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ORIGINAL RESEARCH ARTICLE

Rumen fermentation and microbial profile of Red Sokoto goats fed threshed sorghum top supplemented with browse foliages

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ABSTRACT

Most of the ruminants in Nigeria are found in the Guinea savanna and Sahel zones where crop residues constitute an important feed for ruminants during the long dry season. Threshed sorghum top is the outer coverage of the sorghum grain after the grains have been removed through various threshing methods. The experiment was carried out to evaluate the rumen fermentation and microbial profile of Red Sokoto goats fed threshed sorghum top (TST) supplemented with five indigenous browse foliages (Piliostigma thonnigii, Daniellia oliveri, Afzelia africana, Pterocarpus erinaceus and Annona senegalensis). Twenty growing Red Sokoto bucks of 7 - 9 months old with average weight of 9.00±0.25 kg were used for this study. They were randomly allocated to the five dietary treatments in a completely randomized design. Each treatment was replicated with four animals. Data collected include feed intake, rumen pH, temperature, ammonia nitrogen, volatile fatty acids, microbial count and identification of microbial isolate. Goats fed various browse plants as supplement had higher ($p<0.05$) bacterial population than goats on TST only. Also those fed A. aficana had higher ($p<0.05$) total rumen bacterial count (10.2×10^5CFU/mL), while those on TST had the lowest value (7.2×10^5 CFU/mL). Total coliform count ranged from 0.3×10^5 in D. oliveri supplemented diet to 0.5×10^5 CFU/mL in animals fed TST alone. There was increase in the population of total fungi count from 0.35×10^5 in goats fed TST alone to 0.53×10^5 CFU/mL in those supplemented with P. thonnigii. Protozoa count ranged from 0.33×10^5 CFU/mL in the rumen content of goats on D. oliveri and A. aficana to 0.6×10^5 CFU/mL in rumen content of those fed TST only. The rumen ammonia nitrogen concentration of goats on A. Africana (199.33mg/L) and P. thonnigii (191.33mg/L) were superior ($P<0.05$) to those of goats on other diets. Goats on TST alone had the lowest ammonia nitrogen concentration (104.66 mg/L). The effect of supplementation of TST with browse foliage on total volatile fatty acids (VFA) ranged from 72.33 – 168.67mmol/L. Mean rumen ethanoic acid concentration ranged from 65.33 mol/100mL in animals on TST to 128.33 mol/100mL in animals on A. africana. In conclusion, supplementation of low quality roughages such as Threshed sorghum top with browse foliage led to improvement in rumen fermentation efficiency of rumen microbes, this led to increased production of microbial protein and VFA production (energy supply) which resulted to improved productivity of the goats.

Keywords: Roughages, Nutrition, Improvement, Ruminant, Productivity

INTRODUCTION

The utilisation of forages by ruminants depends on microbial fermentative digestion. The microbes ferment sugars to produce volatile fatty acids (ethanoic acid, propionic acid, and butanoic acid), methane, hydrogen sulphide, and carbon dioxide. The microbial yield is important because it is an index of the amount of microbial protein made available to the animals each day. Tropical grass, fodder and crop by-products available during dry season in the tropics have a low nutritive value due to their low protein and fermentable energy. These constitute an important feed for ruminants during the long dry season (Alhassan, 1987). Threshed sorghum top is the outer coverage of the sorghum grain after the grains have been removed through various threshing methods. Threshed sorghum top is typically a roughage feed, thus characterized by high fibre content (above 40%), low crude protein (less than 60g/kg dry matter) and energy (ME less than 7.5MJ/kg DM). In general, where ruminants are fed roughages, some amounts of extra nutrients are needed to balance nutrient availability to requirements. One of the ways by which the nutritive quality of this crop residue can be improved is through supplementation with browse plants (Adegbola, 1998). There is paucity of information on the supplementation of threshed sorghum top with browse foliages as feedstuff for goat in the Guinea Savanna zone of Nigeria. The objective of this study therefore was to evaluate the rumen fermentation parameters and microbial profile of red

Sokoto goats fed threshed sorghum top supplemented with browse foliages such as *Piliostigma thonnigii, Daniellia oliveri, Afzelia africana, Pterocarpus erinaceus* and *Annona senegalensis*.

MATERIALS AND METHODS

Experimental animals and management

Twenty growing Red Sokoto bucks of 7 - 9 months old with average weight of 9.00±0.25 kg were used for this study. They were randomly allocated to five dietary treatments in a completely randomized design. Each treatment was replicated with four animals. The animals in first treatment (control) were served with basal diet of threshed sorghum top only, while the remaining five treatments were supplemented with *Piliostigma thonnigii, Daniellia oliveri, Afzelia africana, Pterocarpus erinaceus* and *Annona senegalensis.* The experiment lasted for 84 days. The leaves including petioles were plucked, wilted overnight and served to the animals. Known quantity of each browse leaf and the basal feed chopped manually into 10cm pieces were fed to the goats at 09:00 hour and 16:00 hour, respectively. Browse foliage (Table 1) was served before the basal feed. Provision were made for daily feed allowance of 10% above that of previous day`s intake. Clean water was provided *ad libitum* daily.

Rumen p*H*, temperature, ammonia nitrogen and total volatile fatty acids determination

About 100 mL of representative rumen content was collected before morning feeding on the last day of the experiment from each buck with the aid of suction tube as described by Babayemi and Bamikole (2006). Temperature and p*H* of rumen content *were* noted immediately after collection using a digital pH and temperature meter model CT 6020.The content was strained through four layers of muslin cloth to obtained rumen liquor. Strained rumen liquor (SRL) was preserved immediately after adding a few drop of saturated mercury (II) chloride solution and kept in labelled polypropylene bottles at −20 ^{0}C pending analysis. Samples were analysed for ammonia nitrogen using micro Kjedahl method (AOAC, 1995). Volatile fatty acids (ethanoic acid, propionic acid, and butanoic acid) in SRL were determined on HPLC system (Shimadzu 10AD, Japan) as per standard procedure described by Canale *et al*.(1984).

Microbial count

Total viable bacteria and fungi was enumerated by the method of Holdeman *et al*. (1977) and Joblin (1981), respectively, using anaerobic roll tubes. Samples was fixed in methylgreen-formalin-saline (MFS) solution. The determination of DAP (2-6-diaminopimelic acid), AEP (aminoethylphosphonic acid), and chitin concentrations in the rumen fluids as a microbial marker for bacteria, protozoa and fungi was processed according to the method of Olubobokun *et al*. (1988).

Statistical analysis

Data were subjected to a one way ANOVA using version 9.1 of SAS software (SAS Institute, 2003). Significant difference between individual means were separated by Duncan's procedure.

Table 1: Nutrients intake of Red Sokoto goats fed threshed sorghum top supplemented with browse foliage

Parameter	Diets						
	Threshed Sorghum top	*Piliostigma thonningii*	*Daniellia oliveri*	*Afzelia africana*	*Pterocarpus erinaceus*	*Annona senegalensis*	SEM
Threshed sorghum top intake (DM g/day)	197.14^{a}	108.32^{b}	121.06^{b}	120.55^{b}	118.88^{b}	120.31^{b}	7.23
Browse foliages intake (DM g/day)	0.00^{e}	233.03^{b}	216.03^{c}	268.66^{a}	238.73^{b}	201.45^{d}	3.33
Total Dry matter intake (g/day)	197.14^{e}	342.00^{c}	336.85^{d}	398.25^{a}	363.63^{b}	324.43^{d}	3.66
Dry matter intake (g/kg $W^{0.75}$day)	41.70^{d}	63.93^{c}	63.18^{c}	72.38^{a}	67.033^{b}	61.40^{c}	0.99
Crude protein intake (g/kg $W^{0.75}$day)	2.77^{e}	7.61^{c}	6.23^{d}	14.64^{a}	9.75^{b}	5.92^{d}	0.13
NDFintake (g/kg $W^{0.75}$ day)	27.26^{e}	37.47^{b}	35.28^{b}	32.45^{c}	37.25^{a}	29.27^{d}	0.63
ADF intake (g/kg $W^{0.75}$ day)	15.11^{d}	19.33^{b}	19.03^{b}	18.68^{b}	23.94^{a}	16.77^{c}	0.37

^{abc}; means within columns with same superscript are not significantly different ($p<0.05$)
SEM = Standard error of mean, NDF = Neutral detergent fibre, ADF = Acid detergent fibre

Table 2: Rumen fermentation parameters of Red Sokoto goats fed threshed sorghum tops supplemented with selected browse foliage

Parameters	Diets						SEM
	Threshed Sorghum top	*Piliostigma thonningii*	*Daniellia oliveri*	*Afzelia africana*	*Pterocarpus erinaceus*	*Annona senegalensis*	
pH	6.49[b]	7.67[a]	7.21[a]	7.22[a]	7.30[a]	7.17[a]	0.05
Temperature (°C)	31.10[bc]	30.61[c]	31.97[ab]	32.07[ab]	32.63[a]	31.73[ab]	0.72
NH_4 - N(mg/L)	104.66[e]	191.33[a]	170.66[b]	199.33[a]	154.00[c]	137.33[d]	11.05
TVFA (*mmol./L*)	72.33[b]	90.67[b]	74.33[b]	168.67[a]	144.67[a]	83.00[b]	40.79
VFA (mol/100mL)							
Ethanoic acid	65.33[c]	92.00[b]	93.67[b]	128.33[a]	98.67[b]	68.33[c]	24.72
Propionic acid	27.00[c]	40.66[ab]	36.33[bc]	48.00[a]	35.33[bc]	32.00[bc]	9.20
Butanoic acid	5.19[d]	8.35[bc]	6.33[cd]	11.53[a]	9.38[ab]	7.73[bc]	2.24
Ethanoic acid : Propionic acid	2,42[ab]	2.27[b]	2.58[ab]	2.67[ab]	2.79[a]	2.14[b]	0.39

abc…. means in row, with the different superscript are significantly different (P<0.05), SEM = Standard error of mean.

RESULTS

Nutrient intake of Red Sokoto goats fed threshed sorghum top and browses foliages

Table 1 shows nutrient intakes of the experimental animals. The threshed sorghum tops (TST) intake value ranged from 108.32 g/day to 197.14 DM g/day. Animals fed non-supplemented TST diet had highest (p<0.5) intake while those fed browse supplements had similar (p>0.05) intake of TST. Highest intake of browse foliage (268.66 DM g/day) was observed in animals supplemented with *A.* Africana. Least (p<0.5) value was observed in animals that consumed *A. senegalensis* supplement (201.45 DM g/day). Nutrient intake of browse foliage was significantly (p < 0.05) different. Supplementation increased total dry matter intake in animals fed TST from 197.14 DM g/day to 398.25 DM g/day in animals that consumed *A. africana* supplement. Results of crude protein and dry matter intake per metabolic weight of experimental animals followed the same trend with highest intake recorded in goats fed *A. Africana* and least value observed in those fed non-supplemented TST alone. However, *P. erinaceous* supplemented diet had highest neutral detergent fibre (NDF) and acid detergent fibre (ADF) intakes by experimental animals.

Effects of supplementation of threshed sorghum top with browse foliage rumen fermentation parameters of Red Sokoto goats

Table 2 shows the result of rumen p*H*, temperature, ammonia-nitrogen and volatile fatty acids concentrations of Red Sokoto goats fed threshed sorghum top (TST) supplemented with browse foliages. No significant difference was observed in rumen liquor pH among supplemented diets, except (P<0.05) non-supplemented diet (TST only) which was different from the others. Goats on *P. thonniigii* had highest ruminal pH (7.67), while goats on non-supplemented TST had the lowest p*H* value (6.49). The rumen ammonia nitrogen concentrations of goats on *A. Africana* (199.33mg/L) supplemented diet was superior (P<0.05) to those of goats on other diets except for goats supplemented with *P. thonnigii* (191.33mg/L) which was similar. Goats fed threshed sorghum top alone had the lowest ammonia nitrogen concentration (104.66 mg/L).

The effect of supplementation of TST with browse foliage on total volatile fatty acid ranged from 72.33 – 168.67mmol/L. Animals fed *A. africana* supplement had greater (P<0.05) rumen total VFA concentrations than animals on other diets. Goats fed *A. africana* and *P. erinaceous* supplements are not significantly (P > 0.05) different in their rumen total VFA concentrations. Mean ruminal ethanoic acid concentration ranged from 65.33 mol/100mL in animals fed non-supplemented TST to 128.33 mol/100mL in animals fed *A. Africana* supplemented diet. There was similarity (P>0.05) in ethanoic acid concentration in animals fed non-supplemented TST and *A senegalensis supplemented* diets, likewise there was no difference (P<0.05) in ethanoic acid values of animals on *P. thonningii*, *D oliveri* and *P. erinaceus*. Significant (P<0.05) effect was observed in other rumen parameters which ranged from 27 to 48.00 mol/100mL, 5.19 to 11.53 mol/100mL, and 2.14 to 2.79 mol/100mL for propionic acid, butanoic acid, and ratio of ethanoic acid : propionic respectively.

Effects of supplementation of sorghum head with browse foliages on rumen microbial count and identification of Red Sokoto goats

Average microbial count and cultural identification in the rumen liquor of Red Sokoto goats fed various diets are shown in Table 3. Goats fed supplemented diets had higher bacterial population than goats on threshed sorghum top only. Goats fed *A. aficana* supplemented diet maintained its higher nutritive quality by having

(10.2×10^5 CFU/mL) total bacterial count, while those fed sorghum threshed tops diet had the lowest total bacterial count (7.2×10^5 CFU/mL). Diets supplemented with *D. oliveri* and *P. erinaceus* had the same total bacterial count (8.2×10^5 CFU/mL). Total coliform count ranged from 0.3×10^5 to 0.5×10^5 CFU/mL.

There was increase in the population of total fungi count from 0.35×10^5 CFU/mL in goats fed sorghum threshed tops alone to 0.53×10^5 CFU/mL in *P. thonningii*. Protozoa count ranged from 0.6×10^5 in the rumen content of animals on sorghum threshed tops alone to 0.33×10^5 CFU/mL in rumen content of animls fed *D. oliveri* and *A. africana* supplements. Diet supplemented with *D. oliveri* had the most rumen microbial diversity (13 cultured microbes), while the control diet (threshed sorghum top) had the least rumen micro-organism diversity (8 cultured microbes). Six different species (*Bacillus sp, Pseodomonas sp, Streptococcus sp, Penicillum sp, Sacaromyces sp,* and *Micrococcus sp*) were cultured from the rumen liquor of all experimental animals. *Staphylococcus aureus* was found only in animals fed non-supplemented TST diet.

Aspergillus sp was cultured from rumen liquor of all experimental animals except those fed *P. erinaceus* while *Proteos sp* was absent in animals fed non-supplemented TST, *A. Africana* and *A. senegalensis* supplemented diets.

DISCUSSION

Chemical composition of threshed sorghum top (Okunade *et al,* 2014) revealed it as a typical crop residue, because it is well recognized that cereal crop residues are of low nutritive value (Sundstol *et* al., 1979). This is because of their low crude protein content and low content of available minerals and vitamins. These deficiencies combine to make crop residues unpalatable, thus their consumption is also low. In ruminants fed crop residues, such as threshed sorghum top, intake is relatively low due to their high fibre and low nitrogen contents that cannot generally provide sufficient nutrients to meet both rumen microbial and host animal's nutrient requirements (Leng, 1990; Savadogo et al., 2007). This can only be met by supplementation with other feed ingredients of higher crude protein or non-protein nitrogen content. It is known that the best way to increase voluntary intake and digestibility of poor quality forage is to provide supplement with available nutrients which can increase the number and/or activity of rumen micro-organism in order to maximize fibre digestion and optimize microbial protein synthesis. This requires sufficient protein, energy and minerals to support the rumen microbial population. The rumen environment is characterized by strong metabolic activities of the rumen microbes that facilitate the fermentation of plant materials to products useful for the host animal (Silva and Ørskov, 1988). To satisfy these conditions in the present study, TST was supplemented with selected browse species. The supplementation was done mainly to increase the CP content of diets in order to overcome a ruminal N deficiency (Leng, 1990) and to provide fermentable fibre (Pathirana and Ørskov, 1995; Ørskov, 1999). Ruminant animals provide an environment that is conducive for anaerobic microbial activity, maintain a suitable pH and temperature, and remove fermentation end products for its own use.

The rumen fermentation pattern of experimental animals in this study is a typical pattern observed in ruminant animals fed roughage feeds, which is characterized by high rumen pH (Olafadehan, 2013). The pH levels corroborate the pH levels reported by Isah *et al*., (2013). These values are close to the range (6.2-7.2) reported by Van soest (1994) as being optimal for fibre digestion. Problem of acidosis was not observed in the animals fed either TST alone or those fed with supplemented diets. Supplementation with the different browse foliage improved total feed consumed (DM $g/KgW^{0.75}$) by experimental animals. This is essential for optimum rumen fermentation and microbial growth. Rumen temperature values recorded was lower than the mean temperature of 39.30^0C reported by Wora-anu *et al*, (2007). The NH_3-N content in the rumen liquor of animals fed *P. thonningii, D. oliveri, A. Africana* and *P.erinaeus* supplemented diet was above 150 mg/L which has been recommended as minimum acceptable level for optimization of fibre degradation in the rumen (Leng, 1990). Animals fed non-supplemented TST and those fed *A. senegalensis* supplement recorded lower values. This might be responsible for the low values of ethanoic acid, propanoic acid and butanoic acids produced in the rumen. The higher ruminal NH_3-N concentrations in animals fed *Afzelia africana* and *Daniellia oliveri* compared to animals fed other diets indicates more rapid ruminal fermentation of proteins in *Afzelia africana* and *Daniellia oliveri* foliage.

This probably made protein available for rapid proliferation of rumen microbes. The resultant multiple microbes are responsible for degradation of fibrous feed in the rumen. The significantly low pH observed in the rumen liquor of animals fed non-supplemented TST diet might be due to the nature of the TST which contained some leftover grains of sorghum. The grains are fermented to produce propionic acid which is a stronger acid compared to ethanoic acid which is a weaker acid from digestion of forages.

Table 3: Rumen microbial count and cultural identification (cfu/mL) in Red Sokoto goats fed threshed sorghum top supplemented with browse foliage

Parameters	Threshed Sorghum top	*Piliostigma thonningii*	*Daniellia oliveri*	*Afzelia africana*	*Pterocarpus erinaceus*	*Annona senegalensis*	SEM
Total bacterial count	7.2×10^{5c}	7.9×10^{5b}	8.2×10^{5b}	10.2×10^{5a}	8.2×10^{5b}	7.8×10^{5a}	0.32
Total Coliform Count	$0.5\times10^{5\,a}$	0.4×10^{5b}	0.3×10^{5c}	0.4×10^{5b}	0.47×10^{5ab}	0.5×10^{5a}	0.12
Total fungi count	$0.35\times10^{5\,d}$	0.53×10^{5a}	0.50×10^{5a}	0.50×10^{5}	0.48×10^{5bc}	0.374×10^{5cd}	0.12
Protozoa count (ml/100g)	$0.6\times10^{5\,a}$	0.43×10^{5b}	0.33×10^{5c}	0.33×10^{5c}	0.4×10^{5b}	0.4×10^{5c}	0.16
	Staphylococcus aureus, Streptococcus faecum, Bacillus cereus, Micrococcus luteus, Pseudomonas aureginosa, Saccharomyces cervisae, Aspergillus niger. Penicillum oxalicum	*Micrococcus acidophilus, Streptococcus lactis, Pseudomonas aureginosa Bacillus cereus, Bacillus subtilis, Aspergillus niger, Saccharomyces cervisae, Protecus mirabilis, Penicillum oxalicum*	*Micrococcus acidophilus, Protecus vulgaricus, Streptococcus lactis, Micrococcus lactis, Saccharomyces cervisae, Penicillum oxalicum, Aspergillus niger Bacillus macerans, Bacillus subtilis, Bacillus cereus Pseudomonas fragii, Pseudomonas aureginosa, Micrococcus luteus*	*Bacillus macerans, Streptococcus faecum, Micrococcus luteus, Pseudomonas aureginosa, Penicillum oxalicum, Saccharomyces cervisae, Aspergillus niger Bacillus cereus, Streptococcus lactis, Penicillum chrysogenum .*	*Micrococcus acidophilus, Micrococcus luteus, Streptococcus lactis, Streptococcus faecium, Bacillus subtilis, Proteus morganii, Pseudomonas aureginosa, Pseudomonas fragii, Saccharomyces cervisae, Penicillum chrysogenum,*	*Bacillus macerans, Micrococcus luteus, Bacillus subtilis, Pseudomonas, aureginosa, Streptococcus lactis, Saccharomyce cervisae, Penicillum oxalicum, Aspergillus niger, Aspergillus terreus, Streptococcus faecum, Micrococcus acidophilus Bacillus cereus*	

abc…. means in row, with the different superscript are significantly different (P<0.05), SEM = Standard error of mean

Strong acid produced from grain fermentation will reduce the pH of the rumen environment. The total VFA concentrations in rumen fluid of animals supplemented with *A. Africana* and *P. erinaceus* exceeded the normal range (70 to 130 mmol/L) reported in forage-fed ruminants (France and Siddons, 1993), The concentration of VFAs in the rumen fluid is a reflection of the generally high fermentation ability of the browse foliage. Propanoic acid proportion in the rumen is associated with increased growth rate of ruminant animals as Propanoic acid is said to be directly absorbed almost entirely by the liver and used for synthesis of glucose (Forbs and Provender, 2000). Low propanoic acid recorded in animals fed non-supplemented diet might be as a result of low dry matter intake. Ruminants themselves do not have the enzymes to degrade most complex plant polysaccharides, and microbes fill this metabolic role for their hosts. Cellulose and other plant materials are fermented in the rumen by anaerobic microbes to form volatile fatty acids, which, together with microbial protein, are significant nutrient sources for the host animal. The microbial population observed in this study revealed an unequal number among the typical rumen microbes enumerated. Bacterial species had the largest population in all experimental animals. It is therefore likely that bacteria are responsible for the major part of degradation of ingested feed in the rumen and are the main drivers behind the observed differences (Kamra, 2005). The result obtained in this work is comparable to that reported elsewhere (Gabriella and Eric, 1997; Sultan and Kundus, 2011 and Isah *et al*., 2013). Threshed sorghum top supplementation with selected browse plants led to an increase in total bacterial count

and a decrease in protozoa count. Supplementation with selected browse plant foliage increased the utilization of sorghum threshed tops (a poor quality roughage) by increasing the population of bacteria and fungi that colonized and degraded the diets for better fermentation; nutrient availability and nutrient utilization, while decreasing the protozoa biomass which ingest and digest bacteria consequently reducing the protein supply to the animals (Bird and Leng 1985).

CONCLUSION

In conclusion, supplementation of low quality roughages such as threshed sorghum top with browse foliage led to improvement in rumen fermentation efficiency of rumen microbes by creating better substrate environment for fermentation of fibrous materials, this led to increased production of microbial protein and VFA (energy) production leading to improved productivity of the animals.

CONFLICT OF INTEREST

Authors thereby declare that there is no conflict of interest in the submission of this manuscript for publication.

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