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Supplementation with corn oil and palm kernel oil to grazing cows: ruminal fermentation, milk yield, and fatty acid profile

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ABSTRACT - The effect of supplementation with corn oil (CO) and its mixture with palm kernel oil (CO:PKO 75:25) to grazing cows on ruminal fermentation, milk yield, and its fatty acid (FA) profile was evaluated. The treatments were: one control treatment (C) without oil and two treatments with 720 g d^{-1}/cow of CO or CO:PKO (ether extract: 22.7 g kg^{-1} for control treatment, 66 g kg^{-1} for CO, and 65 g kg^{-1} for CO:PKO). Six multiparous Holstein cows (6.3±1.8 yr, 597±11.5 kg body weight (BW), 160±29 d in milk; mean ± standard deviation) were assigned to a double 3 × 3 × 3 Latin square design. Cows grazed (3 kg DM/100 kg BW) a *Cenchrus clandestinus* (previously *Pennisetum clandestinum*) pasture and were supplemented with 0.9 kg d^{-1} DM corn silage, 4.2 kg d^{-1} DM concentrate, and 9 g Cr_2O_3. The mixture of concentrate and oils was offered twice a day. The addition of oils increased milk yield (kg d^{-1}) (C: 21.4, CO: 23.6, CO:PKO: 23.9) and milk fat concentration (g $kg\ milk^{-1}$) (C: 31.5, CO: 34.0, CO:PKO: 34.0). Compared with control, conjugated linoleic acid ($18:2_{c9\ t11}$ CLA) proportion (g 100 g^{-1} FA) in milk fat was higher for oil treatments (C: 0.68, CO: 1.56, CO:PKO: 1.01). Voluntary intake and digestibility were not different among treatments. The molar ratio of acetate, propionate, and butyrate was not different among treatments, but the molar concentration of volatile fatty acids (VFA) was lower for CO and CO:PKO, resulting in a lower estimated methane (CH_4) production (mL/100 mol VFA) for CO and CO:PKO treatments. Supplementing CO and CO:PKO to grazing dairy cows increases milk yield without affecting voluntary intake or diet digestibility. The proportion of conjugated linoleic acid increases more for CO than for CO:PKO.

Key Words: conjugated linoleic acid, methane, milk composition

Introduction

Addition of lipids to diets of grazing dairy cows may increase milk yield and change milk composition and its fatty acid (FA) profile (Khanal and Olson, 2004; Schröeder et al., 2004). Response depends on dose and fatty acid profile. High doses (>50 g kg^{-1}) may depress intake and compromise rumen fermentation (Zinn et al., 2000; Plascencia et al., 2003; Montgomery et al., 2008). Supplementing saturated lipids increases milk fat, while unsaturated rich FA lipids decrease it (Schröeder et al., 2004). High levels of long chain polyunsaturated FA lipids increase trans-vaccenic ($18:1_{t11}$; TVA) and conjugated linoleic acids ($18:2_{c9\ t11}$; CLA) in milk, which are considered functional for their positive effects on human health (Druart et al., 2014; Lim et al., 2014; Yang et al., 2015). Ruminal biohydrogenation of oils rich in linoleic acid ($18:2_{c9\ c12}$) such as corn oil produces higher concentrations of TVA than oils rich in linolenic acid ($18:3_{c9\ c12\ c15}$) *in vitro* and *in vivo* (Matsushita et al., 2007; Castillo et al., 2012). *In vivo*, higher ruminal proportions of TVA have been associated with higher CLA and TVA milk proportions (Harvatine and Bauman, 2006).

Supplementing fats and oils to ruminants reduces methane (CH_4) emission (Martin et al., 2010; Patra and Yu, 2013) and their antimethanogenic effect seems to depend on their FA profile (Beauchemin et al., 2008; Patra, 2014). Oils rich in saturated medium chain FA (coconut and palm kernel oil) seem to exert a more powerful antimethanogenic effect than oils rich in long chain unsaturated FA (Machmüller et al., 2003; Beauchemin et al., 2008; Martin et al., 2010). However, supplementing oils rich in medium chain saturated FA (lauric 12:0, myristic 14:0, and palmitic 16:0) to dairy cows increases their concentration in milk (Storry et al., 1971; Hermansen, 1995; Hristov et al., 2009). Intake of fats rich in these acids increases blood cholesterol (Grundy, 1994; Mensink et al., 2003), risk of heart stroke (Kromhout et al., 1995), and atherosclerotic disorders (Nicolosi et al., 1997).

We hypothesize that supplementation with a mixture of oils rich in $18:2_{c9\ c12}$ (corn) and 12:0 (palm kernel oil) to

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grazing dairy cows may enhance the effect of each FA in CH_4 emissions, when compared with cows supplemented with $18:2_{c9\ c12}$ (corn oil) or a diet without oils. Additionally, supplementation with the mixture of oils could produce a similar milk yield and milk fatty acid profile than cows supplemented with corn oil. The objective of this experiment was to determine the effect of including corn oil (CO) and its mixture with palm kernel oil (CO:PKO 75:25) on ruminal fermentation, milk yield, and its FA composition in grazing cows.

Material and Methods

All the experimental procedures were approved by the Bioethics Committee of Facultad de Medicina Veterinaria y de Zootecnia (School of Veterinary Medicine and Animal Production; Act 004 of 2012). The experiment was conducted in Mosquera, Cundinamarca, Colombia (4°40'89" N latitude and 74°13'13" W longitude, at an altitude of 2540 m) between December 2013 and February 2014. The average temperature is 13 °C (with a 0 to 20 °C range); relative humidity ranges between 80-85%, and precipitation is 900 mm yr^{-1}, with two rainy seasons (April to May and September to November). The experiment lasted 63 days, divided into three periods of 21 days each (14 days of adaptation to treatments and seven days for sampling). Cows grazed a Kikuyu (*Cenchrus clandestinus*) pasture and were supplemented with 3 kg d^{-1} corn silage (30 g kg^{-1} DM) and 4.2 kg d^{-1} concentrate (Tables 1 and 2). The treatments were: control diet C: Kikuyu, corn silage and concentrate, CO: control diet plus 720 g d^{-1} of pure corn oil supplementation, and PKO: control diet plus 720 g d^{-1} of a mixture of corn oil and palm kernel oil at a 75:25 ratio (Table 3).

Six Holstein cows (6.3±1.8 years, 597±11.5 kg weight, 160±29 days in lactation, and 22.1±2.3 kg d^{-1} milk yield; mean ± standard deviation) were randomly assigned to a double Latin square (three periods, three treatments, three cows, two squares). The cows were milked twice a day (5.00 h and 14.00 h) using mechanical milking and strip grazed a kikuyu pasture fenced by an electric cord that was moved twice a day (morning and afternoon). Forage allowance was 3 kg of dry matter (DM) per 100 kg body weight (BW). At each milking (morning and afternoon), each cow received 60 g mineralized salt, 1.5 kg DM of corn silage, 2.1 kg DM concentrate, and 4.5 g chromium oxide. Each cow in the treatment with oils was supplemented twice a day with 360 g (estimating daily dose of 40 g kg^{-1} total diet with an intake of 18 kg DM) of corn oil or a mixture of corn oil and palm kernel oil (75:25).

To calculate forage biomass, samples of three pasture heights (low, medium, and high) were individually harvested using hand shears and a square of 0.5 m^2 by triplicate. The proportion of each height within the pasture was assessed by grading the pasture visually in at least 36 points. The points were evenly distributed within the whole pasture by dividing it in four areas (eight point each). Samples were weighted and dried to determine average forage production for each pasture height. Then, the estimated forage production (kg DM/ha) per each height was multiplied by its proportion in the pasture. A geo-positioning equipment GPSMAP® 76CSX (Garmin Ltda., Kansas, USA) was used to determine the daily area required in each strip.

For each one of the three experimental periods, milk yield was recorded at each milking time (morning and afternoon) between days 15 and 21. Two individual milk samples (100 mL) from each milking were collected on days 15, 18, and 21 in each experimental period. These samples were mixed to obtain one sample per cow on each sampling day and were used for milk FA analysis. On day 21, an additional sample per animal (morning and afternoon) was obtained and divided into two aliquots of 100 mL each; one aliquot was preserved by adding 3 mL of potassium dichromate at 6 g L^{-1} and kept at −20 °C. The other aliquot was sent fresh to the laboratory to determine protein, fat, and total solids by ultrasound (Milk analyzer, Lactan 1-4) (Priev and Barenholz, 2010).

Forage, silage, and supplements: on days 14, 16, 18, and 20 in each period, a sample of kikuyu was collected (500 g approximately) using the "hand-plucking" methodology described by Cook (1964). Daily forage samples were mixed to obtain a unique sample for each day, dried at 60 °C for 48 h and ground in a Romer® mill with a 2 mm sieve. A sample from each supplement was obtained on day 13 in each period (500 g approx.) and a corn silage sample was obtained on days 14, 16, 18, and 20. These samples were processed in the same way as forage.

Ruminal fluid (250 mL) was collected at 16.30 h on day 21 of each period using an oro-ruminal probe (Haumptner®) discarding the first 200 mL for possible contamination with saliva and the remaining volume was filtered using two layers of cheese cloth. An aliquot was used to measure pH using a potentiometer (Beckman). Another sample (50 mL) was acidified with hydrochloric acid 6N (2.5 mL) and frozen at −20 °C for later analysis (Lopez et al., 2016).

A daily sample (300 g approximately) of feces was collected between days 15 and 21 of each period by anus stimulation, avoiding urine contamination after the morning milking. Feces were dried at 60 °C for 48 h, ground in

a Romer® mill with a 2 mm sieve and mixed to obtain a sample per period for each cow.

Milk fat was extracted with the method described by Hurley et al. (1987) and Díaz-González et al. (2002). One hundred milliliters of milk were centrifuged (15 min at 3000 rpm) and the aqueous fraction was removed. The creamy supernatant was mixed with 15 mL of detergent solution (50 g of sodium hexametaphosphate and 24 of Triton X-100 mL dissolved in 1 l of water), stirred, and placed in a water bath (10 min at 90 °C). The fat from the surface layer was removed using a Pasteur pipette, stored at −20 °C, and solubilized in dichloromethane (1:9). The methyl esters were formed according to the method of McCreary, et al. (1978) and quantified by gas chromatography (GC).

Forage, silage, and supplement FA were extracted according to Garcés and Mancha (1993) adapted by Yamasaki et al. (1999). For FA methylation, 50 mg of dry forage, silage, or supplement were weighed and 2150 µL of absolute methanol, 990 µL of toluene, 1000 µL of N, N-dimethylformamide, 66 µL of sulfuric acid 99.9%, and 2 mL of n-hexane were added. The mixture was placed in a water bath (2 h at 80 °C) and left for 5 to 10 min. The supernatant was evaporated under nitrogen and the dried sample was reconstituted with 300 µL of dichloromethane for further analysis by GC.

Methylated FA of forage, silage, supplements, and milk were quantified by GC using a Shimadzu® GC-2014 gas chromatograph with FID detector and a 100 m × 0.25 mm × 0.2 µm Rt 2560 (Restek®) capillary column. The chromatographic conditions were: 260 °C and 270 °C temperature of injection and detection port, respectively; the program was fixed at an initial temperature of 140 °C for 5 min with a further increase of 4 °C per minute up to 190 °C for a total time of 60 min. Helium was used as carrier gas, with 40.4 psi pressure and a split ratio of 1:100. The injected volume was 1 µL.

To determine the proportion of volatile fatty acids (VFA), 800 µL of ruminal fluid and 500 µL of internal solution (100 g L^{-1} of metaphosphoric acid and 0.6 g L^{-1} of crotonic acid as internal standard, 4 °C) were mixed, and then centrifuged three times at 13000 rpm per minute to remove impurities. The VFA (acetate, propionate, butyrate, valerate, and isovalerate) were quantified by GC with a Shimadzu® GC-2014 gas chromatograph with a FID detector and a polyethylene glycol capillary column of 25 m × 0.32 mm × 0.5 µm Agilent® HP-FFAP (Agilent Technologies Inc., Santa Clara, CA, USA). The chromatographic conditions were: 260 °C at injection port, 280 °C at detection port, helium as a carrier with a flow of 42 cm/s and a split gas ratio of 1:50 and 10 min for the program. The injected volume was 1 µL.

Forage, silage, and supplements were analyzed for DM, fat, ash, crude protein (AOAC, 2010), neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest et al., 1991), indigestible acid detergent fiber (iADF) (Sunvold and Cochran, 1991), and FA profile using GC (Garcés and Mancha, 1993; Yamasaki et al., 1999). Non-starch carbohydrates (NSC) and net energy for lactation (NE_L) were determined according to NRC (2001).

Table 1 - Chemical composition of forages and concentrate

	Kikuyu	Corn silage	Concentrate
		g kg^{-1} DM	
Crude protein	161.7	72.7	202.3
Neutral detergent fiber	512.2	568.1	161.6
Acid detergent fiber	223.0	309.3	46.9
Non-structural carbohydrate	174.3	263.0	557.7
Ether extract	33.1	22.6	9.1
Ash	118.7	73.7	69.3
Net energy of lactation (Mcal/kg of DM)[1]	1.88	1.98	2.02

DM - dry matter.
[1] Net energy of lactation at 1x maintenance intake (NRC, 2001).

Table 2 - Concentrate composition

Ingredient	g kg^{-1} as fed
Soybean meal	381.0
Ground corn	266.0
Molasses	100.0
Cassava meal	190.0
Wheat middlings	23.0
Calcium carbonate	39.6
Mineral premix	0.4

Table 3 - Fatty acid profile of forages, concentrate, corn oil, and corn oil and palm kernel oil mixture (75:25) (g 100 g^{-1} of fatty acids)

Fatty acid	Kikuyu	Corn silage	Concentrate	Corn oil	Corn oil + palm kernel oil
8:0	1.01	5.85	0.52	-	-
10:0	2.26	1.65	1.64	-	-
12:0	-	-	-	-	8.40
14:0	-	-	1.05	0.13	2.91
$14:1_{c9}$	-	3.06	-	0.03	0.03
16:0	28.91	23.04	14.91	11.80	10.67
$16:1_{c9}$	13.97	-	10.60	0.03	0.05
16:1	-	-	-	0.21	0.26
18:0	-	-	-	5.18	4.87
$18:1_{c9}$	-	13.75	15.60	20.66	19.65
$18:2_{t9\ t12}$	-	-	-	0.41	0.50
$18:2_{c9\ c12}$	13.41	27.50	40.45	53.99	41.91
$18:3_{c6\ c9\ c12}$	-	-	-	0.26	0.15
$18:3_{c9\ c12\ c15}$	34.06	17.16	12.36	5.51	3.61
Others	6.37	7.99	2.86	1.80	6.99

Milk protein, fat, and total solids were determined by ultrasound (Priev and Barenholz, 2010).

pH was determined in the ruminal fluid using a Beckman® potentiometer.

In the feces, iADF was determined using the Sunvold and Cochran (1991) method and chromium concentration by X-ray fluorescence spectroscopy (S2 PICOFOX® BRUKER®).

Concentration of CH_4 was calculated according to Ramin and Huhtanen (2012): $CH_{4\ (mL/100\ mol\ VFA)} = 22.4 \times (0.5 \times C_2 - 0.25 \times C_3 + 0.50 \times C_4 - 0.25 \times VA)$, in which C_2 = propionic acid proportion; C_3 = acetic acid proportion; C_4 = butiric acid proportion; and VA = valeric acid proportion.

Energy-corrected milk (ECM) was calculated according to Peterson et al. (2012): ECM (kg d^{-1}) = (0.327 × milk yield kg d^{-1}) + (12.87 × fat kg d^{-1}) + (7.65 × protein kg d^{-1}).

For forage intake, chromium oxide (Cr_2O_3) was used as external marker to determine feces production (Holden et al., 1994) and iADF was used as an internal marker (Sunvold and Cochran, 1991). Feces production (kg d^{-1}) was determined according to Holden et al. (1994): $FP = EMD \times R \times [EM_F]^{-1} \times 1{,}000^{-1}$, in which FP = feces production (kg d^{-1}); EMD = external marker dose (g of Cr d^{-1}); R = recovery rate; and $[EM_F]$ = fecal external marker concentration (g of Cr g^{-1} of DM).

Forage intake was calculated according to Aguilar et al. (2009) : $FoI = (FP \times [iADF]_F - SI \times [iADF]_S) \times [iADF]_{Fo}^{-1}$, in which FoI = forage intake (kg of DM d^{-1}); FP = feces production (kg of DM d^{-1}); $[iADF]_F$, $[iADF]_S$, and $[iADF]_{Fo}$ = feces, supplement, and forage iADF concentrations, respectively (g of iADF g^{-1} of DM); and SI = supplement intake (kg of DM d^{-1}).

Atherogenicity index (AI) was calculated according to Ulbricht and Southgate (1991): $AI = (C_{12} + 4C_{14} + C_{16}) \times UFA^{-1}$, in which C_{12} = lauric acid g 100 g^{-1}; C_{14} = myristic acid g 100 g^{-1}; C_{16} = palmitic acid g 100 g^{-1}; and UFA = unsaturated FA g 100 g^{-1}.

Thrombogenicity index (TI) was calculated according to Ulbricht and Southgate (1991): $TI = (C_{14} + C_{16} + C_{18}) \times [0.5_{MUFA} + 0.5\omega6 + 3\omega3 + (\omega3/\omega6)]^{-1}$, in which C_{14} = myristic acid g 100 g^{-1} FA; C_{16} = g 100 g^{-1} of palmitic acid; C_{18} = stearic acid g 100 g^{-1}; MUFA = monounsaturated FA g 100 g^{-1}; ω3 = omega 3 FA g 100 g^{-1}; and ω6 = omega 6 FA g 100 g^{-1}.

Data were subjected to analysis of variance for a double 3 × 3 × 3 Latin square design with a residual effect estimation using PROC MIXED function of SAS (Statistical Analysis System, version 9.0), according to the following model:

$$Yij(k)l = \mu + al + b(a)il + c(a)jl + \beta(k) + eij(k)l,$$

in which: $Yij(k)l$ = dependent variable; μ = overall mean; al = random effect of square l; $b(a)il$ = random effect of period i within square l; $c(a)jl$ = random effect of cow j within square l; $\beta(k)$ = fixed effect of treatment k discriminated in direct and carryover effect; and $eij(k)l$ = random error with mean 0 and variance σ^2. All random effects were considered ~N (0, σ^2e). Significant differences were considered at P<0.05 for main effects. For multiple comparisons among treatment means, the Turkey-Kramer test was used.

Results

Forage DM intake and total intake were not affected by treatments. Silage and concentrate intake were consistent among the three periods and no rejections occurred (Table 4).

Digestibility of DM, organic matter (OM), NDF, ADF, and molar proportions of VFA were not different among treatments. The addition of oils decreased the concentration of VFA (P<0.0001), increased ruminal pH (P = 0.0024), and did not affect total CH_4 production, but decreased the amount of CH_4 produced per mol of total VFA for these treatments (P<0.0001) (Table 5).

Daily milk yield and energy-corrected milk (ECM) were lower for treatment C than for the treatments with oils (P = 0.0046 and P = 0.0021, respectively). Compared with C, the concentration of total solids and milk fat was higher for those cows supplemented with oils (P = 0.0320 and P = 0.0184 respectively). Milk protein concentration was not different among the three treatments. Compared with C, the daily yields of total solids and fat were higher for the cows supplemented with oils (P = 0.0320 and P = 0.0184, respectively) (Table 6).

Lower levels of 8:0, 10:0, 11:0, and 18:2 $_{10\ c12}$ FA were found in milk from cows in the oil treatments than C treatment (P<0.0001, P = 0.0061, P = 0.0476, and P = 0.0155 respectively) (Table 7).

Table 4 - Intake (kg d^{-1}) by grazing cows supplemented with 720 g d^{-1} of corn oil or its mixture with palm kernel oil (75:25) or unsupplemented (control)

Variable	Treatment			SEM	P-value
	Control	Corn oil	Corn oil + palm kernel oil		
Kikuyu	12.2	12.1	12.3	0.258	0.3988
Concentrate	4.2	4.2	4.2		
Corn silage	0.9	0.9	0.9		
Oil	0	0.72	0.72		
Total intake	17.3	17.9	18.1	0.270	0.5129

SEM - standard error of the mean.

The proportion of 12:0, 14:0, and 16:0 FA in milk was higher for C and CO:PKO in relation to CO (P = 0.0241, P = 0.0044, and P = 0.0109 respectively). For $14:1_{t9}$ and $16:1_{t9}$, the previous treatment (carryover effects) affected the response of the treatment. The addition of oils increased milk proportions in 18:0, $18:1_{c9}$, $18:1_{t11}$, and $18:2_{c9\ t11}$ CLA FA in relation to C, but these acids were greater for CO (P = 0.0005, P = 0.0188, P = 0.0145, and P<0.0001, respectively). The addition of oils increased the level of monounsaturated (P = 0.0138), polyunsaturated (P = 0.0027), and preformed (≥18C) (P = 0.0063) FA in milk, but decreased the thrombogenicity and atherogenicity indexes (P = 0.0056 and P = 0.0085) (Table 8).

Discussion

In our work, concentrate and total intakes were similar among treatments, and the only difference in total intake was explained by oil intake (Table 4). Therefore, differences in the response variables such as milk yield and composition and milk FA profile can be attributed to the addition or non-addition of oil and its FA composition. The main differences in nutrient intake (such as energy) were the result of oil supplementation, although other reports suggest that fat supplementation can lower dry matter voluntary intake (Palmquist, 1984; Gagliostro and Chilliard, 1992; Schröeder et al., 2004). Energy supplementation through the use of fats and oils has been widely documented in total mixed ration (TMR) systems. In these systems, most of authors report a decrease in voluntary feed intake even with the use of protected fats (Palmquist, 1984; Gagliostro and Chilliard, 1992; Schröeder et al., 2004). These negative effects are higher when dietary fat concentration exceeds 80-90 g kg^{-1} (Palmquist and Jenkins, 1980), also with the incremental proportion of unsaturated FA of the supplemented lipids (Firkins and Eastridge, 1994;

Table 5 - Effect of supplementing (720 g d^{-1}) corn oil or its mixture with palm kernel oil (75:25) to grazing dairy cows on diet digestibility and rumen fermentation parameters

Variable	Treatment			SEM	P-value
	Control	Corn oil	Corn oil + palm kernel oil		
Digestibility (g kg^{-1})					
Dry matter	599	603	591	6.9	0.1386
Organic matter	626	619	626	5.7	0.0590
Neutral detergent fiber	572	568	541	8.9	0.3652
Acid detergent fiber	255	275	252	6.7	0.0570
Ruminal fluid					
pH	6.50b	6.88a	6.85a	0.4	0.0024
Volatile fatty acids (mmol/L)	94.7a	79.2b	74.0b	30.6	<0.0001
Acetic (mmol 100 $mmol^{-1}$ VFA)	64.0	63.8	63.4	1.9	0.3038
Propionic (mmol 100 $mmol^{-1}$ VFA)	22.6	22.3	22.3	2.03	0.5993
Butyric (mmol 100 $mmol^{-1}$ VFA)	12.8	13.2	13.7	2.3	0.2389
Valeric (mmol 100 $mmol^{-1}$ VFA)	0.6	0.7	0.6	0.4	0.5723
Acetic:propionic	2.8	2.9	2.8	0.3	0.5675
CH_4 (mL/100 mol VFA)	729.7	734.3	735.0	27.1	0.5481
CH_4 corrected (mL/produced mol)	690.6a	581.6b	544.4b	22.3	<0.0001

Values followed by the same letter within rows are not significantly different (P = 0.05).
SEM - standard error of the mean; VFA - volatile fatty acid.

Table 6 - Milk yield and composition of milk from grazing cows supplemented with 720 g d^{-1} of corn oil or its mixture with palm kernel oil (75:25), or unsupplemented (control)

Variable	Treatment			SEM	P-value
	Control	Corn oil	Corn oil + palm kernel oil		
Yield (kg d^{-1})					
Milk	21.4b	23.6a	23.9a	0.326	0.0046
Energy-corrected milk	19.5b	23.2a	23.5a	0.480	0.0021
Total solids	2.31b	2.78a	2.76a	0.061	0.0320
Protein	0.66	0.75	0.74	0.022	0.0566
Fat	0.67b	0.81a	0.82a	0.017	0.0184
Concentration (g kg $milk^{-1}$)					
Total solids	108b	118a	116a	1.48	0.0320
Protein	30.7	31.9	31.1	0.25	0.0566
Fat	31.5b	34.0a	34.3a	0.38	0.0184

Values followed by the same letter within rows are not significantly different (P = 0.05).
SEM - standard error of the mean.

Bremmer et al., 1998). However, Ueda et al. (2003), Zheng et al. (2005), Dai et al. (2011), and Benchaar et al. (2012) found no effect on dry matter intake with the addition of different sources and levels of lipids in TMR systems. In their review on fat supplementation to grazing dairy cattle, Schroeder et al. (2004) and Bargo et al. (2003) found no effect of supplementation with lipids on dry matter intake in grazing dairy cattle.

Several authors have suggested that grazing limits voluntary intake and milk production in dairy cattle (Kolver and Muller, 1998; Bargo et al., 2002; Rego et al., 2016). Therefore, it is possible that under grazing conditions there

Table 7 - Milk fatty acid composition (g 100 g fatty acids^{-1}) of grazing cows supplemented with 720 g d^{-1} of corn oil or its mixture with palm kernel oil (75:25) or unsupplemented (control)

Fatty acid	Treatment			SEM	P-value
	Control	Corn oil	Corn oil + palm kernel oil		
4:0	2.31	2.44	2.54	0.058	0.4013
6:0	1.93	1.64	1.76	0.044	0.2106
8:0	1.24a	0.95b	1.03b	0.043	<0.0001
10:0	3.00a	2.07b	2.26b	0.130	0.0061
11:0	0.40a	0.23b	0.28b	0.026	0.0476
12:0	3.74a	2.48c	3.45b	0.173	0.0241
13:0	0.09	0.05	0.07	0.008	0.1467
14:0	12.55a	9.99c	11.28b	0.299	0.0044
$14:1_{t9}$	0.29a	0.21b	0.22b	0.010	0.0013
$14:1_{c9}$	0.34a	0.28b	0.27b	0.011	0.0079
15:0	1.42a	1.06b	1.15b	0.089	0.2230
15:1	1.31a	0.94b	0.97b	0.047	0.0037
16:0	36.82a	27.55b	30.97b	1.046	0.0109
$16:1_{t9}$	0.27a	0.26a	0.25b	0.011	0.0302
$16:1_{c9}$	1.66	1.16	1.30	0.079	0.4455
17:0	0.33	0.21	0.20	0.021	0.3955
17:1c9	0.81a	0.68b	0.58b	0.031	0.0374
18:0	9.15b	13.65a	12.46a	0.585	0.0448
$18:1_{t4}$ + $18:1_{t10}$	1.27c	3.32a	1.93b	0.217	0.0005
$18:1_{c9}$	17.50b	25.63a	23.11a	1.007	0.0188
$18:1_{t11}$	0.74b	1.86a	1.14ab	0.103	0.0145
$18:2_{c9\ c12}$	0.81	1.12	1.04	0.047	0.0917
$18:3_{c9\ c12\ c15}$	0.32	0.26	0.23	0.012	0.0625
$18:2_{c9\ t11}$ CLA	0.68c	1.56a	1.01b	0.045	<0.0001
$18:2_{t10\ c12}$ CLA	0.11	0.08	0.06	0.006	0.0155
Not identified	0.90	0.60	0.42	0.043	0.1707

Values followed by the same letter within rows are not significantly different (P = 0.05).
SEM - standard error of the mean.

Table 8 - Fatty acid composition of milk (g 100 g^{-1} fatty acids) from grazing cows supplemented with corn oil or its mixture with palm kernel oil (75:25)

Variable	Treatment			SEM	P-value
	Control	Corn oil	Corn oil + palm kernel oil		
Saturated	72.99a	62.32c	67.45b	1.172	0.0141
Unsaturated	26.11c	37.08a	31.92b	1.202	0.0107
Monounsaturated	24.19c	34.04a	29.58b	1.092	0.0138
Polyunsaturated	1.92c	3.03a	2.34b	0.125	0.0027
Odd chain	4.36	3.18	3.25	0.177	0.0705
De novo (≤ 17C)	68.51a	52.22c	58.58b	1.742	0.0065
Preformed (≥ 18C)	30.59c	47.18a	40.79b	1.778	0.0063
Saturated:unsaturated	2.81a	1.69b	2.13b	0.127	0.0083
ω3/ω6	0.35a	0.22b	0.21b	0.024	0.0498
Atherogenicity index	2.05a	1.08b	1.44b	0.106	0.0056
Thrombogenicity index	3.87a	2.16b	2.84b	0.186	0.0085

Values followed by the same letter within rows are not significantly different (P = 0.05).
SEM - standard error of the mean.

is not a feedback to reduce intake when the energy density of the diet increases by the addition of fats, since the cow is under a negative energy balance (Pérez-Prieto et al., 2013).

Lipids have different mechanisms to alter rumen fermentation and reduce CH_4 production. Among them are the reduction of diet digestibility (Beauchemin et al., 2008; Martin et al., 2010), changes in the rumen fermentation routes (Yabuuchi et al., 2006), toxic effects on ruminal microorganisms (Patra, 2013; Patra, 2014), biohydrogenation (BH) of unsaturated FA (Martin et al., 2010), decrease in voluntary feed intake, and change in the proportion of fermentable carbohydrates, such as the substitution of fermentable energy by lipids (McGinn et al., 2004; Martin et al., 2010).

Although in our work CH_4 production was not measured directly, it was estimated using final fermentation products (Ramin and Huhtanen, 2012). Inclusions of 40 g kg^{-1} of oils in diet decreased estimated CH_4 production by almost 16% (Table 5). Giger-Riverdin et al. (2003), Eugene et al. (2008), and Beauchemin et al. (2008) reported that CH_4 production is reduced between 2.2% and 5% per unit of supplemented lipid. Patra (2013) and Patra (2014), in a meta-analysis reported linear decreases close to 4.3% of total production of CH_4 per unit of lipid supplemented in cattle and sheep. In our case, estimated CH_4 production was reduced by about 4% per unit of added oil.

Several authors reported that the addition of oils to ruminant diets reduces the acetic:propionic acid ratio as a result of a lower ruminal degradation of fiber (Machmüller et al., 2000; Beauchemin et al., 2008; Patra, 2014), therefore reducing CH_4 production. In our study, the molar proportion of each VFA did not change due to the addition of oils, but their molar concentration was reduced (Table 5). Several authors have reported that addition of oils to ruminant diets reduces the proportion of VFA (Machmüller, 2006) and increases pH (Ueda et al., 2003) due to a reduction in rumen fermentation as was observed here (Table 5). Unfortunately, rumen digestibility was not measured in our study, but apparent digestibility of DM, OM, NDF, and ADF of total gastrointestinal tract were not different due to the addition of oils as has been reported by several authors (Bateman and Jenkins, 1998; Ueda et al., 2003). It has been suggested that that a lower ruminal digestibility as a result of the use of oils in ruminant diets can be compensated by a higher digestibility in the lower tract (Suttton et al., 1983; Faichney et al., 2002).

The net effect of carbohydrate digestion site (rumen vs. lower tract) on the CH_4 yield per animal cannot be predicted. However, if the addition of lipids decreases starch and fiber ruminal fermentation and this is compensated by enzymatic digestion in the small intestine (starch) and large intestine fermentation (fiber), these would decrease the production of CH_4 as a result of lower total carbohydrates fermentation. The net effect on oil addition may be dependent on the proportion of starch in the diets (Ueda et al., 2003).

In our work, we also compared the effect of FA profile of oils on ruminal fermentation, and in particular, the effects of adding palm kernel oil. The reduction in CH_4 production due to the replacement of 25% of corn oil by palm kernel oil was 5.4%, but was not significant, similarly to that reported by Machmüller et al. (2000) comparing coconut oil (profile similar to palm kernel oil) and sunflower seeds (rich in $18:2_{c9\ c12}$ as corn oil) added to lamb diets. However, several authors suggest a greater antimethanogenic effect of lipids rich in saturated medium chain FA (12:0 and 14:0 mainly) (Eugene et al., 2008; Beauchemin et al., 2008; Patra, 2014). It is possible that higher concentrations of oils richer in these acids than those used in this study should be used to achieve these effects.

We expected that the addition of oils rich in polyunsaturated FA to the diet of grazing cows increased milk yield and decreased milk fat concentration as has been reported by others (Chilliard et al., 2001; Rabiee et al., 2012). In agreement with these reports, milk yield increased due to the addition of oils and was independent of oil source (CO or CO:PKO). Higher milk yield due to dietary addition of oils has been attributed to an increase in energy intake (Van Knegsel et al., 2007; Schröeder et al., 2004). In our study, the oil diets also increased milk fat regardless of the source, resulting in an increase of 19% in ECM yield. Bargo et al. (2003) and Schröeder et al. (2004) suggest that in restricted grazing animals, milk yield increases in proportion to additional energy intake in the diet without changing milk composition. In our study, the increase in milk fat concentration occurred as a result of an increase in the uptake of preformed FA (205, 387, 336 g d^{-1} for C, CO, and CO:PKO, respectively) by the mammary gland more likely of dietary origin. In a recent review, Loften et al. (2014) reported that increasing the flow of FA 16:0, 18:0, and 18:1 to the duodenum increases milk fat yield and milk fat concentration. We suggest that the increase in milk fat yield and milk fat concentration found in our experiment was due to ruminal biohydrogenation of 18:2 and consequently a larger absorption of 18:0 FA in the duodenum.

Different authors indicate that fat supplementation with a high degree of unsaturation such as those used in our study (>60 g 100 g^{-1} FA) decreases milk fat (Garnsworthy, 1990; Bauman and Griinari, 2001; Chilliard et al., 2001).

It has been argued that unsaturated FA are precursors in the rumen of particular FA ($18:2_{t10\ c12}$ CLA and $18:2_{t8\ c10}$ CLA, among others) that inhibit fat synthesis in the mammary gland (Bauman and Griinari, 2001; Baumgard et al., 2002). In this study, milk fat yield (g d^{-1}) from *de novo* synthesized FA (≤ 17 carbons) was similar among treatments (423, 459, 474 g d^{-1} for C, CO, and CO:PKO, respectively); thus, this mechanism seems unlikely, according to the higher level of unsaturated FA for the oil treatments compared with control. Several studies suggest that fat supplementation reduces the concentration of milk protein (Zhang et al., 2006) with an increase in its yield (g d^{-1}) due to a greater milk production (Fearon et al., 2004; Flowers et al., 2008). In this study, protein concentrations in milk were similar among the treatments regardless of the addition of oils to the diet. However, daily protein excreted increased due to a greater volume of milk in cows fed diets including oils.

As regards corn oil, this work ought to increase the proportion of unsaturated FA in milk fat, particularly $18:1_{t11}$ TVA and $18:2_{c9\ t11}$ CLA adding an oil rich in $18:2_{c9\ c12}$ (corn) to the diet of dairy cows. These FA have been associated with beneficial effects on human health (Druart et al., 2014; El Roz et al., 2013; Yang et al., 2015). The addition of corn oil reduced (15%) the saturation of milk fat by increasing 1.42 times the unsaturated FA. The increase was mainly explained by the monounsatured (88%) FA, particularly oleic acid, which represented 89% of these. Polyunsaturated FA also increased 1.58 times in milk fat by the addition of corn oil, but these are a small proportion of it. All unhealthy medium chain (Mensink et al., 2003; Nicolosi et al., 1997) saturated FA (12:0, 14:0 and 16:0) were reduced (Table 7). The only saturated FA that increased was stearic acid (18:0). The proportion of $18:1_{t11}$ TVA and $18:2_{c9\ t11}$ CLA in milk fat increased at least twice due to addition of corn oil. These FA has been associated with beneficial effects on human health (Rosberg-Cody et al., 2011; Druart et al., 2014; El Roz et al., 2013; Yang et al., 2015).

Several authors have reported that addition of vegetable oils rich in polyunsaturated FA ($18:2_{c9\ c12}$ and $18:2_{c9\ c12\ c15}$) increases the proportions of unsaturated FA and $18:2_{c9\ t11}$CLA in milk fat (Dhiman et al., 2000; Harvatine and Bauman, 2006) as was observed in our study. In conditions similar to those in this study, we found similar proportions of $18:2_{c9\ t11}$ CLA in milk fat (1.41 g 100 g FA^{-1}) with supplementation of high-fat rice bran (Castaño et al., 2014).

In laboratory research with animals, a preventive intake of 0.8 g day^{-1} of $18:2_{c9\ t11}$ CLA has been suggested against tumors (Watkins and Li, 2003). On the other hand, the health effects of $18:2_{c9\ t11}$ CLA intake on atherosclerosis may be close to 0.25 g day^{-1} (calculation by extrapolation of effects observed in experiments with laboratory animals to human metabolic weight). We found levels of $18:2_{c9\ t11}$ CLA in milk of 1.56 mg g^{-1} milk fat, which are insufficient to achieve the recommended intake for preventive effects on tumors assuming an average Colombian milk consumption of 0.45 kg d^{-1} (IDF, 2013). However, these levels are enough for the prevention of formation of atheromas.

In our work, milk fat with lower indexes of atherogenicity and thrombogenicity resulted from a diet with addition of corn oil, due to a lower proportion of saturated FA (12:0, 14:0, 16:0, and 18:0) and a higher proportion of unsaturated fatty acids. These indexes have been associated with human health (Lock and Bauman, 2004; Fontecha et al., 2009) and were reduced by more than 50%, suggesting that the inclusion of corn oil to grazing dairy cows is a valid strategy to decrease the risk of atheromas and thrombus in humans associated with milk fat intake.

Partial substitution of corn oil by palm kernel oil (75:25): in our experiment, palm kernel oil was added to corn oil to reduce CH_4 production. The small proportion of palm oil added to corn oil reduced the proportions of $18:1_{c9}$, $18:1_{t11}$ TVA, and $18:2_{c9\ t11}$ CLA, and 12:0, 14:0, and 16:0, were increased. These resulted in an increase of saturation (8.0%) and reduced the unsaturation (14%) of FA in milk fat. However, there was not a significant change in the atherogenicity and thrombogenicity indexes (Table 8) compared with corn oil. Several authors report that dairy cow diets that contain coconut oil, with a similar FA profile to that of palm kernel oil, increase the proportion of 12:0, 14:0, and 16:0 in milk, suggesting that the transfer of these FA from the diet to the product is high and their increase is positively related to their inclusion levels (Hermansen, 1995; Hristov et al., 2009). In our study, the low levels of palm kernel oil (180 g cow d^{-1}) explained the small and not significant changes in health indexes. However, the significant increase in medium chain FA in milk fat due to palm kernel oil addition should be considered negative. These FA are related to human health problems, specifically circulatory system diseases (Nicolosi et al., 1997; Mensink et al., 2003).

Conclusions

The addition of oils to diets of grazing cows is an option to increase milk volume, changing its fatty acid composition and decreasing CH_4 production. However, to achieve adequate levels of $18:1_{t11}$ and $18:2_{c9\ t11}$ conjugated linoleic acid (therapeutic and preventive) in milk, oil inclusion in the diet must be increased, with potential effects on animal productivity.

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