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Research Article

Nutritional, physiological, hematological, and biochemical responses of lambs fed increasing levels of Pornunça silage

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ABSTRACT: Pornunça (*Manihot* sp.) is a potential forage to feed ruminants in drylands worldwide; however, evaluations of animal diets are necessary. This study assessed intake and digestibility of dry matter and nutrients, physiological responses, ingestive behavior, water intake, as well as hematological and biochemical responses of lambs fed diets containing increasing levels of Pornunça silage (PS) replacing Tifton-85 bermudagrass hay (*Cynodon* spp.). Treatments consisted of 0, 33, 66, and 100 % PS considering the roughage portion of the diet (% dry matter – DM). The experimental design was completely randomized with six replicates. Cyanide acid (HCN) levels in fresh Pornunça leaves were 207.7 mg kg^{-1} DM and 76.78 mg kg^{-1} DM in PS. Intake of DM and crude protein (CP), and neutral detergent fiber corrected for ash and protein (NDFap) increased linearly with increasing levels of PS in the diet. Digestibility of DM, NDF, and CP were higher with increasing levels of PS. Idle time decreased linearly, while water balance, water intake via food, and total water in feces increased with PS. Nitrogen balance, physiological, and hematological responses were not influenced by treatments, while the final alanine aminotransferase increased for lambs fed all diets evaluated. The PS up to 100 % of the roughage portion increased DM and nutrient intake and digestibility without altering physiological responses and the hematological and biochemical parameters, resulting in greater water intake via food.

Keywords: *Manihot* sp., feed alternative, forage, semi-arid

Introduction

Pornunça (*Manihot* sp.) is a natural hybrid between cassava and wild cassava used as forage in the Brazilian semi-arid region (Silva et al., 2017), with potential for use in other drylands worldwide. Pornunça shows satisfactory forage yield, considering the Brazilian semi-arid environment (Alencar et al., 2019), and suitable quality of fresh forage or silage (Voltolini et al., 2019).

According to Carvalho et al. (2017), Pornunça silage presents pH = 3.7, N-NH_3/total = 5.5, acid lactic 3.85, acetic 1.13, propionic 0.36, and butyric 0.01 (% dry matter – DM). Thus, demonstrating an adequate fermentation profile and providing considerable productive performance, caracass traits (Campos et al., 2019) and meat sensory properties (Campos et al., 2017), presenting as alternative feed for ruminants.

Pornunça, similar to other plants of the genus *Manihot*, contains cyanogenic glycosides that generate cyanide acid (HCN) when hydrolyzed, which can be toxic to the animal depending on the amount ingested (Beltrão et al., 2015). The conservation of Pornunça as silage reduces HCN concentration and is considered a safe strategy to feed ruminants (Matos et al., 2005).

Tropical grass hay, such as Tifton-85 bermudagrass (*Cynodon* spp.), is a traditional ingredient for ruminant feeding; nevertheless, this grass has limited availability and is costly for purchase. Thus, replacing Tifton-85 with Pornunça silage can reduce production costs for farmers and expand roughage alternatives for animal feeding in dry areas. However, the ideal levels of Pornunça silage to be added to diets for lambs are not well established, requiring studies to define the inclusion level for positive effects on intake and nutrient digestibility as well as biochemical and hematological responses.

We hypothesized that Pornunça silage can replace Tifton-85 hay in lamb diets without reducing productive performance, hematological, and biochemical physiological parameters. This study evaluated the effects of replacing Tifton-85 hay with Pornunça silage in the roughage portion of diets of feedlot lambs on intake, digestibility of dry matter and nutrients, physiological responses, ingestive behavior, water consumption, and hematological and biochemical parameters.

Materials and Methods

Ethical aspects

The project was submitted to the Animal Research Ethics Committee (CEUA) of the Brazilian Agricultural Research Company - Embrapa Semi-arid and approved under registration protocol number 06/2018.

Location, animals, facilities, experimental period

The experiment was carried in the municipality of Petrolina, Pernambuco State, Brazil (9°19′19.6″ S, 40°33′40.8″ W, altitude of 379 m). Twenty-four male, non-castrated, non-defined breed, with 22.28 kg of initial body weight were housed in a covered shed, ventilated, in individual metabolic cages, with individual feeders and drinkers. The study lasted 21 days, 16 days for adaptation and five days for data collection.

Treatments and experimental design

Pornunça was harvested six months after regrowth. The forage material was chopped (2.0 to 2.5 cm of particle size), homogenized, and compacted in plastic drums with a capacity of 200 L, at a density of 660 kg fresh matter m^{-3} and stored for 60 days.

The treatments consisted of levels of replacement of Tifton-85 hay with Pornunça silage (0, 33, 66 and 100 % roughage portion of the diet, in % DM), denominated 0 %PS, 33 %PS, 66 %PS and 100 %PS, in a completely randomized experimental design with six replicates.

Diets were prepared and balanced to provide 200 g d^{-1} average daily gain (ADG) (NRC, 2007). The concentrate was composed of ground corn, soybean meal, wheat meal, and mineral salt. The roughages used (Tifton-85 and PS) were chopped.

Diets were provided as a total mixed ration, twice a day, at 9h00 a.m. and 3h00 p.m. Water was offered *ad libitum*, the animals were fed according to the dry matter intake (DMI) of the previous day and it was calculated to allow for 10 % leftovers.

Chemical analyses

Ingredients, diets, and leftovers samples were pre-dried in an oven at 55 °C. The chemical composition was determined following the recommendations of AOAC (1990) for DM contents (method 934.01), ash (method 930.05), and crude protein (CP) (method 981.10). The ether extract (EE) was determined according to AOCS (2017). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991), and the lignin content according to Van Soest and Wine (1967). The NDF was corrected for protein and ash (NDFap) (Licitra et al., 1996).

Non-fiber carbohydrates (NFC) were calculated according to Detmann and Valadares Filho (2010), and total carbohydrates (TC) were estimated according to Sniffen et al. (1992). Tables 1 and 2 list the chemical composition of ingredients and diets, respectively.

The quantification of cyanide acid (HCN) in leaves of fresh Pornunça and Pornunça silage was carried out according to Ades Totah and Hernandes Luís (1986).

Intake and digestibility of dry matter and nutrients

The DMI and nutrient intake [organic matter (OM), NDFap, CP, EE, TC and NFC] were obtained by the difference of total feed offered daily and daily leftovers (% DM). The DM and nutrient digestibility were evaluated for five consecutive days, using bags for total collection of feces. Feces were collected, weighed, and sampled (10 % total excreted) twice a day. Urine was collected in plastic buckets containing 100 mL 2N hydrochloric acid.

A composite sample from each animal was obtained at the end of the collection period for the chemical analyses. The apparent digestibility coefficients (DC) of DM, OM, NDFap, CP, EE, TC and NFC were calculated using the equation: DC = [(kg ingested fraction – kg excreted fraction)] / (kg ingested fraction) × 100.

Table 1 – Chemical composition (% dry matter-DM) of the ingredients used in the diets.

Fraction	Ingredients, % dry matter (DM)				
	Tifton-85 hay	Pornunça silage	Soybean meal	Corn grain (ground)	Wheat bran
Dry matter *(%)	80.12	27.67	93.09	91.13	92.40
Organic matter	93.13	92.05	93.29	98.57	98.62
Ash	6.47	7.95	6.71	1.43	0.69
Crude protein	7.12	15.0	47.80	8.56	14.26
NDFap	51.37	53.67	13.36	13.52	35.27
ADF	32.10	27.77	9.86	4.11	9.62
Ether extract	2.36	5.23	1.93	4.23	3.91
Total carbohydrate	70.48	71.0	43.56	85.78	73.37
NFC	9.78	13.2	30.20	72.26	32.16

*% as feed; NDFap = neutral detergent fiber corrected for ash and protein; ADF = acid detergent fiber; NFC = non-fiber carbohydrates.

Table 2 – Rates of ingredients and chemical composition of diets.

Ingredient (% dry matter)	Pornunça silage (% dry matter in roughage portion)			
	0	33	66	100
Tifton-85 hay	45.0	30.1	15.2	0
Pornunça silage	0	14.9	29.9	45.0
Ground corn	40.0	39.0	38.0	38.7
Soybean meal	13.0	10.0	8.7	9.5
Wheat bran	1.0	5.0	7.2	5.8
Mineral mix[1]	1.0	1.0	1.0	1.0
Chemical composition (% dry matter)				
Dry matter, %*	87.66	69.72	57.42	56.43
Organic matter	91.88	92.76	92.60	92.88
Ash	8.12	7.24	7.40	7.12
Crude protein	13.00	13.12	14.38	15.00
NDFap	30.61	31.80	32.69	32.70
ADF	18.97	18.44	18.58	16.19
Ether extract	5.72	5.24	6.66	7.05
Total carbohydrate	67.98	71.44	63.20	68.46
NFC	20.00	29.27	29.07	33.60
Lignin	13.90	11.30	9.65	10.70

*% as feed; NDFap = neutral detergent fiber corrected for ash and protein; ADF = acid detergent fiber; NFC = non-fiber carbohydrates; [1]Mineral mix = Calcium (Ca) - 140 g; Phosphorus (P) - 70 g; Magnesium (Mg) - 1,320 mg; Iron (Fe) - 2,200 mg; Cobalt (Co) - 140 mg; Manganese (Mn) - 3,690 mg; Zinc (Zn) - 4,700 mg; Iodine (I) - 61 mg; Selenium (Se) - 45 mg; Sulfur (S) - 12 g; Sodium (Na) - 148 g; Fluorine (F) - 700 mg.

Water and nitrogen balance

Water was supplied *ad libitum*. Additionally, three buckets containing water were randomly distributed in the shed to determine evaporation. The free water intake (FWI) was determined by the difference of water supplied and water remained, discounting the loss of water by evaporation. Water intake via diet (WID) was calculated by the difference of water in the diet and in leftovers.

The water balance was determined based on the following equations: Total water intake (kg d^{-1}) (TWI) = free water intake + water intake via diet; total excretion of water (kg d^{-1}) (TWE) = water excreted in urine + water excreted in feces; absorbed water (g d^{-1}) (AW) = TWI - water excreted in urine; water retained (kg d^{-1}) (Wret) = TWI – TWE; water balance (%WB) = (water retained/TWI) × 100.

Nitrogen balance was determined as the difference of total N ingested (Ning) and total N excreted in feces (Nfec) and in urine (Nur). Nitrogen absorbed (Nabs) was estimated considering: Nabs = Ning – Nfec and nitrogen retained (Nret): Nret = Ning – (Nfec + Nur). Nitrogen balance (NB) was estimated as NB (%) = [Ning – (Nfec + Nur) / Ning] × 100.

Ingestive behavior

Ingestive behavior assessments were performed on the 9th day for 24 h uninterrupted. Observations were recorded every 5 min considering the following activities: intake, rumination, or idle.

Physiological responses and environmental variables

Physiological parameters were measured on the 8th and 15th days, in the morning (8h00 a.m.) and afternoon (2h00 p.m.). Rectal temperature (RT) was measured by introducing a digital clinical thermometer into the rectum of the animals. Respiratory rate (RR) was subjectively measured by a single evaluator, observing the movements of the animal flanks (breath min^{-1}). Heart rate (HR) was measured by auscultating the left side of the animals, on the first rib, using a stethoscope (beat min^{-1}).

The body surface temperature was measured using an infrared laser thermometer on the head, neck, hock, and flank.

Air temperature (°C) and relative humidity (RH, %) were evaluated using a digital thermo-hygrometer HT-700 at three different times every day: at 9h00 a.m., 12h00 p.m., and 3h00 p.m. (Table 3). The temperature-humidity index (THI) was calculated according to Buffington et al. (1981), considering as < 82 = no heat stress; 82 to < 84 = moderate heat stress; 84 to < 86 = severe heat stress, and over 86 = extreme severe heat stress (Marai et al., 2007).

Table 3 – Air temperature, relative humidity (RH), and temperature-humidity index (THI) recorded in the shed (indoor) during the experimental period.

Variable	Morning	Afternoon	Average
Air temperature (°C)	27.2	30.9	29.0
THI	71.5	80.6	75.8
RH (%)	63.2	40.8	52.0

Hematological and biochemical parameters

Blood samples were taken on the 1st day of adaptation and on the 21st day of the experimental period. The jugular vein was punctured and 2 mL blood was drawn, using K50-1302S tubes with EDTA (ethylenediamine tetra-acetic acid) and K50-305M tubes without EDTA. Immediately after collection, blood samples were refrigerated and taken to laboratory for microscopy and the analyses with a magnifying glass. The samples were analyzed for white series (total leukocytes), complete red blood cell (erythrogram), and platelet count.

The analyses were performed using Bioclin analysis kits, determining albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, urea (URE), and creatinine (CREA).

Statistical analysis

Data were checked for normal distribution and tested by the analysis of variance followed by linear and quadratic regression, using the statistical software SAS (Statistical Analysis System). Significance was declared when was $p \leq 0.05$.

Results and Discussion

The HCN in the fresh plant and in the PS were 207.7 mg kg^{-1} DM and 76.78 mg kg^{-1} DM, respectively, demonstrating that ensiling was efficient to reduce HCN levels. Matos et al. (2005) found 972 mg kg^{-1} HCN for fresh maniçoba and reported 162 mg kg^{-1} for maniçoba silage. The final body weight of animals was 22.33, 26.00, 29.35, and 28.33 kg for groups fed 0, 33, 66, and 100 % PS, respectively.

The DMI and the intake of OM, EE, CP, NDFap, TC and NFC increased linearly with the inclusion of PS replacing Tifton-85 hay (Table 4), due to the increase in NFC (Table 2) and high nutrient digestibility (NDFap, TC and CP) (Table 4). Lower fiber digestibility results in rumen filling, which limits feed intake, while high fiber contents reduce ruminal degradation and decrease the passage rate of the fiber fraction. According to Allen et al. (2019), the forage NDF content and the digestibility of forage NDF are the main factors affecting the rumen filling effect.

The highest DMI provided by increasing PS levels in the diets resulted in a greater nutrient intake, increasing intake of CP, NDF, ash, EE, TC, and NFC in feedlot lambs (Table 4).

The inclusion of PS increased linearly the digestibility of DM and nutrients (CP, OM, TC, NFC), except for EE. The PS presented lower lignin and ADF contents (Table 2) as well as ower lignin/NDF ratio. Huhtanen and Kahlili (1991) reported a negative relationship between NDF digestibility and total NDF in the rumen. Therefore, fiber acts limiting the disappearance rate of the material in the digestive tract.

Animals fed 0 % PS diets presented the lowest TC digestibility (Table 4), which is related to the high fiber digestibility and cell content.

The replacement of Tifton-85 hay with PS did not influence the time of feeding and ruminating as well as the efficiency of ingestion and rumination of DM and NDF of lambs (Table 5). However, the increase of PS levels reduced the iddle time. Animals fed 0 % PS spent more time idling. The reduction in idle time is atributed to higher nutritional values of diets containing PS (Table 2) and greater nutrient supply due to greater intake and digestibility (Table 4) provided by PS.

The PS levels incluced to replace Tifton-85 hay did not influence FWI, TWI, WU (water in urine), WF (water in feces), Wret, and WB. There was a positive linear effect of PS inclusion on WID and TWE (Table 6).

The greater water intake via food with increasing PS levels in the diets can be explained by greater water content in this ingredient (27.67 % DM) compared to Tifton-85 hay (80.12 % DM) (Table 1), resulting in diets with lower DM contents (Table 2).

Water is a scarce natural resource in semi-arid regions and its offer via diet, such as the Pornunça silage, saves drinking water (Araújo, 2015). In this study, WID increased proportionally with the PS inclusion in the diets resulting in 19.44 %, 26.33 %, and 28.69 % in relation to the total water ingested daily for 33 %, 66 %, and 100 % PS, respectively. Lambs fed 0 % PS offset the lower WID by the intake of free water; therefore, there was no effect on the TWI.

Diets contatining higher levels of Pornunça silage showed higher water content, promoted greater WID, and provided higher TWE without affecting Wret and WB. All diets resulted in positive WB for feedlot lambs.

The replacement of Tifton-85 hay with PS did not affect Nur, Nfec, and NB. The N ingested, Nabs, and Nret were influenced, presenting higher values with increasing PS levels (Table 6). Greater DMI with increasing PS levels in the diets enhanced CP intake and

Table 4 – Intake and digestibility of dry matter (DM, %) and nutrients from lamb diets based on Pornunça silage replacing Tifton-85 hay, % dry matter.

Variable ($kg\ lamb^{-1}\ d^{-1}$)	% Pornunça silage				SEM	Equation	R^2
	0	33	66	100			
	Intake of DM and nutrients						
DM, % as feed	0.99	1.25	1.22	1.61	0.064	$\hat{Y}$ = 0.96+0.0058x	0.87
Organic matter	0.91	1.16	1.13	1.50	0.060	$\hat{Y}$ = 0.88+ 0.0054x	0.87
Crude protein	0.18	0.20	0.28	0.28	0.010	$\hat{Y}$= 0.18+0.0011x	0.88
NDFap	0.42	0.53	0.53	0.64	0.027	$\hat{Y}$ = 0.41+0.0017x	0.82
Ether extract	0.14	0.13	0.22	0.21	0.004	$\hat{Y}$ = 0.04+0.0005x	0.96
TC	0.67	0.89	0.77	1.11	0.047	$\hat{Y}$ = 0.66+0.0037x	0.70
NFC	0.24	0.37	0.35	0.54	0.024	$\hat{Y}$ = 0.26+0.0023x	0.81
	Digestibility of DM and nutrients						
DM	62.74	67.39	75.14	80.97	2.022	$\hat{Y}$ = 60.90+0.21x	0.96
Organic matter	64.25	68.86	75.66	81.21	1.886	$\hat{Y}$ = 62.62+0.19x	0.95
Crude protein	67.88	67.85	80.06	79.80	1.659	$\hat{Y}$ = 66.75+0.14x	0.79
NDFap	66.07	72.68	71.60	76.64	1.412	$\hat{Y}$ = 65.35+0.086x	0.90
Ether extract	74.22	68.01	86.40	82.16	2.977	$\hat{Y}$ = 77.70	-
TC	62.44	69.15	72.93	81.52	1.936	$\hat{Y}$ = 60.94+0.20x	0.90
NFC	65.28	71.66	74.93	89.96	1.982	$\hat{Y}$ = 64.33+0.19x	0.91

NDFap = neutral detergent fiber corrected for ash and protein; TC = total carbohydrate; NFC = non-fiber carbohydrate. SEM = Standard error of the mean; R^2 = coefficient of determination.

Table 5 – Ingestive behavior of feedlot lambs fed with increasing levels of Pornunça silage (% roughage portion) replacing Tifton-85 hay.

Activity ($min\ d^{-1}$)	% Pornunça silage				SEM	Equation	R^2
	0	33	66	100			
Feeding	213.3	276.7	330.0	293.3	17.00	$\hat{Y}$ = 278.33	-
Rumination	350.0	426.7	541.7	443.3	26.10	$\hat{Y}$ = 440.42	-
Idle	876.7	736.6	568.3	703.4	33.70	$\hat{Y}$ = 549.17+68.33x	0.49

SEM = Standard error of the mean; R^2 = coefficient of determination.

N intake. The higher CP intake combined with higher CP digestibility for 66 % PS and 100 % PS increased Ning and consequently increased Nabs and Nret.

Physiological responses RR, HR, RT, and body surface temperature were not affected by the diets evaluated (Table 7). Indoor temperatures observed during the morning and in the afternoon (Table 3) were above the values considered ideal for sheep (25 °C); however, they were below the upper critical temperature (35 °C). The relative humidity in the afternoon was also below the ideal values (50 to 70 %) (Furtado et al., 2020). In addition, THI (Table 3) indicated no heat stress.

Table 6 – Water and nitrogen balance of lambs fed with increasing levels of Pornunça silage (% roughage portion) replacing Tifton-85 hay.

Variable	Pornunça silage, % DM				SEM	Equation	R^2
	0	33	66	100			
Water balance (kg d^{-1})							
FWI	2.15	2.02	2.20	2.40	0.120	$\hat{Y}$ = 2.19	-
WID	0.09	0.49	0.79	0.97	0.080	$\hat{Y}$ = 0.14+0.0092x	0.98
TWI	2.24	2.52	3.00	3.38	0.132	$\hat{Y}$ = 2.79	-
WU	0.77	0.70	0.86	1.35	0.115	$\hat{Y}$ = 0.92	-
WF	0.36	0.47	0.63	0.62	0.041	$\hat{Y}$ = 0.52	-
TWE	1.14	1.17	1.49	1.98	0.130	$\hat{Y}$ = 1.86+0.0047x	0.45
AW	1.47	1.82	2.14	2.02	0.129	$\hat{Y}$ = 1.86	-
Wret	1.11	1.35	1.51	1.40	0.101	$\hat{Y}$ = 1.34	-
WB	51.84	54.33	49.42	41.85	2.819	$\hat{Y}$ = 49.36	-
Nitrogen balance (kg d^{-1})							
Ning	0.031	0.034	0.048	0.047	0.0025	$\hat{Y}$ = 0.030+0.0002x	0.84
Nur	0.00055	0.00050	0.00040	0.00035	0.000044	$\hat{Y}$ = 0.00045	-
Nfec	0.0088	0.0085	0.0086	0.0088	0.00018	$\hat{Y}$ = 0.0087	-
Nabs	0.022	0.026	0.039	0.039	0.0025	$\hat{Y}$ = 0.022+0.0002x	0.83
Nret	0.022	0.025	0.039	0.038	0.0026	$\hat{Y}$ = 0.02+0.0002x	0.83
NB	68.1	68.5	80.8	80.1	2.10	$\hat{Y}$ = 74.37	-

FWI = Free water intake; WID = Water intake via diet; TWI = Total water intake; WU = Water in urine; WF = Water in feces; TWE = Total water excretion; AW = Absorbed water; Wret = Water retained; WB = Water balance. Ning = Ingested nitrogen; Nur = Nitrogen in urine; Nfec = Nitrogen in feces; Nabs = Nitrogen absorbed; Nret = Nitrogen retained; NB = Nitrogen balance. SEM = Standard error of the mean; R^2 = coefficient of determination.

Table 7 – Physiological responses (respiratory rate, heart rate, rectal temperature, and body surface temperature) measured three times a day in feedlot lambs fed with increasing levels of Pornunça silage (% roughage portion in diets) replacing Tifton-85 hay.

Variable	% Pornunça silage				SEM	Equation	R^2
	0	33	66	100			
Respiratory rate (breath min^{-1})							
09h00 a.m.	58.3	50.0	56.3	52.0	2.37	$\hat{Y}$ = 54.17	-
12h00 p.m.	70.2	68.7	65.7	67.5	2.16	$\hat{Y}$ = 68.00	-
3h00 p.m.	63.3	54.3	56.0	59.3	1.61	$\hat{Y}$ = 58.25	-
Average	63.9	57.7	59.3	59.6	2.05	$\hat{Y}$ = 60.14	-
Heart rate (beat min^{-1})							
09h00 a.m.	113.0	108.7	98.0	118.0	3.76	$\hat{Y}$ = 09.41	-
12h00 p.m.	111.0	98.7	101.7	110.8	2.64	$\hat{Y}$ = 05.54	-
3h00 p.m.	104.3	87.7	93.0	114.3	3.90	$\hat{Y}$ = 99.83	-
Average	109.4	98.3	97.6	114.4	3.43	$\hat{Y}$ = 04.92	-
Rectal temperature (°C)							
09h00 a.m.	36.2	38.9	38.9	39.2	0.73	$\hat{Y}$ = 38.27	-
12h00 p.m.	39.3	39.1	39.1	39.5	0.06	$\hat{Y}$ = 39.25	-
3h00 p.m.	39.3	39.0	38.8	39.3	0.09	$\hat{Y}$ = 39.09	-
Average	38.2	39.0	39.9	39.3	0.29	$\hat{Y}$ = 39.12	-
Body surface temperature (°C)							
09h00 a.m.	31.4	31.1	30.0	31.5	0.14	$\hat{Y}$ = 30.98	-
12h00 p.m.	32.8	32.7	31.2	32.7	0.11	$\hat{Y}$ = 32.33	-
3h00 p.m.	32.2	31.8	33.6	31.5	0.11	$\hat{Y}$ = 32.29	-
Average	32.1	31.9	31.6	31.9	0.12	$\hat{Y}$ = 31.86	-

°C = degrees Celsius; SEM = Standard error of the mean; R^2 = coefficient of determination.

The respiratory rates of 40-60, 60-80, and 80-120 breath min^{-1} are considered low, medium-high, and high stress, respectively. Above 200 breath min^{-1} is considered severe stress (Silanikove, 2000), indicating low thermal stress for lambs fed 33 %, 66 %, and 100 % PS and medium stress for 0 % PS (Table 7), possibly due to the higher DMI and shorter idle time provided for by greater PS levels in the diets. Rectal temperature and body surface temperature were considered normal for lambs fed all diets evaluated. On the other hand, lambs presented tachycardia in the afternoon, considering HR above 70-80 beat min^{-1}.

Hematological parameters (total leukocytes-TL, red blood cells, hemoglobin-HEM, and platelets) were not influenced by increasing PS levels (Table 8). Total leukocytes, red blood cells, hemoglobin, and platelets were normal for lambs, according to Radostits et al. (2000), indicating that an inflamatory process or intoxication did not occur, considering that HCN may increase hemoglobin and red blood cells to offset the oxygen (O_2) transport limitations to tissues.

Alcaline phosphatase (AP) and aspartate aminotransferase (AST) were not affected by diets, while alanine aminotransferase was increased (ALT)

Table 8 – Hematological parameters determined at the beginning (initial) and at the end of the study (final) with sheep fed diets based on Pornunça silage replacing Tifton-85 hay.

Variable	Pornunça silage, %				SEM	Equation	R^2
	0	33	66	100			
Total leukocytes (× 10^3 mm^{-3})							
Initial	12.0	10.0	8.9	10.4	0.12	Ŷ = 10.33	-
Final	9.4	7.4	7.3	6.3	0.07	Ŷ = 7.58	-
Hematocrit (%)							
Initial	38.3	38.5	36.6	34.0	1.39	Ŷ = 36.83	-
Final	30.8	32.0	32.7	32.8	0.60	Ŷ = 32.06	-
Red blood cells (× 10^6 mm^{-3})							
Initial	13.4	13.0	12.5	11.1	0.47	Ŷ = 12.50	-
Final	11.8	11.3	12.0	11.6	0.30	Ŷ = 11.67	-
Hemoglobin (g dL^{-1})							
Initial	9.6	10.1	9.2	8.5	0.29	Ŷ = 9.36	-
Final	10.9	10.8	11.3	11.3	0.21	Ŷ = 11.08	-
Platelets × 10^9 L^{-1}							
Initial	622.0	707.7	585.2	506.1	47.50	Ŷ = 605.30	-
Final	632.5	447.5	623.7	518.0	39.90	Ŷ = 555.43	-

SEM = Standard error of the mean; R^2 = coefficient of determination.

Table 9 – Serum biochemical parameters determined in the beginning (initial) and at the end of the study (final) with sheep fed diets based on Pornunça silage replacing Tifton-85 hay.

Variable	Pornunça silage, %				SEM	Equation	R^2
	0	33	66	100			
Albumin (mg dL^{-1})							
Initial	3.7	3.6	3.9	3.5	0.22	Ŷ = 3.68	-
Final	5.7	5.0	7.5	5.6	0.40	Ŷ = 5.95	-
Alkaline phosphatase (IU L^{-1})							
Initial	81.3	169.1	121.6	159.9	18.72	Ŷ = 132.98	-
Final	344.6	276.2	280.3	314.3	47.21	Ŷ = 303.85	-
Creatinine (mg dL^{-1})							
Initial	0.96	1.52	1.52	1.01	0.110	Ŷ = 1.30	-
Final	0.93	0.92	1.09	0.91	0.060	Ŷ = 0.96	-
Urea (IU L^{-1})							
Initial	64.5	57.8	75.3	63.3	3.37	Ŷ = 65.22	-
Final	115.0	95.6	100.9	105.4	8.80	Ŷ = 104.22	-
Alanine aminotransferase (IU L^{-1})							
Initial	4.1	3.4	5.6	5.8	0.11	Ŷ = 4.72	-
Final	6.7	6.6	7.0	6.5	0.71	Ŷ = 0.66+0.0006x	0.02
Aspartate aminotransferase (IU L^{-1})							
Initial	178	176	121	172	0.01	Ŷ = 161.75	-
Final	136	138	129	135	0.77	Ŷ = 134.50	-

SEM = Standard error of the mean; R^2 = coefficient of determination.

(Table 9). However, the concentrations of these enzimes are considered normal for lambs, according to Radotists et al. (2000), indicating adequate liver function and absence of metabolic disturbances. Cassava plants are rich in carotenoids, precursor of vitamins A, C, and B complex, and may activate ALT, increasing its concentration.

Albumin (ALB) concentration was not influenced by the replacement of Tifton-85 hay with PS in lamb diets (Table 9). Albumin is a sensitive marker for a protein nutritional status, suggesting high protein supply. Pornunça silage presented high protein content resulting in high protein intake. In addition, PS diets provided high protein digestibility.

Urea and creatinine were not affected by increasing levels of PS replacing Tifton-85 hay (Table 9). Values of initial and final urea were high, possibly due to the adequate supply of protein and protein/energy ratio to the lambs, increasing the serum ammonia that is transformed into urea, increasing the circulating urea (Menezes et al., 2012).

The initial and final values of creatinine were below normal, demonstrating that creatinine was not affected by diets. This is used as a reference for correcting changes that may occur in blood urea variations. Therefore, creatinine is generally used to distinghish whether the increase in urea is due to prerenal causes, such as factors related to feed or kidneys. Low creatinine can be explained by compensation or correction of serum urea.

Our results demonstrate that the replacement of Tifton-85 hay with Pornunça silage, including up to 100 % roughage portion in diets for lambs, increased DM and nutrient intake and reduced idle time due to the lower lignin contents and higher NDF digestibility. The greater feed intake and the higher water concentration of Pornunça silage diets increased the amount of WID. Diets composed of Pornunça silage did not promote changes in the main physiological, biochemical, and hematological responses.

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