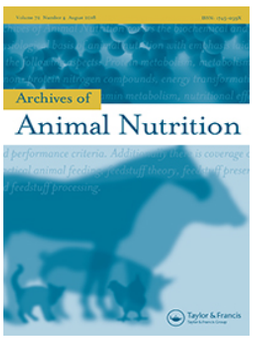

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Effect of soybean oil availabilities on rumen biohydrogenation and duodenal flow of fatty acids in beef cattle fed a diet with crude glycerine

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ABSTRACT

Soybean oil with different ruminal availability (whole soybeans (WS), soybean oil (SO) and calcium salts (CS)) was used to evaluate the fatty acid (FA) intake, rumen biohydrogenation (BH) and duodenal flow of FA in Nellore steers fed diets with crude glycerine (CG). Eight castrated Nellore steers were fitted with a ruminal and duodenal silicone cannula, and distributed in a double, simultaneous, Latin square 4 × 4 design with four diets and four experimental periods. Concentrates contained ground maize, urea, mineral salts, CG (100 g/kg DM) and soybean products with different availability of soybean oil: (1) no additional fat (CO), (2) WS, (3) SO or (4) CS. Fat supplementation was fixed to obtain 50 g ether extract/kg DM. Experimental treatments had no effect on DM intake, DM duodenal flow or ruminal turnover rate of C:16 FA. However, fat addition increased C:18 and turnover rates of total FA rumen ($p < 0.05$). CS resulted in lower C:18 turnover rates and lower ruminal BH of monounsaturated and unsaturated FA (UFA) than WS ($p < 0.05$). SO resulted in a greater duodenal flow of C18:0 (stearic acid), C18:1*t*-11 (vaccenic acid) and saturated FA than the WS and CS diets ($p < 0.05$). CS resulted in a higher duodenal flow of C18:3*n*-3 (linolenic acid) than WS ($p < 0.05$). The association of CG and calcium salts in Nellore steers was the best nutritional strategy to increase duodenal flow of healthier UFA, which may increase the deposition of these FA in meat. However, SO associated with CG association increased the duodenal flow of vaccenic acid, which is main precursor of endogenous synthesis of conjugated linoleic acids in tissues.

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Beef cattle; conjugated linoleic acid; glycerol; lipid metabolism; protected fat

1. Introduction

Rumen microorganisms modify dietary unsaturated fatty acids (UFA) through chemical transformations, such as isomerisation and biohydrogenation (BH) and consequently, the fatty acids (FA) profile incorporated in the tissues is affected (Loor et al. 2004). Therefore, the nutritional characteristics of beef have been criticised due to its relatively

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high concentrations of saturated fatty acids (SFA), which are associated with an increased risk of human coronary heart disease (Zong et al. 2016).

The BH proportion determines the FA profile that reach the intestine (Jenkins 1993), and it is determined by the characteristics of FA source, rumen FA retention time and rumen microbial population (Allen 2000). Thus, different nutritional strategies such as whole soybeans or calcium salts of FA have been used to partially protect UFA from ruminal BH in beef cattle diets (Fiorentini et al. 2015; Carvalho et al. 2017), increasing UFA flow to the intestine and the deposition of healthy UFA in the meat. However, UFA protection is limited in whole seeds due to their rupture during mastication and rumination (Doreau and Ferlay 1995). Additionally, FA calcium salts could be partially generated by acid dissociation in the rumen (Harfoot and Hazlewood 1997), especially when the diet contains high proportions of the concentrate, which can reduce isomerisation or increase the reduction of FA (Abughazaleh and Jacobson 2007).

Another dietary strategy to reduce rumen BH includes the use of vegetables oils associated with crude glycerine (CG). This combination may increase the UFA duodenal flow in high concentrate diets (Granja-Salcedo et al. 2017a), not affecting the dry matter intake (DMI), rumen microbes or ruminal fermentation parameters (Gomez-Insuasti et al. 2014; Granja-Salcedo et al. 2017b). In addition, this alternative may be a useful strategy for the partial replacement of maize in beef cattle diets, resulting in economic and environmental benefit by reducing the faecal nitrogen excretion (Granja-Salcedo et al. 2017b) and increasing the daily gain and feed efficiency (Silva et al. 2013).

It is well known that CG may limit rumen BH *in vitro* and *in vivo* (Krueger et al. 2010; Edwards et al. 2013; Granja-Salcedo et al. 2017a). However, few studies have examined the effects of CG and rumen-protected lipid source association on rumen BH and duodenal flow of UFA in beef cattle. Thus, the hypothesis of this study was that protected lipid sources, such as whole soybeans and calcium salts associated with CG in the diet, may reduce ruminal BH, and then increase the UFA duodenal flow, even with high concentrate proportions. Thus, this study aimed to evaluate the effect of different ruminal availability of soybean oil in the FA intake, rumen BH and FA duodenal flow in feedlot Nellore steers fed a diet with CG.

2. Materials and methods

The experimental procedures used in this study followed the Ethical Principles for Animal Experimentation adopted by Brazilian College of Animal Experimentation, and approved by the Ethics Committee of Animal Experimentation of Faculdade de ciências Agrarias e Veterinárias (FCAV), Universidade Estadual Paulista (UNESP), Jaboticabal campus, Brazil (07784/14). The experiment was conducted at Experimental Feedlot at Animal Science Department in the UNESP, Jaboticabal, Brazil.

2.1. *Animals and diets*

Eight castrated Nellore steers (initial weight 554 ± 18 kg) were used, fitted with a ruminal (diameter: 10 cm) and duodenal silicone cannula (Type T), distributed in a double, simultaneous, Latin square 4 × 4 design with four treatments (diets) and four experimental periods. Ruminal cannulation was conducted 14 months prior to the

experiment under xylazine sedation and local anaesthesia with lidocaine hydrochloride, and all efforts were made to minimise suffering. Postoperative treatment consisted of intramuscular injection of penicillin (sodium, procaine and benzathine 12,000 IU/kg), streptomycin (5 mg/kg) and piroxicam (0.5 mg/kg).

Each period lasted 20 d, with the first 14 d used for adaptation, 5 d for faeces collection and the last 2 d for ruminal and duodenal sampling. Initially, the animals were weighed, identified, treated for ecto- and endoparasites with ivermectin (Ivomec, Merial, Paulínia, BR) and allocated to individual stables (12 m^2) with individual feed troughs and automatic waterers.

Diets were formulated to reach a gain of 1.25 kg/d according to Valadares Filho et al. (2010) and had a roughage:concentrate ratio of 30:70 using maize silage as source of roughage (85.02% DM, 95.16% organic matter (OM), 9.72% crude protein (CP), 2.96% ether extract (EE), 51.57% NDF assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom) and 4.32% acid detergent lignin (ADL)). Concentrates contained ground maize, urea, mineral salts and different soybean oil availabilities as follows: no additional fat (CO), whole soybeans (WS), soybean oil (SO) and calcium salts (CS) (Megalac-E® – Church & Dwight, affiliate Química Geral do Nordeste S/A) associated with 100 g/kg DM of CG. Fat supplementation was fixed to obtain 50 g EE/kg DM (Table 1).

The used CG (83.90% glycerol, 1.75% EE, 4.30% ash and 12.01% water) was acquired from a soybean-oil-based biodiesel production company (Cargill, Três Lagoas, Mato Grosso do Sul, Brazil). All concentrates had to be taken within a maximum of 4 d to prevent the oxidation of fat dietary ingredients. Diets were provided as total mixed ration at 07:00 h and 18:00 h. Throughout the experimental period, the allowance was adjusted to allow refusals of approximately 5% of the total amount consumed on the previous day.

2.2. *Intake and faecal outputs*

Feed and leftovers samples were collected before morning feeding between the 14th and 20th d of each experimental period. Subsamples of □100 g/kg were obtained and frozen at −20°C. At the end of each experimental period, composite samples were collected for each animal based on the weight of leftover.

Between the 15th and 18th d of each experimental period, faeces samples were collected immediately after each spontaneous defecation and stored in 20 l buckets. After each 24 h collecting period, the total amount of faeces produced per animal was weighed and homogenised; a sample (10% of daily faecal production) was removed and dried in a forced-air-circulation oven at 55°C for 72 h. For each animal, in each experimental period, we formed a faecal-composite sample based on the pre-dried weight and used for DM and neutral detergent fibre (NDF) analysis.

2.3. *Ruminal biohydrogenation and duodenal flow*

Rumen fluid and duodenal content samples were obtained using rumen and duodenal cannula, respectively, and were collected at 6-h intervals on the 19th and 20th d of each experimental period. The sample collection during the 20th d was delayed ensuring that

Table 1. Ingredients, nutrient contents and fatty acid (FA) profiles of experimental diets.

	Experimental diet			
	CO (No additional fat)	WS (Whole soybeans)	SO (Soybean oil)	CS (Ca salts)
Ingredients [% of DM]				
Maize silage	30.0	30.0	30.0	30.0
Ground maize	47.9	46.5	45.7	45.4
Soybean meal	8.1	0.00	8.6	8.6
Whole soybean	–	9.52	–	–
Soybean oil	–	–	1.7	–
Rumen-protected fat[†]	–	–	–	2.0
Crude glycerine	10.0	10.0	10.0	10.0
Urea	1.0	1.0	1.0	1.0
Mineral salt[‡]	3.0	3.0	3.0	3.0
Nutrient contents				
Dry matter [% DM]	69.0	68.3	68.0	67.7
Organic matter [% DM]	94.2	94.2	94.2	94.2
Crude protein [% DM]	16.4	17.1	15.5	15.9
Diethyl ether extract [% DM]	3.7	5.3	5.0	4.8
aNDFom [% DM][♦]	25.9	24.6	24.1	24.1
Acid detergent fibre [% DM]	9.0	9.5	8.8	8.9
Acid detergent lignin [% DM]	1.8	3.1	2.4	2.0
Metabolisable energy [MJ/kg DM]	12.2	12.6	12.5	12.5
Fatty acid profile [g/kg of DM]				
C14:0	0.26	0.37	0.37	0.46
C16:0	2.41	4.37	4.22	4.19
C18:0	1.10	2.19	1.45	1.57
C18:1*n*-9	4.51	7.67	7.47	6.68
C18:2*n*-6	7.49	16.63	16.86	17.54
C20:0	1.63	3.76	3.70	2.47
C18:3*n*-3	1.12	1.96	1.56	1.51
Others[§]	4.87	7.35	8.68	8.18
Total UFA*	17.06	34.74	33.68	33.26
Total SFA[‡]	6.34	9.57	10.68	10.33

[†]Megalac-E®; [‡]Provided per kg complete diet: 6.3 g calcium, 0.6 g phosphorus, 1.11 g sulphur, 2.4 g sodium, 147 mg copper, 42 mg manganese, 54 mg zinc, 1.08 mg iodine, 0.87 mg cobalt, 0.27 mg selenium, ≤9.99 mg fluorine; [♦]aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; [§]Sum of C8:0, C10:0, C12:0, C13:0, C14:1 *c*-9, C15:0, C16:1*c*-9, C:17, C18:2*t*-9, 12, C20:1 *c*-11 and C22:1 *c*-13; *UFA, unsaturated fatty acids (sum of C14:1 *c*-9, C16:1 *c*-9, C18:1 *c*-9, C18:2 *c*-9 *c*-12, C18:3 *c*-9 *c*-12 *c*-15, C20:1 *c*-11 and C22:1 *c*-13); [‡]SFA, saturated fatty acids (sum of C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C22:0).

every 3 h slot in a 24 h period was properly represented, after last sample collection total ruminal content mass and volume were determined. A sample of 100 ml of rumen fluid was filtered through a double-layer cotton fabric and stored at –20°C, lyophilised, and used to analyse the profile of long-chain fatty acids (LCFA). Duodenal content samples (200 ml) were divided into two aliquots; a 50 ml sample was stored at –20°C and lyophilised, which was used to analyse the LCFA profile; and a 150 ml sample was dried in a forced-air-circulation oven at 55°C for 72 h to perform DM and NDF analysis.

Indigestible neutral detergent fibre (NDFi), an indicator of the daily DM duodenal flow (Harvatine and Allen 2006), was assessed by *in situ* incubation of offered feeds, leftovers, faeces and duodenum samples for 288 h, following the methodology proposed by Valente et al. (2011). FDNi determination was carried out using α-amylase with no sodium sulphite, as Van Soest et al. (1991) recommendations, and adapted for the ANKOM 200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA). Duodenal DM flow was calculated according to equation:

Duodenal DM = (Faecal DM · Indigestible NDF in faecal DM [g/kg])/Indigestible NDF in duodenal DM [g/kg]

Total FA were extracted from the feed ingredients, leftovers, rumen and duodenum samples using the methylation method described by Palmquist and Jenkins (2003). During this process, 1 ml of methyl nonadecanoate (C19:0) (Sigma-Aldrich, Supelco, USA) at 2.0% was added to each sample, which was used as the internal standard for FA quantification. The FA profile was quantified by gas chromatography (GC Shimatzu model 20-10, with automatic injection; Shimatzu Corporation, Kyoto, Japan) using a SP-2560 capillary column (100 m × 0.25 mm diameter, 0.02 mm thick; Supelco, Bellefonte, PA, USA), and hexane as carrier gas. In order to identify each FA, we used external standards (Supelco, Bellefonte, PA, USA; Nu-Chek Prep, Elysian, MN, USA), whereas an internal standard (C19:0) was used for quantification. The peaks of samples were identified and concentrations calculated based on the retention time and peak area of known standards (Sigma Chemical).

Fractional rates of BH of FA and passage from the rumen were calculated as proposed by Harvatine and Allen (2006), which assumes that unsaturated FA are not oxidised, but are hydrogenated and that BH follows first-order kinetics. FA turnover rates were calculated according to the following equation:

$$FA_{Turnover}\ [\%/h] = (FA_{Intake}\ [g/h]/FA_{Rumen\ mass}\ [g]) \cdot 100\%$$

The rates of ruminal BH were calculated by using the model described by Jenkins and Bridges (2007), which estimates the difference between the consumed FA (initial, [g/d]) and the FA observed in the duodenal flow (final, [g/d]), calculated according to the following equation:

$$BH\ of\ FA\ (BFA)\ [\%] = 100\% \cdot (FA_{Initial} - FA_{Final})/FA_{Initial}$$

2.4. *Experimental design and statistical analyses*

All data were analysed as a double 4 × 4 Latin square design (four treatments and four periods), balanced for residual effects, according to the following statistical model:

$$\gamma_{ijkl} = \mu + \varphi_i + \tau_j + \rho_k + \delta_{l(\varphi i)} + \varphi\tau_{ij} + \varepsilon_{ijkl}$$

where γ_{ijkl} = dependent variable on steers l given treatment j at period k at Latin square i; μ = population average; φ_i = The random effect of the ith Latin square; τ_j = the fixed effect of the jth treatment or diet; ρ_k = fixed effect of the kth period; $\delta_{l(\varphi i)}$ = the random effect of the animal within the Latin square i; $\varphi\tau_{ij}$ = the fixed effect of the interaction between the Latin square i and treatment j; ε_{ijkl} = random error. The errors were normally and independently distributed with mean (0) and homogeneous variance (σ^2).

After verification of mathematical assumptions (Shapiro–Wilk test and Bartlett test), all data were analysed using the MIXED procedure of SAS (Statistical Analysis System for Windows 9.3, SAS Institute Inc., Cary, USA). Diet effects were studied by three orthogonal contrasts: effect of fat supplementation (CO vs. WS+ SO+ CS), effect of rumen-protected soybean oil (SO vs. WS+ CS) and effect of rumen protection type (WS vs. CS). Degrees of freedom correction was performed according to Kenward and Roger (1997), and means were adjusted by LSMEANS.

3. Results

3.1. *Intake of DM and FA*

Diets with different ruminal availability of soybean oil had no effect on DMI (Table 2). DMI supported an average daily of 0.77 kg/d in steers feed CO and WS diets, and 0.52 kg/d to SO and CS diets. As expected, diets supplemented with soybean oil sources (fat supplementation effect) resulted in a higher intake of total FA, SFA, monounsaturated FA (MUFA), polyunsaturated FA (PUFA), UFA and all evaluated individual FA ($p < 0.05$). Steers fed the SO diet had a lower intake of <C15:0, C18:0 (stearic acid) and PUFA ($p < 0.05$), than those fed WS and CS diets. Furthermore, WS increased the intake of C18:0 (stearic acid), C18:1*n*-9 (oleic acid), C18:3*n*-3 (linolenic acid), >C20:0, SFA, MUFA, UFA and total FA ($p < 0.05$) in relation to CS diet.

3.2. *FA rumen concentration and turnover rates*

It was observed that fat supplementation increased the rumen concentration of all quantified FA (Table 3). Rumen-protected soybean oil sources resulted in a lower rumen concentration of <C15:0, C18:0 (stearic acid), C18:3*n*-3 (linolenic acid) and SFA and a higher concentration of C18:1*n*-9 (oleic acid) ($p = 0.010$) and C18:2*n*-6 (linoleic acid) ($p < 0.001$) than SO ($p < 0.05$). Nonetheless, SO resulted in a higher concentration of C18:1*t*-11 (vaccenic acid) ($p = 0.041$) than WS and CS. In addition, CS resulted in a higher rumen concentration of C18:2*n*-6 (linoleic acid), C18:3*n*-3

Table 2. Effect of soybean oil availability on dry matter intake (DMI) and fatty acid (FA) intake in Nellore steers fed a diet with crude glycerine ($n = 8$ per diet).

	Experimental diets*					*p*-Value‡		
	CO	WS	SO	CS	SEM†	Fat effect (CO vs. WS + SO + CS)	Protection effect (SO vs. WS + CS)	Protection type effect (WS vs. CS)
DMI [kg/d]	9.62	9.75	9.10	8.98	0.18	0.131	0.346	0.288
DMI [% of BW]	1.65	1.67	1.56	1.54	0.06	0.175	0.428	0.091
FA intake [g/d]								
<C15:0	19.0	27.5	26.1	29.3	0.79	<0.001	0.007	0.719
C16:0	23.4	43.8	41.4	41.3	1.54	<0.001	0.427	0.635
C17:0	3.7	5.5	5.6	6.6	0.20	<0.001	0.129	<0.001
C18:0	10.7	22.0	14.3	15.5	0.75	<0.001	< 0.001	<0.001
C18:1*t-6*	3.1	4.2	4.2	4.0	0.10	<0.001	0.651	0.427
C18:1*n-9*	48.4	76.8	73.4	65.8	2.45	<0.001	0.372	<0.001
C18:2*n-6*	72.8	166.7	165.7	172.7	7.63	<0.001	0.509	0.426
C18:3*n-3*	10.9	19.7	15.3	14.8	0.59	<0.001	0.211	<0.001
>C20:0	26.8	54.4	51.5	39.6	2.03	<0.001	0.402	<0.001
SFA♦	55.2	94.5	91.3	89.4	3.82	<0.001	0.106	0.017
MUFA§	82.9	152.5	134.0	121.1	9.18	<0.001	0.364	0.032
PUFA‡	83.7	186.4	181.0	187.6	8.32	<0.001	< 0.001	0.477
UFA€	166.6	338.9	315.0	308.6	4.41	<0.001	0.281	<0.001
Total FA	221.7	433.4	406.3	398.1	15.69	<0.001	0.714	0.011

*CO, no additional fat; WS, whole soybeans; SO, soybean oil; CS, calcium salts; †SEM, standard error of mean; ♦SFA, saturated fatty acids (sum of C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C22:0); §MUFA, monounsaturated fatty acids (sum of C14:1*c*-9, C16:1*c*-9, C18:1*t-6*, C18:1*c*-9, C20:1*c*-11 and C22:1*c*-13); ‡PUFA, polyunsaturated fatty acids (sum of C18:2*c*-9 *c*-12, C18:3*c*-9 *c*-12 *c*-15); €UFA, unsaturated fatty acids (MUFA + PUFA).

Table 3. Effect of soybean oil availability ruminal concentration and turnover rates of fatty acid (FA) in Nellore steers fed a diet with crude glycerine (n = 8 per diet).

	Experimental diets*					*p*-Value		
	CO	WS	SO	CS	SEM†	Fat effect (CO vs. WS + SO + CS)	Protection effect (SO vs. WS + CS)	Protection type effect (WS vs. CS)
Ruminal concentration [g/d]								
<C15:0	41.1	64.4	74.5	65.6	2.70	<0.001	0.017	0.736
C16:0	34.5	54.9	50.3	48.4	2.28	0.009	0.387	0.209
C17:0	4.0	4.7	5.1	4.6	0.16	0.047	0.156	0.384
C18:0	28.2	37.8	59.8	32.4	2.22	<0.001	<0.001	0.004
C18:1*t-6*	5.4	7.6	9.4	7.4	0.65	0.043	0.089	0.887
C18:1*c-6*	2.0	2.8	2.9	2.7	0.15	0.028	0.812	0.761
C18:1*n-9*	39.0	56.7	46.9	64.9	2.53	0.001	0.010	0.143
C18:1*t-9*	6.2	8.7	8.8	8.5	0.42	0.011	0.415	0.618
C18:1*t-11*	13.9	16.4	20.2	17.7	0.83	0.024	0.041	0.522
C18:2*c-9t-11*	1.3	1.7	1.9	1.9	0.09	0.079	0.187	0.209
C18:2*n-6*	43.9	61.5	57.3	89.2	3.41	<0.001	<0.001	<0.001
C18:3*n-3*	8.1	10.3	17.1	15.5	0.97	0.002	0.040	0.027
>C20:0	14.9	17.2	16.5	19.4	0.56	0.028	0.373	0.349
SFA♦	242.9	148.6	176.7	140.6	7.58	<0.001	<0.001	0.173
MUFA§	89.4	126.0	121.0	134.2	4.34	<0.001	0.274	0.075
PUFA‡	52.0	71.8	74.4	104.8	4.38	<0.001	0.117	<0.001
UFA€	141.4	197.8	195.5	238.9	8.77	<0.001	0.131	<0.001
Total FA	243.8	346.3	372.2	379.5	11.93	<0.001	0.274	0.306
Ruminal turnover [%/h]								
C:16	2.87	3.53	3.63	3.70	0.168	0.072	0.725	0.674
C:18	4.33	6.46	5.47	5.07	0.453	0.001	0.389	0.003
Total FA	3.80	5.31	4.63	4.44	0.156	0.004	0.446	0.026

*CO, no additional fat; WS, whole soybeans; SO, soybean oil; CS, calcium salts; †SEM, standard error of mean; ♦SFA, saturated fatty acids (sum of C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C22:0); §MUFA, monounsaturated fatty acids (sum of C14:1 *c*-9, C16:1 *c*-9, C18:1*t-6*, C18:1 *c*-9, C20:1 *c*-11 and C22:1 *c*-13); ‡PUFA, polyunsaturated fatty acids (sum of C18:2 *c*-9 *c*-12, C18:3 *c*-9 *c*-12 *c*-15); €UFA, unsaturated fatty acids (MUFA + PUFA).

(linolenic acid), PUFA and UFA than WS ($p < 0.05$). WS resulted in a higher rumen concentration of C18:0 (stearic acid) than CS ($p = 0.004$).

The ruminal turnover rate of C:16 FA was not affected by the diets (Table 3). There was an effect of fat supplementation that increased the rumen turnover rates of C:18 and total FA ($p < 0.05$). An effect of protection type was observed, wherein CS lowered the turnover rates of C:18 and total FA than WS ($p < 0.05$).

3.3. *Duodenal flow of FA and ruminal biohydrogenation rates*

The duodenal flow of DM was similar among diets (Table 4). Diets with fat addition led to a higher duodenal flow of total FA, MUFA, UFA, SFA and most measured individual FA, except C18:2*n*-6 (linoleic acid) and PUFA, which only were stimulated by sources of rumen-protected soybean oil ($p < 0.001$). Moreover, SO induced a greater duodenal flow of C18:0 (stearic acid), C18:1*t*-11(vaccenic acid) and SFA than WS and CS diets ($p < 0.05$). CS resulted in a higher duodenal flow of <C15:0, C18:1 c-6 (petroselinic acid) and C18:3*n*-3 (linolenic acid) than WS ($p < 0.05$).

Additional fat in the diet increased the ruminal BH rates of C18:2*n*-6 (linoleic acid), MUFA, PUFA and UFA (Table 5; $p < 0.05$). In addition, the SO diet exhibited a higher rate of ruminal BH of C18:1*n*-9 (oleic acid), C18:2*n*-6 (linoleic acid) and PUFA ($p < 0.05$). CS diet resulted in lower ruminal BH rates of C18:1*t*-6 (octadecenoic

Table 4. Effect of soybean oil availability on duodenal flow of dry matter and fatty acids (FA) in Nellore steers fed a diet with crude glycerine (n = 8 per diet).

	Experimental diet*					*p*-Value		
	CO	WS	SO	CS	SEM[†]	Fat effect (CO vs. WS + SO + CS)	Protection effect (SO vs. WS + CS)	Protection type effect (WS vs. CS)
Dry matter [kg/d]	3.60	4.66	4.71	3.82	0.331	0.077	0.137	0.082
Duodenal flow of FA [g/d]								
<C15:0	21.6	30.4	36.1	36.4	1.27	<0.001	0.147	0.013
C16:0	21.0	39.2	38.1	39.4	1.56	<0.001	0.326	0.192
C17:0	3.1	4.6	5.1	4.1	0.26	0.003	0.206	0.317
C18:0	56.7	76.1	95.4	75.7	2.97	<0.001	<0.001	0.673
C18:1*t-6*	1.0	1.2	1.6	1.6	0.22	0.025	0.489	0.541
C18:1*c-6*	1.3	1.5	2.1	3.5	0.18	0.002	0.652	<0.001
C18:1*n-9*	27.6	36.9	33.8	37.6	1.64	<0.001	0.218	0.743
C18:1*t-9*	45.2	73.8	67.5	65.2	3.29	<0.001	0.375	0.431
C18:1*t-11*	4.4	9.0	9.2	6.5	0.63	<0.001	0.034	0.142
C18:2*c-9, t-11*	1.1	2.0	2.0	2.1	0.10	0.019	0.529	0.434
C18:2*n-6*	18.5	23.8	10.5	23.9	1.20	0.118	<0.001	0.627
C18:3*n*-3	5.2	8.8	10.7	12.2	0.57	<0.001	0.141	0.001
>C20:0	18.6	41.6	41.4	34.6	1.78	<0.001	0.246	0.002
SFA[♦]	113.2	223.5	249.6	175.5	4.71	<0.001	0.023	0.339
MUFA[§]	80.3	114.3	115.1	114.7	5.04	<0.001	0.162	0.495
PUFA[‡]	23.7	32.6	21.2	36.2	1.80	0.184	<0.001	0.137
UFA[€]	104.0	146.9	136.2	150.9	7.10	<0.001	0.004	0.165
Total fatty acids	21.6	30.4	36.1	36.4	1.27	<0.001	0.340	0.426

*CO, no additional fat; WS, whole soybeans; SO, soybean oil; CS, calcium salts; [†]SEM, standard error of mean; [♦]SFA, saturated fatty acids (sum of C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C22:0); [§]MUFA, monounsaturated fatty acids (sum of C14:1 *c*-9, C16:1 *c*-9, C18:1*t-6*, C18:1 *c*-9, C20:1 *c*-11 and C22:1 *c*-13); [‡]PUFA, polyunsaturated fatty acids (sum of C18:2 *c*-9 *c*-12, C18:3 *c*-9 *c*-12 *c*-15); [€]UFA, unsaturated fatty acids (MUFA + PUFA).

Table 5. Effect of soybean oil availability on the rate of ruminal biohydrogenation (BH) of fatty acids (FA) [% of total ingested] in Nellore steers fed a diet with crude glycerine (n = 8 per diet).

	Experimental diet*					*p*-Value		
	CO	WS	SO	CS	SEM[†]	Fat effect (CO vs. WS + SO + CS)	Protection effect (SO vs. WS + CS)	Protection type effect (WS vs. CS)
C18:1*n*-9	42.97	51.98	53.98	42.84	42.97	0.362	0.041	0.007
C18:2*n*-6	74.56	85.70	93.69	86.14	74.56	<0.001	<0.001	0.264
C18:3*n*-3	52.29	55.46	30.02	17.68	52.29	0.216	0.124	<0.001
MUFA[§]	3.10	25.03	14.15	5.24	5.25	0.011	0.174	<0.001
PUFA[£‡]	71.64	82.50	88.30	80.73	71.64	<0.001	0.027	0.641
Total UFA[€]	37.54	56.65	56.77	50.79	51.12	<0.001	0.137	0.015

*CO, no additional fat; WS, whole soybeans; SO, soybean oil; CS, calcium salts; [†]SEM, standard error of mean; [♦]SFA, saturated fatty acids (sum of C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C22:0); [§]MUFA, monounsaturated fatty acids (sum of C14:1 *c*-9, C16:1 *c*-9, C18:1*t-6*, C18:1 *c*-9, C20:1 *c*-11 and C22:1 *c*-13); [‡]PUFA, polyunsaturated fatty acids (sum of C18:2 *c*-9 *c*-12, C18:3 *c*-9 *c*-12 *c*-15); [€]UFA, unsaturated fatty acids (MUFA + PUFA).

acid), C18:1*n*-9 (oleic acid), linolenic acid (C18:3*n*-3), MUFA and total UFA than WS ($p < 0.05$).

4. Discussion

This study evaluated the effect of different ruminal availability of soybean oil (soybean oil, whole soybeans and calcium salts) associated with CG in the diet of feedlot Nellore

steers as a dietary strategy to limit the ruminal BH and increase the duodenal flow of UFA. Our results showed that rumen-protected soybean oil sources reduced the BH of oleic acid, linoleic acid and PUFA, and that CS was the most efficient soybean oil source to increase the duodenal flow of linoleic acid and PUFA. In this context, the hypothesis that rumen-protected lipid sources associated with CG in the diet may limit ruminal BH and increase the UFA duodenal flow was accepted, even using high concentrate proportions.

Diets containing different ruminal availability of soybean oil associated with CG did not affect the DMI. These results may be linked to two factors: the fat content of diet (<50.3 g EE/kg DM) and CG (100 g CG/kg DM) addition in the diet. Despite the fact that dietary UFA reduced fibre digestibility and diet intake in ruminants due to their deleterious effects on cellulolytic bacteria (Doreau and Chilliard 1997; Oldick and Firkins 2000), several trials have shown that in beef cattle, the DMI reduction by lipid supplementation is observed generally in animals fed diets with a higher lipid content (>60 g EE/kg DM) than used in our study (Hess et al. 2008; Fiorentini et al. 2015; Granja-Salcedo et al. 2017a, 2017b). On the other hand, in feedlot conditions, CG addition to the diet can reduce the adverse effects of UFA on ruminal microorganisms and ruminal fermentation not affecting the intake in diets containing vegetable oils (Granja-Salcedo et al. 2017b).

As expected, lipid supplementation allowed a higher intake of total FA, SFA, MUFA, PUFA and UFA, since soybean oil sources have a higher content of SFA and UFA, and FA intake depends on the concentration in the diet. Nevertheless, even using the same lipid source (soybean oil) and fixing the lipid addition to obtain 50 g EE/kg DM, the fat source and protection type affected the FA profile intake and FA rumen concentration profile. These differences may be due to the source, industrial processing of the raw material, source palatability and animal selection observed during the trial, especially for WS diet.

The FA rumen concentration is important to understand the kinetics of UFA ruminal BH, which is a function of the available ruminal FA pool size, ruminal retention time and bacterial hydrogenation capacity (Allen, 2000). The profiles of rumen FA concentration compared to FA intake presented FA modification in the rumen, even using protected fat sources in the diet with CG. Our results agree with those of previous studies reporting partial dissociation of calcium salts containing soybean oil in the rumen (Lundy et al. 2004; Fiorentini et al. 2015; Bettero et al. 2017).

Lipid supplementation increased rumen concentration of all quantified FA, which was induced by higher intake of total FA, SFA, MUFA, PUFA and UFA, corroborating with Harvatine and Allen (2006) who observed a strong increase in FA ruminal pools in dairy cows fed diets with FA in different saturation. It is worth mentioning that few studies have reported FA rumen concentration in beef cattle, mainly in animals fed diets with different lipid rumen availabilities. FA rumen concentrations have been estimated using grab samples from the rumen at different times to represent the largest and smallest pools experienced during the day. This may not represent the real FA profile of rumen contents, particularly with WS diet, because the FA profile of rumen contents may associate differently with the liquid and solid fractions of the rumen (Harvatine and Allen 2006) and some FA could be contained in the grains.

It was observed that rumen-protected soybean oil sources allowed different FA rumen concentration profiles from SO. Thus, CS and WS diets allowed a lower rumen concentration of SFA and a higher ruminal concentration of oleic acid and linoleic acid. The lower availability of these FA in the ruminal environment for the BH and stearic acid formation may have contributed for these results, which is supported by lower BH rates observed in oleic acid and linoleic acid in steers fed CS and WS diets. This also agrees with the lower rumen concentration and duodenal flow of SFA in rumen-protected soybean oil sources observed in WS and CS diets, suggesting that natural (protein matrix surrounding the lipids fraction of WS) or artificial (calcium soaps) lipid rumen protection was useful in delaying the ruminal bacteria access (Palmquist and Jenkins 1980).

In addition, CS was the most efficient soybean oil source in increasing the duodenal flow of linoleic acid and PUFA, by decreasing ruminal BH rates of octadecenoic acid, oleic acid, linolenic acid, MUFA and total UFA. This is consistent with previous studies conducted in dairy cows (Bettero et al. 2017) and beef cattle (Fiorentini et al. 2015), which described that CS from soybean had a higher UFA ruminal protection than WS. However, BH rates for total UFA and linolenic acid with rumen-protected soybean oil sources were lower than the range reported by those studies, probably due to the CG addition effect and its inhibitory effect on ruminal BH (Edwards et al. 2012; Granja-Salcedo et al. 2017a). In our study, the ruminal BH rates of oleic acid, linoleic acid, PUFA and UFA observed with the SO diet were almost values reported by Granja-Salcedo et al. (2017a) in feedlot Nellore steers fed diets containing CG and SO.

Although the SO diet resulted in higher BH rates and duodenal flow of SFA, it also showed higher accumulation of *cis* and *trans* fatty acids in the rumen, especially vaccenic acid that resulted in greater duodenal flow of this FA (9.17 g/kg DM). Rumen vaccenic acid increment indicates an incomplete BH (AbuGhazaleh et al. 2002), and a greater duodenal flow of vaccenic acid may result in conjugated linoleic acid (CLA) endogenous synthesis in tissues (Bauman et al. 1990; Harfoot and Hazlewood 1997). Our results suggest that soybean oil sources could be used in beef cattle diets to increase duodenal flow of this important FA, but the amount that reaches the intestine would depend on the source and type of diet. Thus, it is plausible that SO, CS and CG in the diet with CG could be a nutritional strategy that would increase the content of healthy UFA in the meat by increasing the duodenal flow of UFA and endogenous synthesis of CLA in tissues, but future studies are required to confirm it.

Although some studies have shown that CLA production could be stimulated with a higher linoleic acid intake (Loor et al. 2002), in this study we observed a higher ruminal BH of linoleic acid (>85%) and a limited duodenal flow of CLA – C18:1 *c*-9, *t*-11 (<2.08 g/kg DM), in rumen-protected sources that showed a higher intake and rumen concentration of linoleic acid. However, several studies in beef cattle have reported that a high proportion of concentrate in the diet reduces the CLA duodenal flow, even using fat rumen-protected sources (Fiorentini et al. 2015). This kind of diet can contribute to lower isomerisation or higher reduction of FA during the final steps of the BH (Abughazaleh and Jacobson 2007).

As expected, additional fat in the diet increased the ruminal BH rates of linoleic acid, MUFA, PUFA and UFA, due to a higher availability of these FA in the ruminal environment (Loor et al. 2002). The higher C:18 and total FA turnover rates in WS

can be explained by the WS particle size; granular forms with a larger particle size, such as whole soybean grains, could be retained in the ruminal fibrous mat (Harvatine and Allen 2006), and are expected to flow from the rumen lesser than FA associated with liquid fraction (oil) or smaller particle size (protected-fat). In the WS diet group, increased ruminal feed retention time could contribute to higher BH rates when compared to the CS diet group. Additionally, with an acid rumen pH, as is usually observed in feedlot diets, a proportion of calcium salts can be dissociated, releasing FA in the non-esterified form into rumen (Sukhija and Palmquist 1990; Harfoot and Hazlewood 1997). Non-esterified FA forms have been able to reduce the rumen motility (Nicholson and Omer 1983) and therefore, reduce the FA turnover rates.

Rumen-protected forms of lipids do not always prevent the ruminal BH in feedlot diets with CG. However, artificial rumen-protected form (calcium salts) in Nellore steers may be a nutritional strategy to decrease the BH of UFA, increasing the duodenal flow of these FA. On the other hand, soybean oil in the unprotected form can increase the vaccenic acid duodenal flow, essential to CLA endogenous synthesis in tissues. These feeding strategies could be resulting, in a qualitative improvement of ruminant products such milk or meat, by a relative increase of UFA content.

Disclosure statement

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