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Apparent digestibility and rumen protozoal profile of sheep fed cassava wastewater

Digestibilidade aparente e perfil de protozoários no rúmen em ovinos alimentados com manipueira

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SUMMARY

The effects of cassava wastewater on nutrient digestibility and rumen protozoa were evaluated in 32 Santa Inês sheep fed a diet with Tifton hay, ground corn cob, and concentrate, containing 0, 500, 1000, or 1500 mL cassava wastewater. Uncastrated sheep at an average age of 167 days and average weight of 25.3 kg were housed in individual stalls and distributed in a randomized complete design with four treatments and eight replicates. The experimental period was 70 days: 15 days for adaptation to the diets and 60 days to collect the ingredients and diets supplied. Prior to this stage, the animals were adapted to the consumption of cassava wastewater, in collective cages, for seven days. Apparent digestibility was determined by partial feces collection and by using the LIPE® external marker in 250-mg capsules. The apparent digestibility of nutrients did not differ (P>0.05) in relation to the cassava wastewater levels tested. Eleven genera of rumen protozoa were identified, and they formed a community that, except for *Ophryoscolex*, did not differ among the cassava wastewater treatments with respect to composition. However, *Entodinium* species were observed at a higher density (P<0.05) in sheep that received 1500 mL of cassava wastewater. The use of cassava wastewater in the sheep diet does not affect the digestibility of nutrients or composition of rumen protozoa; however, it causes a significant increase in *Entodinium* density.

Keywords: alternative feeds, cassava wastewater, ciliate protozoa, *Manihot esculenta*, small ruminants

RESUMO

Os efeitos da manipueira sobre a digestibilidade de nutrientes e protozoários ruminais foram avaliados utilizando-se 32 ovinos Santa Inês submetidos a dietas compostas por feno de Tifton, rolão de milho e concentrado, acrescidas de 0, 500, 1000 ou 1500 mL de manipueira. Foram utilizados ovinos não castrados com idade media de 167 dias e com peso médio e aproximadamente 25,3 kg. Foram alojados em gaiolas individuais e distribuídos em delineamento inteiramente casualizado com quatro tratamentos e oito repetições. O período experimental foi de 70 dias: 15 dias para adaptação às dietas e 60 dias para coleta dos ingredientes e rações. Antes desta fase, os animais foram adaptados ao consumo de manipueira em gaiolas coletivas durante um período de 7 dias. A digestibilidade aparente foi determinada pela coleta parcial de fezes e pelo uso do marcador externo LIPE® em cápsulas de 250 mg.A digestibilidade aparente dos nutrientes avaliados não diferiu (P>0,05) em relação aos níveis de manipueira estudados.

Onze gêneros de protozoários foram identificados, compondo uma comunidade que à exceção de *Ophryoscolex*, não diferiu entre os tratamentos em sua composição. Entretanto, as espécies de *Entodinium* apresentaram maior densidade (P<0,05) em ovinos que receberam 1500 mL de manipueira. O uso da manipueira na dieta de ovinos não afeta a digestibilidade de nutrientes e composição de protozoários ruminais, entretanto, promove aumento significativo na densidade de *Entodinium*.

Palavras-chave: alimentos alternativos, *Manihot esculenta*, manipueira, pequenos ruminantes, protozoários ciliados

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is grown in all Brazilian states (CENI et al., 2009) and used to produce starch and flour. This production generates a large amount of solids (plant shoots, peel, zest, and processing waste bran) and liquid (water used to wash and press the roots) wastes that can cause serious environmental problems (MENEGHETTI & DOMINGUES, 2008).

Cassava wastewater is a liquid waste resulting from cassava flour production or starch extraction (MENEGHETTI & DOMINGUES, 2008). Cassava wastewater has a high residual starch content, medium fiber and protein contents, and low dry matter (DM) (ABRAHÃO et al., 2006), although its nutritional composition may vary according to the quality of cassava and the processing method used.

Because of its chemical composition, cassava wastewater can be considered a food with potential use in animal feeding, and it can also be used to integrate regional strategic programs for ruminant supplementation (SANTANA NETO, 2013). However, cassava wastewater must be maintained for a rest period of three to five days for cyanide and hydrogen cyanide to volatilize to non-toxic levels for ruminants (ALMEIDA et al., 2009).

Although several studies have evaluated the effect of other cassava wastes on the physiology and productivity of ruminants, there are few studies on the use of cassava wastewater in ruminant feed (ALMEIDA et al., 2009). It has also been observed that the use of cassava wastewater in the pre-slaughter diet of sheep did not provide weight gains or changes in nutrient intake or in the quantitative characteristics of the animal carcass. However, water intake can be reduced depending on the amount of cassava wastewater provided, which is favorable in regions such as Northeastern Brazil, where water availability is critical in some months of the year (SANTANA NETO, 2013).

Moreover, there are no studies in the literature evaluating the effects of cassava wastewater on the rumen microbial population, and the few studies with cassava wastewater in ruminant feed address performance, intake, digestibility, and carcass characteristics and indicate a reduction in the above-mentioned variables (ALMEIDA et al., 2009; SANTOS FILHO et al., 2015; URBANO et al., 2015).

Because the protozoal population growth is influenced by the diet, it is important to quantify and evaluate their activity in response to diets provided to ruminants for adjustments in feed formulations (Lima et al., 2012), since these have a large participation in the hemicellulolytic and cellulolytic activities (Franzolin & Franzolin, 2000), directly influencing the digestibility of the feed.

No studies have evaluated the effects of cassava waste, solid or liquid, on rumen microorganisms, although the microorganisms are directly related to

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fermentation and digestion in the rumen. Thus, the present study was conducted to characterize the populations of ciliate protozoa in sheep fed cassava wastewater and determine the effects of different levels of this waste on the density of rumen ciliates and nutrient digestibility.

MATERIAL AND METHODS

Thirty-two uncastrated Santa Inês lambs (average weight, 25.3kg; age, 167 days) were housed in individual stalls in a covered shed and distributed in a randomized complete design with four treatments and eight replicates. The animals received water and mineral salts *ad libitum*, a diet with Tifton 85 hay (*Cynodon* sp.), ground corn cob, soybean meal, and corn (basal diet), and four cassava wastewater levels: 0, 500, 1000, or 1500 mL/day (Table 1; Table 2). Treatments were administered at 08h00 and 16h00, and the daily amount was adjusted to allow for 10% leftovers. Diets were formulated to meet the protein requirements for maintenance and an average daily gain of 150 g, according to the NRC (2007).

Table 1. Chemical composition of ingredients of experimental diets

Ingredient	DM	CP	EE	NDF	ADF	MM	TC	IVDMD	
Tifton hay	90.23	10.77	2.47	78.56	55.94	7.16	79.60	53.03	
Corn	90.13	5.52	0.97	73.09	45.03	4.40	89.11	46.11	
Ground corn cob	89.02	8.86	6.37	43.42	10.72	2.96	81.81	70.80	
Soybean meal	89.09	50.08	2.47	32.01	12.02	6.13	41.32	80.26	
Cassava wastewater[3]	DM	EE	MM	Starch	Sugar	Acidity[1]	HCN[2]	TC%	pH
	4.34	0.66	2.81	1.1	0.18	0.23	16.3	96.53	4,7

DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; MM = mineral matter; TC = total carbohydrates; IVDMD = *in vitro* dry matter digestibility (%).
[1]Acidity, expressed as molar concentration of acid in the sample. [2]Cyanogenic compounds (µg HCN/mL of cassava wastewater). [3]Does not contain significant amounts of CP, NDF, and ADF.

The cassava wastewater was collected from a single flour mill in the region. The wastewater was stored in plastic containers (200-L capacity) for at least 10 days prior to their use, for volatilization of hydrocyanic acid. Immediately before their use, samples were obtained and frozen for subsequent analysis. The cassava wastewater was provided to the animals, in pails secured by a support in the cages, at their respective daily doses. The chemical composition of the diets is described in Table 1. Dry matter, crude protein, ether extract, mineral matter, and *in vitro* DM digestibility were determined as described by Silva & Queiroz (2002). The following fractions were also determined: neutral detergent fiber (NDF) and acid detergent fiber (ADF) (VAN SOEST et al., 1991), total carbohydrates (SNIFFEN et al., 1992), total cyanide (ESSERS et al., 1993), titratable acidity, total sugars, and starch (INSTITUTO ADOLFO LUTZ, 2008).

Table 2. Ingredients and chemical composition of experimental diets

Ingredient	Cassava wastewater level (mL)			
	0	500	1000	1500
Tifton hay (%)	35.0	35.0	35.0	35.0
Ground corn cob (%)	35.0	35.0	35.0	35.0
Soybean meal (%)	18.0	18.0	18.0	18.0
Corn (%)	12.0	12.0	12.0	12.0
Diet composition	Basal diet[1]	Cassava wastewater supplementation (g)[2]		
DM	90.0	21.7	43.4	65.1
CP	15.03	0	0	0
EE	2.38	3.3	6.6	9.9
MM	5.41	14.05	28.1	42.14
NDF	63.57	0	0	0
ADF	34.78	0	0	0
TC	77.18	22.0	44.0	66.0

[1]Dry matter (%); [2]Number of grams of each nutrient supplemented per day at the respective doses of cassava wastewater levels. DM = dry matter; CP = crude protein; EE = ether extract; MM = mineral matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; TC = total carbohydrates.

The experimental period was 75 days: 15 days for adaptation to the diets and 60 days for data collection. Prior to this stage, the animals were adapted to the consumption of cassava wastewater in collective cages for seven days.

Apparent digestibility was determined by partial feces collection and by using the LIPE® external marker in 250mg capsules. The marker was provided for seven days (two days for adaptation), and feces were collected, homogenized, and conditioned at –4°C for further analysis.

The LIPE® in the feces was analyzed using a Varian 099-2243 spectrophotometer with infrared light detector (FTIR) (LANZETTA et al., 2009). In the quantification of LIPE®, the daily fecal output (FO) was obtained using the following formula: FO = LIPE® supplied quantity (g)/LIPE® concentration in feces/total DM. Afterwards, dry matter digestibility (DMD) was determined using the following equation: DMD (%) = DM intake (g/day) – FO (g/day)/DM intake (g/day) × 100. The digestibility of the nutrients (DNut) was calculated based on the ingested and excreted quantities and the percentage of certain nutrients in the feed and feces, as follows: DNut = nutrient consumed (g) – nutrients in feces (g)/nutrient consumed (g) × 100.

At the end of the experiment, the animals were slaughtered after a fasting period of 14 h. Then, samples of the rumen contents were obtained from the center of the rumen mass. Each sample consisted of 50 mL of rumen contents fixed in 18.5% formaldehyde (v/v) (CEDROLA et al., 2015). Protozoa were identified and quantified using a Sedgewick-Rafter chamber (OGIMOTO & IMAI, 1981; CEDROLA et al., 2015).

An Olympus BX-51 light microscope (Olympus, Tokyo, Japan), equipped with an Olympus Evolt E-330 digital camera and Image-Pro Plus 6.0 imaging software (Media Cybernetics, São Paulo, Brazil), was used to take photomicrographs of the ciliates.

Digestibility data were analyzed using the means of variance and regression analyses in accordance with the levels of cassava wastewater (Statistical Analysis System, version 8.2) (2001). The criteria used in choosing the model were the significance of the regression coefficients, the F test (5% probability), the coefficient of determination (r^2), and the linear and quadratic contrasts. The total generic and average density of protozoa were assessed for normality of data and transformed to $\log_{10}$ whenever necessary and then assessed using one-way analysis of variance (R Development Core Team, 2010); P-values greater than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Cassava wastewater is a liquid waste, and therefore has a low DM content. Lambs were supplemented daily with 21.7 to 65.1 g cassava wastewater, but this amount did not lead to changes (P>0.05) in average dry matter intake in grams per day (1135g/day). This result can be attributed to the energy potential of the cassava wastewater, which is rich in starch and, according to Mertens (1994), the control of feed intake is related to the energy requirements and the concentration of fiber in the diet. This aspect may have contributed to the equivalent DM intake of the animals that received cassava wastewater in relation to the diet without this ingredient. The DMI values agree with the NRC (2007), which recommends an intake of 900 g/day for this sheep category.

The digestibility coefficients of DM, OM, and nutrients did not present statistical differences in the contrasts used (linear or quadratic) (P>0.05) for the cassava wastewater levels evaluated (Table 3). Cassava wastewater is a high-starch waste of rapid rumen degradation. However, the high digestibility of this material did not influence the digestibility coefficient of the studied components. A similar result was observed by Abrahão et al. (2006), who used wet cassava waste obtained after starch extraction substituting corn. High NDF and ADF values were negatively correlated with feed intake and digestibility (VAN SOEST, 1994); however, cassava wastewater does not contain significant amounts of these compounds, which could explain the unchanged digestibility coefficients of NDF and ADF.

Table 3. Dry matter intake and apparent digestibility coefficients of nutrients and their regression equations and coefficient of variation (CV) adjusted for the diets supplied to sheep fed cassava wastewater

Variable	Cassava wastewater level (mL)				CV (%)	*P* value		Regression equation
	0	500	1000	1500		L	Q	
DMI (g/d)	1140	1123	1147	1130	14,74	ns	ns	$\hat{Y}$=1135
DMD (%)	78.22	76.26	76.39	76.10	4.82	ns	ns	$\hat{Y}$ = 76.74
OMD (%)	82.26	80.27	83.47	82.81	4.71	ns	ns	$\hat{Y}$ = 82.20
CPD (%)	86.22	86.19	86.90	85.88	1.21	ns	ns	$\hat{Y}$ = 86.30
EED (%)	97.63	97.54	97.57	97.62	0.37	ns	ns	$\hat{Y}$= 97.59
NDFD (%)	33.93	34.99	32.38	34.74	10.49	ns	ns	$\hat{Y}$ = 34.01
ADFD (%)	36.12	36.97	33.31	36.04	13.79	ns	ns	$\hat{Y}$ = 35.61
TCD (%)	26.06	26.26	25.43	26.52	4.30	ns	ns	$\hat{Y}$ = 26.07

ns = not significant. DMI = dry matter intake; DMD = dry matter digestibility; OMD = organic matter digestibility; CPD = crude protein digestibility; EED = ether extract digestibility; NDFD = neutral detergent fiber digestibility; ADFD = acid detergent fiber digestibility; TCD = total carbohydrates digestibility.

The apparent digestibility of NDF was not affected by the cassava wastewater levels (P>0.05), ranging from 32.38% to 34.99%. This finding may have been influenced by the extremely high levels of NDF (63.57%) in the diets, since a reduction of NDF digestibility is more evident when a high level of NDF is used (KOZLOSKI et al., 2006) coupled with high starch doses, due to the larger non-fibrous carbohydrates fraction (OLIVEIRA et al., 2011). Although cassava water had significant starch levels, there was no significant effect (P>0.05) on NDF digestibility. Neutral detergent fiber can be easily fermented in the rumen, which allows for a higher energy intake by the ruminant (NRC, 2007).

The composition, density, and relative abundance (%) of the protozoal community are listed in Figure 1 and Table 4. Important biological and functional diversity was observed, with populations occupying different ecological niches (WILLIAMS & COLEMAN, 1992). The digestion products in the rumen are affected by the density and species of rumen microbiota, and it is important to understand the population dynamics of these microorganisms (DEHORITY, 2003).

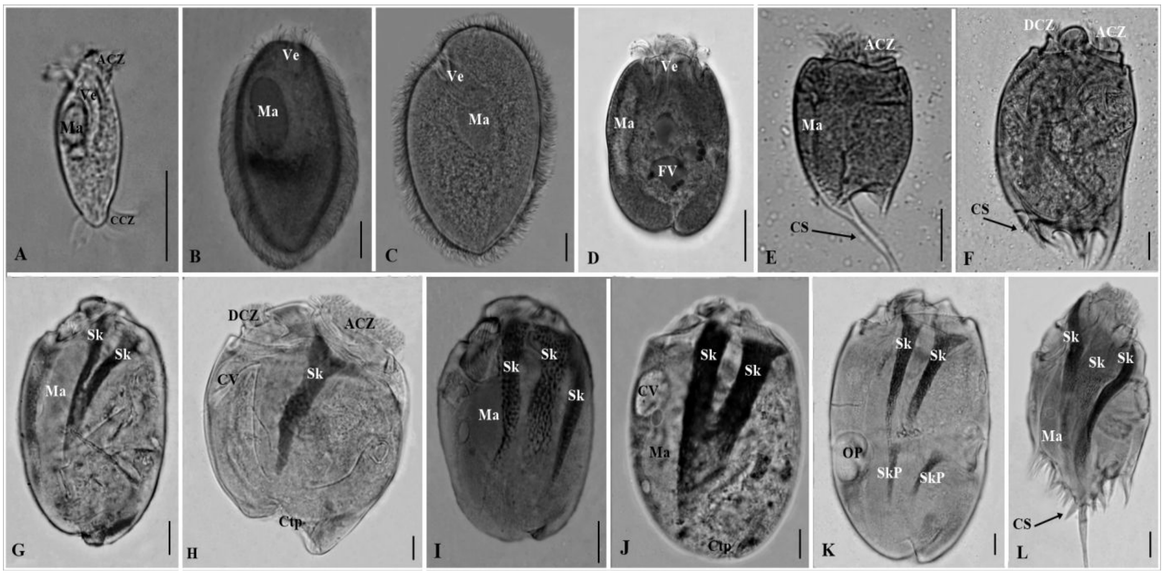

Figure 1. Rumen ciliate protozoa stained with Lugol's solution. A = Family Blepharocorythidae, genus *Charonina*. B-C = Family Isotrichidae, B = *Dasytricha*. C = *Isotricha*. D = Family Ophryoscolecidae, D-E = Subfamily Entodiniinae, genus *Entodinium*. F-L = Subfamily Diplodiniinae, F = *Diplodinium*. G = *Diploplastron*. H = *Eudiplodinium*. I = *Enoploplastron*. J = *Metadinium*. L = *Polyplastron* in binary fission. M = Subfamily Ophryoscolecinae, genus *Ophryoscolex*. ACZ = Adoral ciliary zone; CCZ = Caudal ciliary zone; DCZ = Dorsal ciliary zone. Ma = Macronuclei; Ve = Vestibulum; FV = Food vacuole; CS = Caudal Spine; Sk = Skeletal plate; CV = Contractile vacuole; Ctp = Cytoproct; OP = Oral primordium; SkP = Skeletal plates primordium. Bars: 20 µm

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Table 4. Density and standard error of the mean (SEM) for protozoa/mL ($x10^4$) of rumen contents of sheep fed cassava wastewater

Protozoa	Cassava wastewater level (mL)				SEM	*P* value
	0	500	1000	1500		
Charonina	0.58 (0.24)	0.40 (1.67)	0.02 (0.08)	0.62 (1.85)	0.27	0.5
Dasytricha	2.80 (1.17)	1.32 (0.55)	2.62 (1.05)	2.50 (0.74)	0.84	0.6
Entodinium	226.32[a] (95.07)	230.22[a] (96.63)	237.28[a] (95.95)	326.26[b] (97.42)	21.38	0.01
Isotricha	0.82 (0.34)	0.72 (0.30)	0.91 (0.36)	0.88 (0.26)	0.25	0.9
Ophryoscolex	0.04 (0.01)	0.24 (0.10)	0,04 (0.01)	-	0.08	0.5
Diplodiniinae[1]	7.48 (3.14)	5.34 (2.24)	6.40 (2.50)	4.62 (1.37)	1.00	0.14
Total	238.04 [a]	238.24 [a]	247.29[a]	334.88[b]	22.50	0.02

Means followed by different letters in the same row differ significantly (P<0.05). Values in parentheses indicate relative abundance of rumen protozoa (%).
[1]Protozoa genera observed in this subfamily: *Diploplastron*, *Diplodinium*, *Enoploplastron*, *Eudiplodinium*, *Metadinium*, and *Polyplastron*

The presence of rumen protozoa is associated with an increase in the digestibility of OM and DM, and especially in the digestibility of dietary fiber provided to the ruminants (WILLIAMS & COLEMAN, 1992). However, the digestibility data obtained in this study showed that this improved digestibility also depends on the species present in the rumen and their roles. A high density of protozoa was observed in all treatments, but only *Entodinium* was positively influenced by a higher percentage of cassava wastewater (P<0.05; Table 4). This result is probably due to the greater availability of total carbohydrates in this treatment (Table 1), which favors the growth of amylolytic species such as *Entodinium* (WILLIAMS & COLEMAN, 1992).

Entodinium populations can help contain the fermentation of readily fermentable carbohydrates, since these protozoa digest starch more slowly than other bacteria; thus, they act as moderators of rumen fermentation (NAGARAJA et al., 1992). Furthermore, protozoa are active fermenters that can reduce the depressant effect on the rumen pH with high-starch diets (KOZLOSKI, 2002), resulting in prevention or amelioration of acidosis.

The composition of the basal diet (hay and concentrate) and a continuous supply throughout the course of the treatment may have favored the occurrence and maintenance of the density of other ciliates in the rumen that use water-soluble carbohydrates (*Dasytricha* and *Isotricha*) or cellulose and hemicellulose (Diplodiniinae) (WILLIAMS & COLEMAN, 1992).

Few studies have evaluated the effects of cassava wastewater on rumen fermentation. To date, results have shown that the substitution of corn for cassava flour in the diet of sheep does not compromise nutrient digestibility or rumen parameters such as ammonia N and pH (ZEOULA et al., 2003), and that the use of cassava flakes and peels ensiled with citrus pulp in the diet of steers can provide appropriate rumen pH and NH_3 concentration for microbial synthesis (SILVEIRA et al., 2002).

In this study, the maintenance of cellulolytic species in the four treatments may indicate that the inclusion of cassava wastewater in the diet of sheep did not cause significant changes in rumen pH, despite the low pH of cassava wastewater (Table 2), as cellulolytic species are more sensitive to variations in this parameter (DEHORITY, 2003). However, absence of *Ophryoscolex* in sheep that received 1500 mL cassava wastewater may indicate that the wastewater had a deleterious effect on the species of this genus.

Studies involving the use of cassava wastewater in ruminant diets (ALMEIDA et al., 2009; SANTANA NETO, 2013) have shown that it can be considered a potential ingredient in animal feeding, and this observation was confirmed by our results. However, further studies evaluating other rumen fermentation parameters as well as the physiology and metabolism of ruminants fed cassava wastewater are necessary to determine its effects when added to their diet.

The use of up to 1500mL cassava wastewater in the diet of sheep does not affect nutrient digestibility coefficients or the composition of rumen protozoa. However, the use of 1500mL cassava wastewater promotes an increase in the density of *Entodinium*.

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REFERENCES

ABRAHÃO, J.J.S.; PRADO, I.N.; PEROTTO, D.; ZEOULA, L.M.; LANÇANOVA, J.A.C.; LUGÃO, S.M.B. Digestibilidade de dietas contendo resíduo úmido de mandioca em substituição ao milho para tourinhos em terminação. **Revista Brasileira de Zootecnia**, v.35, n.4, p.1447-1453, 2006.

ALMEIDA, S.R.M.; SILVA, A.M.; LIMA, J.P.; ALMEIDA, A.M.M.; ZACHARIAS, F. Avaliação do potencial nutritivo da Manipueira na dieta de ovinos deslanados. **Revista Brasileira de Agroecologia**, v.4, p.1434-1438, 2009.

CEDROLA, F.; ROSSI, M.; DIAS, R.J.P.; MARTINELE, I.; D'AGOSTO, M. Methods for taxonomic studies of rumen ciliates (Alveolata, Ciliophora): A brief rewiew. **Zoological Science**, v.32, p.8-15, 2015.

CENI, G.C.; COLET, R.; PERUZZOLO, M.; WITSCHINSKI, F.; TOMICKI, L.; BARRIQUELLO, A.L.; VALDUGA, E. Avaliação de componentes nutricionais de cultivares de mandioca (*Manihot esculenta* Crantz). **Alimentos e Nutrição**, v.20, n.1, p.107-111, 2009.

DEHORITY, B.A. **Rumen Microbiology**. Nottingham: Nottingham University Press, 2003. 372 p.

ESSERS, A.J.A.; BOSVELD, M.; GRIFT, R. M.V.D.; VORAGEN, A. G. J**Assay for the cyanogens content in cassava products**. Wageningen Agricultural University, 1965. 9p.

FRANZOLIN, R.; FRANZOLIN, M.H.T. População protozoários ciliados e degradabilidade ruminal em búfalos e bovinos zebuínos sob dieta à base de cana-de-açúcar. **Revista Brasileira de Zootecnia**, v.29, n.6, p.1853-1861, 2000.

INSTITUTO ADOLFO LUTZ. **Normas Analíticas; métodos químicos e físicos para a análise de alimentos**. São Paulo: Instituto Adolfo Lutz, 2008.

KOZLOSKI, G.V. **Bioquímica dos ruminantes**. Santa Maria: UFSM, 2002. 140p.

KOZLOSKI, G.V.; TREVISAN, L.M.; BONNECARRÈRE, L.M.; HÄRTER, C.J.; FIORENTINI, G.; GALVANI, D.B.; PIRES, C.C. Níveis de fibra em detergente neutro na dieta de cordeiros: consumo, digestibilidade e fermentação ruminal. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.58, n.5, p.893-900, 2006.

LANZETTA,V.A.S.; REZENDE, A.S.C.; SALIBA, E.O.S.; LANA, A.M.Q.; RODRIGUÉZ, N.M.; MOSS, P.C.B. Validação do Lipe® como método para determinar a digestibilidade dos nutrientes em equinos. **Revista Brasileira de Zootecnia**, v.38, n.1, p.69-74, 2009.

LEITE, P.M.B.A. **Diferentes relações volumoso: concentrado associadas a manipueira na alimentação de ovinos**. 2013. 39 p. Dissertação. (Mestrado em Zootecnia) - Programa de Pós-Graduação em Zootecnia, Universidade Federal Rural de Pernambuco, Pernambuco.

LIMA, M.E.; VENDRAMIN, L.; HOFFMANN, D.A.C.; LISBOA, F.P.; GALLINA, T.; RABASSA, V. R.; SCHWEGLER, E.; CORRÊA, M.N. Alterações na população de protozoários ruminais, quantificados a partir da adaptação da técnica de Dehority, de ovinos submetidos a uma dieta de confinamento. **Acta Scientiae Veterinariae**, v.40, n.1, p.1-6, 2012.

MENEGHETTI, C. C.; DOMINGUES J. L. Características Nutricionais e Uso de Subprodutos da Agroindústria na Alimentação de Bovinos. **Revista Eletrônica Nutritime**, v.5, p.512-536, 2008.

MERTENS, D.R. Regulation of forage intake. In: FAHEY JUNIOR, G.C. (Ed.) **Forage quality, evaluation and utilization**. Madison: American Society of Agronomy, 1994. p.450-493.

NAGARAJA, T.G.; TOWNE, G.; BEHARKA, A.A. Moderation of ruminal fermentation by ciliated protozoa in cattle fed a high-grain diet. **Applied and Environmental Microbiology**, v.58, p.2410-2414, 1992.

NATIONAL RESEARCH COUNCIL - NRC. **Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids.** Washington: National Academy Press, 2007. 284p.

OGIMOTO, K.; IMAI, S. **Atlas of rumen microbiology.** Tokio: Japan Scientific Societies Press, 1981. 230p.

OLIVEIRA, A.S.; DETMANN, E.; CAMPOS, J.M.S.; PINA, D.S.; SOUZA, S.M.; COSTA, M.G. Meta-análise do impacto da fibra em detergente neutro sobre o consumo, a digestibilidade e o desempenho de vacas leiteiras em lactação. **Revista Brasileira de Zootecnia**, v.40, n.7, p.1587-1595, 2011.

SANTANA NETO, J.A. **Uso da manipueira como suplemento na dieta para cordeiros Santa-Inês**. 2013.64f. Dissertação. (Mestrado em Zootecnia) - Programa de Pós-Graduação em Zootecnia, Universidade Federal de Sergipe, Sergipe.

SANTOS FILHO, H.B.; VÉRAS, R.M.L.; FERREIRA, M.A.; SILVA, J.L.; VASCONCELOS, G.A.; SOARES, L.F.P.; CARDOSO, D.B. Liquid residue of cassava as a replacement for corn in the diets of sheep. **Tropical Animal Health and Production**, v.47, p.1573-7438, 2015.

SILVA, D.J.; QUEIROZ, A.C. **Análises de alimentos**: métodos químicos e biológicos. Viçosa: UFV, 2002. 235p.

SILVEIRA, R.N.; BERCHIELLI, T.T.; FREITAS, D.; SALMAN, A.K.D.; ANDRADE, P.; PIRES, A.V.; FERNANDES, J.J.R. Fermentação e degradabilidade ruminal em bovinos alimentados com resíduos de mandioca e cana-de-açúcar ensilados com polpa cítrica peletizada. **Revista Brasileira de Zootecnia**, v.31, p.793-801, 2002.

SNIFFEN, C. J.; O'CONNOR, J.D.; VAN SOEST, P.J.; FOX, D.G.; RUSSELL, J.B. A net carbohydrate and protein system for evaluating cattle diets: Carbohydrate and protein avaiability. **Journal of Animal Science**, v.70, p.3562-3577, 1992.

URBANO, S.A.; FERREIRA, M.A.; VÉRAS, R.M.L.; AZEVEDO, P.S.; SANTOS FILHO, H.B.; VASCONCELOS, G.A.; OLIVEIRA, J.P.F. Características de carcaça e composição tecidual de ovinos Santa Inês alimentados com manipueira. **Agrária**, v.10, n.3, p.466-472, 2015.

VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, p.3583-3597, 1991.

VAN SOEST, P.J. **Nutritional ecology of the ruminant**. Ithaca, New York: Cornell University, 1994. 476p.

WILLIAMS, A.G.; COLEMAN, G.S. **The Rumen Protozoa**. New York: Springer, 1992. 423p.

ZEOULA, L.M.; CALDAS NETO, S.F.; GERON, L.J.V.; MAEDA, E.M.; PRADO. I.N.; DIAN, P.H.M.; JORGE, J.R.V.; MARQUES, J.A. Substituição do milho pela farinha de varredura de mandioca (*Manihot esculenta* Crantz) em rações de ovinos: consumo, digestibilidade, balanços de nitrogênio e energia e parâmetros ruminais. **Revista Brasileira de Zootecnia**, v.32, p.491-502, 2003.

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