molasses and other cereal by-products which are converted into feeds for poultry, pigs and ruminants in advanced and industrialized countries are often waste in developing countries such as Nigeria these residues when properly could be utilized by livestock animals to produce milk, meat and eggs. Nutrition is the important limitation to livestock production in Nigeria, according to Wilson and Payne (1999), one of the ultimate objectives of any livestock industry is the conversion into animal products of feeds which are either edible to man or surplus to his immediate requirements. Availability of forage in quantities adequate for optimum livestock production to ensure a high level of productivity varies with season, during dry season, there is usually limited and often inadequate supply of forages, which eventually results in retarded growth of the animals. In an attempt to actualize the mandate of better utilization of unconventional feed sources better utilization of the browse species and multipurpose trees as possible source of feed for ruminants especially during dry season is considered, the multipurpose tree legumes are now being utilized as dietary nitrogen supplements for ruminants. Browse trees and multipurpose trees (e.g. *Azadirachta indica* and *Allium sativum*) are known to contain secondary metabolites which are anti-nutritional factor present in plants such as Tannins, Saponins (could be condensed or hydrolyzed) and steroid which could be limiting to their optimum consumption and utilization by man. These anti-nutritional factors when present in such plants could also have an inhibitory effect on methane production in the rumen of ruminant animals (methanogenesis) when consumed due to the suppressive methanogenic effect of Saponins and Tanins. This research work aims at determining the effects of natural additives (*Azadirachta indica* and *Allium sativum*) on feed intake, nutrient digestibility, rumen fermentation, methanogenesis, digestibility, volatile fatty acids and economic efficiency of West African dwarf sheep.

Materials And Method

This was a 37 days experiment carried out at the sheep unit, Teaching and Research farm, University of Ibadan, Oyo State, Nigeria. The system of animal rearing adopted here was there intensive system, the sheep house is characterized by low wall and open side with corrugated iron roofing sheep for proper ventilation, the barn is divided into pens.

Experimental Animal And Diet

Twelve West African dwarf sheep were used. The animals were randomly allotted into a group of 3 animals each. Each animal had a tag on for identification purpose and acclimatization was allowed before the start of the experiment. 4 diets were formulated. Diet 1 contained cassava peels, soyabean meal, corn bran, and mollases. In ratio of 60:20:10:10. In diets 2 there was an inclusion of 1.25% garlic and molasses was reduced to 8.75%. In diets 3, corn bran was replaced with neem fruit (water washed). Also, in diet 4 corn bran was replaced with neem fruit and 1.25% of garlic was also included. All ingredients were thoroughly mixed and kept in a water proof container to prevent mold growth. 3 West African dwarf sheep were placed on each diet for 5 weeks. The gross composition of the experimental diets is presented in Table 1.

In Vitro Gas Production Procedure

Collection of rumen liquor, pH measurement, Buffer preparation

High quality rumen liquor was collected from these animals by suction method, using a suction tube. The rumen liquor was transported the laboratory in pre-warmed insulated flasks to avoid injury or death of constituent rumen microbes and also to avoid contamination of the liquor by maintenance of anaerobic condition. The pH of the liquor was measured at the point of collection using a digital pH meter.

The following reagents were used in the preparation of buffer: NaHCO_3 (9.8g/l), Na_2HPO_4 (2.77g/l), KCl (0.57g/l), NaCl (0.47g/l) + $\text{mgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.12g/l). $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.16g/l). They are the MC Dougall's buffer, they are freshly prepared and placed in the incubator at 39°C to maintain the optimum conditions required for the experiment

Measurement Of Gas Production

The volume of gas produced is determined by the measurement of the piston displacement level during the period of incubation. The reading was taking at intervals of 3 hours for 24 hours after incubation commenced. The readings were taken by inverting the syringes and placing them parallel to the eye to measure the piston displacement as displaced by the space between the piston and the fluid level. The readings were taken and recorded with respect to time (3,6,9,12,15,18,21,24 hours)

Determination Of The Results Of In Vitro Gas Characteristics

The average volume of gas produced by eight blanks in the syringes (contains no substrate: it contains only inoculums and buffer) was subtracted from the volume of gas produced in each test sample syringes throughout the 24 hours of incubation. The difference obtained is the gas produced due to fermentation of the substrate or the net gas produced.

Experimental Design And Statistical Analysis

The experimental design used was completely randomized design and data obtained were subjected to analysis of variance (ANOVA) and where significant differences occurred they were separated by New Duncan multiple range test

Results And Discuss

The result of the volatile fatty acid is shown in table 2 where the pH showed that there was a significant difference ($P < 0.05$) amongst the treatments, treatment I was significantly different from treatment II and treatment II is significantly different from treatment IV. No significant difference between treatment I and other treatment (Findly, 1998). As for the ammonia nitrogen (mg/litter), treatment IV is significantly different from treatment I and from treatment II, also from treatment III. Ammonia is readily absorbed and the rate of absorption is dependent on concentration and pH. It is readily absorbed at a higher pH and absorption decreases as pH decreases. (Feller, 2004). Acetic acid (mM/100ml) indicates there was no significant ($P < 0.05$) difference amongst the means of the 4 treatments, however, the highest mean value of 3.09mM/100ml was recorded for treatment 4, followed by treatment 3 with mean value of 2.51mM/100ml and treatment 1 having the mean value of 2.45mM/100ml with treatment 2 having the lowest mean value of 1.73mM/100ml. Moreover, Butyric Acid (mM/100ml) treatment 4 had the highest mean value of 3.48mM/100ml, followed by treatment 1 with the mean value of 2.76mm/100ml and treatment 3 having the mean value of 2.49. Treatment 2 had the lowest mean value of 2.15mM/100ml. However, there was significant (< 0.05) difference amongst the 4 treatments. Moreover, treatments 1,3 and 4 was not significantly different from each other while treatment 2 is significantly different from treatments 1,3 and 4 respectively. Propionic Acid (mM/100ml) showed that treatment 1 had the highest mean value of 2.61mm/100ml, seconded by treatment 4 with the mean value of 2.53mM/100ml and treatment 3 with the mean value of 2.33mM/100ml. Treatment 2 had the lowest mean value of 1.50mM/100ml. there was no significant (< 0.05) difference amongst the 4 treatments

24 hours in vitro gas production characteristics of a standard substrate (200mg) incubated in rumen liquors obtained from West African Dwarf Sheep fed experimental diets was shown in Table 3 which explains that the gas production from the insoluble but degradable fraction "b" were significantly differences ($P < 0.05$) between the means of each of the 4 treatments. There is no significant difference in treatment 3 and the treatment 4 whereas, treatment 1 is significantly different from treatment 2 having mean values of 50.06ml and 67.07ml. The mean value ranges between 50.06-70.58. The rate of gas production (ml/hr) showed that there is significant difference ($P < 0.05$) in the means of treatment 1 and 4 but no significant difference in the means of treatment 2 and 3,

treatment 1 is significantly different from treatment 2 and it is significantly different from treatment 3 and 4. However they all have the mean values ranging between 0.2- 0.8. Treatment 1 had the highest mean value that is the highest value of gas production and this might be because no additive is included into it, and treatment 4 had the lowest value of gas production and this might be because there is no inclusion of garlic and neem in it. The time of the most rapid increase in gas production” t” ranges between 0.40 to 1.13, there is significant differences between treatment 1 and treatment 2 but there is no significant difference in treatment 3 and 4. There is significant difference amongst treatments 1, 2,3 and 4. The means values for the 4 treatments are: 0.40 for treatment 1, 3.90 treatment 2, 1.60 treatments 3 and 1.1for treatment 4. There is significant difference in the gas volume (GV) produced in the 4 treatments, the mean value of the gas volume ranges between 9.00 - 33.97. Furthermore, the methane (ml/200mg) production ranged from 6.03 – 15.00, the 4 treatments were significantly different from each other, and treatment 1 had the highest value of methane gas production while treatment 4 had the lowest value of methane gas production. Treatment 4 had the inclusion of both garlic and neem this result therefore indicates that garlic and neem have the ability to mitigate methane gas production Feller, (2004). The metabolisable energy values ranges between 3.10 – 7.35. Metabolisable energy mean value for treatment 4 had the highest value and it is not significantly different from treatments 2 and 3 but it is significantly different from treatment 1, this indicates that treatment 1 is significantly different from treatments 2, 3 and 4. (Menke *et al*, 1979; Sommartet *al*, 2000). In the Organic matter digestibility value, treatment 4 had the highest OMD mean value whereas treatment 1 had the lowest value, and the short chained fatty acid showed that there is significant difference in the mean values of treatment 1 and 4 but there were no significant differences in the mean values of treatment 2 and 3. Treatment 4 had the highest mean value of 7.54 and treatment 1 had the lowest mean value of 1.60. Treatment 1 is significantly different from treatments 2, 3 and 4 while treatment 4 is also significantly different from treatments 1, 2 and 3 respectively.

TABLE 1: Gross Composition of the Experimental Diets (%)

INGREDIENTS (%)	T1	T2	T3	T4
Cassava peels	60.00	60.00	60.00	60.00
Soybean meal	20.00	20.00	20.00	20.00
Corn bran	10.00	10.00	0.00	0.00
Neem Fruit	0.00	0.00	10.00	10.00
Garlic	0.00	1.25	0.00	1.25
Molasses	10.00	8.75	10.00	8.75
Total	100	100	100	100

Table 2: This table shows the rumen fermentation characteristics of West African Dwarf Sheep fed supplementary diets containing water-washed neem fruit and garlic

Parameters	T1(0% Neem + 0% Garlic)	T2(0% Neem +1.25% Garlic)	T3(10% Neem +0% Garlic)	T4(10% Neem +1.25% Garlic)	SEM
pH	7.00 ^a	6.63 ^b	6.83 ^a	6.30 ^c	0.08
Ammonianitrogen (mg/litre)	9.18 ^a	9.01 ^a	9.13 ^a	7.76 ^b	0.33
Acetic acid (mM/100ml)	2.45	1.73	2.51	3.09	0.42
Butyric acid (mM/100ml)	2.76 ^a	2.15 ^b	2.49 ^a	3.48 ^a	0.35
Propionic acid (mM/100ml)	2.61	1.50	2.33	2.53	0.35

a, b, c – Means with different superscripts along the same row are significantly different (<0.05)

Table 3: 24 hours In vitro gas production characteristics of a standard substrate (200mg) incubated in rumen liquors obtained from west African Dwarf Sheep fed experimental diets

Parameters	T1(0% Neem + 0% Garlic)	T2(0% Neem +1.25% Garlic)	T3(10% Neem +0% Garlic)	T4(10% Neem +1.25% Garlic)	SEM
b(ml)	50.06 ^c	67.07 ^b	77.47 ^a	70.58 ^a	2.25
c (ml/hr)	0.02 ^c	0.04 ^b	0.05 ^b	0.08 ^a	0.0005
T (hr)	0.40 ^c	3.90 ^a	1.60 ^b	1.13 ^b	0.18
GV	9.00 ^d	31.11 ^c	31.50 ^b	33.97 ^a	0.11
CH ₄ (mM/200mg)	15.00 ^a	10.02 ^b	8.00 ^c	6.03 ^d	0.29

ME(MJ/kg DM)	3.10 ^b	7.07 ^a	7.20 ^a	7.35 ^a	0.23
OMD (%)	28.40 ^c	48.30 ^b	48.40 ^b	50.40 ^a	0.19
SCFA (µml)	1.60 ^c	6.91 ^b	6.93 ^b	7.54 ^a	0.02

KEY:

- b = Gas production of potentially degradable but insoluble fraction
- c = Rate of gas production
- T = Time of most rapid increase in gas production
- GV = Total volume of gas produced
- CH₄ = Methane
- ME = Metabolizable energy
- OMD = Organic matter digestibility
- SCFA = Short chain fatty acid
- SEM = Standard error of the mean

Conclusion

The results from this study showed the potential of the *Allium sativum* and *Azadiractaindicato* reduce the production of methane gas; thereby they have the potential of being a good rumen manipulator, which could result in reduced methanogenesis. This results in reduced energy loss and the protection of the environment by the prevention of global warming.

The treatment with the inclusion of garlic and neem is seen to have the lowest value of ammonia nitrogen and this may indicate that garlic and neem are not feed supplement of good quality protein.

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