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DIET BOTANICAL COMPOSITION AND RUMEN PROTOZOA OF SHEEP IN BRAZILIAN SEMI-ARID AREA

COMPOSIÇÃO BOTÂNICA DA DIETA E PROTOZOÁRIOS DO RÚMEN DE OVINOS NO SEMI-ÁRIDO BRASILEIRO

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ADDITIONAL KEYWORDS

Caatinga. Santa-Ines crossbred sheep. Dry season. Protist. Rainy season. Climatic conditions.

PALAVRAS CHAVE ADICIONAIS

Caatinga. Ovinos mestiços Santa Inês. Estação seca. Protista. Estação chuvosa. Condições climáticas.

SUMMARY

This study aimed to determine the influence of seasonal variations on diet botanical composition and rumen protozoa of five rumen fistulated Santa Ines crossbred sheep kept in natural Caatinga vegetation pasture, Pernambuco State, Brazil. Samples were collected in July 2005 and January 2006, the respective rainy and dry seasons. Botanic composition of animal diet was determined by analysis of ruminal extrusa. The rainy season diet was composed mainly by herbaceous and grass species (88%), with *Herissantia tiubae* making up 51.7%. The dry season diet consisted of shrub species (67.4%), mainly *Capparis yco* (26.3%) and *Bauhinia cheilantha* (22.3%). Under such conditions variations in protozoa populations were observed, with the higher total concentration ($p<0.05$) during the rainy season. Populations of *Entodinium* genus predominated in both seasons, although decreasing ($p<0.05$) during the dry season, as did the *Epidinium* and *Eremoplastron* genera. The *Dasytricha*, *Diploplastron*, *Eudiplodinium* and *Isotricha* genera had the higher concentrations ($p<0.05$) in the dry season.

RESUMO

Objetivou-se neste trabalho determinar a influência estacional sobre a composição botânica

da dieta e nos protozoários do rúmen de cinco ovinos mestiços Santa Inês providos de fístulas ruminais e mantidos em pastagens naturais de vegetação de Caatinga, Estado de Pernambuco, Brasil. As amostras foram coletadas em julho de 2005 e janeiro de 2006, compreendendo, respectivamente, as estações chuvosa e seca. A composição botânica da dieta foi determinada através de amostras de extrusa coletadas via fístula ruminal, que após serem analisadas indicaram que durante a estação chuvosa as espécies vegetais mais comuns na dieta dos animais são herbáceas e gramíneas (88%), com *Herissantia tiubae* representando 51,7% do total. Na estação seca a dieta consistiu de espécies arbustivas (67,4%), comumente *Capparis yco* (26,3%) e *Bauhinia cheilantha* (22,3%). Sob tais condições, foram também verificadas variações nas populações de protozoários ruminais, os quais apresentaram maior concentração total ($p<0,05$) durante a estação chuvosa. Populações do gênero *Entodinium* predominaram em ambas estações, embora tenham apresentado decréscimo ($p<0,05$) durante a estação seca, assim como os gêneros *Epidinium* e *Eremoplastron*. *Dasytricha*, *Diploplastron*, *Eudiplodinium* e *Isotricha* apresentaram maior concentração ($p<0,05$) na estação seca.

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INTRODUCTION

Animal production in Brazil's northeastern semi-arid region is based mainly on pastures of native Caatinga vegetation (Guimarães-Beelen *et al.*, 2006). Caatinga is the low-thorn forest which covers the semi-arid region of North-eastern Brazil, comprising an area of about one million square kilometers. This biome sustains more than 90% of the country's goats and 35% of its sheep production (Araújo Filho *et al.*, 1998).

Both the ovine pasture food habitat and diet composition, are subject to the season changes with local variations in food availability (Molina Alcaide *et al.*, 1997), plant structure characteristics, leaf quality attributes, dry matter content and total protein, and secondary chemical products (O'Reagain, 1993).

In Caatinga pastures, feed availability and quality are influenced by seasonal variations, which are mainly governed by rainfall (Pimentel *et al.*, 1992). In rainy seasons, these pastures contain herbaceous, shrub, and tree species, which have very suitable nutritional characteristics for flock productivity. However, during dry seasons, the herbaceous component practically disappears, reducing the support capacity of the pastures. There is also a considerable reduction in nutritional quality attributes in the shrub and tree food species (Almeida *et al.*, 2006; Araújo Filho *et al.* 1996).

The occurrence and concentration of protozoa population are influenced by various factors, the principal being the nature of the diet, which in the case of roaming flock farming is influenced by seasonal variations. The vegetation of Caatinga is composed of species rich in tannins and other secondary compounds, whose level change according to phenological state, and that influenced the ruminal microbiota. Condensed tannins of *Mimosa hostiles* and *Bauhinia cheillantha* influenced the adhesion conditions, colonization

and activity of the microbial ecosystem, and consequently the ruminal degradation of the different dietary fractions (Guimarães-Beelen *et al.*, 2006). Thus, the objective of this study was to evaluate how the seasons affect diet botanical composition and rumen protozoa ciliate populations of crossbred Santa Ines sheep in natural Caatinga pastures from semi-arid regions of Pernambuco State, Brazil.

MATERIALS AND METHODS

The experiment was conducted between January 2005 and January 2006 with five 10-12 months old Santa Ines wethers with fistulated rumen. Animals were kept in a 37 hectare area of Caatinga pasture (Latitude 8°03'38" S, Longitude 37°13'32" W) belonging to the experimental farm of Empresa Pernambucana de Pesquisa Agropecuária, Sertânia, PE, Brazil; this vegetation was the only food source. The sheep had access to the pasture from 07:00 a.m. to 05:00 p.m. when they were corralled. They were kept in the experimental area for the whole year without any supplements, receiving only water and mineral mixture in dust *ad libitum*. Precipitation was monitored daily throughout the year. All the sampling was performed in July 2005 (30 samples) and January 2006 (26 samples), months which fall in the periods known as rainy and dry seasons, respectively.

Species found in the experimental area were collected and sent to Universidade Federal Rural de Pernambuco, Department of Botany for identification. There were 280 observation points and species samples were grouped as herbaceous, shrub, and tree according to Allaby (2004).

A set of spot ruminal content samples was obtained before the animals had access to the pasture and then at 2, 4, 6, 8, and 10 h after the animals began feeding to obtain an average, in order to decrease the effects of diurnal changes in population density. A maximum of two samplings were made per day so as to minimize the effects on animal

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Table I. Botanical composition of an area of Caatinga pasture (Latitude 8°03'38" S, Longitude 37°13'32" W) in semi-arid of North-eastern Brazil. (Composição botânica de uma pastagem da Caatinga (latitude 8°03'38" S, longitude 37°13'32" W) no semi-árido do nordeste do Brasil).

Herbaceous	Cactus	Shrub	Tree
<i>Alternanthera polygonoides</i>	<i>Arrojadoa rhodantha</i>	<i>Agerantum conyzoides</i>	<i>Amburana cearensis</i>
<i>Aristida purpusii</i>	<i>Bromelia laciniosa</i>	<i>Alternanthera tenella</i>	<i>Anadenanthera macrocarpa</i>
<i>Aristida adscensionis</i>	<i>Cereus jamacaru</i>	<i>Bauhinia cheillantha</i>	<i>Aspidosperma pyrifolium</i>
<i>Boerhaavia coccinea</i>	<i>Neoglaziovia variegata</i>	<i>Calotropis procera</i>	<i>Astronium urundeuva</i>
<i>Cardoispermum corindum</i>	<i>Melocactus bahiensis</i>	<i>Capparis flexuosa</i>	<i>Bumelia sertorum</i>
<i>Cenchrus ciliaris</i>	<i>Opuntia inamoena</i>	<i>Capparis yco</i>	<i>Caesalpinia pyramidalis</i>
<i>Centrosema</i> sp.	<i>Opuntia monacantha</i>	<i>Cissus simsiana</i>	<i>Caesalpinia ferrea</i>
<i>Commelina obliqua</i>	<i>Pilosocereus gounellei</i>	<i>Cnidocolus</i> sp.	<i>Cassia excelsa</i>
<i>Diodia teres</i>	<i>Pilosocereus</i>	<i>Croton sonderianus</i>	<i>Euphorbia tirucalli</i>
<i>Desmanthus virgatus</i>	<i>pachycladus</i>	<i>Croton urticaefolius</i>	<i>Prosopis juliflora</i>
<i>Desmodium asperum</i>		<i>Croton</i> sp.	<i>Schinopsis brasiliensi</i>
<i>Gaya aurea</i>		<i>Gonfrena vaga</i>	<i>Spondias tuberosa</i>
<i>Herissantia tiuba</i>		<i>Jacquemontia bahiensis</i>	<i>Ziziphus joazeiro</i>
<i>Ipomoea</i> sp.		<i>Jatropha mutabilis</i>	
<i>Lamium album</i>		<i>Jatropha pohliana</i>	
<i>Luziola micrantha</i>		<i>Lantana camara</i>	
<i>Macropitilium martii</i>		<i>Manihot dichotoma</i>	
<i>Malvastrum</i> sp.		<i>Mimosa hostilis</i>	
<i>Ocimum campechianum</i>		<i>Piptadenia</i> sp.	
<i>Operculina</i> sp.		<i>Ruelia geminiflora</i>	
<i>Passiflora foetida</i>		<i>Sida</i> cf. <i>cordifolia</i>	
<i>Pavonia cancelata</i>		<i>Tridax procumbens</i>	
<i>Portulaca oleraceae</i>			
<i>Rynchelitrum repens</i>			
<i>Scoparia dulcis</i>			
<i>Selaginella convolute</i>			
<i>Senna obtusifolia</i>			
<i>Sida galheirensis</i>			
<i>Sida spinosa</i>			
<i>Tephrosia cinerea</i>			
<i>Urochloa mosambicensis</i>			
<i>Xylosma ciliatfolium</i>			
<i>Waltheria americana</i>			
<i>Waltheria</i> cf. <i>rotundifolia</i>			

foraging time. These samplings were made one time for each season. Each sample consisted of 20 ml rumen contents, which were immediately fixed in a 1:2 formol mixture (Dehority, 1984). Total and differential quantification of ciliate protozoa were performed in a Sedgewick-Rafter chamber,

using a counting grid in one of the eyepieces to quantify the number of ciliates in 50 fields, and then in another 50 fields after rotating the chamber 180°. Total number of ciliates per ml of content was calculated by multiplying values found by 80 and 20. These values correspond to the total counting

chamber surface and sample dilution (Dehority, 1984). Ciliates were stained with three drops of lugol solution instead of brilliant green (D'Agosto and Carneiro, 1999). Ciliate identification was based on Ogimoto and Imai (1981).

For rumen fistula sample extraction, animals were fasted for 15 h, then released into the pasture for one hour, and recaptured for proceeding manual of complete rumen emptying. The contents were stored in plastic recipients so that they could be returned to the rumen after sample collection. These samplings to analyse extrusa were made after the samplings of ruminal content to protozoal analysis. The diet botanical composition was determined as Heady and Torrel (1959).

The total and differential mean numbers of ciliate protozoa were compared between seasons using the Student's T test ($p < 0.05$). The BIOEST 2.0 program was used to analyze data (Ayres *et al.*, 2000).

RESULTS

Seventy eight species were identified in the experimental area, 34 herbaceous, 9 cactus, 22 shrubs, and 13 trees (**table I**). The families that presented high number of species were Leguminosae (15), Euphorbiaceae (9), Malvaceae (7) and Poaceae (6), contributing approximately to 46% of total species observed. The species identified in animal diets (**table II**), in the rainy season 87.8% were herbaceous while in the dry season 67.4% were shrub and 12.7% tree.

Ciliate protozoa population concentrations varied between rainy and dry seasons (**table III**). The *Eodinium* and *Enoploplastron* genera were only seen in rainy season samples. In the dry season, *Entodinium*, *Epidinium*, *Eremoplastron* genera and mean number of total protozoa decreased ($p < 0.05$), whereas *Dasytricha*, *Diploplastron*, *Eudiplodinium* and *Isotricha* genera showed increases ($p < 0.05$).

DISCUSSION

Pimentel *et al.* (1992) and Araújo Filho *et al.* (1996) reported that sheep are basically dicotyledonous herbaceous plants consumers in Caatinga pasture. However, they are considered moderately selective with high feeding flexibility depending on the time of year, and feed availability and quantity because during dry seasons, they use bush and tree biomass (Araújo Filho *et al.*, 1996; Pimentel *et al.*, 1992). They even select the bush and tree species according to the nutritional value of the available biomass,

Table II. Seasonal botanical composition (%) of food eaten by crossbred Santa Ines wethers grazing on natural Caatinga pasture. (Composição botânica (%) da dieta de ovinos mestiços Santa Inês em pastagem natural de Caatinga).

Rainy season		Dry season	
Grazed species	%	Grazed species	%
Herbaceous		Herbaceous	
<i>Herissantia tiubae</i>	51.7	<i>Sida galheirensis</i>	8.8
Grasses*	22.1	<i>Boerhaavia</i>	
<i>Sida galheirensis</i>	6.3	<i>coccinea</i>	4.6
<i>Diodia teres</i>	2.8	<i>Diodia teres</i>	3.9
<i>Tephrosia cinerea</i>	2.2	Total	17.3
<i>Centrosema</i> sp.	1.8		
<i>Boerhaavia</i>		Shrubs	
<i>coccinea</i>	1.1	<i>Capparis yco</i>	26.3
Total	88	<i>Bauhinia</i>	
		<i>cheilantha</i>	22.3
Shrubs		<i>Mimosa hostilis</i>	9.3
<i>Gonfrena vaga</i>	0.7	<i>Capparis flexuosa</i>	7.9
<i>Capparis flexuosa</i>	6.5	<i>Cordia leucocephala</i>	1.1
Total	7.2	<i>Gonfrena vaga</i>	0.5
		Total	67.4
		Trees	
		<i>Spondias tuberosa</i>	9
		<i>Ziziphus joazeiro</i>	3.7
		Total	12.7
Not identified	4	Not identified	2.5

*Species no determinated.

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Table III. Mean concentration ($\times 10^4$) and composition (%) of ciliate protozoa per ml of rumen contents from crossed sheep Santa-Ines wethers in natural Caatinga pasture. (Concentração média ($\times 10^4$) e composição (%) de protozoários ciliados, por mililitro de conteúdo ruminal de ovinos mestiços Santa-Inês em pastagens naturais de Caatinga).

Rumen ciliates	Rainy season		Dry season		Level of significance
	Mean concentration	%	Mean concentration	%	
<i>Dasytricha</i>	0.43 \pm 0.54 ^a	0.45	1.84 \pm 1.62 ^b	4.49	***
<i>Diplodinium</i>	1.49 \pm 1.18	1.59	1.00 \pm 1.02	2.44	NS
<i>Diploplastron</i>	1.70 \pm 3.01 ^a	1.81	3.04 \pm 2.23 ^b	7.42	*
<i>Entodinium</i>	77.23 \pm 32.77 ^a	82.41	28.66 \pm 22.67 ^b	70.00	***
<i>Eodinium</i>	0.17 \pm 0.77	0.18	- \pm -	-	NS
<i>Elytroplastron</i>	1.25 \pm 1.31	1.33	1.18 \pm 0.96	2.88	NS
<i>Enoploplastron</i>	1.08 \pm 1.33	1.15	- \pm -	-	NS
<i>Epidinium</i>	8.13 \pm 10.21 ^a	8.67	2.34 \pm 2.36 ^b	5.71	**
<i>Eremoplastron</i>	1.61 \pm 2.09 ^a	1.71	0.01 \pm 0.06 ^b	0.02	***
<i>Eudiplodinium</i>	0.04 \pm 0.14 ^a	0.04	0.34 \pm 0.64 ^b	0.83	**
<i>Isotricha</i>	0.19 \pm 0.22 ^a	0.20	1.13 \pm 1.14 ^b	2.76	***
<i>Metadinium</i>	1.19 \pm 2.82	1.26	0.68 \pm 1.07	1.66	NS
<i>Ophryoscolex</i>	0.20 \pm 0.33	0.21	0.63 \pm 1.19	1.53	NS
Total	93.71 \pm 42.87 ^a		40.94 \pm 28.00 ^b		***

Values bearing different superscript (a or b) in a row differ significantly * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$; NS: non significant.

taking into account the seasonal variations in total protein, dry matter, and acid and neutral detergent fiber levels from various species which make up the Caatinga vegetation, and which are used as forage (Almeida *et al.*, 2006; Araújo Filho *et al.*, 1996; Araújo Filho *et al.*, 2002). This is confirmed by the increased levels of *Bauhinia cheilantha* in the animal's diet during the dry season, the use of which as a foodstuff has been recommended due to its high nutritional value (Araújo Filho *et al.*, 1998; Guimarães-Beelen *et al.*, 2006).

The variations in sheep's diet composition due to seasonality, and feeding flexibility to the available vegetation have also been seen in studies on sheep and goats in other semi-arid locations in South Africa (Du Toit, 1998), Spain (Molina Alcaide *et al.*, 1997), Mexico (Lopez-Trujillo and Garcia-Elizondo, 1995) and India (Sharma *et al.*, 1998).

In this study dietary botanical composition was strongly influenced by the seasons which consequently determined protozoa occurrence and concentration. In this way variations in these protozoa concentrations could be linked to seasonal variations or even to the phenological state of the plants used as forage, both being able to trigger changes in soluble sugar and fiber levels which are used by different protozoa populations, according to the enzyme activity shown by each one (Bonhomme-Florentin *et al.*, 1978; Manella and Lourenço, 2004; Nogueira Filho *et al.*, 1992).

The significant reduction in *Entodinium* concentration in dry seasons, when most of the vegetation is in an advanced phenological state corroborates observations by Manella and Lourenço (2004) and by Nogueira Filho *et al.* (1992), and can be attributed to the reduction in total protein content, a reduction in solu-

ble sugars and increase in fiber due to the level of plant maturation. Also *Entodinium* ciliates have lower cellulolytic activity (Takenaka *et al.*, 2004), therefore their populations are at a disadvantage when ruminants are fed fiber-rich diets.

Variations in rumen protozoa concentrations are also attributed to nutritional quality of plants available in pastures (Bonhomme-Florentin *et al.*, 1978) and available dietary protein content, with higher protein content diets tending to produce higher ciliate concentrations (Franzolin *et al.*, 1997). Protein content is known to vary with the phenology of the forage plant, and in characteristic Caatinga species the higher protein levels are seen in the vegetative stage (Araújo Filho *et al.*, 2002), which coincides with the rainy season when the highest number of protozoa are seen.

Diplodiniinae ciliates, *Diplodinium* and *Eudiplodinium*, presented high fibrolytic activity and play an important role in rumen fiber digestion (Takenaka *et al.*, 2004; Williams and Coleman, 1992). Possibly due to their fibrolytic activity, presented significant increases in concentration during the dry season, when the vegetation have higher fiber level (Araújo Filho, 2002).

Guimarães-Beelen *et al.* (2006) observed that the tannin concentration in plants used in Brazilian semi-arid pastures influences degradability, microbial adhesion, and microbial enzymes. So although various

Caatinga plant species have nutritional characteristics which make them potential forages, one must take into account their effect on ruminal microbiota. Using these native species in ruminant diets, it may have unsatisfactory results, not from the lack of productively adequate nutritional characteristics, but because they possess antinutritional factors such as tannins, saponins, lignins, and mimosins which are toxic to rumen microorganisms (Kamra, 2005; McSweeney *et al.*, 2001; McSweeney *et al.*, 2002).

CONCLUSION

The botanical dietary composition of sheep grazing on natural Caatinga vegetation has seasonal variations, with herbaceous species predominating in rainy seasons. In dry seasons, the diet consists mainly of shrub species, with this flexibility linked to food availability; this is linked to seasonal variations, which influence the ciliate protozoa concentrations in the rumen, which amounts vary depending on analyzed population.

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