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Fatty acid profile and in vitro rumen fermentation characteristics of maize silage augmented with canola silage

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Abstract

The objective was to investigate the effect of replacing maize silage (MS) with canola silage (CS) on the chemical composition and fatty acid (FA) profile of total mixed rations (TMR) containing these silages, and on in vitro rumen fermentation and methane production from them. The canola (*Brassica napus* var. Monty) was cultivated on a small-scale agricultural farm and harvested at 148 days after sowing. Maize silage in a TMR was replaced with 0%, 15%, 25%, and 35% CS to make the rations CS0, CS15, CS25, and CS35, respectively. Proximate analyses of the rations were evaluated in a completely randomized design. The results showed that linolenic acid increased linearly with the level of CS, primarily at the expense of linoleic acid. In vitro dry matter digestibility (IVDMD) was similar among treatments. However, in vitro neutral detergent fibre digestibility (IVNDF) decreased linearly (P <0.05) when the CS proportion increased in the TMR. The lowest ammonia nitrogen content (P <0.05) was observed in CS35. The soluble fraction (A) increased (P <0.05) when the CS increased in the TMR from 0% to 35%. In vitro methane (CH_4) production was lowest with CS25 and CS35, decreasing 34% and 23.9%, respectively, compared with CS0. Linolenic acid had a negative correlation with IVNDF (r = -0.94; P <0.05). The IVDMD and methane production were positively correlated (r = 0.60) (P <0.05). In conclusion, 25% and 35% augmentation of MS with CS in a TMR was an important source of linolenic acid (C18:3) and decreased in vitro methane production.

Keywords: *Brassica napus*, fatty acid profile, in vitro digestibility, linolenic acid, total mixed rations
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Introduction

Canola *(Brassica napus)* is a crop that shows drought resistance and uses soil water efficiently (Sánchez-Gutiérrez *et al.*, 2018). In Mexico, canola is sown in autumn and winter in rotation after maize to avoid competition with the maize crop for nutrients. Brassica forage is nutritionally valuable for ruminants owing to its high metabolizable energy, crude protein contents, and low level of neutral detergent fibre (NDF) (Barry, 2013, Reta-Sánchez *et al.*, 2015). Another advantage of using brassicas in diets for ruminant animals is that they increase the content of beneficial linolenic acid in milk compared with that produced when the animals are fed MS. Canola fodder can be used by ruminants as conserved forage (hay and silage) or as grazing (Balakhial *et al.*, 2008; Kincaid *et al.*, 2012; Sun *et al.*, 2015; Limón-Mernández *et al.*, 2019). It can be included in a TMR (Kincaid *et al.*, 2012) as a replacement for maize and oat silages (Burbano *et al.*, 2017). However, there is little information about the optimal inclusion level of CS in TMR for small ruminants.

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Some authors indicated a decrease of methane (CH_4) in vivo from the inclusion in the ration of up to 40% brassicas on a DM basis (Williams *et al.*, 2016). However, in vitro studies have not reported this effect (Limón-Hernández *et al.*, 2019). Several feeding strategies have been proposed to mitigate CH_4 emissions from enteric fermentation, which include feeds with higher digestibility. Sun *et al.* (2015) observed a linear decrease of up to 25% in the in vitro CH_4 production after replacing 0%, 25%, 50%, 75%, and 100% of ryegrass with canola fodder. Canola silage is a good source of linolenic acid (Limón-Hernández *et al.*, 2019), whereas MS provides more linoleic acid (Chilliard *et al.*, 2001). These FAs can promote the synthesis of polyunsaturated fatty acids (PUFAs) such as conjugated linoleic acid (CLA), which is an intermediate in the biohydrogenation of PUFAs, and is desirable for human health (Lock & Bauman, 2004). Increasing the concentration of linoleic and linolenic acids in the diet for dairy cows, sheep and goats can modify the content of CLA in milk (Lock & Garnsworthy, 2002; Sanz Sampelayo *et al.*, 2007). Therefore, the objective in this study was to investigate the effects on the chemical composition and FA profile of the diets of replacing MS with three levels of CS, and on in vitro ruminal fermentation and methane production of TMR.

Material and methods

The study was conducted from May to July 2018 at the Bromatology and Metabolism Laboratory of the Animal Nutrition Department, Universidad Autónoma del Estado de México. The corn crop was cut and chopped, compacted, and sealed in a trench silo that was opened 60 days after sealing. Briefly, the canola crop was harvested and chopped on day 148 after sowing. Two microsilos were used for each of the CS treatments. Sugarcane molasses was diluted in water (1 : 1) and added to canola forage to promote a desirable fermentation process. The microsilos consisted of polyvinyl chloride tubes 10 cm in diameter and 20 cm in length, packed with 220 kg DM of silage per cubic metre, sealed with plastic bags and adhesive tape, and kept at room temperature. The microsilos were opened 28 days post incubation and taken to the laboratory for later analysis. The samples of CS were dried at 55 °C until they lost no further weight, milled (2-mm particle size) and stored at room temperature. The TMR with MS and no CS was the control diet (CS0). As shown in Table 1, three other TMRs were formatted in which a portion of the MS was replaced with 15%, 25%, and 35% of CS (CS15, CS25, CS35, respectively).

Table 1 Chemical composition of total mixed rations with mixtures of canola and maize silages

	Treatments			
Ingredients, g/kg	CS0	CS15	CS25	CS35
Canola silage	0	150	250	350
Maize silage	500	350	250	150
Maize stover	100	100	100	100
Soybean meal	257	222	202	181
Ground sorghum	20	70	123	184
Wheat bran	108	93	60	20
Mineral mix[2]	15	15	15	15

CS0: total mixed ration without canola in the silage, CS15: total mixed ration with 15% canola silage, CS25: total mixed ration with 25% canola silage, CS35: total mixed ration with 35% canola silage
Phosphorus: 120 g, copper 1.33 g, iron: 2 g, magnesium: 10 g, cobalt: 11 mg, iodine: 0.080 g, zinc: 4 g, sulfur, 12.5 g, selenium: 0.022 g, calcium: 120 g, salt, 200 g; manganese: 4 g

The analyses of chemical composition, pH (OAKTON®), gas production, IVDMD, organic matter (IVOMD) and in vitro neutral detergent fibre digestibility (IVNDFD) were carried out as described in AOAC (2012), Pell and Schofield (1993) and Theodorou *et al.* (1994). The in vitro ammonia nitrogen (NH_3-N) content was quantified at 72 hours post incubation (Broderick & Kang, 1980; Theodorou *et al.*, 1994). The in vitro gas production technique was used to determine the kinetics of ruminal fermentation (Theodorou *et al.*, 1994). Each sample

was assayed in triplicate, and three blanks with ruminal fluid and buffer only were used in each incubation run. Two dairy cows in lactation were used as donors of ruminal fluid, which were fed with a diet based on CS and concentrate. Rumen fluid was extracted with a nasogastric tube filtered in a triple layer of cheesecloth gauze, and homogenized with CO_2 for five minutes, then mixed and used as inoculum. The flasks were incubated in a water bath at 39 °C. Gas volume was recorded at 1 to 8, 9, 12, 24, 36, 40, 44, 48, 52, 56, 60, 72, 84, and 96 hours of incubation, using a Delta pressure transducer (model 8804 HD). Methane (CH_4) gas was quantified according to the gas production technique (Theodorou *et al.*, 1994) by measuring the accumulated CH_4 concentration in the headspace of the bottles at 24 hours post incubation with an electrochemical methane sensor coupled to a portable analyser (Aeroqual Series 500®). The FA profiles of the TMR were determined (Palmquist & Jenkins, 2003) and assessed for gas chromatography (Clarus 500 Perkin Elmer chromatograph, equipped with a capillary column of 100 m× 0.25 mm× 0.2 µm) (SULPELCO TM-2560), with nitrogen as carrier gas. The chromatograms were identified according to the retention times of FA methyl ester standards (Supelco37, FAME MIX analytical).

Gas production was fitted to the model described in Jessop and Herrero (1996). The relative gas production (GRY) was calculated as the ratio of DM degradability (g) in vitro to the volume (ml):

GRY = total gas production (GP 96 h) / IVDMD.

Where: GP 96 h = in vitro gas production at 96 hours (ml / g DM).

Gas yield (GY_{24}) was calculated as the volume of gas (ml gas / g DM) produced after 24 hours of incubation divided by the amount of IVDMD (Blümmel *et al.,* 1997):

$$GY_{24} = \frac{ml\ gas/g\ DM}{i\ IVDMD}$$

Short-chain fatty acid (SCFA) concentration was calculated (Getachew *et al.,* 2002) as SCFA (mmol/200 mg DM) = 0.0222GP - 0.00425, where: GP is the 24 hour net gas production (ml / 200 mg DM). Microbial crude protein (MCP) biomass production was calculated (Blümmel *et al.,* 1997) as MCP (mg/g DM) = mg IVDMD – (ml gas x 2.2 mg/ml), where: 2.2 mg/ml is a stoichiometric factor, which expresses mg of the C, H, O required for the production of SCFA gas associated with production of 1 ml of gas.

The effects of the treatments were tested with a general linear model:

$$Y=\mu + T_i + E_{ij},$$

Where: Y = the response variable,
µ = general mean,
T_i = treatment effect, and
E_{ij} = experimental error.

The effect of CS inclusion was analysed using orthogonal contrast to evaluate linear and quadratic responses. The Tukey test was applied when significant differences (P <0.05) were observed. Pearson correlations were used to evaluate the relationships between the variables.

Results and Discussion

A Saanen doe in the eighth week of lactation, which produces 5.3 kg milk per day, which contains 3.2% fat and 2.7% protein, and consumes 3.33 kg of dry matter, requires 15% crude protein and 72.3% total digestible nutrients (NRC, 2007). Table 2 shows the average chemical composition of the diets. Each of these diets would satisfy the requirements for protein but would be slightly deficient in energy for the doe at this particular point in lactation.

Table 2 Chemical composition (g/kg DM) of canola and maize silages and total mixed rations formulated with various proportions of canola silage

Variable	Treatments				Silages	
	CS0	CS15	CS25	CS35	Canola	Maize
DM	593.04 ± 1.33	583.43 ± 0.59	576.93 ± 0.10	569.61 ± 0.26	204.2 ± 0.80	265.8 ± 3.89
OM	934.14 ± 1.71	929.41 ± 1.81	924.61 ± 0.18	923.35 ± 1.25	892.9 ± 0.10	949.6 ± 0.40
CP	164.57 ± 0.02	165.38 ± 0.46	165.34 ± 1.07	164.61 ± 0.22	141.5 ± 0.50	47.70 ± 0.70
EE	14.41 ± 1.67	15.04 ± 0.63	15.39 ± 0.04	15.94 ± 0.05	35.7 ± 1.50	15.8 ± 0.30
NSC	325.30 ± 5.40	358.13 ± 4.80	387.13 ± 6.50	416.58 ± 10.80	351.2 ± 0.80	291.1 ± 13.3
NDF	429.86 ± 2.00	391.07 ± 1.58	361.53 ± 0.12	332.23 ±1.19	364.5 ± 1.90	594.6 ± 10.2
ADF	211.62 ± 1.03	200.95 ± 0.43	187.94 ± 0.56	176.79 ± 1.06	212.4 ± 3.20	206.1 ± 17.0
ADL	50.66 ± 1.53	53.77 ± 0.17	51.95 ± 1.00	52.54 ± 0.12		
GE	4.18 ± 0.01	4.15 ± 0.01	4.13 ± 0.01	4.07 ± 0.01	4.02 ± 0.30	4.24 ± 0.08

CS0: total mixed ration without canola in the silage, CS15: total mixed ration with 15% canola silage, CS25: total mixed ration with 25% canola silage, CS35: total mixed ration with 35% canola silage, DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, NSC: nonstructural carbohydrates, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, GE: gross energy

The FA profiles that characterize the treatments are shown in Table 3. The CS inclusion in the TMR had an effect (P >0.05) on the long-chain FA (>C18) content. The contents of linolenic acid (C18:3) and heneicosanoic acid (C21:0) increased (P <0.05) with the proportion of CS in the TMR. On the contrary, as the CS proportion increased in the TMR, its linoleic acid (C18:2) concentration decreased. The contents of lauric (C12:0) (0.12 ± 0.04 g/100 g FA), myristic (C14:0) (0.22 ± 0.03), and palmitic (C16:0) (18.46 ± 0.69) FAs were similar (P >0.05) among treatments.

Table 3 Fatty acid profile (g/100 g fatty acids) of the total mixed rations with various proportions of canola silage

Fatty acid	Treatment (Trt)				SE	*P*-values		
	CS0	CS15	CS25	CS35		Trt	Linear	Quadratic
Lauric acid	0.11	0.10	0.16	0.10	0.02	0.050	1.000	1.000
Tridecanoic acid	0.18	0.23	0.14	0.15	0.03	0.060	0.027	0.093
Myristic acid	0.22	0.23	0.21	0.19	0.02	0.060	0.384	0.018
Palmitic acid	18.6	18.7	18.6	17.9	0.40	0.451	1.000	0.443
Palmitoleic acid	0.29	0.30	0.38	0.36	0.05	0.400	0.874	0.668
Stearic acid	2.77^{b}	2.96^{ab}	2.94^{ab}	3.03^{a}	0.05	0.019	0.157	0.527
Oleic acid	23.8^{a}	22.2^{ab}	21.4^{b}	22.1^{ab}	0.41	0.019	0.028	0.093
Linoleic acid	48.5^{a}	44.6^{b}	39.6^{c}	36.2^{d}	0.38	0.001	0.003	0.510
Linolenic acid	5.01^{d}	8.37^{c}	12.1^{b}	14.0^{a}	0.38	0.001	0.001	0.278
Heneicosanoic acid	0	1.89^{c}	4.07^{b}	5.38^{a}	0.12	0.001	0.001	0.737
Others	0.52	0.42	0.40	0.59	0.08	0.192	0.001	0.347

^{a,b,c} Within a row, means with a common superscript were not different with probability P =0.05

CS0: total mixed ration without canola in the silage, CS15: total mixed ration with 15% canola silage, CS25: total mixed ration with 25% canola silage, CS35: total mixed ration with 35% canola silage

Fermentation characteristics of the TMR are shown in Table 4. The soluble fraction (A) of the diet increased with the proportion of CS in the TMR. Gas production from the insoluble fraction (B) was lower (P <0.05) in CS25 and CS35 compared with CS0. No significant differences (P >0.05) were detected in the rate (c_a) at which the soluble fraction was digested. The rate (c_b) at which the insoluble fraction was degraded increased with the amount of CS. Lag time was affected linearly (P <0.05), decreasing as the proportion of CS in the TMR increased.

Table 4 Estimates of rumen fermentation parameters for total mixed rations with various proportions of canola silage

Parameter	Treatment				SE	*P*-value		
	CS0	CS15	CS25	CS35		Trt	Linear	Quadratic
pH	6.67	6.69	6.69	6.70	0.020	0.737	1.000	1.000
A, ml gas/g DM	28.2^{b}	37.9^{a}	36.8^{a}	40.8^{a}	1.58	<0.001	1.000	1.000
c_a/h	0.166	0.165	0.167	0.171	0.004	0.683	0.007	0.010
B, ml gas/g DM	232.6^{a}	232.8^{a}	223.5^{b}	222.4^{b}	3.89	0.043	0.961	0.717
c_b/h	0.045^{b}	0.049^{b}	0.053^{a}	0.056^{a}	0.001	<0.001	0.133	0.361
Lag time, hours	3.19^{a}	2.87^{b}	2.89^{b}	2.99^{ab}	0.067	0.008	0.001	0.865
IVDMD	841.9	833.1	828.7	828.4	6.36	0.423	1.000	1.000
IVOMD	906.6^{a}	924.2^{a}	869.6^{b}	896.4^{ab}	7.18	<0.003	0.158	0.783
IVNDFD	649.4^{a}	617.0^{b}	576.4^{c}	553.9^{c}	5.78	<0.001	0.001	0.001
RGY	312.1	317.3	311.9	317.1	6.22	0.869	0.990	0.495
GY24	198.5^{b}	211.9^{ab}	221.3^{a}	220.2^{a}	4.59	0.155	0.004	0.731
SCFA, mmol/200 mg DMD	0.742^{b}	0.785^{ab}	0.825^{a}	0.807^{a}	0.015	0.014	0.002	0.948
MCP, mg/g DMD	476.7^{a}	448.2^{ab}	432.6^{b}	428.9^{b}	10.01	0.021	0.008	0.610
NH_3-N, mg/dL	33.1^{a}	33.7^{a}	34.3^{a}	22.6^{b}	2.41	<0.001	1.000	1.000
CH_4, mmol/g OMD	0.346	0.263	0.227	0.264	0.034	0.115	0.142	0.707

^{a,b,c} Within a row means with a common superscript were not different with probability P =0.05

A: gas produced from quickly degradable carbohydrates; c_a: rate of gas produced from rapidly degradable carbohydrates; B: gas produced from insoluble fraction; c_b: rate of gas produced from insoluble fraction; lag: time (hours) before fermentation of insoluble fraction begins; IVDMD: DM degraded at 96 hours (mg/g DM); IVOMD: OMD degraded at 96 hours (mg/g DM); IVNDFD: NDF degraded at 96 hours (mg/g DM); RGY (ml gas 96 h / g IVDMD96 h); GY24: gas yield at 24 hours (mL gas/g DMD); SCFA: short-chain fatty acid; MCP: microbial CP biomass production; NH3-N: ammonia nitrogen at 72 hours post incubation; CH_4: methane at 24 hours post incubation

There were no significant differences (P >0.05) in IVDMD, RGY, and pH. The highest IVOMD and GY24 were observed in CS15, whereas the IVNDFD decreased (P <0.01) 14.7% in CS35 compared with the control (Table 4). The CS35 treatment produced the least (P <0.05) NH_3-N and MCP. Furthermore, CS35 and CS25 reduced in vitro CH_4 production by 23.9% and 34% at 24 hours post incubation compared with CS0 (Table 4).

Significant estimates of Pearson correlation between TMR characteristics and digestion kinetics are shown in Table 5. C18:2 and C18:3 FAs had strong correlations with IVNDFD and c_b and slightly weaker relationships with the B fraction, pH and the amount of NH3-N that was produced. In vitro CH_4 production was correlated negatively with the rates of digestion, but correlated positively with IVDMD (P <0.05).

Various factors influence feed fermentation in the rumen, including the proportion of starch, NDF, and the lignification of the cell wall (Nagani *et al.*, 2000). Beauchemin *et al.* (2000) stated that the (c_b) fraction is related to the chemical composition and amount of NDF in the diet. This observation may be manifest in the negative correlations of (c_b) with NDF (r = -0.96) and GE (r = -0.87) that were observed in the present study. In vitro dry matter digestibility might indicate the nutritional quality of the diet, with IVDMD higher than 700 g/kg DM being

ideal for ruminants (Chamberlain & Wilkinson, 2002). In the present study, IVDMD had an average value of 833.07 ± 6.29 g/kg DM when CS was included in the TMR. Kincaid *et al.* (2012) reported IVDMD of 759 g/kg DM in a TMR containing CS (15% on DM), MS (8.6%), and alfalfa hay (34%). IVNDFD decreased with the inclusion of CS in the diet owing to heterogeneity in the cell wall components of brassicas and the bacterial content population (Keim *et al.*, 2018).

Table 5 Significant estimates of Pearson correlation of chemical composition, in vitro digestibility, and fatty acids of total mixed rations with various proportions of canola silage

Variable	In vitro *gas* production and digestibility					Fatty acids		
	A	c_a	B	c_b	IVDDM	C18:1	C18:2	C18:3
Neutral detergent fibre			0.62	-0.96				
Acid detergent fibre			0.62	-0.96				
Nonstructural carbohydrates			-0.62	0.96				
Gross energy				-0.87				
IVNDFD	0.77		0.66	0.80		0.57	0.95	-0.94
B				-0.52			0.69	-0.65
c_b							-0.87	0.81
pH	0.40		-0.43			-0.64	-0.75	0.72
NH_3-N							0.62	-0.60
Methane production at 24 hours		-0.42		-0.55	0.60			

C18:1, oleic acid; C18:2, linoleic acid, C18:1, linolenic acid; A: gas produced from quickly degradable carbohydrates; c_a: rate of gas produced from rapidly degradable carbohydrates; B: gas produced from insoluble fraction; c_b: rate of gas produced from insoluble fraction; lag: time (hours) before fermentation of insoluble fraction begins; IVDDM: in vitro digestibility of dry matter; IVDNDF: in vitro digestibility of neutral detergent fibre; NH_3-N: in vitro ammonia nitrogen at 72 hours post incubation, correlations of magnitude greater than 0.40 were significant (P <0.05) and those of magnitude greater than 0.7 were highly significant (P <0.01)

There is little information about the effects of CS and methane production. Compared with CS0, the CS25 and CS35 treatments showed 34% and 23.9% in vitro CH_4 production, respectively. Parven *et al.* (2005) commented that brassicas possess glucosinolates and tannins that may affect the activity of methanogenic bacteria. However, the mechanisms of action for these compounds remain unknown. On the other hand, Sun *et al.* (2015) stated that fermentable carbohydrates of canola promote a high synthesis of propionate, which is associated with lower production of hydrogen gas and, consequently, lower production of CH_4. Limón-Hernández *et al.* (2019) added 0%, 1%, 2%, 3%, and 4% molasses (a fermentable carbohydrate) to CS and observed decreased CH_4 production at 24 hours and 48 hours of digestion. In the present study, similar effects might explain the negative correlation of the (c_a) and (c_b) fractions with in vitro gas production.

The uptake of NH_3-N by rumen microorganisms is based on protein and carbohydrate degradation. Therefore, the NH_3-N concentration in the rumen is a good predictor of nitrogen use by rumen microorganisms and its transformation into microbial protein (Hall, 2017). The diet with the highest NH_3-N uptake was CS35, which resulted in the highest values of MCP (Table 4). The amount and degradation rate of CP can influence the NH_3-N contents in the rumen (Oh *et al.*, 2008). This degradation of protein that is associated with starch fermentation could explain the differences in NH_3-N among the treatments in the present study. Ruminal digestion depends on a dynamic sequence of synergic events that influence fermentation products and the production of carbohydrate sources (Gallo *et al.*, 2016). Short-chain fatty acids increased with the inclusion of CS in the TMR, whereas MCP decreased, possibly because of poor synchronization between energy and nitrogen availability.

The presence of PUFAs in the diet tends to decrease the concentration of NH_3-N in the rumen. However, it does not affect the flow of digesta to the duodenum, so the impact on the microbial protein synthesis is minimal (Doreau & Ferlay 1995). In contrast, NDF digestibility is affected by the long-chain FA content in the diet, probably owing to the inhibition of growth of cellulolytic microorganisms (Doreau & Chilliard, 1997). Indeed, a strong negative correlation ($r = -0.94$, $P < 0.05$) between IVDNDF and C18:3 was observed in this study. However, similar relationships were not evident with the other long-chain FAs.

The total content of long-chain unsaturated FAs was similar among the CS diets. However, CS35 and CS0 differed notably in their content of C18:2 and C18:3. Both C18:2 and C18:3 are essential for the animal, and are precursors of vaccenic acid in the biohydrogenation process (Harfoot & Hazlewood, 1997). High content of C18:3 could favour the biohydrogenation (BH) synthesis of MUFA such as C18:1 trans and particularly vaccenic acid. Thus, C18:3 is also a precursor of functional components with positive effects on human health (Pariza *et al.*, 2001; Wang *et al.*, 2012). Ruminal pH could affect the activity of cellulolytic bacteria negatively, which could result in a reduction of ruminal BH of PUFA. Low pH could affect the production of vaccenic acid and conjugated linoleic acid (CLA) in the rumen (Harfoot & Hazlewood, 1997). In the present study, the authors observed higher amounts of products from biohydrogenation of PUFA at pH values between 6.9 and 6.4 in agreement with Troegeler-Meynaider *et al.* (2003). At lower pH (6.2 - 5.4) a decrease of C18:2 and C18:3 was observed and quantities of C18:1 *trans*, and CLA decreased (Martin & Jenkins, 2002). C18:2 and C18:3 are hydrogenated by the same bacteria, and thus similar conditions might affect their BH favourably (Kepler & Tove, 1967).

Total mixed rations based on MS are typically fed indoors and usually result in milk with reduced FA contents compared with milk from cows in other feeding systems such as TMR plus grazing, grazing only, and TMR plus legume silages (Morales-Almaráz *et al.*, 2010; 2011). One of the main reasons for the improved FA composition of milk was the higher level of dietary C18:3, consequent production of vaccenic acid, followed by its desaturation in the mammary gland to produce CLA. Thus, it may be possible to improve milk fat composition by incorporating CS in a TMR for dairy animals.

Conclusion

Augmenting a TMR with more than 15% CS affected MCP, IVOMD, and IVNDFD negatively, decreased in vitro methane production, and increased the C18:3 content of the ration. Additional studies are required to evaluate the effect of CS on animal performance and the nutritional quality of the fat in ruminant products.

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Author's Contributions

LAM-U edited and maintained the data, conducted laboratory analyses, and wrote the original draft of the article; IAD-V designed the research, analysed the data, and reviewed and edited the article; FH reviewed and edited the article; AAR-A provided instrumentation and analysis tools, and reviewed and edited the article; EM-A administered the project, participated in its design, acquired the necessary funding, and reviewed and edited the article.

Conflicts of Interest declaration

None of the authors has a conflict of interest regarding the content of this manuscript.

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