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Research Article

Effect of browse plants' formulated feed on rumen microbial population and growth performance of Red Sokoto goat breed (*Capra aegagrus hircus*)

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A B S T R A C T

This research appraised the effect of browse Plants formulated feed on rumen microbial population and the animal performance of Red Sokoto goat for 14 weeks. Eight browse plants; Acacia alibido, Piliostigma thonnigi, Teminalia jigosona, Vitellaria paradoxa, Afzelia africana, Detarium microcarpum, Daniellia oliveri and Khaya senegalensis were pre-screened for its phytochemical composition out of which four Afzelia africana, Detarium microcarpum, Daniellia oliveri and Khaya senegalensis were selected and used to formulate the animal diets. Three groups of goats were assigned to each of the browse plant diets, while the control group was placed on a basal diet only. Rumen liquor were collected from the goats intermittently for three periods, and analyzed for microbial population. The results showed that browse plant formulated feed have a significant effect ($p > 0.05$) on the population of the rumen microbes. Significant ($p < 0.05$) reductions in fungal count was recorded in all the formulated diets treatments except the control diets. Besides, significant ($p < 0.05$) higher concentration was recorded in Protozoa and Bacteria count in all treatments except the control diets. Significant growth performances of 28.2%, 28.2%, 28.0% and 26.2% were recorded for T2, T3, T1 and T4, consecutively, relative to 15.8% in the control (T5). Overall, this study demonstrated that browse plant feed is able to significantly improve animal performance and as well,alter the microbial popullation in ruminant animals.

INTRODUCTION

The omasum, abomasum, reticulum, and rumen made up the ruminant digestive system. The rumen is a sizable frothing room that offers an anoxic system, uniform pH, temperature, including adequate coalescence (An *et al.*, 2020). The primary location for the main fermentation processes is the rumen (Hinsu *et al.*, 2021). Microorganisms produce the enzymes found in the rumen. Rumen is regarded as a frothing vat because these enzymes are used to break down the feed that ruminants eat (Mizrahi *et al.*, 2021).

Ruminants are herbivorous that are regarded as latecomers in darwinism (Zheng *et al.*, 2020). Ingested plant materials are broken down and fermented anaerobically by the billions of complex microbiota that made up the rumen microbial population. Ruminant complex environments of the stomach enable them to transform plant tissues into useful and nutritious products (Okunade *et al.*, 2014; Attwood, 2019). Environmental situation such as redox potential, temperature, saliva's buffer-controlled pH, and osmolarity create good technique for microbes to break down the plant materials (Chen *et al.*, 2021). The interactions between the different microbial populations that live in the rumen hydrolyze and ferment fibrous components, primarily generating acetate, propionate, butyrate, CO_2, H_2, and CH_4 (Douglas *et al.*, 2020; Cox *et al.*, 2021). Volatile fatty acids (VFAs) are very crucial energy source for the ruminant, i.e., 75% of the entire digested energy. Moreover, the primary root of amino acids

and protein is the microbial cell biomass (*Rinninella et al.*, 2019; Cui et al., 2020).

Ruminant gut environment contains high microbial population density, predominantly obligate and facultative anaerobic microorganisms (Emerson and Weimer, 2017; Duarte *et al.*, 2019).

The first phase in the enteric fermentation process, is the hydrolysis of plant polysaccharide material. Bacteria and fungi are responsible for 80% of the degradation of plant cell material, with protozoa accounting for the remaining 20% (Gunnu *et al.*, 2016; Elghandour *et al.*, 2017). Approximately 2–12% of gross energy intake (GEI), produced in the rumen, as a result of fermentation is transformed to methane, which not only causes feed energy loss but also emissions and, subsequently, the warming effect (Hu *et al.*, 2018; Kim *et al.*, 2020).

A thorough understanding of ruminant digestion and rumen microbiome manipulation has been considered an effective method of ruminant feed utilization (Patra *et al.*, 2017; Palevich *et al.*, 2020; Mu *et al.*, 2021).

Better ruminant production at a reasonable cost requires this understanding. Improving feed efficiency is crucial for adequately controlling production costs in animal agriculture, particularly when feed prices are high. Additionally, improving feed efficiency may be a useful strategy for lowering feed consumption while preserving animal performance (Rowland *et al.*, 2018; Shi *et al.*, 2021). Similarly, rumen gut flora and feed efficiency working together could make it easier to choose more productive breeding stock while reducing the stress in data collection from individual feed intake and other demanding tasks. There has not been much published work on this area, despite how crucial it is to the scientific field of environmental ecology as it relates to animal feed productivity with rumen microbial flora (Ellison *et al.*, 2017; Sha *et al.*, 2019). However, with more advanced and reasonably priced sequencing technologies, the research in this field has become more interesting (Tran *et al.*, 2019; Wang *et al.*, 2021).

Most of the browse plant foliage containing tannins, saponins, and other phenolic compounds has not been strategically used to alter the rumen environment for the best feed utilization. Therefore, this research assessed the consequence of forage plant foliage fed as an additive on rumen microbiome, growth performance, and *in vitro* fermentation in goats (*Capra aegagrus hircus).*

MATERIALS AND METHODS

Experimental Site

This work was conducted at the Federal College of Wildlife Management's Animal Production Technology Department research farm in New-Bussa, Niger State. The experimental station (New Bussa) sits at 9º 53'N ,9.883ºN and 4º 31'E, 4.517ºE (NIPOST Archives, 2009).

Ethical Clearance

Ethical approval for the study was collected from College management Ethical Committee.

Sample Collection

Browse plant sample was harvested from the Villages around the premises of the Federal College of Wildlife Management, New Bussa, Niger State. The plants were identified at the forestry department of the College. Fifteen (15) red Sokoto goat weighing between 6.8kg - 9.6kg were purchased from Dogongari in Borgu local government area, Niger State, Nigeria. They were allowed to adapt to the experimental site conditions within 14days and were given water and feed *ad libitum. One hundred* millilitres {100mls} of rumen content were collected, on 3 different occassions, from each goat into a sample bottle and.kept in a pre-heated flask at 39ºC with the aid of suction tube as described by Olafadehun and Okunade, (2016) This was done before morning feeding on each period of the sample collection.

Screening of Browse plant

Plant samples were subjected to phytochemical analysis according to the official methods of analysis described by the Association of Official Analytical Chemist (AOAC, 2005). The browse plant foliage (*Acacia alibido (*Ac), *Piliostigma thonnigi (*Pt), *Teminalia jigosona (*Tj), *Vitellaria paradoxa (*Vp), *Afzelia africana* (Aa) *Detarium microcarpum* (Dm), *Daniellia oliveri* (Do) and *Khaya senegalensis* (Ks) were analyzed for phytochemical and proximate composition The fibre fractions; acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were determined according to AOAC official method 988.05. The basic intake consist of (Maize, offal 25kg, Groundnut cake (GNC) 20kg, cowpea husk 45,00kg, Dried grain brewers 5kg, , Dicalcium phosphate 2kg, mineral-vitamim Premix 1kg, Table Salt 1kg and sulphur powder 1kg = 100kg) boost with acceptable selected pasture plants were also examined as stated by the standard methods of (AOAC, 2005).

Experimental management and design

Fifteen growing Red Sokoto bucks, 6–8months old, with an average starting weight of 8.5.00±0.25 kg, were used for the study. Before their arrival at the farm, the goats' pen was cleaned and disinfected. Each pen was furnished with wood shavings. Each goat was housed in an individual pen (1.20 m × 0.80 m × 0.70 m) furnished with drinking and feeding facilities on the concrete floor. On their arrival, they were given prophylactic treatment which include vaccination and they were injectecd with oxytetracycline and vitamin B – complex intramuscularly at the dosage of 1ml/10kg weight of the body kg. They were also dipped in diazintol solution and dewormed with ivomec against endoparasites and ectoparasites earlier before the start of the research. They were arbitrarily assigned to five nutritional treatments using a fully randomized design. Three animals were used for each treatment. The experimental diets were formulated to satisfy the goats' nutritional needs as they grew.The basic diet was given to the animals in the final treatment (control), whereas the other four treatments included supplements of *Detarium microcarpum*, *Khaya senegalensis Afzelia aaricana and Daniellia oliveri.Two equal meals containing the entire ration mix (concentrate and fodder) were provided as the*

experimental diets. The trial lasted for 3 months, 2weeks. There was Provision for a daily feed allowance of 15% above that of the previous day`s intake. Clean water was provided *ad libitum*. The weight of the feed consumed and that of the feed left over were used to determine each goat feed intake. Weight of individual goat was measured at the beginning of the experiment and afterwards on weekly basis with a hanging scale until the termination of the experiments. On the days of weighing, it was done before the goats were offered feeds. The average weekly weights of the animals for the 104 days trial period were used to calculate their metabolic weights (LW). Data derived from initial and final live weight of goats were used to compute total weight gain.

Quantitative examination of Protozoa

Five milliliters of Lugol's iodine solution and fifteen milliliters of saline solution were added to one milliliter (1 ml) of the strained sample, and the mixture was gently shaken. A 22 x 50 mm section of the glass slide was covered with 0.1 ml of the mixture. Low power field (X10) was used to estimate the 30 fields on the slide.

Isolation Procedure

Conventional base techniques of serial dilution of rumen sample were prepared up to 10^{-10} with repeated tubing of the selectively enriched microbial cultures by using the specific media.During the culture process, strict anaerobic conditions were followed. On each agar medium, one hundred 100 µL aliquot of the serially diluted rumen sample was plated twice. For three days, the plates were incubated in the anaerobic chamber at 39 °C. For the first control, 2 plates each from different media type were not inoculated, yet they were incubated along with the additional plates. In additiom, all the buffers utilized for the dilutions were inoculated without the rumen samples for the second control. Following incubation, each plate was covered with 1 mL of sterile anaerobic 0.85% NaCl solution, which was then used to mechanically scrape and collect all of the microorganisms from the plate. Colony counter was used to enumerate the total number of viable bacteria and fungi.The growth media included: 15 ml Mineral Solution I $(NH_4)_2SO_4$ 6.0 g; KH_2PO_4 3.0 g; $MgSO_4$ 0.6 g; NaCl 6.0 g; $CaCl_2 \cdot 2H_2O$ 0.795 g per Litre), 15 ml Mineral Solution II 0.25 g Yeast Extract, (K_2HPO_4 3 g/litre)1 g Tryptone, 0.2 ml Hemin (0.05%), 0.1 ml Resazurine (0.1%), 0.5 g Microcrystaline Cellulose, 0.4 g Sodium Carbonate, 0.1 g Cellobiose, 100 ml Distilled Water, 20 ml Clear Rumen Fluid and Cysteine Hydrochloride (50mg) were prepared and used. Little adjustment was made and in addition the last medium were comprised of (2.5µ g mL^{-1}) Amoxicillin and 22.75 µL Grifulvin to forestall rumen bacteria and ruminal fungi from growing on the cultured plates. Additional 310 µL volatile fatty acid (VFA) was included for defined medium, The media was sterilized using anaerobic bottles for 20 minutes at 121°C.

In vitro fermentation

Handling of the rumen fluid were done under carbon IV oxide influence. Into each experimental diet, two hundred milligrams (200 mg) of the air-dried and powdered leaves were placed into a calibrated 60 ml glass syringe with plunger. The samples underwent triplicate *in vitro* incubation. Ten milliliters (10ml) of rumen fluid and twenty milliliters (20ml) of buffer solution (g/liter of 1.302 KH_2PO_4, 1.985 (Na_2) HPO_4, 0.105 $MgCl_2.6H_2O$, 5.418 $NaHCO_3$, 1.407 NH_2HCO_3, 0.100 NaOH) and 0.390 Cystene HCl were used to fill the syringes. The system were placed in a rotor inside the incubator at 39°C with approximately one rotation per minute (rpm). Three blank samples containing 30mls of medium (inoculums and buffer) only were incubated simultaneously. The gas production was recorded at 3, 6, 9, 12, 18, 24, 36 and 39hours respectively At the post-incubation period, 4 ml of (10M) sodium hydroxide (NaOH) was dispensed into each incubated sample in order to absorb carbon (iv) oxide generated during the fermentation process, The remaining volumes of gas is reported as methane, according to Isah *et al.*, (2014) report.

Data analysis

The obtained data were statistically analyzed using Analysis of Variance (ANOVA), and the significance mean value was determined using Dunnet posthoc analysis at $P < 0.05$ on SPSS version 21 software.

RESULTS

The results of the phytochemical constituent of browse forage samples pre-screened for preference were presented in Figure 1. The condensed tannin level in all the plant sample forage analysed in the present research are within the typical range of 3-7% for the measure ruminant animals can condone without any injurious effect (Okunade *et al.*, 2014; Tiwari *et al.*, 2020; Ye *et al.*, 2021). Saponin concentration of all the browse plants investigated also fall within the tolerable level according to Isah *et al.*, (2014). Baker *et al.*,, (2021) further stated that chemical composition of browse plant foliage is exposed to vast variation depending on soil characteristics and climate. Phytic acids value recorded were within the range of 3.8 - 8.2% while the volatile oil fall within the range of 10.6 - 15.1%. The selected browse plant's forage has a sufficient nutrient density in terms of NFC, NDF and CP to satisfy the growing goat's nutritional needs (Zhao *et al.*, 2020).

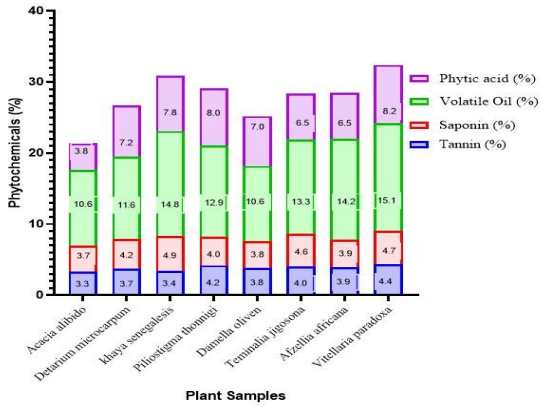

Figure 1: Phytochemical composition of browse plant sample

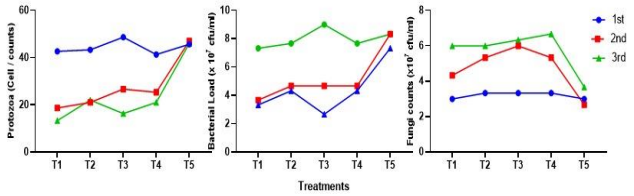

Figure 2: Microbial Load (Bacteria, Protozoa and Fungi) in the rumen contents

T1 = Concentrate diet + *Afzelia Africana*, T2= Concentrate diet + *Detarium macrocarpum*, T3 = Concentrate diet + *Danniellia oliveri*, T4 = Concentrate diet + *Khaya senegalensis*, T5 = Concentrate (Control) diets.

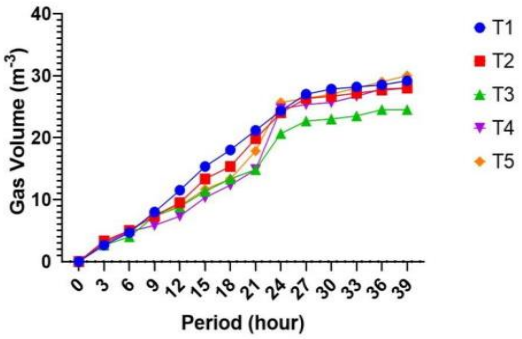

Figure 3: I*n vitro* fermentation (200ml/mg Dried Matter) of goats fed with experimental diets

T1 = Concentrate diet + *Afzelia Africana*, T2= Concentrate diet + *Detarium macrocarpum*, T3 = Concentrate diet + *Danniellia oliveri*, T4 = Concentrate diet + *Khaya senegalensis*, T5 = Concentrate (Control) diet.

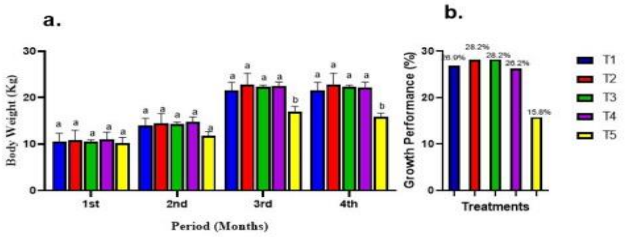

Figure 4: Growth performance of the experimental Animal

T1 = Concentrate diet + *Afzelia Africana*, T2= Concentrate diet + *Detarium macrocarpum*, T3 = Concentrate diet + *Danniellia oliveri*, T4 = Concentrate diet + *Khaya senegalensis*, T5 = Concentrate (Control) diet; The difference in the alphabet on the charts indicate significant difference at P< 0.05.

DISCUSSION

The condensed Tannins and saponins concentration as seen in this research helped to slow down the degradation process, saves energy loss for the animal, make more protein available for improved animal performance and productivity. This suggested that these chosen browse plants could be useful as dietary supplements for ruminants in regions where shortage of feed is one of the main factors limiting productivity. The findings of (Patra *et al.*, 2017) are consistent with this observation. According to Isah *et al.* (2014), the molecular weight of tannins affects both the amount and rate of gas produced *in vitro.*

There was no significant difference ($p<0.05$) in the rumen microbial population between supplemented diets. The study's findings demonstrated that adding browse plants had a major impact on the rumen microbial population. This supports previous findings by Abdul-Majeed (2011) and Isah *et al.* (2014) that plants with saponins and tannins have inhibitory qualities. The ability of tannins to bound with the cell membrane and cell wall of microorganisms, which results in structural modification of the cell wall and the release of extracellular enzymes, has been linked to the tannins' inhibitory effect against microbes (Elghandour *et al.*, 2017). But according to Zhao *et al.* (2020), supplementation of swamp buffalo feed with plants high in tannins and saponins had no effect on the microbe population. These authors' findings may be the result of variations in tannin dosage, diet sources, feeding practices, animal species, and environmental factors between the current study and earlier research, On the other hand, supplementation with browse plants increased the fungal population ($p<0.05$). A higher pH and more fiber could have contributed to the results, due to increase in the digestibility of the fiber and, in turn, the colonization of fungi (Fu *et al.*, 2019). Protozoa count reduction was recorded in the browse plant supplemented diet as against the control diet treament This results differs from that of Inyang and Ososanya (2017). where the probiotic-supplementation does not have any significant increase on rumen microbes. But agreed with (*Ismael et al.*, 2014; Lagier *et al.*, 2018). Because archaea and protozoa in the rumen have a symbiotic relationship, Diets supplemented with browse plants differed significantly ($p<0.05$) from the control diet (T5). This suggested that the presence of tannins and saponnins in plant foliage reduces the number of protozoa.

Tannin exhibited a depressing effect on plant fermentability and digestibility in the current study. The most dietary treatment, capable of fermentation was the control diet (T5), which did not contain browse plant supplements. This could be attributed to the absence of phenolic compounds (tannins and saponin), which likely allowed for the faster degradation of high NDF, NCF, and CP that resulted in an elevated *in vitro* fermentation relative to other diets. Conversely, diets supplemented with browse plants (T1, T2, T3, and T4) recorded reduced *in vitro* fermentation in that sequential order relative to control diets, which may have been caused by the influence of condensed tannin in the browse plants (Duarte *et al.*, 2019; Kim *et al.*, 2020; Cox *et al.*, 2021). The formation of tannin–protein and tannin–carbohydrate complexes may be linked to the impact of tannins on fermentation and digestion (Gharechahi *et al.*, 2018; Jha *et al.*, 2019; Mu *et al.*, 2021).

CONCLUSION

The findings indicated that the condensed tannins concentration present in the browse plants formulated feed has led to inhibitory activities of microbes in breaking down feedstuff and reduction in microbial population which in turn led to improved feed efficiency and total animal productivity. Plant secondary metabolites contained in

browse plants can be used diplomatically to manipulate rumen microbial environment in order to enhance ruminant performance and productivity contributing to addressing food safety and promote sustainable development in animal agriculture.

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