Archivos de Zootecnia
Journal website: https://www.uco.es/ucopress/az/index.php/az/

Increasing dietary doses of cashew nut shell liquid on rumen and intestinal digestibility of nutrient in steers fed a high-grain diet

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Additional keywords

Additive.
Cardol.
Cardanol.
Digestibility.
Ruminal fermentation.

SUMMARY

The objective was to evaluate the inclusion of technical cashew nut shell liquid (tCNSL) in the concentrate for cattle fed on a high-grain diet on feed intake, ruminal and total apparent digestibility coefficients of nutrients, fermentation characteristics and microbial efficiency. Four Holstein steers (445 ± 23.39 kg of body weight) fitted with ruminal and duodenal cannulae were used. The diets had (in DM basis) 144 g kg-1 of crude protein and 799 g kg-1 of total digestible nutrients, with a forage to concentrate ratio of 15:85. The experimental design was a 4 x 4 Latin square with 10-day experimental periods. The diets consisted of 0; 300; 600 and 1200 mg of tCNSL in DM of the concentrate. Intake and total apparent digestibility coefficient of nutrients, on DM basis, were not affected by experimental diets. However, ruminal digestibility of ether extract showed quadratic response in which animals fed 0 and 300 mg tCNSL kg-1 of concentrate had the lowest values for ruminal digestibility. Ruminal ammonia nitrogen, plasma urea nitrogen and microbial efficiency were not affected by treatments. However, ruminal pH tended to increase when animals were fed with tCNSL.

Aumento das doses dietéticas de líquido de casca de castanha de caju na digestibilidade ruminal e intestinal de nutrientes em novilhos alimentados com uma dieta rica em grãos

Palavras-chave adicionais

Aditivo.
Cardol.
Cardanol.
Digestibilidade.
Fermentação ruminal.

RESUMO

Objetivou-se avaliar os efeitos da inclusão do líquido da casca da castanha de caju (tCNSL) na dieta de bovinos alimentados com dietas ricas em grão, sobre a ingestão e o coeficiente de digestibilidade ruminal e total dos nutrientes; as características de fermentação ruminal, eficiência de síntese microbiana no rúmen e parâmetro sanguíneo, através do nitrogênio ureico plasmático. Foram utilizados quatro novilhos da raça Holandesa (445 ± 23,39 kg de peso vivo) providos de cânula ruminal e duodenal, recebendo uma dieta com 144 g kg-1 de proteína bruta e 799 g kg-1 de nutrientes digestíveis totais, com uma relação volumoso:concentrado de 15:85. O delineamento experimental foi o quadrado latino 4 x 4 com 10 dias de períodos experimentais. As dietas foram compostas de 0, 300, 600 e 1200 mg de tCNSL na matéria seca (MS) do concentrado. A ingestão e os coeficientes de digestibilidade aparente total dos nutrientes, com base na MS, não foram influenciados pela inclusão de tCNSL na dieta. O coeficiente de digestibilidade ruminal (CDR) do extrato etéreo apresentou resposta quadrática, em que os animais que consumiram 0 e 300 mg tCNSL d-1 apresentaram menores valores de CDR. O uso do LCCt não influenciou o nitrogênio amoniacal no rúmen, o nitrogênio ureico plasmático e a eficiência de síntese microbiana. No entanto, houve tendência de maior pH ruminal quando os animais consumiram o LCCt.

Information

Cronología del artículo.
Recibido/Received: 12.07.2016
Aceptado/Accepted: 06.03.2017
On-line: 15.07.2017
Correspondencia a los autores/Contact e-mail:
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INTRODUCTION

The search for alternatives to ionophores, which are antibiotics that improve the performance of ruminants through the manipulation of ruminal fermentation by changing the rumen microbiota and enhancing the synthesis of products from feed digestion, is continuously increasing.

Among the alternatives, the cashew nut shell liquid (CNSL) is a by-product of cashew nut processing that

has a variety of industrial uses. This product contains cardanol, cardol, anacardic acid, and 2-methylcardol (Vasapollo *et al*., 2011), and its mode of action on ruminal fermentation appears to be similar to ionophores because they act primarily on Gram-positive bacteria, which produce hydrogen fumarate and butyrate. The supply of CNSL to ruminants can cause changes in rumen fermentation, such as increase of propionate production and reduction in methane production (Shinkai *et al*., 2012; Watanabe *et al*., 2010).

Technical-grade CNSL (tCNSL) is the most abundant form of the product, and the roasting process of CNSL converts the anacardic acid to cardanol, resulting in the production of cardanol (67.8 to 94.6%), cardol (3.8 to 18.8%) and 10 to 15% of polymers (Mazzetto and Lomonaco, 2009). These compounds are the main active constituents of tCNSL and have antibacterial, antiprotozoal, and antifungal properties (Stasiuk and Kozubek, 2010).

Thus, tCNSL could be used as feed additive to modify the rumen environment and improve animal performance. However, the responses to their use in ruminant diets have been assessed in a limited number of studies and the ideal dosage to be provided has not been established. Branco *et al*. (2015) suggested the need of studying the effect of tCNSL, which is more available commercially, on animal productivity *in vivo*. Therefore, the objective of this study was to evaluate different levels of the inclusion of tCNSL in cattle consuming high-grain diets on apparent digestibility of nutrients, ruminal fermentation and efficiency microbial synthesis.

MATERIAL AND METHODS

Animals, diets and experimental procedure

The experiment was conducted at Fazenda Experimental de Iguatemi, of the Universidade Estadual de Maringá, Maringá, Paraná, Brazil. Four Holstein steers weighing 445 ± 23.39 kg of body weight, and 20 months of age, fitted with ruminal and duodenal cannulae were distributed in a 4 x 4 Latin square design. The animals were kept in individual indoor installation, equipped with feeder and automatic drinker. All animals' procedures are in accordance with Law 11.794 of October 8, 2008. Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and were also conducted in accordance with ethical standards and approved by the Ethics and Biosafety Committee (CEUA 023/2015).

The diets **(table I)** were composed of corn silage as roughage source, and concentrate (corn, soybean meal, minerals and urea) and were formulated according to NRC (2000) to contain a 150 g kg^{-1} of CP and 799 g kg^{-1} TDN and estimated average daily gain of 1.5 kg. All diets consisted of 150 g kg^{-1} of corn silage and 850 g kg^{-1} of concentrate. The tCNSL was provided by Usibras Company (Aquiraz city, state of Ceará, Brazil) and contained cardanol (73.3%), cardol (16.4%), and 2-methylcardol (3.0%), and it was included to the concentrate at 0; 300; 600 and 1200 mg kg^{-1} of DM, to compose the treatments. The animals were fed a total mixed ration, *ad libitum*, twice a day (08.00 h and 16.00 h). The tCNSL was mixed with the ingredients for the preparation of concentrate.

Each experimental period lasted 10 days (Castillejos *et al*., 2007), and the last three days of each period were used for collection of rumen fluid, duodenal digesta and feces. On the last day of each period, after weighing the animals and before changing the diets, they were submitted to washing rumen technique and the exchange of rumen contents among animals according to the diet change (Kim *et al*., 2013; Kim *et al*., 2014). This procedure was done to reduce the adaptation period to the diets.

Nutrient intake was determined from the feed offered and orts, which were collected and weighed daily before the morning feeding. To determine the ruminal, intestinal and total digestibility of nutrients, duodenal digesta samples were collected (~ 300 mL) via duodenal cannula, and feces were collected directly from the rectum. To determine the duodenal and fecal output, all animals were provided with a daily dose of 10 g of titanium dioxide (TiO_2) directly into the rumen, from the first to the last day of each experimental period. Duodenal and fecal samples were collected from day 7^{th} of each experimental period, during three days, at different times. Collection times were: 0, 3, 6, 9, 12, 15, 18 and 21h after the morning feeding, making a total of eight samples of duodenal contents and eight samples of feces per animal per period. The samples were stored in plastic bags and kept at -20ºC for subsequent chemical analysis. Before chemical analysis, samples were thawed, dried at 55ºC for 72h and ground to pass through a 1 mm sieve. The composite samples of duodenal digesta and feces by animal and period were made considering the dry weight, taking the same percentage of each sample.

Rumen fluid (~150 mL) was collected between day 7 and 9 of each period for pH and ammonia (NH_3-N) determination. The first collection was performed before the morning feeding (0h) and the following collections were 3, 6, 9, 12, 15, 18 and 21h after the morning feeding. The pH was measured by using digital pH-meter (Digimed DM20; Digimed Instrumentação Analítica, São Paulo, SP, Brazil). For NH_3-N determination, 50 mL of rumen fluid was acidified with 1 ml of H_2SO_4 (1:1) and kept at -20ºC, for subsequent chemical analysis.

Blood samples were collected twice a period, on 4^{th} and 10^{th} day of each experimental period, 4h after morning feeding by jugular vein puncture. The samples were collected twice a period to improve the number of samples and reduce the error. After collection, both samples of each animal were used to do a composite sample. To obtain the plasma, the samples were centrifuged at 2 500 x g for 15 min at 4°C.

Microbial synthesis efficiency, used to estimate the flow of microbial nitrogen compounds to the intestine, was determined by collection of 1.5 kg of rumen content of each animal, on day 9, 4h after the afternoon feeding and on day 10 before the morning feeding of each experimental period. Samples were mixed with

Table I. Chemical composition, percentage of feed and experimental diets (g kg^{-1}) (Composição química, percentagem de alimento e dietas experimentais (g kg^{-1}).

Feed	DM[1]	CP	EE	Ash	NDF	ADF	Starch	TMR*
Corn silage	303	62	20.2	37.1	523	218	199	150
Ground corn	959	82	11.4	13.7	106	32	645	779
Soybean meal	966	510	61.6	34.8	131	91	86	37
Urea	990	2810	-	-	-	-	-	14
Mineral mixture[2]	990	-	-	990	-	-	-	21
				Chemical composition of experimental diets (g kg^{-1})				
	DM	CP	EE	Ash	NDF	NFC[2]	Starch	TDN[2]
TMR (total mixed ration) on DM basis	961	144	34	37.5	103	682	595	799

DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber corrected for ash and protein; ADF = acid detergent fiber. ²NFC = Non-fiber carbohydrates and (TDN) total digestible nutrients were calculated according NRC (2000). MM: Mineral Mixture²: Calcium carbonate; 258.4 g kg^{-1}; kaolin; 24.8 g kg^{-1}; sulfur flower; 0.059 g kg^{-1}; calcium iodate; 157.9 g kg^{-1}; common salt; 0.020 g kg^{-1}; sodium selenite; 0.110 g kg^{-1}; cobalt sulphate; 2.672 g kg^{-1}; copper; 2.461 g kg^{-1}; manganese sulphate; 2.286 g kg^{-1} zinc sulphate.

500 mL saline solution (9 g of NaCl L^{-1}) for 1 minute in a blender followed by filtration through cheesecloth and frozen at -20ºC until the end of the experiment. At the end of the experiment, samples were thawed and the bacteria were isolated by differential centrifugation (500 g and 27 000 g), according to Cecava *et al.* (1990). The bacterial pellet resulted from the centrifugation was dried at 55°C for 48 hours and macerated. The estimates of bacterial nitrogen production were done according to the technique of the purines in the bacterial pellet and duodenum digesta. The efficiency of the microbial synthesis was calculated from the protein and organic matter degraded in the rumen and estimated by the difference between the protein or organic matter intakes and the duodenum flow of these fractions (Ushida *et al*., 1985).

Chemical analyses

Samples of feed, orts, duodenal digesta and feces were analyzed for dry matter (DM; #934.01), ash to determine organic matter (OM; #924.05), crude protein (CP) obtained by total N determination using the micro Kjeldahl technique (#920.87) multiplying to a fixed conversion factor (6.25), and ether extract (EE) was determined gravimetrically after extraction using petroleum ether in a Soxhlet instrument (#920.85; AOAC, 1990). For analysis of neutral detergent fiber (NDF), samples were treated with alpha thermostable amylase with no sodium sulfite and corrected for ash residue (Van Soest *et al*., 1991).

The tCNSL chemical composition was determined by gas chromatography-mass spectrometry, performed on a Shimadzu GCMSQP-2010 Ultra system, equipped with an AOC-20i autoinjector. The column used was a Restek RTX- 5ms (30 m, 0.25 mm i.d., 0.25 µm df), coated with 5% diphenyl-95% polydimethylsiloxane and was operated with the following conditions: oven temperature program: 50°C, rising at 10°C min^{-1} to 200°C; rising at 2°C min^{-1} to 320°C; injection temperature and volume, 250°C and 1.0 µL, respectively; injection mode, splitless; carrier gas, helium; ion source, 300°C.

Percentage of total digestible nutrients (TDN) and non-fiber carbohydrates (NFC) of diets were determined by the equation : TDN (g kg^{-1}) = (digestible CP (g kg^{-1}) + digestible NDF (g kg^{-1}) + digestible NFC (g kg^{-1}) + (2.25 * digestible EE) (g kg^{-1})) / (dry matter intake (DMI)*100) and NFC (g kg^{-1}) = 100 – (CP (g kg^{-1}) + NDF (g kg^{-1}) + EE (g kg^{-1}) + ash (g kg^{-1})). Titanium dioxide concentration was determined in samples of duodenal digesta and feces according Myers *et al*. (2004) to determine intestinal and total solids flow.

Feed samples, orts, duodenal digesta and feces were sent to the Laboratory of Physical and Chemical Analysis of ABC Foundation in the city of Castro, located in state of Paraná, Brazil, for starch determination by the acid method (IAL, 2008). Fecal N (g d^{-1}) was estimated by chemical analysis of fecal CP and the value was divided by 6.25. Urinary N (g d^{-1}) was estimated by the equation: Urinary N (g d^{-1}) = 0.56 * (N intake (g d^{-1})) – 21.18 (Waldrip *et al*., 2013).

Rumen fluid samples were thawed at room temperature and centrifuged at 3,000 x *g* for 15 minutes before determining the NH_3-N concentration by potassium hydroxide (2N) distillation (Fenner, 1965). Urea concentration in plasma was determined according to the modified method diacetyl (GoldAnalisa®) and plasma urea nitrogen (PUN) was obtained by the product of the urea concentration value, which corresponds to the 0.466 N content in urea.

Statistical analyses

Statistical analysis was performed as an ANOVA, followed by linear, quadratic and cubic regression analysis using the PROC MIXED of SAS (Statistical Analysis System, version 9.1) and α=0.05. The model was: Yijk = µ + Ai + Pj + Tk + eijk, where: Y_{ijk} = observed variable; µ = the overall mean; Ai = the effect of animal i between 1 and 4; Pj = effect of j period between 1 and 4; Tk = treatment effect k between 1 and 4; eijk = random error. All effects were considered fixed, except the effect of animal that was considered random.

Data of ruminal fermentation was analyzed as repeated measures adding to the previous model the fixed effect of ruminal digesta collection (0, 3, 6, 9, 12, 15, 18 and 21h) and the fixed effect of treatment by time

interaction. The differences among treatments were tested to linear, quadratic, and cubic contrasts and significance level was set at 0.05

RESULTS AND DISCUSSION

Nutrient intake, expressed either in g day^{-1} or in relation to body weight (g kg^{-1} BW), and the metabolic weight (g kg^{-1} $BW^{0.75}$) were not affected by tCNSL levels consumed by animals **(table II)**. In agreement with our results, Branco *et al.* (2015) and Coutinho *et al.* (2014) found no effect of adding tCNSL on the dry matter intake (DMI) of dairy cows. The average dry matter intake was 10.79 g day^{-1} and the estimated DMI (NRC, 2000) for steers in the same conditions is 10.25 kg day^{-1}, being the values similar to those observed in the experiment.

Functional oils might have properties that influence the feed intake (Yang *et al*., 2010; Rodríguez-Prado *et al*., 2012). In contrast, Tassoul and Shaver (2009) found that the use of functional oil decreased feed palatability and, consequently, decreased DMI. The DMI when expressed by kg 100 kg^{-1} BW (g kg^{-1} BW) and g kg^{-1} $BW^{0.75}$ (% metabolic weight) showed high values, probably due to breed used, since Holstein cattle have well-developed bone structure, allowing a greater volume of rumen, and leading to greater DMI.

As the tCNSL did not affect the DMI, it also did not change the CP and NDF intake (p>0.05), showing means of 1.4 and 1.8 kg d^{-1}, respectively. The fiber intake data **(table II)** are in agreement with Shinkai *et al.* (2012) who evaluated the methane mitigation in cattle fed with CNSL. Similarly, Branco *et al.* (2015) showed that tCNSL had no effects on DM, CP and NDF and starch intake, although the information about the use of tCNSL in animal nutrition is limited. There are also little data about the effect of tCNSL on digestibility in steers and no information on animal fed high-grain diets. The diets in the present study were high in grains, and the animals had average intakes of 13 and 15.3 g kg^{-1} BW for starch and non-fiber carbohydrates, respectively. Coutinho *et al.* (2014) did not find any influence of tCNSL on dairy cow performance and production. However, the non-fiber carbohydrates intake was similar to present experiment (15.5 g kg^{-1} BW).

The use of tCNSL did not affect the ruminal, intestinal and total digestibility of DM, starch and NFC, as well as TDN levels (p>0.05; **table III**), which is in agreement with the results of Coutinho *et al.* (2014). However, the intestinal digestibility of CP was influenced by tCNSL supplies, showing a cubic response with higher value when animals fed 300 mg tCNSL. Shinkai *et al.* (2012) that found total digestibility coefficient of DM was affected by CNSL (p<0.05) and there was a decrease in some bacteria population as *Butyrivibrio fibrisolvens*; but in this study CNSL was included in the ration as a pelleted product. The heat converts anacardic acid into cardanol (Mazzetto; Lomonaco, 2009), but the effect of pelleting CNSL on its anacardic acid content has not been reported. The supply of tCNSL to lactating dairy cows tended to decrease bacteria populations but had no effect on apparent total-tract digestibility of nutrients (Branco *et al*., 2015).

Higher rumen digestion of protein and consequent N disappearance might produce higher ammonia absorption through the rumen wall or lower N fixation in microbial N form, being desirable ruminal digestibility values to be negatives or near zero (Maeda *et al*., 2007), which did not happen, since the ruminal digestibility of CP was 315.5 g kg^{-1}, as it is shown in **table III**. McIntosh *et al.* (2003) suggest some beneficial effect of plant extracts on protein metabolism when high-fiber diets are provided, mainly due to their influence on cellulolytic bacteria, but in the present experiment it was used a high-grain diet.

The main effects of plant extract in the rumen are a reduction of protein and starch degradation, and an inhibition of amino acids degradation by a selective action on certain rumen microorganisms, especially some bacteria (Hart *et al*., 2008). Thus, the use of tCNSL

Table II. Nutrient intake of Holstein steers fed on diets supplemented with different levels of tCNSL (Consumo de nutrients de novilhos da raça Holandesa alimentados com dietas suplementadas com diferentes níveis de tCNSL).

Intake	Levels of tCNSL (mg kg^{-1})				SEM*	p		
	0	300	600	1 200		L	Q	C
DM (g d^{-1})	1 1018	10 874	10 784	10 467	0.586	0.47	0.97	0.97
DM (g kg^{-1} BW)	24.88	23.82	24.51	23.76	0.066	0.36	0.85	0.31
DM (g kg^{-1} $BW^{0.75}$)	1 140.56	1 100.07	1 121.57	1 088.13	3 115	0.31	0.90	0.44
CP (g d^{-1})	1455	1424	1424	1 378	74.469	0.45	0.99	0.87
CP (kg 100 kg^{-1} BW)	0.33	0.31	0.32	0.31	0.009	0.36	0.82	0.25
NDF (g d^{-1})	1 829	1 875	1 794	1 7515	108.624	0.48	0.83	0.68
NDF (g kg^{-1} BW)	4.13	4.10	4.07	3.97	0.012	0.36	0.94	0.98
EE (g d^{-1})	354.80	349.86	347.15	333.72	18.331	0.38	0.92	0.95
Starch (g d^{-1})	5 917	5 786	5 784	5 606	297.866	0.46	0.99	0.87
Starch (g kg^{-1} BW)	13.36	12.68	13.16	12.72	0.033	0.35	0.80	0.20
NFC (g d^{-1})	6 965	6 815	6 814	6 610	374.043	0.50	0.99	0.88
NFC (g kg^{-1} BW)	15.73	14.93	15.49	15.00	0.044	0.41	0.80	0.25

*SEM= Standard error of mean; P= linear (L), quadratic (Q) and cubic (C) probabilities (p).

Table III. Ruminal (RD), intestinal (ID) and total (TD) digestibility coefficient of cattle fed on diets supplemented with different levels of tCNSL (Coeficientes de digestibilidade ruminal (RD), intestinal (ID) e total (TD) de bovinos alimentados com dietas suplementadas com diferentes níveis de LCCt).

Item	Levels of tCNSL(mg kg^{-1})				SEM[1]	p		
	0	300	600	1200		L	Q	C
Dry matter								
RD (g kg^{-1})	451	365	434	454	6.14	0.71	0.53	0.39
ID (g kg^{-1})	578	659	620	626	4.10	0.65	0.43	0.33
TD (g kg^{-1})	774	795	785	801	1.75	0.34	0.88	0.49
Crude protein								
RD (g kg^{-1})	350	244	320	347	3.72	0.61	0.22	0.12
ID (g kg^{-1})	627	709	661	667	1.96	0.60	0.18	0.04[1]
TD (g kg^{-1})	759	783	769	781	1.36	0.38	0.74	0.29
Neutral detergent fiber								
RD (g kg^{-1})	586	562	576	594	5.23	0.83	0.74	0.84
ID (g kg^{-1})	99	186	131	132	3.84	0.99	0.35	0.22
TD (g kg^{-1})	636	641	636	640	4.26	0.97	0.99	0.93
Ether extract								
RD (g kg^{-1})	287	198	342	345	3.26	0.09	0.70	0.02[2]
ID (g kg^{-1})	660	699	634	591	5.02	0.20	0.66	0.47
TD (g kg^{-1})	757	758	758	735	3.52	0.62	0.78	0.95
Starch								
RD (g kg^{-1})	606	492	562	587	8.48	0.90	0.51	0.47
ID (g kg^{-1})	703	806	769	797	3.71	0.20	0.33	0.20
TD (g kg^{-1})	887	910	897	923	1.89	0.24	0.99	0.43
Non-fiber carbohydrate								
RD (g kg^{-1})	478	369	443	438	9.31	0.82	0.56	0.51
ID (g kg^{-1})	659	735	714	729	3.65	0.38	0.34	0.22
TD (g kg^{-1})	828	856	840	867	2.23	0.29	0.96	0.44
TDN* (g kg^{-1})	784	804	793	810	1.68	0.36	0.93	0.47

*TDN = total digestible nutrients calculated according NRC (2000).SEM= standard error of mean, p= linear (L), quadratic (Q) and cubic (C) probabilities. [1]$\hat{Y} = 62.66 + 6.31\ x - 1.42\ x^2 + 0.076\ x^3$ $R^2 = 42.77$; [2]$\hat{Y} = 28.66 - 9.53\ x + 2.65\ x^2 - 0.15\ x^3$ $R^2 = 52.41$.

might improve the total digestibility of CP, due to higher N flow to small intestine and as a consequence of a decrease of peptides and amino acids fermentation, due to lower deamination. Another mode of action suggests that functional oils affect the pattern of substrate colonization, mainly those rich in starch, or might inhibit the ammonia' hyper producers' bacteria, involved in the deamination process (Patra, 2011).

Total digestibility of NDF, on average, was 639 g kg^{-1} and was not affected by tCNSL supply ($p>0.05$). Total digestibility of EE was not influenced by tCNSL levels ($p>0.05$), but its ruminal digestibility coefficient showed a quadratic response, being lower for animals fed 300 mg tCNSL kg^{-1} of concentrate ($p<0.05$) when compared to the other diets. Negative ruminal digestion of EE or close to zero would be expected because there is no ruminal microorganism able to use lipids as an energy source, which was not verified in the current experiment.

The diets supplemented with tCNSL also did not influence the starch and non-fiber carbohydrates responses **(table III)**. Van Soest (1994) affirms the high intake of starch might provide negative effects to the animals due its fast fermentation and development of high lactic acid amount. Therefore, the rumen pH decreases by its inadequate buffering capacity and might provide metabolic disorders to the animal, like acidosis. With the high intake of starch **(table III)** it was expected lower intestinal digestibility and ruminal pH of animals **(tables III** and **IV)**.

No difference was observed in ruminal parameters, except that pH tended to increase ($p= 0.05$) by tCNSL supply **(table IV)**, with no interaction between diets and time of collection. The mean value of 6.17 was similar than of 6.2 proposed by Van Soest (1994) as optimal for maximum activity of the microorganisms, ruminal fermentation and degradation of NDF. Diaz (2013) observed that tCNSL inclusion increased linearly the *in vitro* ruminal pH. Probably, the effect on pH is due to the antimicrobial activity of CNSL against Gram-positive bacteria, which improves Gram-negative growth, as *Selenomonas ruminantium* and *Megasphae-*

Table IV. Ruminal pH, ruminal N-NH_3, faecal N, urinary N, plasma urea N (PUN) and microbial efficiency (ME) of cattle fed on diets supplemented with different levels of tCNSL (pH do liquido ruminal, N-NH_3 ruminal, N fecal, N urinário, N ureico plasmático e eficiência de síntese microbiana de bovinos alimentados com dietas suplementadas com diferentes níveis de tCNSL).

Item	tCNSL (mg kg^{-1})				SEM[1]	P<[2]		
	0	300	600	1200		L	Q	C
pH	6.05	6.17	6.25	6.21	0.028[3]	0.05[3]	0.07	0.08
N-NH_3 (mg dL^{-1})	14.53	12.74	12.11	13.70	0.863	0.41	0.15	0.48
Fecal N (g d^{-1})	55.82	49.63	52.38	48.36	4.186	0.29	0.99	0.43
Urinary N (g d^{1})	109.22	106.46	106.44	102.25	6.672	0.45	0.78	0.87
PUN (mg dL^{-1})	13.91	14.16	12.84	13.27	1.256	0.60	0.79	0.57
ME(g N_m kg^{-1} OMDR)*	23.10	33.01	26.17	22.94	5.773	0.69	0.39	0.34

*g microb N kg^{-1} OMDR= grams of microbial nitrogen kg^{-1} organic matter degraded in the rumen (OMDR). [1]SEM= standard error of mean, [2]P= linear (L), quadratic (Q) and cubic (C) probabilities. [3]y = 6.10 + 0.012 x; r^2 = 53.00.

ra elsdenii (Watanabe *et al*., 2010), the main bacteria that use lactate as an energy substrate.

Phenolic compounds such as cardol and methylcardol, from tCNSL under alkaline conditions form liposomal structure, providing a controlled release (Stasiuk and Kozubek, 2010). Spanghero *et al.* (2008) suggest the effects of functional oils may be pH dependent, acting more effectively when rumen pH is close to 5.5 which were not observed in the present study. Thus, this mechanism dependence on a more acidic ruminal pH to express the maximum effect of some plant extracts, can also affect the responses recorded for the use of tCNSL **(table IV)**.

The inhibitory activity of functional oils in relation to the bacteria population involve ruminal ammonia production, which was found *in vitro* by McIntosh *et al.* (2003) and Newbold *et al.* (2004), but in this research, the inclusion of tCNSL did not influence the concentration of NH_3-N concentration in rumen **(table IV)**, in agreement with results *in vitro* (Spanghero *et al*., 2008) and *in vivo* (Khiaosa-Ard and Zebeli, 2013).

Castillejos *et al.* (2007) suggest that a long period of adaptation of rumen bacteria to functional oils (at least 4 weeks) may be required to achieve the depression in the concentration of NH_3-N concentrations, which was not achieved in this experiment. The authors confirm that for changes in the concentrations and ratios of short chain fatty acids is required more than 24 hours and less than six days to be modified by functional oils. However, Coutinho *et al.* (2014) report that the 21 days trial using levels of tCNSL in lactating dairy cows were sufficient to provide adaptation of rumen microorganisms to these compounds and influenced the lack of effect in almost all parameters evaluated. This shows that the results of tCNSL are still very incipient and deserve to be studied, especially *in vivo* studies, as much as the literature refers to *in vitro* data.

The N output by feces and urine was similar between levels of tCNSL ($p>0.05$; **table IV**), such as observed by Branco *et al.* (2015). However, when supplemented the animals with 1200 mg tCNSL kg^{-1} of concentrate, there was a reduction of 63.1 g kg^{-1} in N excretion, in relation to diet control. So, during one year, just one animal, in the same conditions of this experiment, would decrease in 2.520 kg the excretion of N to the environment, and could be interesting new researches with tCNSL in ruminant supplementations.

The average plasma urea nitrogen (PUN) for all treatments was 13.26 mg/dL PUN **(table IV)**, being close to the ideal range between 13.5 and 15 mg/dL, which provides maximum microbial efficiency (Valadares *et al*., 1997).

Functional oils might be more effective in intensive systems of feeding, such as cattle diets with high levels of concentrate or finishing high production of dairy cows, where the rumen fluid is more acidic. In some situations, low acetate:propionate ratio could increase the food utilization efficiency, especially in finishing animals and also in high production of dairy cows, if the scarcity of ruminal acetate is not limiting for the synthesis of fat (Spanghero *et al*., 2008).

For Hart *et al.* (2008) the effects of functional oil are large and often contradictory, and this occurs by differences in plant extracts used. The variation of the concentration and quality of the active compounds within the same species grown in different locations, the amount provided and the type of basal diet provided to the animals may be responsible for these contradictions in the responses.

CONCLUSION

The tCNSL (containing cardanol and cardol as main active ingredients, but no anacardic acids) had no effect on intake and total nutrients digestibility. The product increased the ruminal pH, for cattle fed high-grain diets, but had no effect on ruminal ammonia concentration. However, the best dosage to be provided should be continuously evaluated to validate the use of tCNSL since it still presents contradictory responses in the literature.

ACKNOWLEDGMENTS

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e

Tecnológico (CNPq) for financial support and fellowships granted.

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