



Effect of concentrate supplement to ewes on nutritive value of ingested Caatinga native forage nutritive value as affected by season

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Abstract

The objective of this study was to identify by microhistological technique the reference chemical components for use as indicators of the nutritive value of Caatinga plants forage grazed by sheep throughout the year. A flock of twenty mixed-race meat ewes, multiparous, in production, with an average 34.84 ± 1.75 kg live weight and 36 months of age was assigned to supplement treatment of 0, 200, 350, and 500 g concentrate/head/day for 3 years. The experiment was designed as a randomized complete design with repeated measures over time. Supplementation with concentrate did not influence chemical composition of selected forage. In contrast, season heavily influenced diet chemical composition. Canopy stratum, season, and plant botanical family of selected species affected forage chemical composition selected by ewes. The ewes selected forage with greater nutritive value during the rainy season. Based on principal component analysis of the nutritive value of the primary forage species selected, ewes preferentially grazed plants contained greater neutral detergent fiber, acid detergent fiber, crude protein (CP), C fraction of nitrogenous compounds, and carbohydrate fractions A + B1 and C compared to the average native Caatinga herbage. Rangeland botanical composition and ewe diet varied during the year, affecting forage nutrients on offer, with the best diet selected during the rainy season because of the presence of dicotyledonous herbaceous species rich in CP as well as soluble carbohydrates and nitrogenous compounds. Concentrate supplement strategies for ewes on rangelands, such as Caatinga, should be determined by herbaceous species nutritive value during the rainy season and deep-rooted perennial dicotyledons during the dry season. These include the need to monitor ewe selection of forage species and their nutritive value, which is effectively accomplished with fecal microhistological techniques.

Keywords Carbohydrate fractions · Nitrogenous compound fractions · Forage on offer · Native pasture · Selective grazing · Semi-arid

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Introduction

The Caatinga biome is found entirely in Brazil's northeastern semiarid region and is characterized by high temperatures, elevated evapotranspiration rates, irregular rainfall over time and space, and long drought periods. As a result, native forage availability varies within and among years. Small ruminant production in Brazil's semiarid region is generally extensive and, therefore, dependent on native vegetation forage for nutrients (Formiga et al., 2011). Improving production indexes of flocks in Caatinga depend on genetic selection, health management, and adequate nutrition (Askar et al., 2014).

Native herbage encompasses diverse herbaceous annual and perennial browse species, many of these nutritionally valuable forage (Santana et al., 2011). Approximately 70% of the plant species present in Caatinga rangeland contribute to small ruminant diets (Araújo Filho et al., 1998). Among these, however, chemical composition varies widely. Diets selected by small ruminants depend largely on season and differ greatly from the botanical composition and chemical characteristics of the herbage on offer, especially when compared to more uniform cultivated pastures (Formiga et al., 2011; Silva et al., 2017).

More efficient use of Caatinga vegetation for small ruminant production requires greater knowledge of species preferentially grazed, their availability, and the nutritive value over different seasons (Oliveira et al., 2016). During seasons of less rainfall, forage availability and nutritive value can decline, particularly the crude protein content (Silva et al., 2017). This is a yearly cycle resulting in low flock production when sheep are fed exclusively rangeland herbage, with resulting economic challenges and food insecurity for producers (Pereira et al., 2010).

The fecal microhistological technique (Sparks and Malechek, 1968) is a viable methodology for determining the diet of animals in the Caatinga (Araújo et al., 2019), as it is less laborious and less invasive, although it presents some inaccuracies when sampling, due to the interaction of factors involved in grazing by animals in the Caatinga environment (Rogério et al., 2017). We hypothesize that the selectivity of Caatinga pasture species by sheep, as a function of the meteorological conditions of the environment, can be determined by the microhistological technique and reveal the impact of the animal-plant interaction on the nutritional value related to the chemical constituents of the forage and to provide information to Caatinga sheep producers, and comparable systems around the world, to be used for rangeland vegetation management. Thus, our study was carried out with the objective of identifying by microhistological technique the reference chemical components for use as indicators of the nutritional value of forage of Caatinga plants grazed by sheep throughout the year.

Material and methods

Location and season of experiment

The Experiment took place from February 2015 to August 2017 at the Fazenda Lagoa Seca, Cariré, Ceará State, Brazil, located at 3°57' latitude south and 40°28' longitude west. The climate is type BSh hot semiarid according to the Köppen classification, with a rainy season from January to June and a dry season from July to December. During the 3 years of the experiment, an average 764-mm rainfall fell during the rainy season and 84 mm during the dry season with an average 26.5 and 28.6 °C, respectively, measured by the Fundação Cearense de Meteorologia e Recursos Hídricos (FUNCEME, 2019) weather station, located in Cariré – CE, Brazil (Fig. 1).

The ranch has 240 ha of Caatinga rangeland with no history of vegetation management. These are divided into five paddocks in which the owner rotated sheep stocking rate adjusted to utilize a maximum 60% of the available herbaceous forage in an effort to preserve Caatinga floristic diversity (Araújo Filho, 2013). Grazing was managed as continuous with put-and-take stocking rates (Mott and Lucas, 1952). The native Caatinga vegetation was dominated by low-growing browse species with twisted stems and variable canopy densities that allowed herbaceous vegetation growth (Giulietti et al., 2004). The entire Caatinga area was used during the trial. These were mapped based on ecosystem characteristics (Stoddart et al., 1975). Primary characteristics included browse density versus the presence of grasses and herbaceous legumes, making it possible to maximize use during the rainy, transition and dry seasons.

Species frequency, bare soil, and forage dry matter availability were determined by point sampling based on macro- and micro-plots established based on paddock size. Macro- and micro-plots were distributed uniformly across paddock transects with an average 100 m between sampled areas consisting of 1.0 by 0.25-m quadrats (Araújo Filho et al., 1986). Herbaceous vegetation collected was divided into fractions consisting of grasses, forbs (including legumes) and leaf litter. These were dried at 55 °C until weight loss ceased and weighed to determine forage availability (Table 1).

Browse up to 1.5-m height, considered within animal reach, was evaluated by a quadrat system described by Araújo Filho (2013). The same point at which herbaceous vegetation was collected was used as the center from which two crossing metal bars indicated four cardinal directions at 90° angles. In each direction indicated by the bars, the height, diameter, and distance to the closest browse species were determined. This allowed an estimate of total plant density calculated based on distance to the nearest browse species from the sample point center. Relative density was

Fig. 1 Total monthly rainfall (mm) and average temperatures (°C) at Cariré, Estado do Ceará, Brazil, from January 2015 to December 2017 (data obtained from FUNCEME)

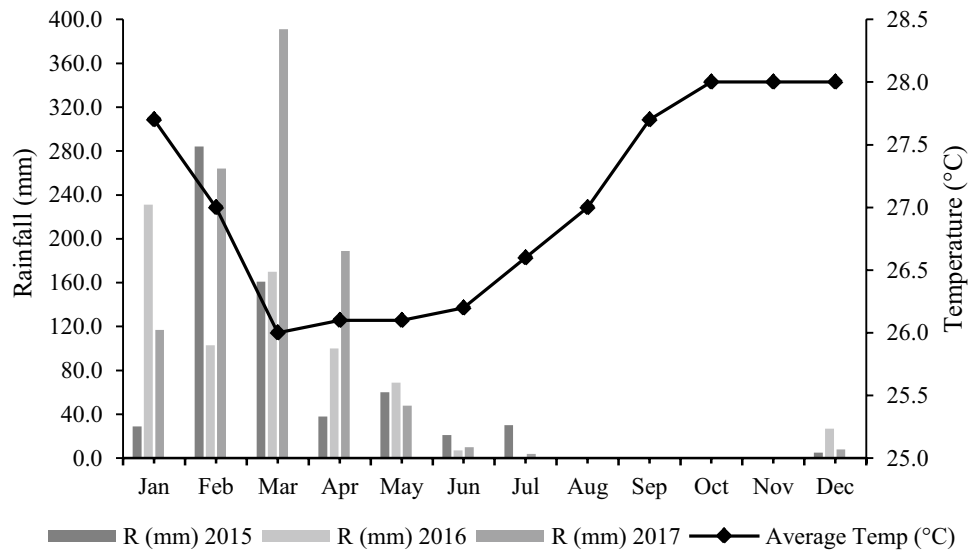


Table 1 Soil cover, vegetation productivity, and ewe stocking rate during three seasons in Caatinga rangeland near Cariré, Ceará state, Brazil

Season	Soil cover (%)	Vegetation productivity (kg dry matter (DM)/ha) ²				Total productivity (kg DM/ha)	Stocking rate (ha/AU/year)
		Grasses	Legumes	Other dicotyledons	Leaf litter		
Rainy ¹	64.5	292.5	189.6	268.3	210.1	960.7	7.2
Transition	54.0	170.1	154.3	274.2	334.0	932.7	7.1
Dry	35.8	88.5	1423	278.2	532.6	1041.5	6.4

DM dry matter, AU animal unit

¹Rainy season, March; transition, may; dry season, July

²Estimated based on 60% consumption of total herbage available in rangeland during 365 days (Araújo Filho, 2013)

determined by dividing plant number of each species by the total of all species, while specific density was obtained by multiplying individual species density with relative density (Table 2).

Animals and supplements

The use of animals in our research followed the norms established by Embrapa's Commission for Ethical Use of Animal protocol no. 009/2015 and by the Federal University of Piauí protocol no. 496/2018. A flock of twenty ($n=5$) mixed-race meat ewes, multiparous, in production, with an average 34.84 ± 1.75 kg live weight and 36 months of age was assigned to supplement treatment of 0, 200, 350, and 500 g concentrate/head/day from 2015 to 2017. Data were collected within rainy, transition, and dry seasons as defined by Rogério et al. (2017). These coincided with the final third of ewe gestation (March), initiation of lactation (May), and weaning (July), respectively.

The maximum supplement level of 500 g/head/day was selected based on the maximum generally used in sheep production systems in the Brazilian semiarid region. The intervals were defined considering the total dry matter intake of 3.6% of live weight (1250 g/animal/day), as verified by Barbosa et al. (2003). Thus, it was established the supplementation with 200, 350, and 500 g/animal/day, corresponding to 16, 28, and 40% of the predicted total dry matter consumption. Forage in the diets consisted of ad libitum native Caatinga vegetation. Supplement consisted of 72.5% ground maize, 6.7% soybean meal, 18.0% cottonseed meal, 1.6% calcium carbonate, and 1.1% mineral (Ovinofós with monensin; Tortuga®), on a dry matter basis (Table 3). This was fed once daily to individual animals as they returned from the pasture, where they remained from 8:00 to 16:00 h. All ewes had access to the same rangeland throughout the experiment and were separated only when fed supplements. Animals were adapted to supplement for 21 d prior to the start of the trial.

Table 2 Species density (SD, plants/ha), relative density (RD, %), total density, and arboreal canopy in native Caatinga vegetation during the rainy, transition, and dry seasons near Cariré, Ceará state, Brazil

Arboreal species	Rainy ¹		Transition		Dry	
	SD	RD	SD	RD	SD	RD
<i>Cenostigma pyramidale</i> (Tul.) E. Gagnon & G. P. Lewis	141.7	7.4	95.2	8.0	-	-
<i>Amburana cearensis</i> F. Allemão	-	-	-	-	38.1	6.5
<i>Libidibia ferrea</i> (Mart. ex Tul.) L. P. Queiroz	133.3	6.9	-	-	-	-
<i>Piptadenia stipulacea</i> (Benth.) Ducke	147.6	7.6	-	-	-	-
<i>Mimosa tenuiflora</i> (Willd.) Poir	200.0	10.3	-	-	-	-
<i>Croton sonderianus</i> Muell. Arg	347.6	18.0	319.1	26.7	161.9	27.6
<i>Combretum leprosum</i> Mart	323.8	16.8	219.1	18.3	123.8	21.2
<i>Bauhinia cheilantha</i> (Bong.) Steud	57.1	2.9	38.1	3.2	-	-
<i>Auxemma oncocalyx</i> (Allemão) Baill	142.9	7.4	181.0	15.1	109.5	18.7
<i>Handroanthus impetiginosus</i> (Mart. ex DC.) Mattos	23.8	1.2	-	-	-	-
<i>Luetzelburgia auriculata</i> (Allemão) Ducke	38.1	2.0	-	-	-	-
<i>Jatrophamo lissima</i> L	57.1	3.0	-	-	-	-
<i>Mimosa caesalpiniaefolia</i> Benth	290.5	15.0	342.9	28.7	138.1	23.6
<i>Mascagnia rigida</i> (Juss.) Griseb	28.6	1.5	-	-	14.3	2.4
Total density (plants/ha)	1932.1		1195.5		575.7	
Arboreal canopy area (% of total area)	68.5		43.3		27.5	

¹Rainy season measured in March; transition season measured in June; dry season measured in July

Table 3 Chemical composition, in vitro dry matter digestibility (IVDMD), and total digestible nutrients (TDN) of supplement concentrate fed to ewes

DM g/kg	Ash (g/kg DM)	CP	EE	apNDF	ADF	Lignin	TDN	IVDMD kg/kg DM	NDIP g/kg CP	ADIP
910.5	40.8	149.7	29.1	207.0	87.8	61.3	747.9	0.83	600.0	337.5

DM dry matter, g/kg green matter; CP crude protein; EE ether extract; apNDF neutral detergent fiber corrected for ash and protein; ADF acid detergent fiber; NDIP protein insoluble in neutral detergent; ADIP protein insoluble in acid detergent

Forage nutritive value

The forage selected and ingested by ewes was identified according to fecal microhistological techniques described by Sparks and Malecheck (1968). Forage available in the rangeland was sampled after observing ewe plant species selection. Fecal samples for microscope slides were collected at the same time in order to observe microhistological characteristics as described by Rogério et al. (2017). Diet nutritive value was estimated by the equation proposed by McInnis and Vavra (1987), calculated using values of species ingested according to those identified on microscope slides using the equation:

$$Ni = \sum_{i=1}^n aijxj$$

where Ni is the contribution of nutrient i in feed composition, aij is the content of nutrient i of forage species j, and xj is the percentage composition, by dry matter, of forage species j.

Ewes selected a wide diversity of forage species during the 3-year experiment. This necessitated grouping forages to facilitate inferences of selected and ingested diet nutritive value. Seasons consisted of rainy, transition, and dry; canopies were divided into herbaceous or bush/arboreal; and botanical groups included grasses, dicotyledons, and others. Each was evaluated by 1st and 3rd quartile median distances and minimum and maximum nutritive value observed in each group presented as box plots.

Forage chemical composition

Dry matter (DM; AOAC 934.01), ash and organic matter (OM; AOAC 942.05), crude protein (CP) via the Kjeldahl method (AOAC 920.87), and ether extract (EE; AOAC 920.85) herbage content were determined according to AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were obtained using methods described by Van Soest et al. (1991). Using these data, NDF was estimated following adjustment for ash and CP (apNDF; Hall et al., 1999). Herbage lignin content (%)

was determined by treating ADF with 72% sulfuric acid according to Van Soest et al. (1991).

Herbage insoluble N in NDF (NDFN) and ADF (ADFN) were calculated according to Licitra et al. (1996). Dry matter in vitro digestibility (IVDMD) was obtained as described by Tilley and Terry (1963). Herbage total digestible nutrients (TDN) for each species was estimated according to Weiss et al. (1992) using the equation: $TDN = tdNFC + tdCP + (tdFA \times 2.25) + tdNDF - 7$, FA = fatty acids (i.e., EE - 1). Metabolizable energy (ME) was estimated with the equation: $ME = 0.82 \times \text{digestible energy (DE)}$ which was in turn calculated by multiplying TDN by a factor of 4.409 (Crampton et al., 1957).

Carbohydrate fractions and nitrogen compounds

Herbage total carbohydrate (TCHO) content and carbohydrate nutritional fractions were estimated according to Sniffen et al. (1992) using the equation $TCHO (\%) = 100 - (\%CP + \%EE + \%ash)$. Carbohydrates were classified into four fractions according to degradation rate in IVDMD; fraction A corresponded to rapidly degraded TCHO, fraction B1 included starch and pectin of intermediate degradability, B2 was slowly degraded cellular wall, and C corresponded to the cell wall associated to lignin and not available to rumen microorganism degradation. Fractions A + B1 were estimated by subtracting TCHO from apNDF. Fraction C was estimated by multiplying lignin content by 2.4. Fraction B2 was the difference between apNDF and fraction C (Sniffen et al., 1992).

Nitrogen compounds were divided into five fractions, according to rumen degradation rate as described by Sniffen et al. (1992) and modified by Licitra et al. (1996). Fraction A corresponded to non-protein N; fraction B1 to truly digestible protein of rapid degradation in the rumen; B2 to N compounds of intermediate degradation rate; B3 to protein insoluble in neutral detergent, of slow degradability; and fraction C to indigestible N fractions. Fraction A was determined from the difference between TN and residual, insoluble N after exposure to trichloroacetic acid (10%). Fraction B1 was obtained by treating herbage with borate-phosphate buffer and 10% sodic azide, while fraction B1 was estimated by subtracting this soluble portion from fraction A. Fraction B3 was obtained from the difference between NDFN and ADFN. Fraction C corresponds to ADFN, while fraction B2 was the difference between TDN and the sum of A, B1, B3, and C (Licitra et al., 1996).

Experimental design and statistical analyses

The experiment was set up in a totally random design for nutrient selection with measurements repeated over time and

five replications. The means resulting from supplements (0, 200, 350, and 500 g of concentrate/animal/d) and seasons (rainy, transition, and dry) were submitted to analyses of variance (PROC MIXED) and orthogonal polynomials (PROC REG) in SAS Version 15.1. Means were compared by Tukey's test. Differences were considered significant at $P \leq 0.05$, and specific probabilities were included in the "Results" section only if $P > 0.05$.

The statistical model was $Y_{ijk} = \mu + ti + e(i)k + sj + tsij + E_{ijk}$ with Y_{ijk} the observation from the subplot according to supplement effect i ($i = 0, 200, 350, \text{ and } 500 \text{ g ewe}^{-1}$) of factor t (supplement level) and season j ($j = \text{rainy, transition and dry}$) of factor s (seasons) in replication r , μ the overall mean, ti the fixed effect of supplement i , and $(i)k$ the error associated with plots, sj the effect of season j , $tsij$ the interaction among supplements and season, and E_{ijk} the experimental error associated with subplots.

The forage species were grouped by multivariate analyses based on chemical composition as well as carbohydrate and N fractions. The relative influence of each variable in the groups was obtained by principal component analysis (PCA) using PRINCOMP function from R software, version 4.0.3.

Results

Diet chemical composition

Feed supplement did not influence ($P = 0.85$) chemical composition of herbage selected by ewes, and there was no interaction ($P = 0.36$) between supplement level and season. Herbage selected by ewes during the rainy season had greater CP, IVDMD, and energy content. During the dry season, selected herbage had greater DM, OM, EE, apNDF, ADF, lignin, NDIN, and ADIN. The non-fibrous carbohydrates selected by ewes was stable ($P = 0.16$) throughout the 3 years of evaluation (Table 4).

Nutritive value of herbage selected by ewes

Ewes selected 22 Caatinga plant species, of which six were grasses, nine dicotyledons, and seven arboreal or brush. Of these, 54.5% were preferentially selected vis-à-vis what was available to the animals in the paddocks and contributed 70.0% of the diet. Preferred grasses included *Aristida longiseta* Steud., *Cynodon dactylon* (L.) Pers., *Aristida adscensionis* L., and *Eleusine indica* (L.) Gaertn. Preferred dicotyledons included *Arachis dardani* Krapov. & W. C. Greg., *Alternanthera tenella* Colla, *Alternanthera brasiliiana* (L.) Kuntze, *Stylosanthes humilis* H.B.K., and *Sida cordifolia* L., while brush/arboreals included *Croton sonderianus* Muell. Arg., *Combretum leprosum* Mart., and *Mimosa caesalpiniaefolia* Benth.

Table 4 Chemical composition, in vitro dry matter digestibility (IVDMD), and total digestible nutrients (TDN) of forage selectively grazed by ewes on Caatinga rangeland in Cariré, Ceará state, Brazil

Item	Supplement (g/animal/day)				Season			SEM	p-value season ¹
	0	200	350	500	Rainy	Transition	Dry		
DM (g/kg green)	357.9	360.0	373.4	362.7	290.7c	353.5b	446.2a	0.63	<0.001
OM	897.2	900.3	895.2	894.6	887.5b	882.4c	920.5a	0.17	<0.001
CP	136.3	135.7	132.0	143.1	146.6a	140.5b	123.3c	0.16	<0.001
EE	22.7	22.2	21.6	22.8	18.3b	18.5b	30.3a	0.05	<0.001
apNDF	607.1	605.0	622.7	615.2	604.7b	602.2b	630.6a	0.33	<0.001
ADF	368.9	374.6	376.6	372.6	337.7c	366.4b	415.6a	0.36	<0.001
Lignin	87.5	90.7	88.3	85.0	54.1c	85.2b	124.4a	0.26	<0.001
NFC	232.8	239.9	223.0	223.3	234.5	229.1	225.7	0.22	0.16
IVDMD	0.50	0.51	0.49	0.51	0.54a	0.50b	0.48b	0.42	<0.001
TDN	559.5	561.6	551.6	563.5	595.8a	535.2b	546.1b	0.28	<0.001
NDIP (g/kg CP)	481.3	493.0	478.0	485.7	482.9b	465.5c	507.7a	0.07	<0.001
ADIP (g/kg CP)	174.6	176.9	179.5	171.2	124.8c	160.9b	252.2a	0.05	<0.001
ME (Mcal/kg DM)	2.02	2.03	1.99	2.03	2.15a	1.96b	1.93b	0.01	<0.001

SEM standard error of the mean, DM dry matter on green plant basis, g/kg green plant material, OM organic matter, CP crude protein, EE ether extract, apNDF neutral detergent fiber corrected for ash and protein, ADF acid detergent fiber, NFC non-fibrous carbohydrates, IVDMD in vitro DM digestibility, TDN total digestible nutrients, NDIP neutral detergent insoluble protein, ADIP acid detergent insoluble protein, DE digestible energy, ME metabolizable energy

¹Different letters in lines under season headings differ by Tukey's test ($p \leq 0.05$)

Greater overall herbage DM, OM, apNDF, ADF, ADIN, and lignin were measured during the dry season with greater DM, apNDF, and ADF values for grasses. In the rainy season, ewes selected herbage with greater CP, IVDMD, NDIN, and energy, with greater contributions of CP and NDIN from arboreal dicotyledons, while IVDMD and energy were associated with herbaceous dicotyledons which were more tender and had greater soluble compounds (Fig. 2). The primary species selected by ewes had greater energy content associated with elevated CP content, as exemplified by *S. cordifolia*, *S. humilis*, and *A. brasiliensis* (Fig. 3).

Based on the chemical composition of the herbage selected by ewes, PCA indicated that the two primary components explain 73.4% of data variability, allowing herbage species to be grouped into three distinct groups (Fig. 4). The first consisted of brush/arboreal species *M. caesalpiniaefolia*, *C. leprosum*, and *C. Sonderianus*. The second was constituted by grasses *A. adscensionis*, *E. indica*, and *A. longisetata* while the third by herbaceous dicotyledons *A. dardani*, *S. cordifolia*, and *S. humilis*.

Chemical constituents that influenced the selected herbage species in PC1 (45.6% of variability) included ADF (0.859) and apNDF (0.857) with individual characteristics that had the most significant positive scores. By contrast, CP (0.80%) presented a negative significant score. These four were most influential in classifying herbage species by chemical composition in PC1. For the formation of CP2 (27.8% of represented variability), NDIN (0.947), lignin

(0.779) and ADIN (0.702) contributed the most positive and significant scores (Fig. 4).

Fractionation of plant nutrients selected by ewes

The dicotyledonous herbage species presented the greatest fraction of insoluble carbohydrates A + B1 during the rainy season, most notably *A. dardani*, *S. cordifolia*, and *S. humilis* (Fig. 5). The grasses contributed most to carbohydrate fraction B2, associated with NDF, representing 630 g/kg of TCHO, mostly from *A. longisetata*, *E. indica*, and *C. dactylon*. The brush/arboreal species contributed most to fraction C, associated with ADF, during the dry season, primarily from lignin in *C. sonderianus*, *C. leprosum*, and *M. caesalpiniaefolia* (Fig. 6).

Herbaceous species contributed most to soluble N fractions A and B1 during the rainy season, most notably *A. brasiliensis* and *E. indica* (Fig. 7). Fraction B2 represented the greatest proportion of N within the plant species selected by ewes, with the greatest contribution from grasses during the dry season, especially *A. longisetata* (500 g/kg), while B3 contributed most during the rainy season. Arboreal species contributed most to fraction C during the dry season, mostly *M. caesalpiniaefolia*, *C. leprosum*, and *C. sonderianus*, up to 400 g N/kg in *M. Caesalpiniaefolia* (Fig. 8).

Based on carbohydrate and N compounds in herbage selected by ewes, PCA indicated that the two primary

components explained 68.4% of total variability, allowing the grouping of plant species into three distinct groups (Fig. 9). The first consisted of arboreal species *M. caesalpiniaefolia*, *C. leprosum*, and *C. sonderianus*. The second consisted of herbaceous dicotyledons *S. cordifolia*, *S. humilis*, and *A. brasiliensis*, while the third by grasses *A. adscensionis*, *E. indica*, and *A. longisetata*.

Among the carbohydrate and N fractions that most influenced the formation of herbage species groups selected by ewes, 45.8% of variability was represented by CP1, which had as individual characteristics of the greatest score fraction C of N compounds (0.931) and carbohydrates (0.926). Fractions B2 of carbohydrates (0.768) and B3 of N compounds (0.701) had negative and highly significant scores, contributing the most to classifying selected species based on carbohydrate and N fractions. The PC2 represented 22.6% of variability, with carbohydrate fractions A + B (0.879) the greatest positive score, while carbohydrate fraction B2 (0.607) had a negative score (Fig. 9).

Discussion

Chemical composition of selected diet

The increase in DM and OM as well as the variation in their proportion in diets selected by ewes (Table 4) resulted from physiological processes in plants over time. Primary among these were physiological progression from vegetative to reproductive to senescent stages with accompanying conversion of soluble compounds into less digestible structural components (Pellegrini et al., 2016).

Independent of season, animals selected herbage with an average 136 ± 16 g CP/kg DM (Table 4). This value was greater than the minimum 7 to 8 g CP/kg DM widely considered minimum to maintain rumen microbial activity and herbage consumption by ruminants (Van Soest, 1994). This indicates that the CP in Caatinga vegetation sampled in our study, based on ewe selection, was superior than the average CP in other Caatinga floristic compositions. In Caatinga of the Pernambuco scrubland, northeastern Brazil, Oliveira et al. (2015) obtained lower CP contents, 9.1 and 8.9%, in herbage selected by sheep in March and June, respectively.

With season progression, herbage CP declined from the rainy to the dry season. As tropical forage species mature or leaves senesce, herbage CP tends to decline precipitously (Silva et al., 2017). Besides that, herbaceous annual and short-lived perennial legume species, such as *A. dardani*, *S. humilis*, and *Phaseolus patyroides* offer foliage only during the rainy season and therefore contributed to ewe diet during that season only. As the rainy season transitioned to the dry, these short-lived, shallow-rooted legumes disappeared, resulting in less CP available to the ewes. Although

perennial and annual grasses, such as *A. Adscensionis*, were present in the dry season herbaceous layer, because of shallow, fibrous root systems, they did not maintain green foliage so their contribution to ewe diet nutritive value was not as great as during the rainy season when they were growing.

There was an increase in plant fiber content (apNDF, ADF, and lignin) as the rainy season progressed to the dry season (Table 4). This is typical of herbaceous Caatinga vegetation which undertakes the majority of its photosynthesis and leaf formation when soils are moist but rapidly transition to mostly fibrous stems, high in ADF and lignin, as rainfall declines during the transition from rainy to dry seasons (Formiga et al., 2011).

The influence of the period of the year on the concentration of NFC was expected, due to the variation in the selection of NDF, and also of the supplementation, as there was an input of CNF in the diet with the supplementation, on the order of 286 g/head/day (573.4 g/kg of NFC in DM) (Table 4). Concentrate supplementation is an important driver of ruminant selection for CP and fiber in pasture herbage. In general, as supplement increases, ruminants select for less CP and more fiber while unsupplemented animals in the absence of concentrate supplement select pasture herbage diets with more CP and less fiber than is present on average in available herbage (Araújo et al., 2019).

Of the CP available in dry season herbage, 508 g/kg was associated with NDIN, while 225 g/kg was bound in ADIN (Table 4). As these N fractions bound to fiber increase, forage digestibility can be negatively affected. As such, even if overall herbage CP is sufficient to meet ewe nutritional needs during the rainy season, its unavailability to rumen microorganisms could mean that the proportion of available N bound to cell-wall fiber is such that ewe nutritional needs are still not met as undigested fiber passes through the gastro-intestinal tract without absorption (Santos et al., 2017).

Botanical composition and phenological changes in the pasture contributed to IVDMD and available energy in ewe diets (Table 4). Herbaceous dicotyledons predominated in the rainy season (Table 1), exemplified by *S. humilis*, *A. dardani*, and *P. patyroides*, species with greater digestibility compared to grasses. By contrast, these species disappeared in the dry season, thereby increasing the proportion of lower nutritive value grasses, leaf litter, and arboreal dicotyledons such as *C. leprosum* and *M. Caesalpiniaefolia* in ewe diets.

Ambient factors, such as high temperatures and low precipitation, increase plant cell wall and diminish soluble cell content proportions, resulting in lower forage digestibility in ruminants (Boufennara et al., 2012). Crude protein and non-fibrous CHO correlate to TDN, while fiber increases reduce available energy (Pereira et al., 2010). As a consequence, lower dietary energy selected by ewes in the dry season on Caatinga pasture is associated with lower CP, increase in fiber and reduction in IVDMD.

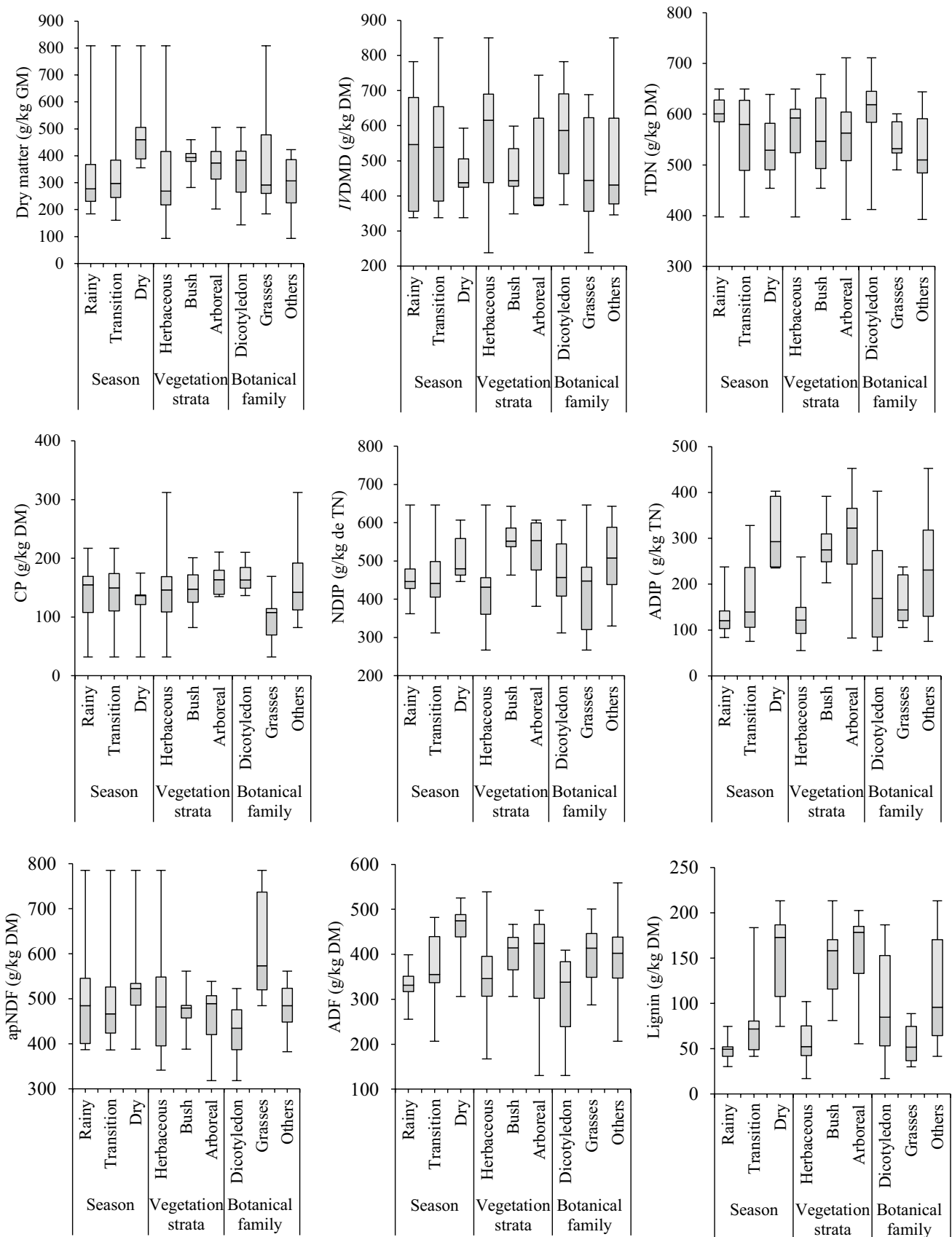


Fig. 2 Chemical composition of forage by season, vegetation strata, and botanical family selectively grazed by ewes on native Caatinga rangeland near Cariré, Ceará state, Brazil

Concentrate feed supplementation level did not change ewe grazing selectivity or forage nutrient intake. This was likely because the fecal microhistological technique directly reflected ewe forage selection. Consequently, ewe nutrient intake during various seasons was more effectively characterized without supplements skewing animal forage selection. This technique is therefore recommended when formulating supplemental feed strategies for ewes on Caatinga and similar rangelands.

Nutritive value of plants selected by ewes

The inferences derived from groupings based on ewe-selected forage chemical composition can be partially explained by small ruminant feeding preferences for browse and grasses, the only plants available to them in Caatinga during the dry season (Boufennara et al., 2012). That reduction in plants available for grazing can also alter Caatinga botanical vegetation composition over years of continuous selective grazing and browsing by ewes. This emphasizes the importance of determining individual plant species contribution to ewe diet vis-à-vis their relative contribution to available vegetation. This information can be used to guide herd grazing management over seasons when attempting to preserve native rangeland diversity and species contribution.

Grasses predominated in ewe diets during the dry season, explaining the increase in dietary DM and fibers of groups (Fig. 2). Tropical grasses have elevated photosynthetic efficiency, leading to rapid vegetative growth and changes in vegetation structure when rainfall occurs, all of which contribute to rapid NDF accumulation (Formiga et al., 2011).

Forage digestibility and energy content selected by ewes relate directly to selected diet nutritive value (Fig. 2). During the rainy season, the herbaceous canopy consisted of dicotyledons offering more tender green forage of greater digestibility, as exemplified by *W. amplissima*, *S. humilis*, *A. dardani*, and *S. Cordifolia*. These contributed to increased average IVDMD and TDN forage values (Fig. 3). This was likewise observed by Santos et al. (2008) studying sheep diet selection in Caatinga of Pernambuco State, Brazil.

Crude protein, ADIN, NDIN, and lignin were more elevated in arboreal specie during the dry season. This was associated primarily with *C. sonderianus*, *C. leprosum*, *A. oncoalix*, and *M. caesalpiniaefolia* (Fig. 2). Despite greater CP content in these species relative to dormant grasses and other species in the dry season, a large part of that protein was associated with ADF which can make CP a limiting nutritional factor even in the rainy season (Silva et al., 2017).

The connection between botanical groups and nutritive value of forage selected by ewes for PCA (Fig. 4) has been reported by several studies (Santos et al., 2008; Formiga et al., 2011; Boufennara et al., 2012; Silva et al., 2017). The botanical groups in our study are similar to those obtained by Oliveira et al. (2016) who reported sheep dietary botanical composition correlation to forage chemical composition. This indicates the possibility of strategies for rangeland, such as Caatinga, sheep feeding management based on forage nutritive value and availability over seasons.

The greater contribution of herbaceous dicotyledons during the rainy season, which contributed greater forage nutritive value due to CP and TDN (Fig. 3), could result in less economic return of supplements during that time of the year. However, during the dry season, arboreal dicotyledons with greater ADIN and lignin contents, as well as senescing grasses with greater apNDF, will likely result in greater efficacy of protein and energy feed supplements to ewes.

Across seasons, the grass *C. dactylon* offered greater CP (169 g/kg DM) and chemical composition similar to herbaceous dicotyledons. These characteristics made it a dissimilar dietary component from the other plants comprising PCA groups (Fig. 4). The genera *Cynodon* is generally characterized as productive and highly nutritious. As a consequence, our observations indicated that they were disproportionately selected by ewes which, in turn, would likely keep it nutritious due to regrowth, even during the dry season.

Among the forage plants most selected by ewes, herbaceous dicotyledons were the most important in the rainy season. Grasses, despite being largely dormant, and arboreal dicotyledons, which maintained some green leaves, were the most important dietary components in the dry season. Their respective nutritive values should be considered when elaborating concentrate supplements for ewes grazing Caatinga during the rainy and dry seasons, respectively.

Carbohydrate and nitrogen fractions of Caatinga plants selected by ewes

Herbaceous dicotyledons contributed disproportionately to soluble carbohydrates in ewe diets (Fig. 5). This implied that conserving these in the Caatinga floristic composition is important because the association of soluble carbohydrates to nitrogenous compounds can contribute to greater energy availability in the rumen which, in turn, stimulates rumen microbial population growth and subsequent forage fiber degradation (Santos et al., 2017).

Grasses predominated in the Caatinga during the rainy season although they also contributed disproportionately to ewe diet in the dry season despite being mostly dormant. During the dry season, herbaceous dicotyledons tended to disappear, indicating the need for greater supplementation during that season because of their disproportionate

Fig. 3 Total digestible nutrients (TDN) (column) and crude protein (CP) (line) of native Caatinga rangeland vegetation selectively grazed by ewes near Cariré, Ceará state, Brazil. DM dry matter

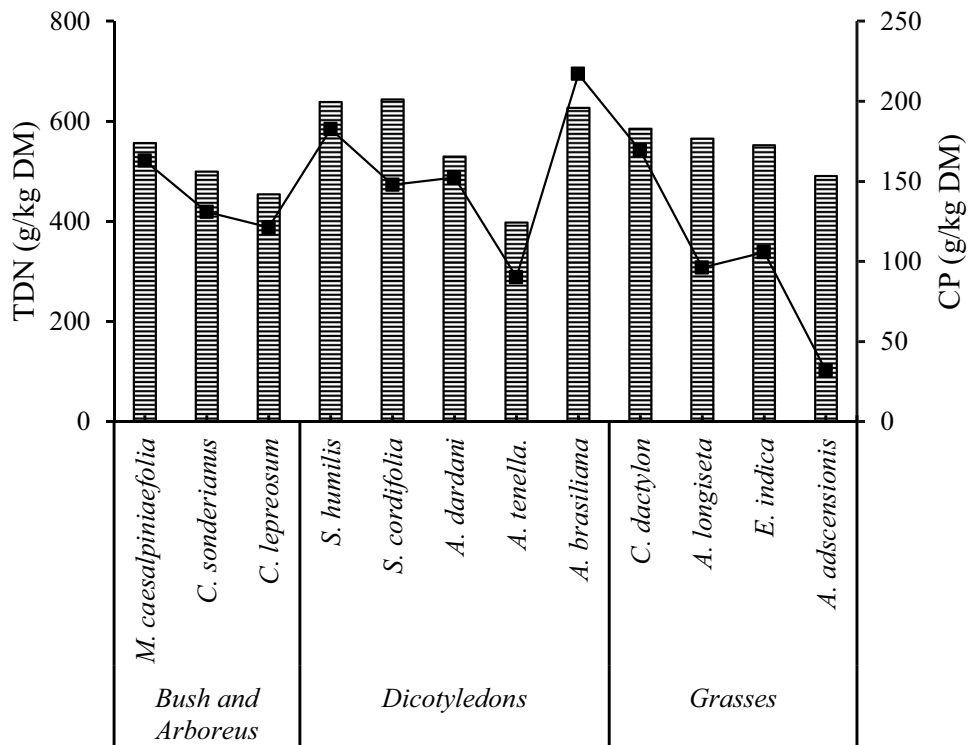
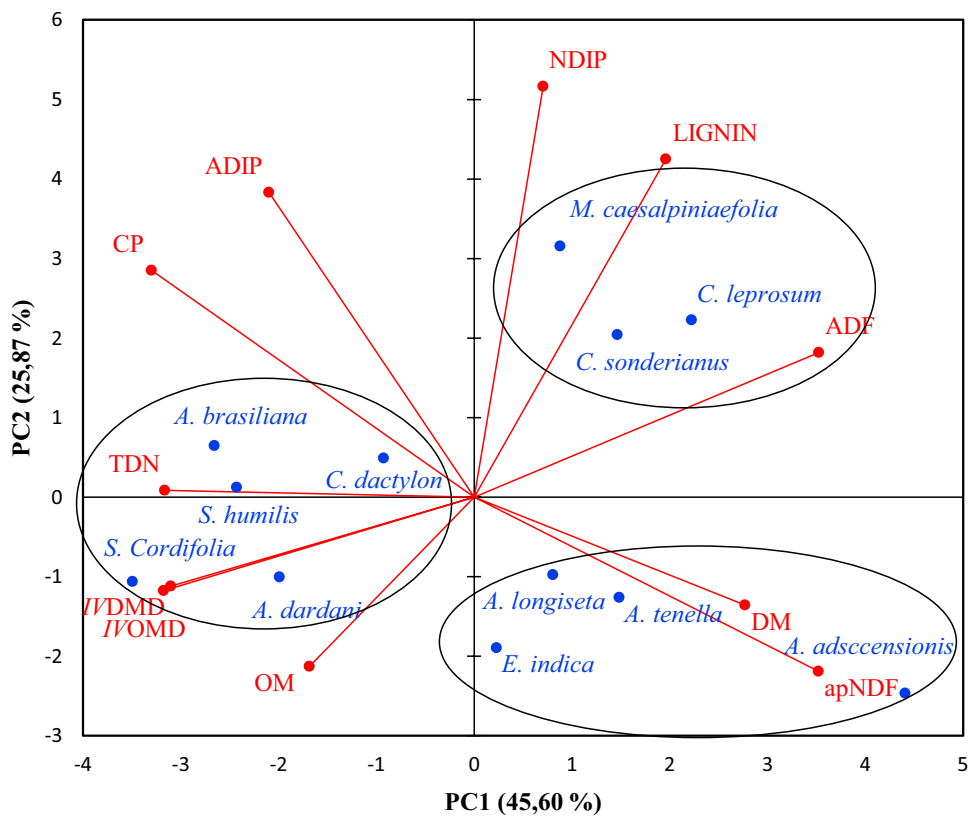


Fig. 4 Principal component analysis of chemical composition of native Caatinga rangeland forage species selectively grazed by ewes near Cariré, Ceará state, Brazil. CP crude protein, ADF acid detergent fiber, ADIP CP insoluble in ADF, apNDF neutral detergent fiber corrected for ash and CP, NDIP CP insoluble in apNDF, DM dry matter, IVDMD in vitro digestible DM, OM organic matter, IVOMD in vitro digestible OM, TDN total digestible nutrients



contribution of B2 carbohydrates (Fig. 5). The B2 fraction of carbohydrates is associated with the NDF content of the forage species, and greater B2 carbohydrates generally

provide more slowly to the rumen with consequent less energy available for ruminant nutrient requirements. This is because fibrolytic rumen microbes are key to ruminant

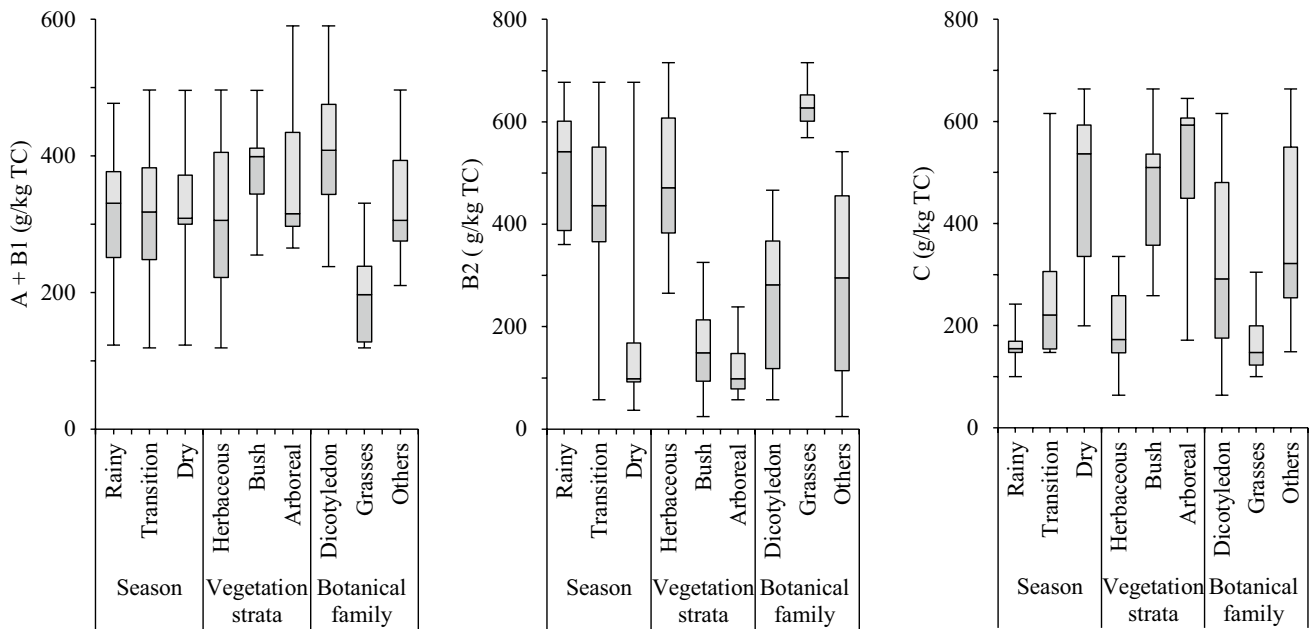


Fig. 5 Total carbohydrate fractions of forage by season, vegetation strata, and botanical family selectively grazed by ewes on native Caatinga rangeland near Cariré, Ceará state, Brazil. TC total carbohy-

drate; Carbohydrate fractions: A=carbohydrate rapidly degradable; B1=intermediate degradation; B2=slowly degradable; C=unavailable fraction (Sniffen et al., 1992)

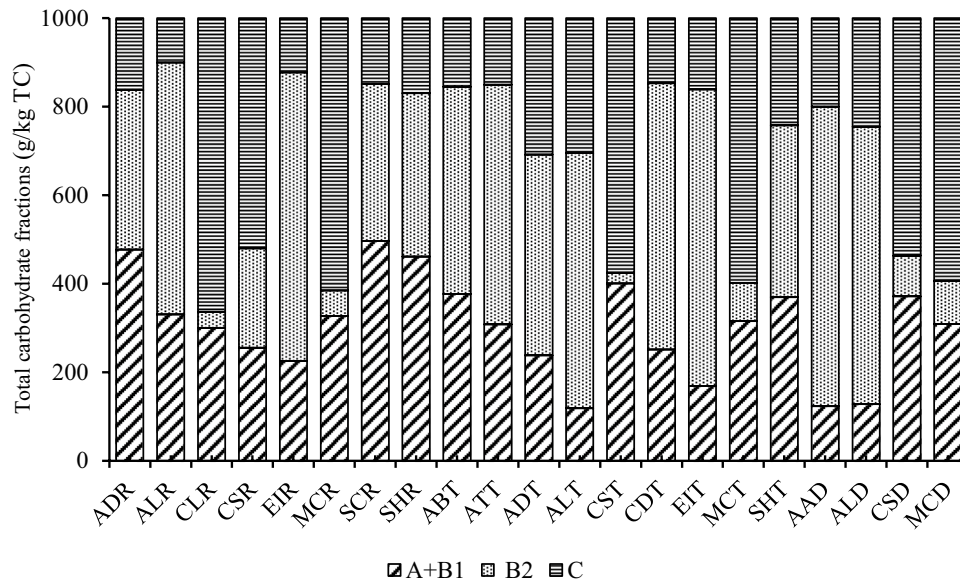


Fig. 6 Total carbohydrate fractions of forage species selectively grazed by ewes on native Caatinga rangeland during the rainy, transition, and dry seasons near Cariré, Estado do Ceará state, Brazil. TC total carbohydrate; rainy season: ADR *A. dardani*, ALR *A. longiseta*, CLR *C. leprosum*, CSR *C. sonderianus*, EIR *E. indica*, MCR *M. caesalpiniaefolia*, SCR *S. cordifolia*, SHR *S. humilis*. Transition season: ABT *A. brasiliana*, ATT *A. tenella*, ADT *A. dardani*, ALT *A. longi-*

seta, CST *C. sonderianus*, CDT *C. dactylon*, EIT *E. indica*, MCT *M. caesalpiniaefolia*, SHT *S. humilis*. Dry season: AAD *A. adscensionis*, ALD *A. longiseta*, CSD *C. sonderianus*, MCD *M. caesalpiniaefolia*. Carbohydrate fractions: A=carbohydrate rapidly degradable; B1=intermediate degradation; B2=slowly degradable; C=unavailable fraction (Sniffen et al., 1992)

nutrition and subsequent development (Pereira et al., 2010). The grass *A. adscensionis* contributed disproportionately to ewe diets, especially during the dry season,

and contained greater B2 fractions than other plant species (Fig. 6). This species can contribute up to 71.0% of sheep diet in Caatinga rangelands (Araújo Filho et al., 1996).

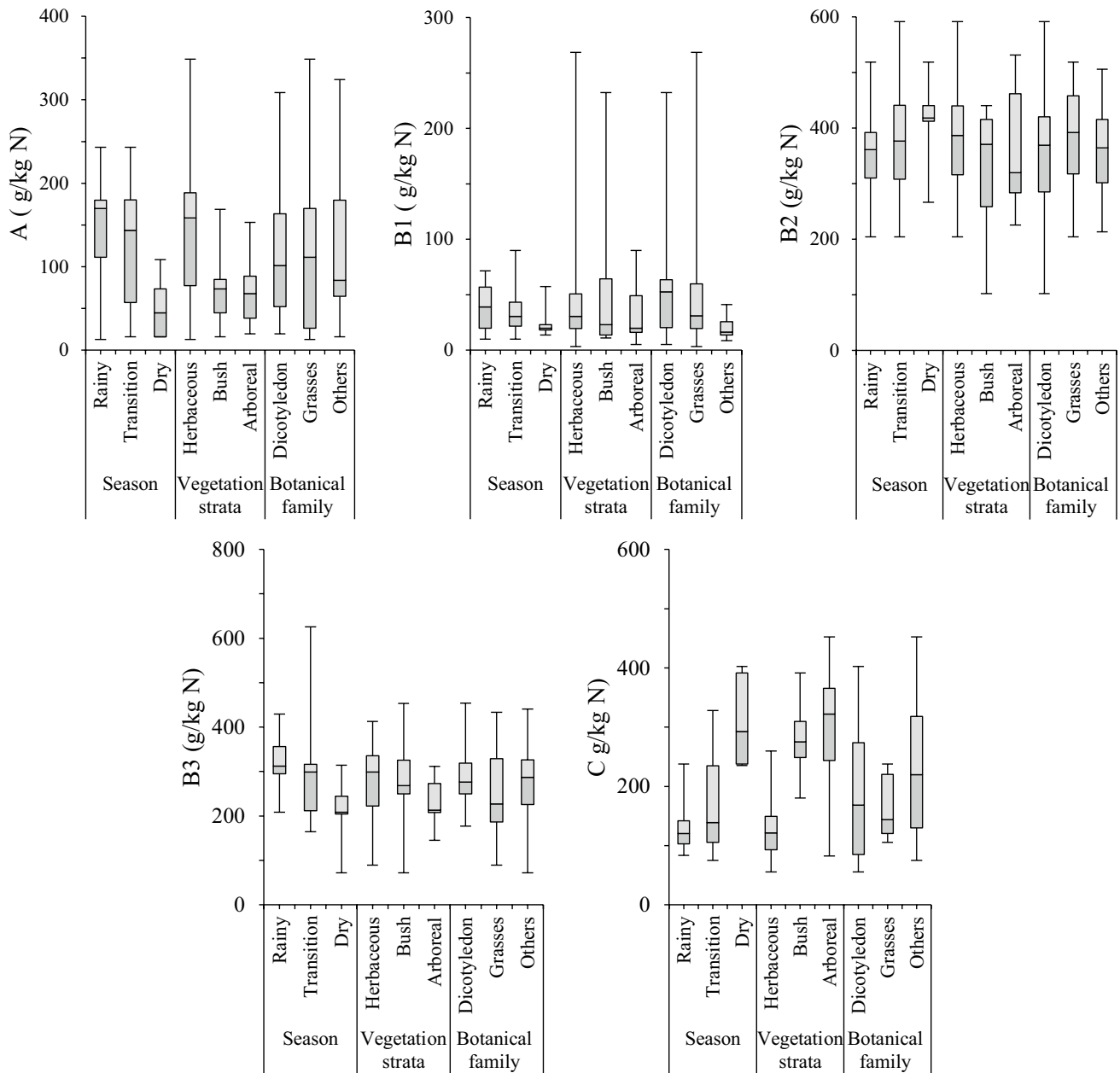


Fig. 7 Nitrogenous fractions of forage by season, vegetation strata, and botanical family selectively grazed by ewes on native Caatinga rangeland near Cariré, Ceará state, Brazil. Nitrogenous fractions:

A=non-protein N; B1=truly digestible protein; B2=N of intermediate degradation (Sniffen et al. (1992), modified by Licitra et al. (1996))

Arboreal species contained high C carbohydrate fractions, primarily during the dry season (Fig. 5). Forage available to ewes from those plants also contained high levels of ADF and lignin which can limit consumption and DM degradability, with negative consequences on dietary energy available to ruminants. One of the management alternatives to overcome this limitation when forages comprise the primary dietary component is to feed high-energy supplements (Pereira et al., 2010) as we did in our study.

The IVDMD of the selected forage, composed mainly of leaves, stems and leaf litter, during the dry season, was less than 0.50 kg/kg forage DM, likely because of high C fraction content. This result is compatible with the one obtained in the Caatinga do Semi-arid area in the dry season by Araújo et al. (2019), 0.43 kg/kg forage DM, which indicates high forage lignification and unavailability of N, with a negative impact on IVDMD. Thus, strategies for the maintenance and production of herds under these conditions

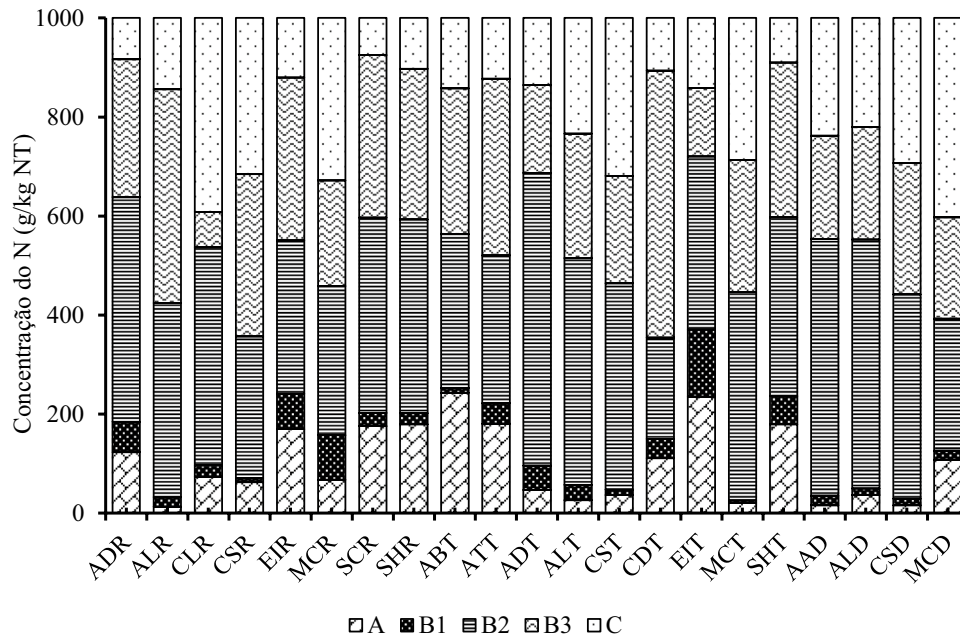
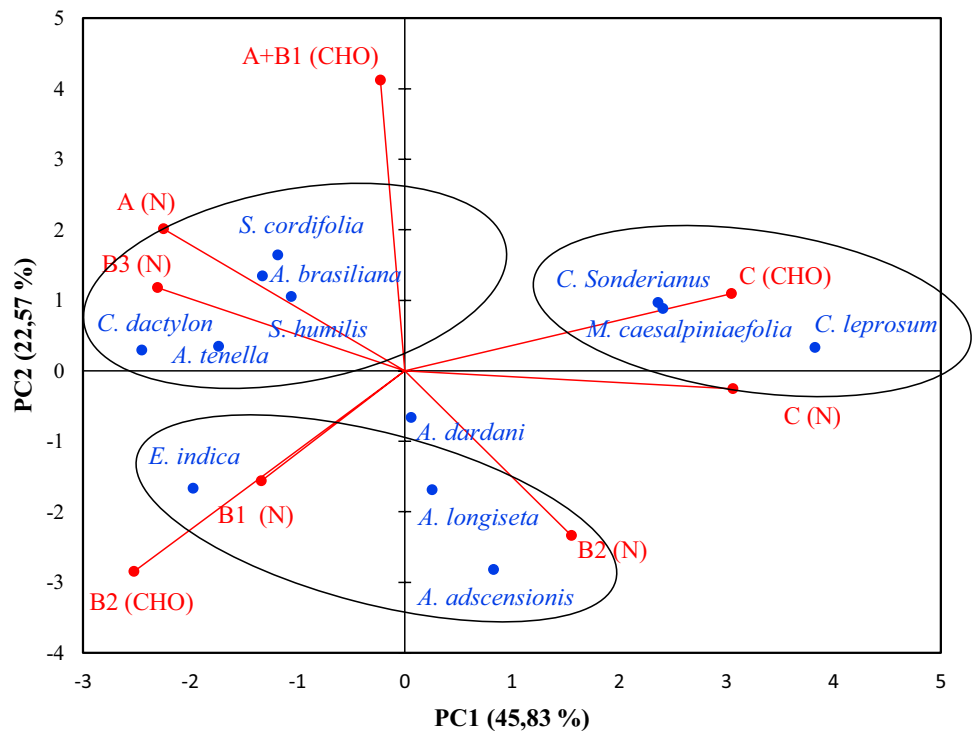


Fig. 8 Nitrogenous fractions of forage species selectively grazed by ewes on native Caatinga rangeland during the rainy, transition, and dry seasons near Cariré, Ceará state, Brazil. TN total nitrogenous; Rainy season: ADR *A. dardani*, ALR *A. longiseta*, CLR *C. leprosum*, CSR *C. sonderianus*, EIR *E. indica*, MCR *M. caesalpiniaefolia*, SCR *S. cordifolia*, SHR *S. humilis*. Transition season: ABT *A. brasiliana*, ATT *A. tenella*, ADT *A. dardani*, ALT *A. longiseta*, CST *C. sonde-*

rianus, CDT *C. dactylon*, EIT *E. indica*, MCT *M. caesalpiniaefolia*, SHT *S. humilis*. Dry season: AAD *A. adscensionis*, ALD *A. longiseta*, CSD *C. sonderianus*, MCD *M. caesalpiniaefolia*. Nitrogenous fractions: A=non-protein N; B1=truly digestible protein; B2=N of intermediate degradation; B3=protein insoluble in neutral detergent; C=undigestible N (Sniffen et al. (1992), modified by Licitra et al. (1996))

Fig. 9 Principal component analysis of total carbohydrate (CHO) and nitrogenous fractions (N) of native Caatinga rangeland forage species selectively grazed by ewes near Cariré, Ceará state, Brazil. Nitrogenous fractions: A = non-protein N; B1 = truly digestible protein; B2 = N of intermediate degradation; carbohydrate fractions: A = carbohydrate rapidly degradable; B1 = intermediate degradation; B2 = slowly degradable; C = unavailable fraction (Sniffen et al. (1992), >modified by Licitra et al. (1996))



can be recommended, with the adoption of supplementation alternatives with an emphasis on the potential availability of protein and energy for the rumen microbiota.

Forage species with greater CP content also contained greater proportions of soluble N fractions. This can result in greater N losses to volatilization and less efficient supply of amino acids to rumen microorganisms (Russell et al., 1992). It can also compromise true protein bypass from the rumen to the ruminant gastrointestinal tract, a loss of important amino acids for animals grazing native forages such as Caatinga (Van Soest, 1994). That characteristic of forage available to ewes supports our findings that non-fibrous carbohydrate supplements to ewes are justified when they graze Caatinga.

Due to the elevated proportion of B1 fractions, primarily during the rainy season, in some dicotyledonous species such as *A. dardani* and grasses such as *E. indica* (Fig. 8), the maintenance of these species in the definition of pasture management strategy may favor the use CP and animal performance. Fraction B1 consists of soluble proteins (peptides and oligopeptides) and tends to be mostly degradable by rumen microorganisms, thereby contributing to their N requirements (Sniffen et al., 1992).

The biomass of bacteria that ferment carbohydrates in the rumen can increase when there are greater forage A and B1 N fractions in herbage in the rumen (Soltan et al., 2012). As such, when Caatinga or other rangeland pastures contain large proportions of species such as *A. brasiliana*, *S. humilis*, *S. cordifolia*, *A. dardani*, and *E. indica* and these are ingested by ewe, concentrate supplement feeding strategies should favor the digestion of these nutrients.

B2 represented the greatest N fraction in herbaceous dicotyledons, primarily in the rainy season (Fig. 7). This fraction has an intermediate rumen degradation rate. According to Sniffen et al. (1992), fraction B2 can be either a source of amino acids and peptides for rumen microbes or it can escape rumen degradation and provide true digestible protein in ruminant intestines.

Forage species *A. dardani*, *S. humilis*, and *S. cordifolia* contained high fractions of N B2, primarily during the rainy season (Fig. 8). As such, understanding this fraction is important to ewe nutritional management because of its effectively degradable characteristics which relate to passage rate and, consequently degradation/passage rate ratios (Singh et al., 2014). The extent of degradation is an important indicator of availability and absorption of rumen and intestinal N compounds. As such, it should be considered during forage evaluation when defining feed supplement strategies for small ruminants in Caatinga and other similar rangelands around the world.

The increase of N fraction B3 can result in more non-degradable CP in the rumen and inadequate ammonial N, reducing microbial population growth and causing

subsequent decreases in forage fiber fermentation (Soltan et al., 2012). The proportion of N B3 fraction was directly proportional to N fraction B2 in the same forages. Because fraction B3 represents protein adhered to cell walls, with some potential for slow degradation in the rumen (Pereira et al., 2010), forage species with greater B3 N fractions, such as *C. dactylon*, *A. tenella* and *A. longiseta* (Fig. 8), can release N slowly in the rumen. As such, an increase in N B3 can result in greater content of protein not degradable in the rumen which reduces the growth of rumen microbial populations and fiber fermentation (Soltan et al., 2012).

The forage species *C. sonderianus*, *C. leprosum*, and *M. caesalpiniaefolia* contained greater proportions of fraction C protein compounds (Fig. 8) than other species. Fraction C consists of protein associated to lignin, tannins and Maillard products, all highly resistant to rumen microbial and enzymatic degradation (Licitra et al., 1996). As such it provides few to no amino acids for absorption in the ruminant intestinal system (Vieira et al., 2000). The Euphorbiaceae *C. Sonderianus*, a brushy perennial that heavily colonizes many Caatinga rangelands, was observed in high densities in our trial (Table 2) and is a species of low forage value because of C proteins (Carvalho et al., 2001).

Among forage species present in ewe diets, herbaceous dicotyledons had greater soluble carbohydrate and N fractions compared to other species. Grasses, by contrast, were generally of more intermediate IVDMD, while arboreal species had greater undegradable carbohydrates and N compound fractions (Fig. 9).

The only components with dissimilar chemical composition and groupings consisted of *C. Dactylon* which was most similar to the herbaceous dicotyledons and *A. dardani* which most approximated the grasses (Fig. 9). *C. dactylon* exhibited a high proportion of N B3, likely a result of lower CP degradability than the other grasses grazed. *A. dardani* had greater degradability potential compared to other legumes selected by ewes, possibly because it contained high B2 levels. These phenomenon may have arisen from ecosystem alterations in some of the areas evaluated in our trial. In contrast to other grasses, *C. dactylon* developed in shaded areas. The Fabaceae *A. dardani* was more abundant in humid areas where soil fertility was also greater, both of which may have affected its chemical composition.

In general, forage species in the same botanical family which grew in the same season had similar phenotypical cycles. However, Caatinga forage species can have disparate growth cycles, likely associated to different plant survival strategies and environmental conditions such as conservation management strategies or soil chemical and physical properties (Silva et al., 2011).

The Caatinga forage composition in our trial and ewe selection from the species available to them varied over seasons and reflected various plant adaptation strategies

to rainfall patterns. These strategies impacted nutrients on offer as well as ewe diet selection with the greatest nutritive value measured during the rainy season. Based on these results, fiber fractions, CP, NDIN, and ADIN were the most important chemical components for measuring Caatinga forage nutritive value among the plant species selected by ewes. Soluble and insoluble carbohydrate fractions as well as N compounds also contributed to our understanding. We recommend that these forage characteristics be included in herbage evaluation when determining supplement strategies in Caatinga and similar rangelands elsewhere around the world.

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Author contribution WFC: Investigation, Data curation, Methodology, Writing — original draft, Writing — review and editing. AAA: Investigation, Methodology, Supervision, Writing — original draft, Writing — review and editing. RCFPP: Investigation, Methodology, Writing — original draft, Writing — review and editing. ARA: Investigation, Methodology, Writing — review and editing. FEPPF: Investigation, Methodology, Writing — review and editing. CSC: Data curation: Formal Analysis, Software. DSO: Investigation, Writing — review and editing. HQM: Investigation, Methodology. LFG: Investigation, Writing — review and editing. JPM: Investigation, Writing — original draft, Writing — review and editing. MCPR: Investigation, Conceptualization, Project administration, Methodology, Resources, Writing — original draft, Writing — review and editing.

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Data availability The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval The experiment was conducted following the guidelines for animal well-being provided by CONCEA (National Council for Animal Experimentation Control) of Brazilian government. All procedures and protocols involving the use of animals were approved by the ethics committee on animal use of the Brazilian Agricultural Research Corporation – Embrapa Sheep and Goats (protocol number 009/2015) and Federal University of Piauí (protocol number 496/2018).

Consent to participate All authors were participated this manuscript.

Consent for publication All authors have consented to the submission of the manuscript in this journal.

Conflict of interest The authors declare no competing interests.

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