Effect of Canola oil Supplementation Level on Total Tract Digestion, Ruminal Fermentation and Methane Emissions of Cows Grazing *Brachiaria* sp Complemented with Fixed Amount of Concentrate

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Abstract

Four rumen-cannulated cows (*Bos taurus* × *Bos indicus*, 631 ± 88 BW) were assigned in a 4×4 Latin Square design to test the effects of different canola oil inclusion levels (CO; 0.0, 0.40, 0.80 and 1.2 g/kg shrunk body weight, SBW = BW×0.96) on the reduction of CH₄ emissions and the energy value of the diet based on *Brachiaria sp.* under rotational grazing supplemented with a fixed amount of concentrate. The CH₄ emission was analysed by an infrared analyser. Grass consumption and faecal production were estimated using Cr₂O₃ as external marker. The CO supplementation increased (linear effect, P < 0.05) DM (16.6%), organic matter (OM, 18.0%) and gross energy intakes (26.3%) and decreased the total digestibility of neutral detergent fibre (NDF) by 26% (linear effect, P < 0.05); also increased digestible energy value (Mcal/kg) by 7.1%. In the rumen, the maximum oil inclusion level decreased the total protozoa population by 89%, the acetate: propionate ratio by 25% and the enteric methane production by 18.9%, all without negatively affecting any of the other variables of fermentation and digestion evaluated. It is concluded that canola oil at supplementation levels of up to 1.2 g CO/kg SBW (5.3% of the total diet) for cows grazing tropical forages supplemented with concentrates increases the total and energy consumption and the efficiency of ruminal fermentation, thereby reducing the emission of enteric methane by up to 18.9%.

Introduction

Cattle are among the major contributors to greenhouse gas (GHG) emissions, mainly methane (CH₄) from enteric fermentation and from extensive grazing production systems. The emission of methane represents a significant energy loss, ranging from 2 to 12% of energy intake (Audsley and Wilkinson, 2014; Beauchemin, 2015; Hristov et al., 2015). Therefore, the challenge is to develop diets and strategies for CH₄ emission reduction by optimising diet energy use, which should improve productive performance and reduce the impact on global warming (McGeough et al., 2010, Wu et al., 2016).

Previous reports have pointed out the addition of lipids to diets as a strategy to reduce CH₄ emissions in ruminants (Grainger and Beauchemin, 2011; Hristov et al., 2013; Knapp et al., 2014; Martin et al., 2016). Unsaturated fatty acids (UFA) reduce CH₄ by increasing propionic acid production, inhibiting protozoa survival and UFA hydrogenation (Bonilla and Lemus et al., 2012, Mata e Silva et al., 2017). For this purpose, long-chain (C18) UFAs, i.e., vegetable and fish oils, are more effective; the most abundant ones are oleic (C18:1) and linoleic (C18:2) FAs, and their proportions change according to the oil source (Orsavova et al., 2015). *In-vitro* studies have shown that the addition of UFAs to the ruminal inoculum can decrease CH₄ production (Castro-Montoya et al., 2015; Wu et al., 2016). However, oil supplementation needs to be performed carefully, especially in grazing cattle, because of the negative effects on the ruminal degradation of NDF (Jenkins and Palmquist, 1984) and the low intestinal digestibility (as well as the usable energy value of lipids) when supplemented at high levels (Zinn and Jorquera, 2007). Piñares-Patiño et al. (2016) added canola oil directly to *Lolium perenne* meadow grass (733 g of oil/animal/day), which resulted in a lower ruminal acetate/propionate ratio and an 11% decrease in CH₄ compared to non-

supplemented animals. Angus heifers on a barley silage diet (75% of diet DM; Beauchemin and McGinn, 2006), supplemented with 4.6% canola oil (CO; equivalent to 1.2 g oil/kg SBW), showed a 32% decrease in methane emissions. However, this reduction was due to a decrease in feed intake and total tract DM digestibility as a direct consequence of NDF digestion decrease.

To date, the effects of oil supplementation have mostly been reported for temperate conditions, whereas reports for tropical grazing conditions are lacking. Under tropical conditions, the diet is based on grasses with lower NDF digestibility, nitrogen (N) content and available energy; these characteristics decrease N and carbon retention efficiency, favouring an increase in enteric CH₄ production. Therefore, a feasible GHG mitigation strategy is to use concentrates containing adequate amounts of vegetable oils to increase energy intake, with a minimum effect on NDF forage digestibility, to significantly decrease enteric CH₄ emissions. Mate and Silva et al. (2017) reported a 23% reduction in CH₄ emissions in dairy cows (Holstein × Gyr) grazing *Urochloa* sp. supplemented with 2.86 kg/day concentrate containing 13.4% sunflower oil (0.73 g of oil/kg of live weight). Although in their study, milk production was evaluated, the variables of ruminal digestion and fermentation were not considered; however, a decrease in CH₄ reduces the loss of gross energy intake by the animal from 2 to 12% (Johnson and Johnson, 1995). Furthermore, in beef cattle, a maximum lipid intake of 0.96 g/kg LW has been determined for optimal FA digestibility, which represents an adequate fat usable energy value (Zinn & Jorquera, 2007). Therefore, the level used by Mata and Silva (2017) was 24% lower than the theoretical optimal inclusion level. Consequently, it is necessary to determine the optimum level of vegetable oil inclusion to reduce methane emissions without negatively affecting DM intake and dietary energy use in cattle grazing under tropical conditions. In this context, the objective of this study was to evaluate the effect of canola oil inclusion level on nutrient digestibility, ruminal fermentation and enteric CH₄ emissions in grazing cattle under tropical conditions and complemented with a fixed amount of concentrate.

Materials And Methods

Location

The study was carried out at the Center for Teaching, Research and Extension in Tropical Livestock (CEIEGT, FMVZ-UNAM), located in Tlapacoyan, Veracruz, Mexico (20°03'N and 93°03'W). The climate is hot and humid, Af (m) w "(e) (García, 2004), with an average daily temperature of 23.9 ± 0.5°C and an average annual rainfall of 1,931 ± 334 mm.

The study used four non-pregnant rumen-cannulated adult cross-bred (*Bos taurus* × *B. indicus*) cows with an average BW of 631 ± 88 kg. Cows were weighed daily at 1100 h for 3 consecutive days, and body weight adjusted to the Shrunk Body Weight (SBW) by multiplying body weight with a correction factor of 0.96 to account for gut fill, according to the National Academies of Sciences, Engineering, and Medicine (NASEM; 2016).

Animals, feeding and experimental design

The cows were subjected to a 4 × 4 Latin square design to test four levels of canola oil (CO) inclusion: 0.0, 0.40, 0.80 and 1.20 g CO/kg SBW. Cows were housed in 1 hectare of a *Brachiaria* spp. grazing paddock, which was divided into four pasture sections of 50 × 50 m. Feeding was based on a rotational grazing system of 7 days per paddock, with a recovery period of 21 days, ensuring an average height of the pasture of 40 cm before grazing and 10 cm of residual. To achieve the level of intake, the required CO was mixed into pelletised commercial concentrate to obtain final concentrations of 0, 40, 80 and 120 g CO/kg concentrate. This combination was offered individually at 6.3 kg/animal so that the total daily intake of CO was 0, 250, 502 and 752 g, corresponding to 0.0, 0.40, 0.80 and 1.20 g CO/kg SBW, respectively (Zinn and Jorquera 2003; Bayat et al., 2018). The concentrate was offered in equal amounts (3.51 kg) at 0600 and 1500 h. Residual concentrate was introduced directly to the rumen via the ruminal cannula to ensure correct oil consumption.

Sampling and measurements

The trial consisted of four periods of 19 days each, corresponding to 13 days of gradual adaptation to the treatment and 6 sampling days. Dry matter intake (DMI) was estimated using the chromium oxide technique (Cr_2O_3 ; Schneider and Flatt, 1975). The animals received 10 g of Cr_2O_3 absorbed into cellulose paper envelopes and daily placed directly into the rumen via the cannula, from day 8 to 17 of each period at the same time (1530 h). Individual faecal samples (200 g) were collected directly from the rectum every 8 hours during the last 4 days of each treatment period, starting at 0000h on day 1, 0200 on day 2, 0400 on day 3 and 0600 on day 4. Samples were kept refrigerated (1°C) and homogenised at the end of each period to generate a composite sample, which was frozen (-18°C) until analysis.

Ruminal fluid samples were collected every 4 hours on days 18 and 19 of each period, starting at 0300h; samples were filtered through eight gauze layers, and their pH values were measured using a portable potentiometer (Oakton, pHTestrs®). For protozoa estimation, 5-mL filtered ruminal fluid samples were collected, and 5 mL iodine solution was added (5 mL; 1.5 g potassium iodine and 0.5 g iodine re-sublimated volumetric 100 mL). Samples were kept under refrigeration (1°C) until analysis. The filtered ruminal fluid (40 mL) corresponding to 4 h post-feeding (1900 h concentrate and 0300 h forage) was preserved with 10 mL metaphosphoric acid at 25% (v/v) and frozen (-18°C) for the estimation of ammonia nitrogen ($\text{NH}_3\text{-N}$) and volatile fatty acids (VFA).

To evaluate CH_4 emissions, animals were moved to individual stalls with a closed trough, and methane was estimated using an infrared methane analyser (Guardian Plus; Edinburg Instruments Ltd., Livingston, UK) with the “sniffer method”, as reported by Garnsworthy et al. (2012). The CH_4 emissions were estimated during the concentrate feeding time at 0600 and 1500h for a period of ~30 min.

The energy loss due to CH_4 emission (Y_m) was estimated with the formula proposed by Sauvant and Giger-Reverdin (2009):

$$Y_m = 10.8 - 2.99 \times \text{LI} + 0.40 \times \text{LI}^2 + 7.23 \times \text{CPr} - 8.71 \times \text{CPr}^2 - 0.98 \times \text{CPr} \times \text{LI},$$

where Y_m represents CH_4 energy as a percentage of gross energy; LI indicates intake level as a percentage of body weight; CP_r is the proportion of concentrate in the diet.

Using the methodology of the Intergovernmental Panel on Climate Change (IPCC, 2006), CH_4 emissions were estimated using the GE and CMS values obtained during the experiment, according to the following equation:

$$EF = \left[\frac{GE \cdot \left(\frac{Y_m}{100} \right) \cdot 365}{55.65} \right],$$

where EF = emission factor, $kg\ CH_4\ head^{-1}\ yr^{-1}$; GE = gross energy intake, $MJ\ head^{-1}\ day^{-1}$; Y_m = methane conversion factor, percentage of feed gross energy converted to methane. The constant value 55.65 ($MJ/kg\ CH_4$) was used as the energy content of methane.

Representative samples of concentrate and forage for the grazing paddock were taken. Faecal, forage and concentrate samples were dried at $60^\circ C$ for 72 h, ground and sieved (1 mm) for further analysis.

Sample analysis and calculations

Samples were subjected to all or part of the following analyses: dry matter (DM, oven-drying until no further weight loss, method 934.01; AOAC, 2015), crude protein (CP, method 2001.11; AOAC, 2015), ash (method 942.05; AOAC, 2015), neutral detergent fibre (NDF) and acid detergent fibre (ADF), as described by Van Soest et al. (1991), using alpha-amylase without correction for ash and an ANKOM 2000 analyser, gross energy (GE), using the calorimetric pump approach (Parr, 6400; Illinois, USA). Chromium oxide (Cr_2O_3) in faecal samples was determined using atomic absorption spectrometry following Williams et al. (1962). Medium- and long-chain FA determination was performed by gas chromatography (Perkin Elmer, model AutoSystem XL) using a CP-7420 Select column $100\ m \times 0.2\ mm \times 0.25\ \mu m$ FAME capillary column (Agilent Technologies). To carry out the methylation of the FA, the methodology AOAC (2019) Official Method 969.33 (2005) was used. For the calculations, we used the AOAC (2019) Official Method 963.22. The chromatograph conditions were as follows: flow rate, $1.5\ mL/min$; temperature, $80^\circ C$; injector temperature, $220^\circ C$; detector temperature (FID): $275\text{--}280^\circ C$; Conditions: $80^\circ C$ (1 min), $20^\circ C/min$, $160^\circ C$, $1^\circ C/min$, $198^\circ C$, $5^\circ C/min$ $250^\circ C$ (15 min). Chromatographic analysis was performed with the Supelco TM 37 Component FAME MIX standard, catalogue number 47885-U.

Protozoa were microscopically counted (US Micro 2, US Micro products, Austin, TX, USA) using a haemocytometer (Neubauer improved, Marienfeld, Germany) to estimate the number per mL. Six observations were made per sample/hour/animal/treatment/period, with the total number and genre recorded (Holotrichidae and Entodinidae). Ammonia nitrogen (NH_3-N) was estimated using the Conway and O'Malley (1942) technique, and the VFA profile was measured by gas chromatography (Perkin Elmer, AutoSystem XL model).

Faecal excretion of DM and individual nutrients were calculated based on marker ratios using Cr₂O₃.

Statistical analysis

Data were analysed as a 4 × 4 Latin Square design using the MIXED procedure of SAS 9.1 (Statistical Analysis System, Inc., Cary, NC, USA), according to the following model:

$$Y_{ij} = \mu + H_i + C_j + T_k + \varepsilon_{ij}$$

where Y_{ijk} = variable response, μ = overall average, H_i = effect of the i-th animal (row), C_j = effect of the j-th period (column), T_k = effect of the k-th treatment and ε_{ijk} = experimental error. The effects of treatment means were analysed using polynomial orthogonal for linear and quadratic components of the level of CO (Cochran and Cox, 1992). For ruminal fermentation (VFAs, protozoa number estimation and methane emission), sampling period/time was included as a repeated measure; differences with P values ≤ 0.05 were considered statistically significant.

Results

The fatty acid profile and the chemical compositions of CO, concentrate and grass offered are shown in Table 1. The lipid concentration was 2.26, 3.00 and 99% for the *Brachiaria* spp. grass, the commercial concentrate and the canola oil, respectively.

Table 1
Chemical compositions and fatty acid profile of the ingredients.

Items	Canola oil	<i>Brachiaria</i> sp.	Concentrate
Crude Protein	ND	6.4	17.5
Ashes	ND	11.1	20.7
Neutral Detergent Fiber	ND	66.6	41.8
Acid Detergent Fiber	ND	41.4	14.6
Ethereum Extract	99.0	2.26	3.0
Gross Energy	9.5	3.7	3.7
<i>Fatty acids (%)</i>			
C6: 0, Caproic	0.13	0.00	0.00
C8: 0, Caprylic	0.12	0.00	0.00
C12: 0, Lauric	0.14	0.00	5.77
C14: 0, Myristic	0.37	0.00	2.61
C16: 0, Palmitic	10.6	41.78	26.5
C16: 1, Palmitoleic	0.18	0.00	0.00
C17: 0, Margaric	0.02	0.00	0.00
C18: 0, Stearic	0.99	3.42	1.21
C18: 1, Oleic	70.32	39.2	45.84
C18: 2, Linoleic,	5.09	8.28	15.42
C18: 3, α -Linolenic acid	0.14	0.00	0.00
C20: 0, Arachidonic,	9.84	0.00	0.00
C20: 1, Eicosenoic,	0.81	7.33	2.65
C21: 0, Heneicosenoic,	0.23	0.00	0.00
C22: 1, Behenic and C22: 0, Erucic	0.70	0.00	0.00
C23: 0, Tricosylic	0.06	0.00	0.00
C20: 5, Eicosapentaenoic	0.12	0.00	0.00
C24: 1, Nervonic	0.14	0.00	0.00

Dry matter intake and digestion

The basal diet (concentrate + grass) contained 2.73% lipids. Addition of CO to the concentrate at levels at 4, 8 and 12% oil resulted in a total daily CO intake of 0, 250, 502, and 752 g, corresponding to 0, 1.9, 3.8 and 5.3% CO, respectively, of the total diet (concentrate + grass) consumed during the experiment for each of the treatments (Table 2).

Table 2

Contribution of oil and total fat in the diet of cows fed *Brachiaria* sp and supplemented with canola oil.

Items	Canola oil g/kg SBW ¹				SEM ²	P-value ³	
	0.00	0.40	0.80	1.20		L	Q
Canola oil g/kg DMI	0.0	19.1	38.8	54.1	11.8	<0.01	0.32
<i>Oil intake, g/cow/d</i>							
Canola oil	0.0	250	502	752	163.46	<0.01	0.99
Grass	146	170	157	175	0.54	0.62	0.47
Concentrate	189	189	189	189	0.03	0.99	0.99
Total Fat	335	609	847	1117	163.23	<0.01	0.94
<i>Contribution of total fat, %</i>							
Canola oil	0.0	41.1	59.2	67.4	15.02	<0.01	<0.01
Grass	43.6	27.9	18.5	15.7	4.31	<0.01	<0.01
Concentrate	56.4	31.0	22.3	16.9	3.56	<0.01	<0.01
<i>Fat contribution to the diet, %</i>							
Canola oil	0.00	1.84	3.80	5.26	1.18	<0.01	0.32
Grass	1.19	1.25	1.18	1.22	0.10	0.02	0.87
Concentrate	1.54	1.39	1.43	1.32	0.06	0.02	0.86
Total fat	2.73	4.49	6.41	7.80	1.03	<0.01	0.64
¹ SBW, shrunk body weight, BW × 0.96 (NASEM, 2016); ² SEM, standard error means; ³ P-value linear and quadratic.							

The effects of the treatments on feed intake and digestion are shown in Table 3. An increment in CO intake from 0 to 1.20 g/kg SBW increased (linear effect, $P < 0.05$) DM (16.6%), organic matter (OM, 18.0%) and gross energy intakes (26.3%). While no change in OM and CP digestion was observed, the addition of CO caused a linear decrease ($P < 0.05$) of NDF at 2.5, 15.4 and 26.5% for levels 0.0, 0.40, 0.80 and 1.20 g CO/kg SBW, respectively. Furthermore, the digestible energy (Mcal/kg) of the diets increased linearly ($P < 0.01$) by up to 7.1%.

Table 3
Effect of canola oil supplementation on dry matter intake, fecal excretion, and digestibility

Items	Canola oil g/kg SBW ¹				SEM ²	P-value ³	
	0.00	0.40	0.80	1.20		L	Q
^a Intake, g/d							
Concentrate	5787	5784	5785	5788	0.93	0.56	0.07
Canola oil	0	250	502	752	163	< 0.01	0.99
<i>Brachiaria</i> sp	6480	7519	6927	7758	289	0.11	0.80
DM	12267	13557	13219	14306	423	0.02	0.81
OM	10356	11541	11251	12221	387	< 0.01	0.77
NDF	7852	7689	5994	6331	470	0.21	0.81
ADF	3565	3980	3745	4156	130	0.11	0.99
CP	1428	1495	1456	1511	19	0.11	0.82
GE, Mcal/d	45.3	51.5	51.7	57.2	2.43	< 0.01	0.79
<i>Fecal Excretion, g/d</i>							
DM	4907	5423	5288	5722	169	0.02	0.81
OM	3406	3445	3512	3627	48.4	< 0.01	0.13
NDF	2553	2760	2550	3017	11.1	0.08	0.34
ADF	1890	1991	1861	2149	65.0	0.22	0.41
CP	424	470	447	482	12.8	0.17	0.82
GE, Mcal/d	16.5	18.6	19.2	20.9	0.91	< 0.01	0.78
<i>Digestibility, %</i>							
OM	66.9	69.3	68.0	69.2	0.57	0.28	0.57
NDF	65.6	64.0	55.5	48.3	4.02	0.05	0.62
ADF	46.2	49.1	50.6	45.2	1.25	0.94	0.28
CP	70.3	68.6	69.5	68.5	0.42	0.34	0.74
DE	63.5	63.7	62.8	63.0	0.21	0.52	0.96
DE, Mcal/d	28.8	32.5	32.9	36.3	1.53	<0.01	0.86
DE, Mcal/kg	2.34	2.42	2.46	2.52	0.04	<0.01	0.79

Items	Canola oil g/kg SBW ¹				SEM ²	P-value ³	
	0.00	0.40	0.80	1.20		L	Q
¹ SBW, shrunk body weight, BW × 0.96 (NASEM, 2016); ² SEM, standard error means; ³ P-value linear and quadratic; DM, Dry Matter; OM, Organic Matter; NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; CP, Nitrogen; GE, Gross Energy; DE, Digestible Energy; Mcal, Megacalories; ^a Intake, was estimated based on fecal output (using Cr ₂ O ₃ as marker).							

Ruminal fermentation

The effects of CO supplementation on NH₃ -N, pH, protozoa and VFA are shown in Table 4. The N-NH₃ and pH showed values between 25.53 and 34.37 mg/dL and 6.47 and 6.50, respectively, and were not affected (P > 0.05) by CO supplementation. The total ruminal protozoa population decreased (29.7, 69.5 and 88.6%; linear effect P < 0.01) with the addition of CO at the different levels (0.4, 0.8 and 1.2 g/kg SBW). This decrease was reflected by the Entodinidae (31.4, 68.8 and 88.8%, respectively) and Holotrichidae (11.2, 77.5 and 86.4%) populations.

Table 4
Effect of canola-oil supplementation on ammoniacal nitrogen, pH, protozoa, volatile fatty acids, and methane.

Items	Canola oil g/kg SBW ¹				SEM ²	P-value ³	
	0.00	0.40	0.80	1.20		L	Q
NH ₃ -N, mg/dL	25.5	26.7	33.0	34.4	2.23	0.27	0.99
pH	6.49	6.50	6.47	6.48	0.006	0.783	0.986
<i>Protozoa, x10⁴ cells/mL</i>							
<i>Entodinidae</i>	1844.6	1265.4	575.2	206.9	364.048	<0.01	0.048
<i>Holotrichidae</i>	166.5	147.9	37.5	22.7	37.008	<0.01	0.792
Total Protozoa	2011.0	1413.3	612.7	229.6	399.867	<0.01	0.052
<i>Volatile fatty acids, molar%</i>							
TVFA, mM	77.45	74.52	74.25	70.67	1.388	0.183	0.924
Acetic	60.08	58.45	57.59	55.47	0.960	<0.01	0.728
Propionic	19.13	20.9	22.64	23.84	1.028	<0.01	0.498
Butyric	11.82	11.68	10.81	11.1	0.239	0.758	0.361
Valeric	3.32	3.33	3.44	3.42	0.031	<0.01	0.384
Iso-butyric	2.60	2.58	2.51	2.68	0.035	0.904	0.408
Iso-valeric	2.10	2.07	2.01	2.14	0.027	0.056	0.790
A:P	3.18	2.84	2.59	2.38	0.172	<0.01	0.470
<i>Infrared methane emissions</i>							
MER, mg/min	0.92	0.85	0.82	0.49	0.096	0.001	0.252
CH ₄ , g/d	304.8	300.9	299.1	280.1	5.512	0.001	0.252
CH ₄ , g/kg DMI	25.03	22.75	23.26	20.30	0.976	0.001	0.652
CH ₄ , g/kg GEI	10.70	9.42	9.47	8.10	0.529	0.001	0.882
Y _m	7.04	6.78	6.78	6.58	0.094	0.005	0.692
¹ SBW, shrunk body weight, BW × 0.96 (NASEM, 2016); ² SEM, standard error means; ³ P-value linear and quadratic; NH ₃ -N, ammonia nitrogen; TVFA, Total Volatile Fatty Acids; A:P, Acetic acid to propionic acid ratio; MER, Methane Emission Rate; CH ₄ , Methane; DMI, Dry Matter Intake; GEI, Gross Energy Intake; Y _m , GE loss, % GEI.							

No differences were observed in total ruminal concentrations of VFA as well as butyric, iso-butyric and iso-valeric acids: 74.2, 11.4, 2.6 and 2.1 mM, respectively. Furthermore, acetic acid decreased linearly ($P < 0.01$) to 7.7%, whereas propionic and valeric acids increased ($P < 0.01$) to 24.6 and 3.0%, respectively, with CO supplementation compared to the control. Consequently, the acetate propionate ratio decreased by 10.7, 18.6 and 25.2% for the levels of 0.40, 0.80 and 1.20 g CO/kg SBW, respectively.

Methane emissions

The enteric emissions of CH_4 are shown in Table 4. Compared with the diet without canola oil supplementation, the CH_4 emission rate (MER) decreased linearly (7.61, 10.87 and 46.74%, $P < 0.05$) as the CO level in the diet increased. For CH_4 expressed in g/d, the observed decrease (linear effect, $P < 0.05$) was 1.29, 1.87 and 8.12%, whereas for CH_4 expressed as g/kg DMI, the decrease (linear effect, $P < 0.01$) was 9.09, 7.08 and 18.91%. For CH_4 expressed in g/kg GHG, the linear decrease ($P < 0.01$) was 11.93, 11.48 and 24.23% for 0.0, 0.40, 0.80 and 1.20 g CO/kg SBW, respectively.

The estimated values of the loss of energy by the emission of CH_4 (Y_m) were 7.04, 6.78, 6.78 and 6.58%, whereas those estimated using the methodology of the IPCC (2006) were 140.5, 160.8, 158.8 and 177.3 g/kg of CH_4 for the consumption levels of 0.40, 0.80 and 1.20 g CO/kg SBW, respectively.

Discussion

Chemical composition

The chemical composition of *Brachiaria sp.* agrees with that reported in previous studies considering a similar state of maturity (Avellaneda et al., 2008). The concentration of nutrients in the commercial concentrate closely conformed to that specified on the label provided by the food manufacturer. Similarly, the fatty acid profile of the canola oil used agrees with that reported by the AFOA (1988), confirming that canola oil is an important source of oleic FA (70.32% C18:1).

Intake and digestibility

The inclusion of 4, 8 and 12% CO to the concentrate, plus the lipid content of the basal diet (concentrate + grass), corresponded to a total lipid concentration (DMB) of 2.7, 4.5, 6.4 and 7.8% in the final diet for the treatments with 0.0, 0.40, 0.80 and 1.2 g CO/kg SBW, respectively. Generally, lipid supplementation above 7% DM may decrease intake due to an increase in circulating unsaturated FAs that cause activation of receptors in the hypothalamus satiety centre and a reduction in fibre digestibility, resulting in reduced DMI (Allen, 2000; Kumar, 2014). Several mechanisms may explain how dietary lipids interfere with ruminal fermentation. In this study, a decrease was observed in the digestibility of NDF, most likely because long-chain FA present in the CO and those who escape biohydrogenation are potentially toxic for the bacteria responsible for fibre degradation (cellulolytic, gram +) and for methanogens and protozoa (Desbois and Smith, 2010; Zeitz et al., 2013). Also, fats form a physical coating on the fibre, preventing microbial degradation, which has a negative effect on voluntary consumption (Jenkins, 1993). However, the effects

of the addition of supplementary oils on the consumption and digestibility of fresh forages are inconsistent. Some studies involving oil supplementation with fresh forages indicate reductions in both feed intake and overall dietary digestibility (Pavan et al., 2007; Cosgrove et al., 2008), whereas other studies (King et al., 1990; Woodward et al., 2006) found no effect on food intake. On the contrary, Ueda et al. (2003) reported a higher digestibility and feed intake of a high-forage diet supplemented with 3% flaxseed oil. In grazing cows, supplementation reduces the consumption of grass due to a substitution effect but increases the total DMI (Vazquez and Smith, 2000). In the present study, a reduction in DMI was not observed; on the contrary, an increase in DMI and OMI occurred when increasing the CO inclusion level (Table 3). Thus, the low ruminal digestion of NDF can be explained more plausibly by an increase in the rate of fibre passage than by a direct reduction of oil. of the consumption of green pastures supplemented with oil has been previously investigated by Piñares-Patiño et al. (2016), who observed that steers grazing *Lolium perenne* increased their DMI ($P < 0.05$) when roseate CO was added to the pasture. Similarly, Beauchemin et al. (2006) observed an increase in DMI and the intake of digestible energy when a diet based on barley silage supplemented with 3.8% sunflower oil; compared with other sources of fat, such as tallow or whole sunflower seed, sunflower oil had a minimal effect on fibre digestibility. These studies attribute that the increase in DMI of the forage can be explained by the possible increase in the rate of passage of the fibre fraction in forage rich in moisture and supplemented with moderate amounts of oil, considering that the lipid contribution of green pastures is low (Harfoot and Hazlewood, 1997), as observed in the present experiment (2.26% lipids on average).

Ruminal fermentation

The addition of FA can have a toxic effect on rumen protozoa when offered in large quantities (Machmüller and Kreuzer, 1999). According to Gawad et al. (2015), this is mainly observed in protozoa of the family Holotrichidae. In the present study, the ratio of acetate/propionate decreased significantly with the addition of canola oil, which is consistent with the findings of Beauchemin and McGinn (2006). As mentioned above, unsaturated FA can change the ruminal microbiota, and the resulting changes may be the reason for changing the rumen fermentation towards propionate (Guyader et al., 2015). Most likely, this occurs because gram negative (propionate-producing) bacteria are not specifically inhibited by FA (Van Nevel and Demeyer, 1988); thus, the reduction in CH₄ emissions observed when FAs are included in the ruminant diet mainly results from a shift towards propionate production.

Methane emissions

Enteric methane emission, expressed as g CH₄/kg DMI, for the cows that consumed the pasture without oil supplementation in this study was 19.3 g CH₄/kg DM (range 15.8–22.4 g CH₄/kg DMI), which was similar to the values reported by Kennedy and Charmley (2012) for cattle consuming pastures and tropical legumes (19.6g/kg DMI) and the 20 g CH₄/kg DMI recorded by Beauchemin (2006) for a base diet of 75% barley silage and 19% steam-rolled barley. However, it was lower than the 28 g of CH₄/kg DMI reported for cattle grazing leucaena (McGinn et al., 2011). Variations in the values of methane emissions among studies are mainly due to the technique used to measure methane and the chemical composition of the forage consumed. Beauchemin et al. (2007) found that sunflower oil, which is rich in linoleic acid

(C18:2), was effective in reducing the emissions of CH₄ by 14% (177.4 vs. 152.7g/day and 20 vs. 17.7g/kg DMI). The authors concluded that adding 3.3% lipids to forage diets in the form of unsaturated FA limits CH₄ emissions and could have substantial effects on CH₄ inventories. Various studies have suggested that, through different mechanisms of action, lipids reduce the CH₄ production in the rumen (Dohme et al., 2001; Machmüller et al., 2001; Bayat et al., 2018; Muñoz et al., 2019; Nogueira et al., 2020). Reductions in the emission of methane (CH₄/kg DM/d) of 11.5% have been reported for 3.4% sunflower oil supplementation fed to cattle consuming diets based on barley silage (Beauchemin et al., 2006). Moate et al. (2011) report that the addition of fat in diets of lactating cows (60:40 ratio of forage: concentrate) containing 51, 52 and 65 g fat/kg DM decreases the emissions of CH₄ (g/day) by 7.6, 4.5 and 10.2%, respectively, compared to a control diet. In another study, Moate et al. (2014) recorded a reduction in emissions of CH₄ at about 20% with an add grape marc (high fat source and tannins) diet for lactating cows. The same research group (Moate et al., 2016) performed a meta-analysis of 362 individual measurements in lactating cows from 12 different experiments and estimated that for a typical diet containing 30 g of lipids/kg DMI, for each 1% increase in the concentration of fat in the diet, the methane emissions will decrease by approximately 3.5%. A study by Woodward et al. (2006), carried out with lactating dairy cows fed pasture forage dominated by perennial ryegrass (13.5–15.0 kg DMI/d) supplemented with 500 g of fish oil, reported a reduction (compared to the control) in the CH₄ yield of 27%. Martin et al. (2010), in a metanalysis with 67 *in-vivo* diets for sheep, beef and dairy cattle, estimated an average 3.8% decrease in enteric CH₄ (g/kg DMI) with each 1% added fat, which is consistent with the 3.6% decrease per 1% oil added observed in the present study.

The estimated values of Y_m decreased (P < 0.05) by up to 6.5% with the addition of up to 1.20 g CO/kg PMr, taking into account that the formula used in the experiment (Sauvant and Giger-Reverdin, 2009) considers the proportion of the concentrate; for all treatments, it was 47%, and the decrease in energy loss by the emission of CH₄ is attributed to the addition of CO. Gerber et al. (2013) consider that concentrates can influence the decrease of enteric CH₄ from 35–40% of inclusion; at inclusion levels > 40%, there could be a decrease in fibre digestibility.

Regarding the estimation of CH₄ emissions with the IPCC methodology (2006), an increase in methane emissions of 14.4, 13.0 and 26.2% g/d was observed for treatments with 0.0, 0.40, 0.80 and 1.2 g CO/kg SBW, respectively, which is explained by the increase in the GE of the diets. Escobar-Bahamondes et al. (2017) mention that the use of a constant GE value results in the equation being only sensitive to changes in DMI and not to changes in the composition of diets, digestibility and rumen fermentation. Therefore, using the IPCC model, changes in CH₄ are strictly due to changes in DMI, which is a limitation of the IPCC 2006 model; although in the 2019 refinement, the DMI estimates are modified in some categories, the estimate of energy is not modified. We therefore conclude that canola oil supplemented at 1.2 g CO/kg SBW (5.4%) and a contribution of up to 7% total fat in the diet (DMB) of cows grazing tropical fodder, supplemented with a fixed amount of concentrate, can increase the total energy intake and efficiency of rumen fermentation, thereby reducing the emission of enteric methane by up to 18.9%.

Although CO supplementation decreased the total digestibility of NDF by 26%, the DE value (Mcal/kg) of the diet increased by 7.1%

Declarations

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Conflict of interest The authors declare no competing interests.

Ethicals approval The protocols used in this study were reviewed and approved by the Universidad Nacional Autónoma de México, Institutional Committee for the Care and Use of Experimental Animals, according to official animal care normativity (NOM-062-ZOO-1999) (Diario Oficial de la Federación, 2001).

Consent to participate All authors have consented to participate.

Consent for publication All the authors consent to publish the manuscript.

Data availability The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Code availability Not applicable

Author contribution L.C., C.C.M.M. and A.P. conceived and design research. J.N.A.N. and E.C.G., conducted experiments. L.C., J.N.A.N. E.C.G and J.H.H.H. analysed data. J.A.N.A.N., L.C., A.P., F.A.C.P., J.H.H.M. and J.E.R.B. wrote the manuscript. All authors read and approved the manuscript.

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