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Microbial isolation and digestion of some non-leguminous browse plants in semi-arid environment of North-East Nigeria

Abstract

Non-leguminous browse plants such as *Ziziphus spina-christi* (T1), *Ziziphus mauritiana* (T2), *Azadirachtaindica* (T3), *Moringa oleifera* (T4), *Ficus polita* (T5) and *Annona senegalensis* (T6) can produce high yield biomass. *Z. spina-christi*, *Z. Mauritiana* and *M.oleifara* contain high CP content and as such can be used to improve the performance of ruminants in the dry season during feed scarcity. The bacteria isolated from the rumen liquor of the feed samples were *Bacillus subtilis, Streptococcus faecalis, Staphylococcus aureus, Corynebacterium species, Micrococcus species* and *Escherichia coli,* while the isolated fungi include *Aspergillus niger* and *Rhizophus species.* Microbial analysis during their degradation in the rumen shows that *B. subtilis* and *S. faecalis* were found to be present as major bacterial species during their digestion. At the end of 48 hours period of incubation, the degradability rate of all the browse plants was more than 60%- T2 (67.60%), T1(66.10%), T4 (65.95%), T6 (65.50%) and T5 (63.55%). Their high CP content makes them good choices as feed ingredients and can be used for supplementation in ruminant feeding to improve feed quality of livestock feed production in Borno State, Nigeria. Their availability all year round, high protein level and high degradation characteristic can support and meet the nutrient requirements of ruminants during the dry season.

Keywords: browse-plants, degradation, non-leguminous, microbial, ruminants

Volume 9 Issue 5 - 2020

Phychwemillah JS,[1] Baiyi KL,[2] Marte AM,[1] Ishaya T,[1] Mohammed ID,[1] Malgwi IH,[1]
[1]Department of Animal Science, University of Maiduguri, Nigeria
[2]Department Agricultural Engineering, Faculty of Agricultural and Environmental Sciences, Szent István University, Hungary
[3]Department of Animal Nutrition, Faculty of Agricultural and Environmental Sciences, Szent István University Kaposvár Campus, Hungary

Correspondence: Malgwi IH, Department of Animal Nutrition, Faculty of Agricultural and Environmental Sciences, SzentIstván University Kaposvár Campus, 7400 Kaposvár, Hungary, Tel +36 302810384, Email zeekofficial@yahoo.com

Received: September 18, 2020 | **Published:** October 26, 2020

Introduction

Inadequate nutrition and feed supply are a major constraint to ruminant production in the tropics especially during the dry season (Nov–Mar). Therefore, drought-feeding strategies, which include the use of browse plants, is practised in order to meet up with nutritional requirements of the ruminants. Browse plants contain protein, vitamins and mineral elements that are lacking in grassland pastures during the dry season.[1] Several tree species could be an effective source of fodder which provide good nutrition during feed scarcity.[2] Although there are many advantages of forage and leguminous crops over the tree crops, nevertheless, the leaves of certain trees can be as nutritive as those of fodder legumes.[3] Browse plants can be either leguminous or non-leguminous. The non-leguminous browse plants form a natural part of ruminant diet meeting more than half of their forage requirement because even after reaching full maturity, the nutrients in the plants remain in the branches and leaves and as well as the roots. They also have low fibre content and higher crude protein (CP)compared to grasses.[4] Generally, most farmers utilize these non-leguminous browse plants to feed ruminants. The extent to which these plants contribute to the dietary need of ruminants depend on the type and quality of the browse available, performance of the animal, palatability and presence of anti-nutrient contents such as saponins, tannins and phytate.[5,6] However, recent studies have shown that these anti-nutrients can be beneficial if supplied at low concentration to animals.[7,8] In this experiment, we determine by the proximate nutrient composition of six non-leguminous browse plants and observed their rumen degradation characteristics of the feed ingredients while trying to identify microorganism (bacteria and fungi) present in the rumen liquor when ruminants consumed such feed ingredients.

Materials and methods

Experimental site

The experiment was conducted at the University of Maiduguri Livestock Teaching and Research Farm in May 2014. This area is located at the semi-arid region of the Sahelian part of Nigeria on latitude $11^0.5'$N and longitude $13^0.5'$Ewith an altitude of 354m above sea level and an annual rainfall of 500–600 mm. the ambient temperature ranges between 35–45^{0}C with relative humidity at 5–45% depending on the period of the year.

Sample collection and preparation

The feed ingredients used for the experiments were *Ziziphus spina-christi* (T1), *Ziziphus mauritiana* (T2), *Azadirachtaindica* (T3), *Moringa oleifera* (T4), *Ficus polita* (T5) and *Annona senegalensis* (T6). The feed ingredients were collected from trees in the vicinity of the University. They were then dried for five days and pounded with mortar and pestle to a fine coarse size 2.2 mm.

Rumen degradation

Nylon bags used were of the dimension of 80mm x 20mm with pore size of 5.2 x 10^{-2}mm, Cotton wool, Antiseptic (Dettol), thread, and forceps were used. Around 3g of each sample from the feed ingredient (T1-T6) were placed into the nylon bag for incubation in the rumen of the bull. Wadara bull weighing 550 kg for a period of 3, 6, 12, 24, 48 and 72 hours. Using the Sequential addition method, the bags were removed following each incubation period, bags were then washed thoroughly, dried and their weight measured. The amount of degraded feed as dry matter is the difference between the sample

weight before incubation and the weight after incubation expressed as a percentage. In addition, the amount of organic matter was also measured as the difference between before and after incubation.

$$\%DMdegradability = \frac{sampleweightbeforedegradation - sampleweightafterdegradation}{sampleweightbeforedegradation} * 100$$

Sample preparation for microbial analysis

Samples of the rumen liquor and the fermented rations were screened for microorganisms. Sterile containers were used from the laboratory and labelled for easy identification. A small amount of each of the fermented ration was picked using a spatula and put into the corresponding container, while a small amount of the rumen liquor was also poured into a labelled sterile container and taken to the Veterinary Medicine and Research Laboratory for microbial analysis. The samples were cultured on Blood, Sabouraud dextrose and MacConkey agar by streaking method and incubated at 37ºC for 24hours. The cultured plates were later examined for growth and identification of microorganisms.

Culture media

MacConkey Agar: 52g of commercially prepared powdered MacConkey agar was weighed and transferred into a conical flask. To dissolve it, 1L of distilled water was added and then the solution was boiled. The content was sterilized by autoclaving at 121ºC for 15minutes. It was allowed to cool before being transferred to plates. This agar solution is selective for gram-negative bacteria such as *E. coli*.

Blood agar: 37g of commercially prepared powdered blood agar base was measured and dissolved in 1L of distilled water. It was boiled and sterilized by autoclaving at 121ºC for 15minutes. The medium could cool in a water bath at 55ºC. 50ml of sheep blood was added to the medium and mixed and then aseptically poured into plates. This media is used for fastidious microorganisms such as *Streptococci*.

Sabouraud Dextrose Agar (SDA): 65g of plated commercially prepared SDA was weighed and dissolved in 1L of distilled water. The medium suspension was heated, dissolved completely and then sterilized by autoclaving at121ºC for 15minutes. It was poured into plates aseptically after cooling in a water bath. SDA is useful in growing yeast, moulds etc.

Identification of bacteria

The isolates were identified using conventional methods as described by Cowan & Steel.[9] Briefly, using a sterile wire loop, a drop of normal saline was placed at the centre of a grease-free slide and a portion of colony was picked and emulsified into the drop sample and allowed to air dry before fixing. Crystal violet was then applied for 3minutes to gram stain. It was then replaced with grams iodine for 1minprior to rinsing with water and application of 95% of alcohol until no colour appeared on the flow. Safranin was applied for 1–2 minutes after slides were rinsed with water. This was followed by rinsing and air drying before been observed microscopically under x100 emersion oil objective. Growth was interpreted as described by by Cowan & Steel.[9] .

Isolation of mould and fungi

The samples were cultured on SDA and incubated at room temperature checking only for growth. A small portion of mycelia was transferred to a drop of Lacto phenol cotton blue stain using two needles to tear the mycelium in the solution and cover with a colour slip. It was then examined under x10 and x40 of the objective lens of the microscope and identified by their hyphae.

Results

Table 1 shows the proximate analysis of different feed contents employed in this study. T2 had the highest DM of 97.3% followed by T1 and T4 with 97.0% and 96.0% respectively. The least DM content was found in T6 (94.7%).

Figure 1 shows the degradation characteristic of the feed samples at different hours of incubation. At 3 hours of incubation, a large variation in degradation was recorded ranging from 40.9% (T2) to 1.4% (T5). These two feed ingredients had the highest and lowest degradation even after 72 hours with T2 (74.1%) andT5 (66.8%) respectively.

Table 1 Chemical composition of proximate analysis (%)

Feed samples	DM (dry matter)	CP (crude protein)	CF (crude fibre)	EE (ether extract)	Ash	NFE (nitrogen-free extract)
T1 (*Z. spina-christi*)	97.0	11.0	12.0	2.1	2.9	72.1
T2 (*Z.mauritiana*)	97.3	11.4	12.1	3.1	2.0	71.5
T3 (*A.indica*)	95.0	9.27	27.0	1.0	3.0	59.7
T4 (*M. oleifera*)	96.0	15.6	7.1	3.0	11.1	66.3
T5 (*F.polita*)	94.8	8.40	26.0	2.0	6.0	57.6
T6 (*A.senegalensis*)	94.7	4.37	23.0	3.0	2.0	67.6

Table 2 Microorganism isolated from the rumen liquor of feed samples

Feed samples	Bacteria	Fungi
T1 (*Z. spina-christi*)	Bacillus subtilis,Corynebacterium species, Staphylococcus albus, Streptococcus faecalis	Rhizophus species,Aspergillus niger
T2 (*Z.mauritiana*)	Bacillus subtilis, Staphylococcus albus, Streptococcus faecalis	Rhizophus species,Aspergillus niger
T3 (*A.indica*)	Bacillus subtilis, Streptococcus faecalis, Micrococcus species	Rhizophus species,Aspergillus niger
T4 (*M. oleifera*)	Bacillus subtilis, Streptococcus faecalis, Micrococcus species	Rhizophus species,Aspergillus niger
T5 (*F.polita*)	Bacillus subtilis,Corynebacterium species, Staphylococcus albus, Streptococcus faecalis	Rhizophus species,Aspergillus niger
T6 (*A. senegalensis*)	Bacillus subtilis,Corynebacterium species, Staphylococcus aureus, Streptococcusfaecalis, Escherichia coli	Rhizophus species,Aspergillus niger

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The bacteria species, *B. subtilis* and *S. Faecalis* were found present in all the feed samples T1–T6 (Table 2). *S. albus* was only present in three feed samples T1, T2 and T6, while *C. species* was found in three feed sample, T1, T5 and T6. *E. coli* and *S. Aureus* were found only in T6 whereas *M. Species* was found only in T3. The fungi species *R. species* and *A. niger* were found present in all the feed samplesT1–T6 (Table 2).

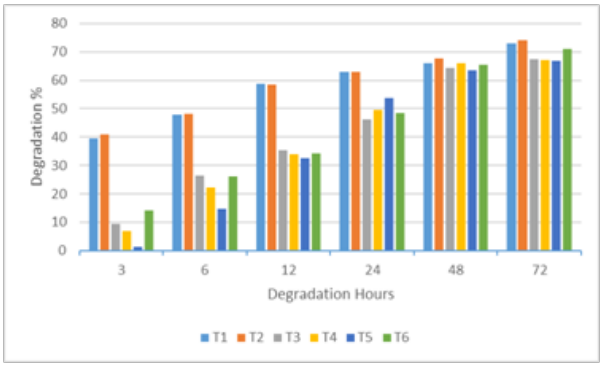

Figure 1 Degradability of samples (%).

Discussion

The highest CP content was found in T4 with 15.6%, this value is greatly higher than that reported in the studies of who recorded CP as 1.4% in *M. oleifera*. Also, the CP in T2 and T1 are quite similar (11.4 and 11.0% respectively). A possible explanation can be because they are of the same genus *Ziziphus*. The CP content of T2 and T2 obtained from this study are quite lower than the amount reported by Orwa et al,[10] to be 15.4%. Furthermore, the least CP was found in T6 estimated at 4.4% which is half the amount estimated in the study of Yisa et al,[11] which recorded CP of *A. senegalensis* as 8.8%. The variations of CP contents from this study in comparison with other research work could be due to the method employed. T3 had the highest CF (27.0%) among all feed ingredients which was slightly higher than that reported by Atangwho et al,[12] where CF was found to be 20.1% where as Esonu et al,[13] reported CF of leaf meal of *A. indica* as 16.6%. CF content from of T5 was similar to that reported by Abegunde et al,[7] with values of 26.0 and 22.0% respectively. The least CF was observed in T4 as 7.1%, however, this is contrary to the findings of Aja et al,[14] who recorded CF to be as high as 35.0% in *M. oleifera*. It is important to also note that CP and CF are negatively correlated Mongeau et al.[15]

T4 and T6 had the same EE content of 3.0%, whereas T2 recorded a slightly high amount of 3.1%. The least EE was found in T3 to be 1.0%, this amount is quite lower than that reported by Esonu et al,[13] and Atangwho et al,[12] having a value of 4.3% and 5.2% respectively in *A. indica*. The ash content was highest in T4 (11.1%), whereas, the studies of Aja et al,[14] estimated the ash content as 20.0%. Similarly, the ash content in T5 (6.0%) differs from that reported by Abegunde et al,[7] as (10.0%. The minimum ash content was 2.0 recorded in both T2 and T6. The maximum and minimum NFE was recorded in T1 (71.5%) and T3 (59.7%).[16]

Conclusions

These selected browses (*Ziziphus spina-christi* (T1), *Ziziphus mauritiana* (T2), *Azadirachtaindica* (T3), *Moringa oleifera* (T4), *Ficus polita* (T5) and *Annona senegalensis* (T6) for having high CP can be used for supplementation in ruminant feeding to improve feed quality of livestock feed production in Borno State of Nigeria. Their availability all year round, high protein level and high degradation characteristic can support and meet the nutrient requirements of ruminants during the dry season.

Acknowledgments

The authors wish to thank the University of Maiduguri for providing the technical and academic support in carrying out this research.

Conflicts of interest

Author declares that there are no conflicts of interest.

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