Different Sources Of Fat In Supplements For Beef Cattle At Pasture

Lucien Bissi da Freiria (lucien.freiria@ifro.edu.br)
Instituto Federal de Rondonia: Instituto Federal de Educacao Ciencia e Tecnologia de Rondonia
https://orcid.org/0000-0003-3715-5228
Joanis Tilemahos Zervoudakis
Universidade Federal de Mato Grosso
Nelcino Francisco de Paula
Universidade Federal de Mato Grosso
Mozart Alves Fonseca
University of Nevada Reno
Pedro Ivo José Lopes da Rosa e Silva
Universidade Federal de Mato Grosso
Yasmim Rodrigues Vilas Boas e Silva
Universidade Federal de Mato Grosso
Adriano Jorge Possamai
Cargill Animal Nutrition

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Abstract

The aim of this study was to investigate the consequences of the fat supplementation source (free oil and rumen-protected fat) on the nutrient intake and digestion of beef cattle at pasture. Five rumen-cannulated Nelore bulls, with an average 467.8±32.8 kg of body weight (BW) and age of 26 months, were distributed in a Latin square design (5 x 5). The treatments were as follows: WF: without fat, PS: rumen-protected fat soybean oil, PA: rumen-protected fat palm oil, SO: soybean free oil, and CO: corn free oil. Nutrient intake and digestibility, ruminal pH and ammonia (NH_3-N), serum urea, and nitrogen balance were analyzed. The supplements with different fat sources did not alter (P>0.05) the intake and digestibility of dry matter (DM), forage, organic matter (OM), crude protein (CP), neutral digestibility fiber (NDF), neutral digestibility corrected ash and protein (NDFap), nonfiber carbohydrates (NFC) or total digestible nutrients (TDN), except ether extract (EE). An increase (P<0.05) in the intake and digestibility was observed with the inclusion of a fat supply, independent of the fat source. Differences were observed between the WF and other supplements with regard to ruminal parameters (pH and NH_3-N) (P>0.05) and serum urea (P>0.05). The nitrogen balance was not affected by the fat source (P>0.05). Supplementation of grazing beef cattle with 2 g/kg BW low-level free oil (130 g/kg DM supplement) or rumen protection (160 g/kg DM supplement) did not interfere with the characteristic nutrient intake and digestibility.

Introduction

Increasing attention has been placed on enhancing the use of fat in the diet of ruminants, and according to Jenkins et al. (1993), the emphasis on ruminal lipid metabolism is associated with manipulating physicochemical events in the rumen to produce two practical outcomes: 1) control of the antimicrobial effects of fatty acids so that additional fat can be fed to ruminants without disrupting ruminal fermentation and digestion and 2) regulation of microbial biohydrogenation to change the absorption of selected fatty acids that might enhance performance or improve the nutritional qualities of animal food products.

In beef, soybean and corn oil represent sources of unsaturated fatty acids and enhance the content of CLA, the cis-9,trans-11 isomer (Duckett et al., 2009; Cooke et al., 2011; Choi et al., 2013), which has anticarcinogenic and antiatherogenic effects (Scollan et al., 2006; Shingfield et al., 2011). Early studies showed that soybean oil has the potential to provide direct energy for muscle gain and decrease in the size of adipose cells in subcutaneous tissue (Choi et al., 2013).

Using palm oil as the source of saturated fatty acid requires a longer period of supplementation time, and it may significantly increase carcass adiposity and has the potential to increase marbling scores without increasing the palmitic acid or reducing the oleic acid content of beef (Choi et al., 2013).

However, fat supplementation for animals at pasture may decrease the forage dry matter intake and performance of the animals. Lipids inhibit the growth of fibrotic bacteria and show high potential

reactions with ruminal microorganism membranes, which has lethal toxicity (Patra &Yu, 2012; Huws et al., 2010).

When including fat supplements in the diet, it is important to avoid promoting negative effects on the growth of fibrotic bacteria; moreover, rumen-protected fat should be used because it is less harmful for ruminal bacteria and only becomes available in the intestine (Cooke et al., 2011).

The aim of this study was to investigate how the source fat (oil free or rumen protected) in supplements for beef cattle at pasture affects nutrient intake and digestion.

Material And Methods

Experimental design and treatments

The experiment was developed during the transitional rainy-dry season at the beef cattle facility (15°47'5" S, 56°04' W, and 140 m above sea level) of the Sector Nutrition of Beef Cattle in Pasture, UFMT, from March 2017 to June 2017. The climate is classified as a tropical climate (Aw in the Köppen international system), the average maximum temperature is 32.8°C, and the minimum average temperature is 19.7°C.

Five rumen-cannulated Nelore bulls, with an median 467.8±32.8 kg of body weight (BW) and age of 26 months, were used in the experiment to evaluate the inclusion effects of fat-free or fat-protected supplements on nutrient intake and digestibility, ruminal pH and NH_3-N, and N utilization efficiency of the Nellore bulls over five 19-d periods. The rumen-cannulated Nelore bulls were allocate in a Latin square design (5 x 5, five treatments × five periods). The experimental period lasted 95 days and was divided into five periods of 19 days each. Each period consisted of 14 d for adaptation to the supplement and 5 d for sampling.

Initially, the rumen-cannulated Nelore bulls were identified, weighed and distributed into 5 paddocks of 0.24 ha each. The paddocks consisted of *U. brizantha cv.* Marandu and were surrounded with smooth wire fencing, more, water and feed bunks. Before allocate in the paddocks, was administered of Ivermectin (Ivomec, Merial, Paulínea, BR) to the animals, for control ecto and endoparasites.

The effects of different fat-free and fat-protected sources in the supplements were evaluated in the following treatments: **WF**: protein-energetic supplement without lipidic source; **PS:** rumen-protected fat soybean oil; **PA:** rumen-protected fat palm oil; **SO:** soybean free oil; and **CO:** corn free oil (Table 1).

The supplements used, contain 280 g of crude protein (CP) in the dry matter, and they additional support the protein for requirements of Nellore bulls in pasture, with an average body weight of 498.5±28.2 kg and weight gain of 0.620 kg/animal/day (Valadares Filho et al., 2016). The animals were supplemented at 1.0 kg DM/animal/day at 10 a.m. In each period, the ingredients were sampled. The formulation of ingredients and chemical composition of the supplements are showed in Table 1.

Table 1. Experimental supplement and chemical composition of supplements

Item[1]	Supplement (g/kg of DM)				
	WF	PS	PA	SO	CO
Ingredient					
Fine ground corn	640	450	450	480	480
Soybean meal	210	240	240	240	240
Urea	100	100	100	100	100
Mineral Mixture[2]	50	50	50	50	50
Soybean oil free	-	-	-	130	-
Corn oil free	-	-	-	-	130
Rumen-protected fat soybean oil	-	160	-	-	-
Rumen-protected fat palm oil	-	-	160	-	-
	Chemical composition Supplement (g/kg of DM)				
Item	WF	PS	PA	SO	OC
DM					
OM	877	840	834	877	877
CP	280	280	280	280	280
NDF	89	74	74	76	76
NDFap	72	60	60	62	62
NFC	510	359	353	399	399
EE	14	141	141	136	136
TDN	716	867	867	868	868

[1]Treatment: WF, without fat; PS, rumen-protected fat soybean oil; PA, rumen-protected fat palm oil; OS, soybean oil free; CO, corn oil free. [2]Mineral Mixture: 198 g calcium; 60 g phosphorous; 117 g sodium; 5.1 g magnesium; 12.6 g sulfur; 17.7 mg iodine; 425 mg iron; 10;4 mg selenium; 80 mg cobalt; 527 mg manganese; 600 mg fluorine; 1000 mg copper and 3000 mg zinc. [3]DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent insoluble fiber; NDFap, neutral detergent insoluble fiber corrected for contaminant ash and protein; NFC, nonfiber carbohydrate; EE, ether extract; TDN, total digestive nutrients.

Every 19 days, the average sward height was randomly measured by reading 25 sampling points in each paddock with the aid of a graduated measuring stick in centimeters (Barthram, 1985). The forage mass in each paddock was estimated for each period (19 d). Three forage samples and the average sward height were collected by clipping all forage within a 0.25 m^2 frame in each paddock at each sampling to 5.0 cm stubble height with hand shears. The clipped samples were dried to a constant weight under forced air at 55°C for 72 h. The forage mass of paddock to estimate by dry matter weights of these clippings were multiplied by the paddock area (Detmann et al., 2016).

The forage samples using in the herbage chemical composition analyses were collected manually using a plucking method (Johnson, 1978) that mimicked forage selected by grazing steers. Samples, collected from each pasture during each period, were dried to a constant weight at 55°C under forced air. Subsequently, samples were sent to the laboratory for analysis (chemical composition).

Chemical composition analysis

For the proximate analysis, supplement ingredient samples, forage samples, and feces samples were dried at 55°C for 72 h and then ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 2-mm screen for indigestible neutral detergent fiber (iNDF) analysis (Valente et al. 2011). Twenty grams of each sample was ground to pass through a 1-mm screen for further analyses.

The samples were analyzed following procedures described by the AOAC (1995) for dry matter (DM, method 934.01), organic matter (OM, method 942.05), ether extract (EE, method 920.85) and N using the Kjeldahl method (method 981.10). Crude protein was calculated as the percentage of N in the sample multiplied by 6.25.

The neutral detergent fiber content was corrected for contaminant ash and protein (NDFom(n), index INCT-CA F-002/1, INCT-CA M-002/1, and INCT-CA N-004/1) according to Detmann et al. (2012). Nonfiber carbohydrates (NFCs) were quantified according to Hall (2000). Based on the feedstuff chemical composition, the TDN (total digestive nutrients) contents were assessed according to the NRC (2000).

The potentially digestible dry matter (pdDM) was estimated using the second pasture sample collected in each period as previously described using the following equation (Detmann et al. 2016):

pdDM (%; dry matter basis) = 0.98* (100-NDF) + (NDF-iNDF)

where 0.98 is the true digestibility coefficient of intracellular content; NDF is the forage content of neutral detergent fiber corrected for residual ash and nitrogen; and iNDF is the forage content of indigestible neutral detergent fiber.

Intake estimation

Intake and nutrient digestibility were estimated throughout the period between days 15 and 18 using the marker method: titanium dioxide and indigestible NDF (iNDF). To estimate the excretion of fecal matter (as dry weight), the supplement intake and forage intake were used.

Fecal samples were collected directly from the rectum on the 16^{th} day from 0600 h and 1400 h, 17^{th} day from 0800 h and 1600 h, 18^{th} day from 1000 h to 1800 h, and 19^{th} day from 1200 h and 2000 h. The fecal samples were dried (55°C for 72 h) and composited throughout the day for each animal.

An external marker, titanium dioxide (15 g/animal/day), was used to estimate the DM fecal excretion and, estimated based on the ratio between the amount of marker supplied and its concentration in the feces according Holleman and White (1989), to the equation described in: FE (fecal excretion, g/d) = (TiO_2 provided (g/d)/TiO_2 concentration feces (g/kg)) x 100. Titanium dioxide was provided for 11 d by into the rumen, with the first 7 d used to stabilize fecal excretion of the marker and the last 4 d used to collect the samples (Titgemeyer et al., 2001).

An internal marker, iNDF, using for estimated the dry matter voluntary intake (DMI)
(Valente et al. 2011) by the following equation:

DMI (kg/day) = {[FE × fecal iNDF) – supplement iNDF] ÷ forage iNDF} + SDMI

where fecal iNDF = iNDF in the feces (%); supplement iNDF = iNDF in the supplement (kg/day); forage iNDF = iNDF in the forage (kg/kg); and SDMI = supplement dry matter intake (kg/day).

Samples of feces (0.5 g), forage, and supplement were placed in 5 by 5 cm polypropylene bags (nonwoven fabric, weight 100 g/m^2) to determine the iNDF. The samples were weighed to allow 20 mg DM/cm^2 of surface area (Nocek, 1988) and incubated in the rumen of a cannulated Nellore bull for a period of 288 h (Valente et al. 2011).

Nitrogen retained

Urine collection was fulfilled on the 16^{th} to 18^{th} day of each experimental period and collected at the same time as the fecal samples by spontaneous urination. Eight urine samples were stored in the form of spot samples, kept cool in a polystyrene cooler with ice, and then formed a compost aliquot to analyze the concentration of creatinine and urea, which were analyzed using the colorimetric method according to the method of Fujihara et al. (1987) as described by Chen and Gomes (1992).

Urine volume was estimated in relation the animal body weight (kg of BC), daily creatinine excretion (mg/kg BC) and, creatinine concentration (mg/L) in the urine (Chizzotti et al., 2008). To calculate the daily creatinine excretion per kg of BW, the mean of 27.76 mg/kg LW obtained by Rennó (2003) was adopted. Daily excretion was calculated as the product of the urea concentration and the urinary volume after 24

hours, which was then multiplied by 0.466, which corresponds to the nitrogen content in the urea (Rennó et al., 2000).

The amount of nitrogen retained was obtained based on the difference between the nitrogen ingested and the nitrogen excreted in the feces and urine.

Blood urea serum

Blood samples were collected on the 15th day at 0600 h and 1400 h in each experimental period. Blood samples were collected of the caudal vein, by puncture, using test tubes and kept cool in a polystyrene cooler with ice. After that, serum samples were taken (centrifuge 2000 x *g*), send on to the laboratory and analyzed to determine the urea content.

Ruminal fermentation

The concentration of ammonia nitrogen (NH_3–N) in the rumen fluid was measured on day 19 of each period. Ruminal contents were manually obtained from several sites within the rumen at 0 (before supplementation) and 3, 6 and 9 h after supplementation. Rumen fluid was obtained from strained through 2 layers of cheesecloth. Additionally, pH was measured, using a digital pH meter, immediately after collection, and samples were poured into 50-mL plastic flasks, 1 mL of 9.3 M H_2SO_4 was added, and then the contents were frozen at –20°C for subsequent analysis of NH_3–N concentration. Ruminal fluid NH_3–N was analyzed by distilling in a micro-Kjeldahl system, according to procedures of Detmann et al. (2012).

Statistical analysis

Tukey's test was used to analyze the forage of the GLM procedure of the SAS (Statistical Analysis System, version 9.3) software package, model: $Y_{ij} = \mu + T_i + e_{ij}$, where $Y_{ij\ is}$ an observation of unit *j* in treatment *i*; μ is the overall mean; T_i is the random effect of treatment *i*, with mean 0 and variance σ^2_t; and e_{ij} is the random error, with mean 0 and variance σ^2. In the analysis of variance, a value of 0.05 was considered significant.

The nutrient intake and digestibility and nitrogen balance were analyzed using a mixed model in the SAS software package (Statistical Analysis System, version 9.3) as follows: $y_{lm(i)} = \mu + A_l + P_m + \tau_i + \varepsilon_{lm(i)}$, where $y_{lm(i)}$ is the observation *lm(i)*; μ is the overall mean; A_l is a random effect animal; P_m is a random effect period; τ_i is the fixed effect of treatment *i*; $\varepsilon_{lm(i)}$ is the random error, with mean 0 and variance σ^2; and *lmi* represents five animals, periods and treatments.

Repeated measures (ruminal pH and ammonia) analyses were performed using the mixed model of the SAS software package (Statistical Analysis System, version 9.3). Ruminal pH was analyzed using the variance structure in unstructured mode, ruminal ammonia was analyzed using the variance structure in antedependence mode, and urea serum was analyzed using the variance structure in compound symmetry mode according to the AIC (Akaike Information Criteria) and BIC (Schwarz's Bayesian Information Criteria) values. The model used was $y_{ijk} = \mu + \tau_i + \delta_{ij} + t_k + (\tau * t)_{ik} + \varepsilon_{ijk}$, where i represents five supplements (treatments), j is five animals (subjects), k is four times, y_{ijk} *is* observation ijk, μ is the overall mean, τ_i is the effect of treatment i, t_k is the effect of times k, $(\tau * t)_{ik}$ is the effect of interaction between treatment i and period k, δ_{ij} is the random error with average 0 and variance σ^2 δ. The variance between animals (subjects) within treatment ijk was equal to the covariance between repeated measurements within animals. In addition, ε_{ijk} is the interaction between treatment i and period k.

Results

The average forage masses for dry matter, potentially digestible dry matter, green leaves, green stems and senescence were 4,054.4, 2,668.3, 1,141.2, 1,124.5 and 1,788.7 kg/ha, respectively (Table 2). Dry matter availability was close to the recommended value of 4,262 kg/ha, and the green leaf mass was greater than the recommended value of 1,108 kg/ha to avoid animal selectivity (Euclides et al., 1992).

The 3rd and 4th periods corresponded to April and May, and higher pdDM and green leaf mass ($P<0.05$) was observed in these periods, which was probably due to the higher rain levels influencing the proportion of green leaves in the same period (Table 2). The value and proportion of senescent leaves were higher than those of green leaves and stems in all periods due to the height of the forage of 19.6 cm.

The average content of crude protein of hand-plucked forage (Table 2) during the experimental period was 120.5 g/kg DM, which was higher than the value of 70 g/kg DM recommended by Van Soest (1994) as the limit value of basal forage fiber carbohydrates according to the rumen microorganism digestion capacity (Mathis et al., 2002).

Table 2. Forage mass and morphological components in the experimental periods

Item[1]	Periods						
	1	2	3	4	5	SEM	P value
Forage mass (kg/ha)[1]							
DM	3,605.2	3,628.3	4,534.8	4,693.4	3,810.2	315.57	0.06
pdDM	2,324.8^{b}	2,318.3^{b}	3,062.0^{ab}	3,164.5^{a}	2,246.8^{b}	294.84	<0.01
Green leaf	823.5^{b}	1,189.4^{ab}	1,515.6^{a}	1,268.2^{ab}	909.4^{b}	169.92	<0.01
Green stem	984.2	832.5	1,162.9	1,515.3	1,127.5	157.61	0.07
Senescent	1,797.2	1,606.4	1,856.32	1,909.9	1,773.3	167.3	0.75
Proportion (%)							
Green leaf (% DM)	22.8^{b}	32.8^{ab}	33.4^{a}	27.0^{ab}	23.9^{b}	3.27	0.01
Green stem (% DM)	27.3	22.9	25.6	32.3	29.6	2.13	0.10
Senescent (% DM)	49.9	44.3	40.9	40.7	46.5	2.83	0.24
Leaf: stem	0.8^{b}	1.4^{a}	1.3^{ab}	0.9^{ab}	0.8^{b}	0.18	<0.01
Bromatological and chemical composition (hand-planking; g/kg)							
DM[2]	360.25^{a}	268.56^{c}	294.11^{bc}	344.75^{a}	327.06^{ab}	16.59	<0.01
OM	911.6^{b}	914.8^{c}	921.2^{a}	916.5^{ab}	926.9^{b}	1.80	<0.01
CP	123.4^{ab}	126.1^{ab}	116.1^{bc}	105.8^{c}	130.9^{a}	2.94	<0.01
NDF	639.0^{ab}	673.5^{a}	609.5^{b}	623.3^{b}	643.3^{ab}	12.86	<0.01
NDFap	599.2^{b}	631.6^{a}	582.9^{b}	594.6^{b}	614.3^{ab}	9.38	<0.01
NFC	168.6^{bc}	140.0^{c}	202.5^{a}	191.1^{ab}	154.6^{c}	10.51	<0.01
EE	20.3^{abc}	17.1^{c}	19.7^{bc}	24.8^{ab}	27.2^{a}	1.75	<0.01

Means with the same letters in the row are not different according to Tukey's test at P>0,05. [1] DM, dry matter; pdDM (%; dry matter basis) = 0,98* (100-NDF) + (NDF-iNDF); OM, organic matter; CP, crude protein; NDF, neutral detergent insoluble fiber; NDFom(n), neutral detergent insoluble fiber corrected for contaminant ash and protein; NFC, nonfiber carbohydrate; EE, ether extract. [2] DM dry matter of forage availability.

The different lipidic sources evaluated in the supplements did not alter the intake of DM, forage, OM, CP NDF, NDFap, NFC and TDN (P>0.05) (Table 3). For ruminants at pasture, Hess et al. (2008) determined that a limit of supplemental fat of 2% of the dietary DM will prevent negative associated effects for ruminants fed high-forage diets, which was observed in the current study. The same authors affirmed that the energy density of a high forage diet will not be increased if the supplemental fat exceeds 4% of the DM.

This affirmation was confirmed in the trial of Pavan et al. (2007), who found increased fat intake of 340 to 840 g in the diet using corn oil free supplements in animals at pasture, which is higher than that observed in our experiment, and decreased forage and NDF intake. Negative effects on fiber digestibility with the inclusion of fat in the diet occur because lipids inhibit the growth of fibrotic bacteria (Patra &Yu, 2012; Huws et al., 2010).

In this study, the lipid level of the diet based on rumen-protected fat soybean oil or oil free in the supplement was 34 g/kg DM, and greater amounts of DM had deleterious effects on nutrient intake. In Carvalho et al. (2017), supplementation of beef cattle at pasture was performed with fat sources (palm and soybean oil) that provide a fat content higher than 40 g/kg DM of diet, and they indicated that this value was sufficient to decrease the DM, forage and NDF intake.

Table 3. Dry matter and nutrient intake according to source of fat supplementation for grazing bulls

Item[2]	Treatment[1]					SEM	P value
	WF	PS	PA	CO	SO		
	kg/day						
DM	9.44	9.34	8.55	9.44	9.41	1.39	0.97
Forage	8.44	8.34	7.55	8.44	8.41	1.39	0.97
OM	8.62	8.48	7.77	8.62	8.60	1.27	0.97
CP	1.28	1.28	1.18	1.29	1.29	0.16	0.97
NDF	5.40	5.39	4.97	5.48	5.38	0.89	0.99
NDFap	5.12	5.11	4.68	5.19	5.09	0.85	0.98
EE	0.21[b]	0.32[a]	0.31[a]	0.31[a]	0.32[a]	0.03	0.01
NFC	2.01	1.76	1.59	1.81	1.88	0.25	0.57
TDN	4.92	4.91	4.37	4.81	5.07	0.81	0.94
CP:OMD	0.275	0.298	0.287	0.279	0.267	0.01	0.26
	g/kg of PC						
DM	18.85	18.65	17.07	18.93	19.39	2.89	0.96
Forage	16.84	16.65	15.06	16.93	17.34	2.89	0.96
OM	17.22	16.93	15.52	17.28	17.71	2.63	0.96
apFDN	10.23	10.18	9.34	10.42	10.51	1.76	0.98

Averages with the same letters in the row did not show differences in F test at the level of P>0,05.

[1]Treatment: WF, without fat; PS, rumen-protected fat soybean oil; PA, rumen-protected fat palm oil; SO, soybean free oil; CO, corn free oil. [2]DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent insoluble fiber; NDFap, neutral detergent insoluble fiber corrected for contaminant ash and protein; NFC, nonfiber carbohydrate; EE, ether extract.

Jenkins (1993) and Patra & Yu (2012) emphasize that the elevated content of unsaturated fatty acids (C18:2) with the use of soybean oil sources or an increase in saturated short chains (C14:0 mistiric and C:12 lauric) with the use of palm oil sources in the diet have high potential for reaction with the membrane of ruminal microorganisms, which will lead to a decrease in the microbial population and is toxic to microorganisms that ferment fiber, which may reduce the intake of DM.

However, in this study, supplementation with oil-free or fat-protected levels did not have a negative effect on nutrient intake but increased (P<0.05) the fat intake in animals supplemented with fat (34 g EE/kg

DM) relative to the animals that were not supplemented with fat (22 g EE/kg DM) (Table 3). According to Doreu et al. (2009) and Ueda et al. (2003), because fat supplementation provides less than 30 g EE/kg DM of diet, the expected negative effect on the intake of forage or DM was not observed.

In relation to nutrient digestibility, DM, OM, CP, NDF, NDFap and NFC were not affected (P>0.05) by fat supplementation with free oil or rumen-protected fat (Table 4). Jenkins and Palmquist (1984) affirmed that less than 10% added fat can decrease the ruminal digestibility of structural carbohydrates by as much as 50% or more. However, this decrease was not observed here because the level of fat supplementation was consistent with the recommendation by Hess et al. (2008), who summarized previous results and indicated that an optimal inclusion rate for supplemental fat is less than 3% of DM if the goal is to maximize the intake of forage-based diets.

Table 4. Dry matter and nutrient digestibility according to the source of fat supplementation in grazing steers

Item[2]	Treatment[1]					SEM	P value
	WF	PS	PA	CO	SO		
	g/g						
DM	0.512	0.490	0.506	0.502	0.526	0.02	0.62
OM	0.558	0.534	0.548	0.548	0.570	0.02	0.61
CP	0.556	0.542	0.568	0.552	0.558	0.03	0.96
NDF	0.574	0.536	0.548	0.536	0.562	0.02	0.38
NDFom(n)	0.590	0.552	0.566	0.562	0.588	0.02	0.42
EE	0.136[b]	0.444[b]	0.456[b]	0.402[b]	0.426[b]	0.03	<0.01
NFC	0.524	0.492	0.470	0.518	0.548	0.05	0.73
TDN	0.512	0.506	0.518	0.516	0.538	0.02	0.66

[1]Treatment: WF, without fat; PS, rumen-protected fat soybean oil; PA, rumen-protected fat palm oil; SO, soybean free oil; CO, corn free oil. [2]DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent insoluble fiber; NDFom(n), neutral detergent insoluble fiber corrected for contaminant ash and protein; NFC, nonfiber carbohydrate; EE, ether extract; TDN, total digestible nutrient.

Fat supplementation increased (P<0.05) the ether extract digestibility compared with the animals that did not receive lipidic supplementation in the diet (Table 4). A previous study showed that lipid intake greater than 500 g/day per animal could exceed the maximum intestinal capacity to digest lipids (Brandt, 1995); however, later studies by Andrae et al. (2000) and Hales et al. (2017) did not confirm this finding and

indicated that an increase in lipid intake over 500 g/day in the diet linearly increased the digestibility in the total gastrointestinal tract.

The results of this study (Table 5) indicate that supplementation without or with fat did not affect (P>0.05) the ruminal parameters, such as ruminal pH and NH_3-N.

Fat supplementation may reduce the ruminal pH due to an increase in fat levels, which could enhance the glycerol content in the rumen for microbial fermentation and promote a greater release of volatile fatty acids by the potential reduction in ruminal pH (Nagaraja et al., 1997; Patra & Yu, 2012). However, in this study, the lipid levels in the diet did not change the ruminal pH (Table 5).

The ruminal ammonia concentration (Table 5) was appropriate for optimizing microbial growth and further use of fiber substrate on the forage (Sales et al., 2011) because the mean value (17.8 mg/dl) obtained in the experiment was greater than 5.0 mg/dl, which limits microbial fermentation, and close to 15.0 mg/dl, which maximizes forage intake according to Detmann et al. (2010). However, the ruminal ammonia values observed in this study were below 20.0 mg/dL, which is recommended by Mehrez et al. (1977) and Leng (1990) for maximizing the fermentation rate.

Table 5. Ruminal pH and NH_3-N and urea serum according to the source of fat in grazing steers

	Treatment					SEM	P value		
Item	WF	PS	PA	CO	SO		Treat	Time	TreatxTime
pH	6.35	6.42	6.40	6.32	6.23	0.084	0.22	<0.01	0.94
NH_3-N, mg/dl	16.05	19.82	17.19	19.70	16.06	1.724	0.30	<0.01	0.69
Serum urea, mg/dl	29.01	28.95	29.52	28.39	28.56	2.411	0.95	<0.01	0.63

[1]Treatment: WF, without fat; PS, rumen-protected fat soybean oil; PA, rumen-protected fat palm oil; SO, soybean free oil; CO, corn free oil.

The curve of ruminal pH in relation to time (Figure 1a) was quadratic (P<0.05), with a minor value of reserved time of greater intake forage. The curve of ruminal NH_3-N in relation to time (figure 1b) was cubic (P<0.05) and presented two peaks, with the first in relation to supplementation and second in relation to the greater intake of forage at the end of the day.

Nitrogen intake and nitrogen excretion in the feces and urine were not affected by fat supplementation (Table 6). At greater fat contents in the diet (>30 g EE/kg DM of diet), nitrogen retention may decrease because of the decreased microbial synthesis in the rumen under higher dietary lipid contents due to the disruption of ruminal degradation (Hales et al. 2017). Some lipidic sources are known to be toxic to ruminal microorganisms through the detergent action of fatty acids on the microbial cell membrane

(NRC, 2016) and through inhibition of enzymatic digestion. However, in this study, the inclusion of fat in the supplement (free oil or protected fat) was not enough to promote deleterious effects.

Table 6. Nitrogen balance with the source of fat supplemented to grazing steers

Item[2]	Treatment[1]					SEM	P value
	WF	OS	PA	CO	SO		
N Intake g/day	204.74	204.89	188.68	207.69	207.45	32.89	0.97
N Feces g/day	89.62	89.40	81.84	96.06	91.42	17.01	0.94
N Urine g/day	45.20	45.30	45.72	46.66	49.34	5.51	0.95
N Retained g/day	69.90	70.17	61.12	64.95	64.95	19.07	0.98
N Retained (%)	32.94	30.34	30.56	31.67	29.31	5.84	0.97

[1]Treatment: WF, without fat; PS, rumen-protected fat soybean oil; PA, rumen-protected fat palm oil; SO, soybean free oil; CO, corn free oil.

Thus, there is potential for fat use as a supplement for beef cattle at pasture at a level below 40 g EE/kg DM using fat free or rumen-protected fat sources. At this level, the lipid sources did not influence nutrient intake, nutrient digestibility or nitrogen balance. Strategies can be explored with different sources of fat. Studies have shown that palm oil, which is rich in saturated fatty acids (C16:0 palmitic acid), promotes an increase in the synthesis of subcutaneous fat while sources such as corn or soybean oil, which are rich in unsaturated fatty acids (C18:2 linoleic acid), promote decreased cell adiposity and alter energy for muscle growth (Choi et al., 2013).

Conclusion

Supplementation of grazing beef cattle with 2 g/kg BW low-level free oil (130 g/kg DM supplement) or rumen-protected oil (160 g/kg DM supplement) did not interfere with the characteristic nutrient intake and digestibility.

Declarations

Funding information is not applicable / No funding was received.

Conflicts of interest/Competing interests - The authors declare no conflict of interest

Availability of data and material – the trial is the availability of data and material

Code availability Not applicable

Author's contributions - LF, JZ, PS, and YS conceived and designed research. LF, PS, and YS conducted experiments. JZ and NP contributed new reagents or analytical tools. LF, JZ, MF and NP analyzed data. LF, JZ, NP, MF and AP wrote the manuscript. All authors read and approved the manuscript.

Ethics approval - The trial was performed at the Faculdade de Agronomia e Zootecnia, Universidade Federal de Mato Grosso (UFMT; Cuiabá, Mato Grosso, Brazil), and it followed humane animal care and handling procedures based on UFMT guidelines (Protocol 23108.060964/13-6).

Consent to participate All authors consented to participate in the study.

Consent for publication All authors consented to the publication of the study.

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References

1. Andrae, J.G., Hunt, C.W., Duckett, S.K., Kennington, L.R., Feng, P., Owens, F.N., and Soderlund, S. 2000. Effect of high-oil corn on growth performance, diet digestibility, and energy content of finishing diets fed to beef cattle, Journal of Animal Science, 78, 2257–2262.
2. AOAC. 1995. Official methods of analysis. 16th ed. AOAC Int., (Arlington, VA).
3. Barthram, G.T. 1985. Experimental techniques: The HFRO sward stick. In: The Hill Farming Research Organization biennial report 1984/1985. Hill Farming Research Organization, Penicuik, Scotland. 29–30.
4. Brandt, Jr., R.T. 1995. Use of supplemental fat to optimize net energy intake by feedlot cattle. In: Proc. Intake by Feedlot Cattle. P- 942. (Oklahoma State University, Stillwater), 303 – 311.
5. Carvalho, I.P.C. de, Fiorentinia, G., Castagninoa, P. de S., Jesusa, R.B. de, Messanaa, J.D., Granja-Salcedoa, Y.T., Detmann, E., Padmanabhad, J., McSweeney, C.S., Berchielli, T.T. 2017. Supplementation with lipid sources alters the ruminal fermentation and duodenal flow of fatty acids in grazing Nellore steers, Animal Feed Science Technology, 227, 142–153.
6. Chizzotti, M. L., S. C. Valadares Filho, R. F. D. Valadares, F. H. M. Chizzotti, and L. O. Tedeschi. 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livestok Science, 113, (2–3):218–225.
7. Choi, S.H., Gang, G.O., Sawyer, J.E., Johnson, B.J., Kim, K.H., Choi, C.W., Smith, S.B. 2013. Fatty acid biosynthesis and lipogenic enzyme activities in subcutaneous adipose tissue of feedlot steers fed supplementary palm oil or soybean oil, Journal of Animal Science, 91, 2091–2098.

8. Cooke, R.F., Bohnert, D.W., Moriel, P., Hess, B.W., and Mills, R.R. 2011. Effects of polyunsaturated fatty acid supplementation on ruminal in situ forage degradability, performance, and physiological responses of feeder cattle, Journal of Animal Science, 89, 3677–3689.
9. Detmann, E., Gionbelli, M.P., Paulino, M.F., Valadares Filho, S.C., Rennó, L.N., 2016. Considerations on research methods applied to ruminants under grazing, Nutritime Revista Eletrônica, 13, 4711–4731.
10. Detmann, E., Souza, M.A., Valadares Filho, S.C., Queiroz, A.C., Berchielli, T.T., Saliba, E.O.S., Cabral, L.S., Pina, D.S., Ladeira, M.M., Azevedo, J.A.G., 2012. Métodos para análise de alimentos – INCT – Ciência Animal, (Suprema Gráfica: Visconde do Rio Branco).
11. Detmann, E.; Paulino, M.F.; Valadares Filho, S.C. 2010. Otimização do uso de recursos forrageiros basais. In: SIMPÓSIO DE PRODUÇÃO DE GADO DE CORTE, 7, 2010, Viçosa, MG: DZO-UFV, 191–240.
12. Doreau, M., Aurousseau, E., Martin, C. 2009. Effects of linseed lipids fed as rolled seeds, extruded seeds or oil on organic matter and crude protein digestion in cows. Animal Feed Science and Technology, 150, 187–196.
13. Duckett, S.K.; Pratt, S.L., Pavan, E. 2009. Corn oil or corn grain supplementation to steers grazing endophyte-free tall fescue. II. Effects on subcutaneous fatty acid content and lipogenic gene expression, Journal of Animal Science, 87, 1120–1128.
14. Euclides, V.P.B.; Macedo, M.C.M.; Oliveira, M.P. 1992. Avaliação de diferentes métodos de amostragem [para se estimar o valor nutritivo de forragens] sob pastejo, Revista da Sociedade Brasileira de Zootecnia, 21, 4, 691–702.
15. Chen, X.B.; Gomes, M.J. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-an overview of the technical details. Buchsburnd Aberdeen: Rowett Research Institute, 21.
16. Fujihara, T.; Ørskov, E.R.; Reeds, P.J., Kyle, D.J. 1987. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition, Journal Agricultural Science, 109, 1, 7–12.
17. Hales, K. E., Foote, A. P., Brown-Brandl, T. M., Freetly, H. C. 2017. The effects of feeding increasing concentrations of corn oil on energy metabolism and nutrient balance in finishing beef steers, Journal of Animal Science, 95, 939–948.
18. Hall, M.B., 2000. Calculation of non-structural carbohydrate content of feeds that contain non-protein nitrogen. Gainesville: University of Florida, 25–32.
19. Hess, B.W., Moss, G.E., Rule, D.C. 2008. A decade of developments in the area of fat supplementation research with beef cattle and sheep. Journal of Animal Science, 86, 188–204.
20. Holleman, D.F., White, R.G. 1989. Determination of digesta fill and passage rate from non-absorbed particulate phase markers using the single dose method. Canadian Journal of Zoology, 67, 488–494
21. Huws, S.A., Lee, M.R., Muetzel, S.M., Scott, M.B., Wallace, R.J., Scollan, N.D. 2010. Forage type and fish oil cause shifts in rumen bacterial diversity, FEMS Microbiology Ecology, 73, 396–407.
22. Jenkins, T.C., Palmquist, D.L. 1984. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations, Journal of Dairy Science, 67, 978–988.

23. Jenkins, T.C., 1993. Lipid Metabolism In the Rumen, Journal of Dairy Science, 76, 381–3863.
24. Johnson, A.D. 1978. Sample preparation and chemical analysis of vegetation. In: Manejte, L.T., editor, Measurement of grassland vegetation and animal production. Commonweath Agricultural Bureaux, Aberustwysth, UK, 96–102.
25. Lazzarini, I.; Detmann, E.; Sampaio, C.B., Paulino, M.F., Valadares Filho, S. de C., Souza, M.A. de, Oliveira, F. A. 2009. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. Revista Brasileira de Zootecnia, 38, 10, 2021–2030.
26. Leng, R.A. 1990. Factors affecting the utilization of “poor-quality” forages by ruminants particularly under tropical conditions. Nutritional Research and Review, 3, 277–303.
27. Mathis, C.P., Cochran, R.C., Heldt, J.S., Woods, B.C., Abdelgadir, I.E., Olson, K.C., Titgemeyer, E.C., Vanzant, E.S. 2002. Effects of supplemental degradable intake protein on utilization of medium-to low-quality forages. Journal of Animal Science, 78, 1, 224–232.
28. Mehrez, A.Z., ØRskov, E.R., Mcdonald, I. 1977. Rates of rumen fermentation in relation to ammonia concentration. British Journal of Nutrition, 38, 3, 437–443.
29. Nagaraja, T.G., Newbold, C.J., Van Nevel, C.J., Demeyer, D.I. 1997. Manipulation of ruminal fermentation. In: P.N. Hobson and C.S. Stewart, editors, The Rumen Microbial Ecosystem. Chapman and Hall, London, UK, 523–632.
30. Nocek, J.E. 1988. In situ and other methods to estimate ruminal protein and energy digestibility: A review. Journal of Dairy Science, 71, 8, 2051–2069.
31. NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.
32. NRC. 2016. Nutrient requirements of beef cattle. 8th ed. Natl Acad. Press, Washington, DC.
33. Patra, A.K., Yu, Z. 2012. Effects of essential oils on methane production, fermentation, abundance and diversity of rumen microbial populations, Applied Environment. Microbiology, 78, 4271–4280.
34. Pavan, E.; Duckett, S.K., Andrae, J.G. 2007. Corn oil supplementation to steers grazing endophyte-free tall fescue. I. Effects on in vivo digestibility, performance, and carcass traits, Journal of Animal Science, 85, 1330–1339.
35. Rennó, L.N., Valadares, R.F.D., Leão, M.I., Valadares Filho, S. de C., Silva, J.F.C. da, Cecon, P.R., Dias, H.L.C., Costa, M.A.L., Oliveira, R.V. de. 2000. Estimativa da produção de proteína microbiana pelos derivados de purina na urina em novilhos, Revista Brasileira de Zootecnia, 29, 4, 1223–1234.
36. Rennó, L.N. Consumo, digestibilidade total e parcial, produção microbiana, parâmetros ruminais e excreções de uréia e creatinina em novilhos alimentados com dietas contendo quatro níveis de uréia ou dois de proteína. 2003. 252f. Tese (Doutorado em Zootecnia) - Universidade Federal de Viçosa, Viçosa, MG.
37. Sales, M.F.L., Paulino, M.F., Valadares Filho, S. De C., Figueiredo, D.M.de, Porto, M.O., Detmann, E. 2011. Supplementation levels for growing beef cattle grazing in the dry-rainy transition season, Revista Brasileira de Zootecnia, 40, 4, 904–911.

38. Scollan, N.D., Choi, N.J., Jurt, E., Fisher, A.V., Enser, M., Wood, J.D. 2001. Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle, British Journal Of Nutrition, 85, 115–124.
39. Shingfield, K.J., Lee, M.R.F., Humphries, D.J., Scollan, N.D., Toivonen, V., Beever, D.E. 2011. Effect of flax oil and fish oil alone or as an equal mixture on ruminal fatty acid metabolism in growing steers fed maize silage-based diets, Journal of Animal Science, 89, 3728–3741.
40. Titgemeyer, E.C., Armendariz, C.K., Bindel, D.J., Greenwood, R.H., Löest, C.A. 2001. Evaluation of titanium dioxide as a digestibility marker for cattle. Journal of Animal Science, 79, 1059–1063.
41. Ueda, K., Ferlay, A., Chabrot, J., Loor, J.J., Chilliard, Y., Doreau, M. 2003. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage: concentrate ratios. Journal of Dairy Science, 86, 3999–4007.
42. Valadares Filho, S.C., Silva, L.F.C., Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F. 2016. Nutrient requirements of zebu and crossbred cattle BR-CORTE, 3ed. UFV-Departamento de Zootecnia, Viçosa, 314.
43. Valente, T.N.P., Detmann, E., Queiroz, A.C., Valadares Filho, S.C., Gomes, D.I., Figueiras, J.F., 2011. Evaluation of ruminal degradation profiles of forages using bags made from different textiles, Revista Brasileira de Zootecnia, 40, 2565–2573.
44. Van Soest, P.J. 1994. Nutrition ecology of the ruminant. 2.ed. Ithaca: Cornell University, 476.

Figures

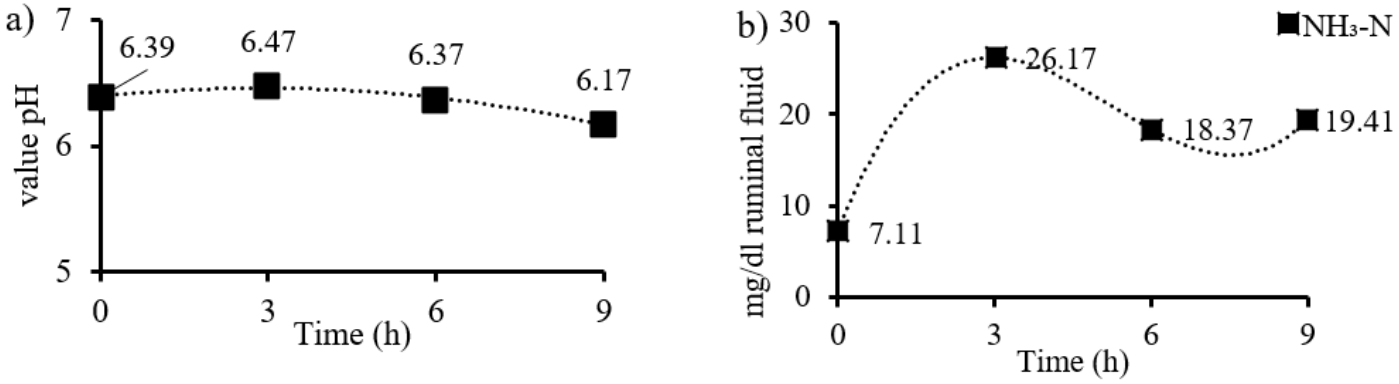

Figure 1

Curve profile of the ruminal pH (a) and ammonia (NH3-N, b) of bulls in pasture supplemented with different sources of fatty acid in relation to collection time. Equation for pH: y = - 0.0078x2 + 0.0447x + 6.394, r2 = 0.994; and equation for ruminal NH3-N: y = 0.2204x3 – 3.4756x2 + 14.797x + 7.11, r2 = 0.999.