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Intake, digestibility of nutrients, milk production and composition in dairy cows fed on diets containing cashew nut shell liquid

Diogo Antignani Coutinho, Antonio Ferriani Branco*, Geraldo Tadeu dos Santos, Milene Puntel Osmari, Ana Lucia Teodoro and Tatiana Garcia Diaz

*Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: afbranco@uem.br*

ABSTRACT. The study evaluated the effects of supplementing cashew nut shell liquid (CNSL) in the diet of lactating dairy cows on the intake, nutrient digestibility, milk yield and composition, and fatty acids composition of milk fat. Four Holstein cows (600±43 kg) were used in a 4 x 4 Latin square design with 21 days experimental periods. Experimental treatments consisted of CNSL inclusion in a total mixed ration (dry matter basis), as follows: 1) CNSL 0% (control, CON), 2) CNSL 0.012% (0.012), 3) CNSL 0.024% (0.024) and 4) CNSL 0.036% (0.036). Dry matter and nutrients intake, apparent digestibility of nutrients, fat corrected milk yield and milk composition were not affected ($p > 0.05$) by the inclusion of CNSL in the ration. CNSL linearly decreased the C6:0 concentration ($p < 0.02$), produced a quadratic response on C13:1n-5 concentration ($p < 0.03$) and a linear increase on C16:1n-7 concentration ($p < 0.04$). Results show that an intake of up to 7 g cow^{-1}day^{-1} (0.036%) of CNSL may alter the milk fatty acid composition but does not influence intake, nutrient digestibility and milk yield.

Keywords: cardol, cardanol, methylcardol, CNSL, polyphenols.

Consumo e digestibilidade dos nutrientes da dieta, produção de leite e composição em vacas leiteiras alimentadas com dietas contendo líquido da casca de castanha de caju

RESUMO. O estudo avaliou os efeitos da suplementação com líquido da casca da castanha de caju (LCCC) em ração de vacas em lactação sobre o consumo, a digestibilidade dos nutrientes, a produção e composição do leite, e a composição de ácidos graxos da gordura do leite. Foram usadas quatro vacas Holandesas (600±43 kg), e o delineamento estatístico foi o quadrado latino 4 x 4, com períodos experimentais de 21 dias. Os tratamentos experimentais consistiram na inclusão de LCCC na ração total misturada, como segue: 1) LCCC 0% (controle, CON); 2) LCCC 0,012% (0,012); 3) LCCC 0,024% (0,024) e 4) LCCC 0,036% (0,036). O consumo de matéria seca e de nutrientes, a digestibilidade aparente dos nutrientes, a composição e a produção de leite corrigida para gordura não foram influenciados pelos tratamentos ($p > 0,05$). O uso de LCCC diminuiu linearmente a concentração de C6:0 ($p < 0,02$), produziu uma resposta quadrática na concentração de C13:1n-5 ($p < 0,03$) e um aumento linear no C16:1n-7 ($p < 0,04$). Os resultados mostram que o consumo de até 7 g dia^{-1} de LCCC por vacas leiteiras pode alterar a composição de ácidos graxos da gordura do leite, mas não influencia o consumo, a digestibilidade dos nutrientes e a produção de leite.

Palavras-chave: cardol, cardanol, metilcardol, LCCC, polifenóis.

Introduction

Ionophore antibiotics are widely used to modify the microbial population of the rumen, improve the efficiency of energy and protein metabolism, decrease the incidence of digestive disorders and increase animal productivity. However, the safety of ionophore antibiotics is constantly questioned and Regulation 1831/2003/EC banned its use in animal feed in EU countries. The search for greater food safety has increased worldwide and the consumer market is increasingly demanding to that effect. These facts have led scientists in the field of ruminant nutrition to increase research with natural substances capable of modulate the rumen fermentation.

According to Tager and Krause (2011), functional oils could be an alternative because some of these oils have shown different functions, such as anti-inflammatory, antioxidant and antimicrobial features. Additives based on plant extracts tend to be underscored as new alternatives to replace synthetic additives and thereby reduce the restrictions imposed by some consumer markets. Essential oils, an important group from plant extracts, have been studied for ruminant nutrition to understand effects on the rumen (MOLERO et al., 2004; NEWBOLD et al., 2004; CASTILLEJOS et al., 2006) and milk production (BENCHAAR et al., 2006; BENCHAAR et al., 2007; TASSOUL; SHAVER, 2009; TAGER; KRAUSE, 2011).

Studies on cashew nut shell liquid (CNSL) are scarce. In fact, it is known to contain a significant amount of anacardic acid which is active against gram-positive bacteria (KUBO et al., 1993). Results from *in vitro* studies conducted by Watanabe et al. (2010) showed that the use of CNSL inhibited methane production by 70% and stimulated the production of propionate by 44%. More recently, Shinkai et al. (2012) found an increase in rumen propionate production and 38% reduction in methane production in cows fed on hay and concentrate (60:40 ratio), with an inclusion of 4 g CNSL 100 kg^{-1} of body weight.

Since most published research on CNSL for ruminants has been performed in vitro, its acceptance by industries is rather difficult since they require *in vivo* studies. Current research has been conducted to evaluate the use of cashew nut shell liquid (CNSL) in diets of lactating cows and its effects on intake and digestibility of nutrients, milk production, milk composition and fatty acids composition of milk fat.

Material and methods

The experiment was conducted from October 2011 to January 2012, at the Iguatemi Experimental Farm of the State University of Maringá, Maringá, Paraná State, Brazil. Four multiparous lactating Holstein cows (600 ± 33 kg), with an average of 100 ± 20 days in milk (DIM), were used. The experimental design was a 4 x 4 Latin square, with 21-days periods, of which 16 days were for adaptation and 5 days for sampling. The cows were kept in a dairy facility, using a tie-stall system, with individual spots per cow, provided with an individual feeder and bowl, and fed twice a day *ad libitum* on a total mixed ration (TMR).

The TMR was formulated with 16.5% crude protein and 1.6 Mcal NE_L kg^{-1} of dry matter, meeting the cows' requirements according to NRC (2001) and shown in Table 1. Experimental treatments consisted of four levels of cashew nut shell liquid (CNSL) and included in the total mixed ration, as follows: 0% CNSL (CON), 0.012% CNSL (0.012), 0.024% CNSL (0.024), and 0.036% (0.036). The CNSL was obtained during the industrial processing of *Anacardium occidentale* nuts, and the technical grade of CNSL from Usibras was adopted.

Table 1. Chemical composition of feeds and experimental total mixed ration (TMR, 100% DM).

	Nutrient[1] (%)							
Feed	DM	ASH	CP	EE	NDF	NFC	NE_L (Mcal kg^{-1} DM)	TMR[3] (%)
Corn silage, %	31.0	5.3	7.5	2.4	50.3	34.5	1.40	40.0
Ground corn, %	89.9	1.3	9.5	4.4	15.2	69.6	1.80	32.8
Soybean meal, %	88.6	7.3	49.9	1.7	15.7	25.4	1.80	20.1
Wheat bran, %	88.0	5.5	17.8	3.2	40.1	33.4	1.60	4.4
Mineral mixture, %	99.3	99.3	-	-	-	-	-	2.7
TMR, %	66.2	6.9	16.9	2.8	30.0	43.7	1.58	100.0

[1] DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; NFC: non-fiber carbohydrates; NE_L: net energy of lactation.

Technical CNSL chemical composition (73.3% cardanol, 16.4% cardol and 3.0% methylcardol) was determined by gas chromatography-mass spectrometry, performed on a Shimadzu GCMS-QP2010 Ultra system, equipped with an AOC-20i autoinjector. The column used was an Restek RTX-5ms (30 m, 0.25 mm i.d., 0.25 μm d_f), coated with 5% diphenyl-95% polydimethylsiloxane, operated with the following 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.

From day 4 to day 21 of each experimental period, all cows received a daily dose of titanium dioxide (20 g day^{-1}) as an external marker, by mixing with a small amount of concentrate and top-dressing. Fecal samples were collected from the rectum from day 10 to day 14 of each period, four times a day, at three-hour intervals (9, 12, 15, and 18 hours) to determine the total tract apparent digestibility of the nutrients.

Feeds were sampled weekly, orts were weighed, sampled daily and frozen at -20°C for subsequent chemical analysis. Daily orts samples were prepared to obtain a sample per animal per period.

Samples of feed, orts and feces were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash (AOAC, 1990) and neutral detergent fiber (NDF), according to Van Soest et al. (1991). The titanium dioxide content was determined following Myers et al. (2004). The percentage of total digestible nutrients (TDN) of the diets was determined by the equation described by Sniffen et al. (1992):

$$TDN = (CP_i - CP_f) + (CHO_i - CHO_f) + ((EE_i - EE_f) \times 2.25)$$

where:

CP_i = protein intake;
CP_f = fecal crude protein;
CHO_i = carbohydrate intake;
CHO_f = fecal carbohydrate;

EE_i = ether extract intake;

EE_f = fecal ether extract.

Milk yield was measured at every milking time in the collecting milk system. The 4% fat-corrected milk yield (FCM) was obtained using the equation: FCM (kg day^{-1}) = (0.4 x milk yield) + (15 x fat production), as recommended by NRC (2001). During day 15 and day 16 of each experimental period, milk samples were collected in the morning and afternoon milking, and were pooled proportionally to the morning and afternoon yield. The composite samples were stored in properly labeled plastic vials containing bronopol (2-bromo-2-nitro-1.3-propanediol) for conservation and for subsequent analyses of fat, protein, lactose, total solids and somatic cell count. These analyses were performed in the laboratory of the Associação Paranaense de Criadores de Bovinos da Raça Holandesa.

Two other samples of milk per animal/period were collected on the 15th and 16th day of each experimental period; they were stored in plastic vials and frozen at -20°C for later analysis of milk urea nitrogen (MARSH et al., 1965) and fatty acids. For the analysis of fatty acids in milk, fat was extracted after thawing the samples and esterified according to the method by ISO 5509 (1978), using KOH/methanol and n-heptane. Fatty acid esters were analyzed by gas chromatography Thermo Scientific® (Thermo Fisher Scientific Inc., USA), model Trace GC Ultra equipped with auto-sampler TriPlus, with flame ionization detector and a fused silica capillary column CP- 7420 (100 m, 0.25 mm i.d. and 0.39 mM, 100% cyanopropyl). Gases flow rates (Praxair, Praxair Technology, Inc., USA) were 1.4 mL min.$^{-1}$ for the carrier gas (H_2), 30 mL min.$^{-1}$ for the auxiliary gas (N_2), and 30 mL and 300 mL min.$^{-1}$ for H_2 and flame synthetic air, respectively. Injector and detector temperatures were 230 and 240°C, respectively. Column temperature was programmed at 65°C for 4 minutes, followed by the first ramp 16°C min.$^{-1}$ up to 185°C, with duration of 12 minutes. The second gradient was programmed from 20°C min.$^{-1}$ to 235°C, maintained at this temperature for 9 minutes, with 35 minutes as total time for analysis. The peak areas were determined by ChromQuest 5.0. The injections were performed in triplicate, with injection volume at 2 μL. Identification of fatty acids (FA) was based by comparing retention times of the standards of methyl esters of fatty acids. The quantification of the methyl esters of CLA was performed by comparing retention times of a mixture from Sigma-Aldrich® standards. The quantification (mg fatty acid g^{-1} total lipid) was performed by methyl ester of tricosanoic acid as an internal standard (23:0), described by Joseph and Ackman (1992). The concentration of FA was accomplished by a theoretical correction factor (VISENTAINER, 2012) for flame ionization detector (FID).

Statistical analysis was performed with ANOVA, followed by polynomial regression analysis using SAS (2004) for each independent variable. Data were analyzed considering the Latin design 4 x 4, and the value of $\alpha = 0.05$. The mathematical model used was:

$$Y_{ijk} = \mu + A_i + P_j + T_k + e_{ijk}$$

where:

Y_{ijk} = observed variable;

μ = the overall mean;

A_i = the effect of animal i ranging between 1 and 4;

P_j = effect of j period between 1 and 4;

T_k = treatment effect k between 1 and 4;

e_{ijk} = random error.

All effects were considered fixed, except the effect of animal that was considered random.

Results and discussion

The dry matter intake in absolute value (21.3 kg DM day^{-1}) and as a percentage of body weight (3.6%) were not influenced ($p < 0.05$) by CNSL in the diet (Table 2). The estimated dry matter intake (NRC, 2001) for cows in the same conditions is 20.6 kg day^{-1}, similar to that observed in the experiment. As a consequence of lack of effect on DMI, the intake of crude protein and NDF was not affected too ($p > 0.05$) by the addition of CNSL in the diet, averaged 3.5 and 6.0 kg day^{-1}.

Table 2. Dry matter and nutrient intake in dairy cows fed on a control diet and diets supplemented with cashew nut shell liquid (CNSL).

	Treatments						
Item[1]	COM	0.012	0.024	0.036	P<	Mean	SEM[2]
DMI, kg day^{-1}	20.5	21.7	22.2	20.8	0.11	$\hat{Y} = 21.3$	0.50
DMI, %BW	3.4	3.7	3.6	3.5	0.13	$\hat{Y} = 3.6$	0.10
CPI, kg day^{-1}	3.5	3.4	3.9	3.5	0.12	$\hat{Y} = 3.6$	0.10
NDFI, kg day^{-1}	5.8	6.2	6.3	6.0	0.20	$\hat{Y} = 6.0$	0.20
NDFI, %BW	1.3	1.4	1.4	1.4	0.22	$\hat{Y} = 1.4$	0.10
EEI, kg day^{-1}	0.6	0.4	0.8	0.6	0.20	$\hat{Y} = 0.6$	0.20
NFCI, kg day^{-1}	9.0	9.3	9.5	9.3	0.19	$\hat{Y} = 9.3$	0.20

[1]DMI: dry matter intake; CPI: crude protein intake; NDFI: neutral detergent fiber intake; EEI: ether extract intake; NFCI: non-fiber carbohydrate intake; [2]SEM: standard error of the mean.

Dry matter intake is highly important to evaluate the quality of the diets fed to lactating cows, and the results of this experiment showed that feeding up to 7 g day^{-1} of CNSL did not affect intake. These results have also been observed in several studies with essential oils (EO). Benchaar et al. (2006) used a product containing a mixture of EO (Crina Ruminants, Akzo Nobel Surface Chemistry Ltd) at a 2 g cow^{-1} day^{-1} in the diet of lactating cows and found no effect on dry matter and nutrient intake.

This result was confirmed when a mixture of EO (750 mg cow^{-1} day^{-1}) was used by Benchaar et al. (2007), and by Yang et al. (2007) who used garlic (5 g cow^{-1} day^{-1}) and juniper oil (2 g cow^{-1} day^{-1}). More recently, Tager and Krause (2011) using a high EO dose (10 g cow^{-1} day^{-1}) also confirmed these results. Tassoul and Shaver (2009) reported a different result, or rather, a reduction of 7% in dry matter intake during the first 15 weeks of lactation in cows with higher milk yield (48 kg day^{-1}), when using EO supplementation. The authors suggested that a possible explanation for the decreased dry matter intake could be that EO adversely influenced the palatability of the TMR fed in the assay.

The apparent digestibility of DM, OM, CP, NDF, NFC and EE were not affected ($p > 0.05$) by the use of CNSL in the diet, and mean rates were 64.8, 67.5, 70.2, 59.2, 76.3, and 73.7%, respectively. The TDN of the diets was not affected ($p > 0.05$) by treatments, with an average rate of 68.8% (Table 3).

Table 3. Total tract nutrient digestibility in dairy cows fed on a control diet and diets supplemented with cashew nut shell liquid (CNSL).

Item, %	Treatments						
	CON	0.012	0.024	0.036	P<	Mean	SEM[1]
Dry matter	63.5	65.8	64.6	65.1	0.32	$\hat{Y} = 64.8$	0.80
Organic matter	67.0	68.3	66.1	68.5	0.39	$\hat{Y} = 67.5$	0.80
Crude protein	70.0	69.6	70.6	70.8	0.89	$\hat{Y} = 70.2$	0.70
Neutral detergent fiber	59.0	60.4	58.2	59.4	0.65	$\hat{Y} = 59.2$	0.70
Non-fiber carbohydrate	75.4	78.6	74.9	76.5	0.23	$\hat{Y} = 76.3$	1.00
Ether extract	74.5	73.9	73.0	73.4	0.68	$\hat{Y} = 73.7$	0.60
Total digestible nutrients	67.9	69.7	68.3	69.4	0.37	$\hat{Y} = 68.8$	0.60

[1] SEM: standard error of the mean.

Studies to determine the effects of CNSL inclusion in the diets of ruminants and of other farm animals are still fledging, and thus little information is available. Shinkai et al. (2012) conducted two experiments and verified the occurrence of variability in the effect on DM digestibility when CNSL was included in the diet. Although there was no effect in the first experiment, in the second the dry matter digestibility decreased ($p < 0.05$) by 4.4% when CNSL was included. The same variability observed for DM digestibility was observed for CP and NDF digestibility (SHINKAI et al., 2012). In the case of CP, there was a decrease trend ($p = 0.076$) in apparent digestibility (68.2 to 63.2%) in one experiment; however, in another experiment there was an increase trend ($p = 0.063$) in apparent digestibility of CP (67.4 to 73.1%) by including CNSL in the diet. With regard to the apparent digestibility of NDF, the authors found no difference in one experiment but a tendency ($p = 0.054$) to increase in another (57.3 to 60.8%). Shinkai et al. (2012) suggested that the decrease in DM and CP digestibility in one experiment and not in the other was due to a reduction in the abundance of some important rumen bacteria (*B. fibrisolvens* and *Ruminococcus* species).

The variability in nutrient digestibility due to the inclusion of CNSL may be partly explained by the inhibitory effect of phenolic compounds in CNSL over different types of microorganisms (KUBO et al., 1993) and the same may occur with regard to microorganisms in the rumen.

When the use of the active principles derived from plants for feeding ruminants is taken into consideration, the studies are more advanced with EO. Results from *in vivo* experiments, conducted with lactating cows, showed the same variability when EO was used. While Benchaar et al. (2006) observed an increase in ADF digestibility with the use of EO, other authors (BENCHAAR et al., 2007; YANG et al., 2007; TAGER; KRAUSE, 2011) observed no effect on nutrient digestibility. However, the number of experiments with these oils is still low and mostly performed *in vitro*. It is also important to note that EO are composed from more than 100 components (GUILLÉN; MANZANOS, 1998) that could affect differently nutrient digestibility.

There were no differences ($p > 0.05$) between treatments with regard to milk yield (25.8 kg day^{-1}), and 4% fat-corrected milk yield (23.8 kg day^{-1}). Milk composition was also not affected ($p > 0.05$) by the inclusion of CNSL in the diet (Table 4) and the average concentration of protein and fat were 3.1 and 3.5%, respectively. The daily production of protein (810 g), fat (910 g) and total solids (3,160 g) were not affected ($p > 0.05$) by treatments. Milk urea nitrogen (17.1 mg dL^{-1}) was also not influenced ($p > 0.05$) by CNSL inclusion (Table 4).

Table 4. Milk yield and milk composition from dairy cows fed on a control diet and diets supplemented with cashew nut shell liquid (CNSL).

	Treatments						
Item	CON	0.012	0.024	0.036	P<	Mean	SEM[1]
Milk yield, kg day^{-1}	25.7	26.0	25.3	26.3	0.51	$\hat{Y} = 25.8$	0.60
FCMY, kg day^{-1}[2]	23.9	23.2	23.4	24.5	0.32	$\hat{Y} = 23.8$	0.50
Protein, %	3.07	3.10	3.17	3.14	0.68	$\hat{Y} = 3.12$	0.40
Protein, g day^{-1}	788	806	802	826	0.28	$\hat{Y} = 810$	101.30
Fat, %	3.55	3.56	3.50	3.55	0.54	$\hat{Y} = 3.54$	0.20
Fat, g day^{-1}	912	926	886	934	0.38	$\hat{Y} = 910$	122.85
Lactose, %	4.63	4.67	4.63	4.59	0.32	$\hat{Y} = 4.63$	0.20
Lactose, g day^{-1}	1189	1214	1171	1207	0.46	$\hat{Y} = 1170$	121.70
Total solids, %	12.30	12.23	12.25	12.18	0.44	$\hat{Y} = 12.24$	0.20
Total solids, g day^{-1}	3160	3180	3099	3203	0.52	$\hat{Y} = 3160$	363.40

[1] SEM: standard error of the mean; [2] FCMY: 4% fat-corrected milk yield.

Recently, Watanabe et al. (2010) and Shinkai et al. (2012) used CNSL in diets for cattle, but the experiments were not conducted with lactating cows. Shinkai et al. (2012) used 4 g cow^{-1} day^{-1}, reaching 30 g CNSL day^{-1}, or rather, a higher dose than that in current experiment. No results have been published so far on the yield and composition of milk with CNSL inclusion in the diet of cows.

In general, there has been no response in relation to milk yield in the few studies with lactating cows and use of EO, but changes in the composition may occur. Benchaar et al. (2006) and Yang et al. (2007) experimented with cows with a higher production (32 kg day^{-1}) than those used in current experiment and they failed to find any effect on milk yield and composition. In another research with EO, although no change occurred in the milk yield, there was an increase in the milk lactose concentration (BENCHAAR et al., 2007) and a decrease in the milk protein concentration (TASSOUL; SHAVER, 2009). Thus, besides the need for further studies to evaluate the effects of these compounds of plant origin on milk yield, another important area to be studied is related to changes in milk composition.

Table 5 shows the concentration of esters of fatty acids in milk fat. The use of CNSL decreased linearly the concentration of caproic acid (C6:0, $p < 0.02$), produced a quadratic response for tridecanoic acid concentration (C13:1n-5, $p < 0.03$) and increased linearly the concentration of palmitoleic acid (C16:1n-7, $p < 0.04$).

Since research on these plant-derived compounds is recent, there are no studies on their inclusion in the diet of lactating cows and their effects on the composition of milk fatty acids. Caproic acid decreased 41% when compared CON and the highest CNSL diet level (0.06); increased 44% in the case of palmitoleic acid. Palmitoleic acid (C16:1n-7), a monounsaturated omega-7, recently denominated lipocine, emerges as a new hormone produced by adipose tissue which protects mice consuming rich lipids diets for a long time, from their harmful effects (CAO et al., 2008). It has been demonstrated that a higher level of C16-1n-7 in serum suppresses hepatic lipogenesis, and significantly improves systemic insulin action and glucose metabolism (CAO et al., 2008).

Results of current study do not confirm the beneficial effects of CNSL in lactating dairy cows' diet. CNSL had no effects on digestibility, milk production and milk composition at the concentrations evaluated under the current study´s experimental conditions up to 0.036% of dry matter. It had only a slight effect on the fatty acid composition of fat milk.

Table 5. Milk fatty acids composition (mg g^{-1}) in milk fat from dairy cows fed on a control diet and diets supplemented with cashew nut shell liquid (CNSL).

Fatty acid	CON	0.012	0.024	0.036	P<	Mean	SEM[1]
4:0	3.35	3.49	3.29	3.04	0.86	$\hat{Y} = 3.3$	0.17
6:0	2.46	2.41	1.79	1.45	0.02	★	0.13
8:0	6.00	6.00	6.00	6.00	0.57	$\hat{Y} = 6.1$	0.09
10:0	17.00	13.00	14.00	15.00	0.19	$\hat{Y} = 14.9$	0.21
12:0	21.00	21.00	21.00	21.00	0.70	$\hat{Y} = 21.1$	0.02
13:0	1.12	1.13	0.90	0.80	0.29	$\hat{Y} = 1.0$	0.01
13:1n-5	0.91	1.32	1.08	1.00	0.03	★★	0.49
14:0	77.00	78.00	77.00	78.00	0.27	$\hat{Y} = 77.0$	4.70
14:1n-5	8.52	11.06	12.73	12.72	0.33	$\hat{Y} = 11.3$	0.13
15:0	7.00	7.00	8.00	8.00	0.50	$\hat{Y} = 7.5$	0.11
15:1n-5	4.29	4.65	4.05	4.70	0.22	$\hat{Y} = 4.4$	0.80
16:0	325.00	314.00	319.00	321.00	0.45	$\hat{Y} = 319.8$	0.01
16:1n-7	8.35	11.27	11.70	12.06	0.04	★★★	0.01
16:1n-5	4.36	4.12	4.05	4.42	0.23	$\hat{Y} = 4.2$	1.40
17:0	9.40	10.70	8.00	9.50	0.29	$\hat{Y} = 9.4$	0.04
17:1n-7	1.10	1.70	1.80	1.70	0.47	$\hat{Y} = 1.4$	0.05
18:0	111.50	121.00	106.00	103.00	0.53	$\hat{Y} = 110.4$	0.40
18:1n-9t	31.70	32.00	32.20	32.60	0.48	$\hat{Y} = 31.2$	4.60
18:1n-9c	309.00	311.00	321.00	279.00	0.25	$\hat{Y} = 305.0$	18.3
18:2n-6c	36.00	32.00	32.00	29.00	0.39	$\hat{Y} = 32.3$	4.20
18:3n-3	4.00	4.00	4.00	3.00	0.38	$\hat{Y} = 3.8$	0.07
9c, 11t	5.56	5.19	6.82	5.43	0.79	$\hat{Y} = 5.8$	0.10
10t, 12c	2.00	2.00	2.00	3.00	0.65	$\hat{Y} = 2.3$	0.06
Others	3.60	3.60	3.60	3.50	0.16	$\hat{Y} = 3.6$	0.08
SFA[2]	580.3	586.2	565.0	566.8	0.29	$\hat{Y} = 574.6$	0.04
UFA[3]	367.3	377.1	387.6	348.2	0.19	$\hat{Y} = 370.1$	0.18
PUFA[4]	40.0	36.0	36.0	32.0	0.70	$\hat{Y} = 36.0$	0.05
SFA/UFA	1.42	1.41	1.32	1.49	0.23	$\hat{Y} = 1.4$	0.57

[1]SEM: standard error of the mean; [2]SFA: saturated fatty acids; [3]UFA: unsaturated fatty acids; [4]PUFA: polyunsaturated fatty acids.

Conclusion

CNSL´s lack of effect may be due to an adaptation of rumen microbes to these compounds. Possibly CNSL concentration was not enough to produce rumen impact, and consequently to affect milk composition and production. Cashew nut shell liquid has been shown to favorably alter the rumen fermentation in vitro, but more research is required to validate its *in vivo* efficiency in normal feeding doses.

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