Abstract
We investigated the consequences of fat supplementation (free oil and rumen-protected oil) on the nutrient intake and digestion of beef cattle at pasture. Five rumen-cannulated Nelore bulls, with a median body weight (BW) of 467.8 ± 32.8 kg and an age of 26 months, were distributed in a Latin square design (5 × 5). The treatments were as follows: WF, no additional fat; PA, rumen-protected palm oil; PS, rumen-protected soybean oil; SO, soybean-free oil and CO, free corn oil. Nutrient intake and digestibility, ruminal pH and ammonia (NH₃-N), serum urea and nitrogen balance were analysed. The supplements with different oil sources did not alter (P > 0.05) the intake and digestibility of dry matter (DM), forage DM intake (DMI), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), neutral detergent fibre-corrected ash and protein (apNDF), nonfibre carbohydrates (NFC) or total digestible nutrients (TDN) compared to WF. An increase (P < 0.05) in the intake and digestibility of EE was observed with the inclusion of fat, independent of the source. No differences were observed between WF and other supplements with regard to ruminal parameters (pH and NH₃-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 (2 g/kg BW) with free oil (130 g/kg DM supplement) or rumen-protected oil (160 g/kg DM supplement) did not interfere with nutrient intake and digestibility.

Keywords Corn · Free oil · Palm · Rumen protected · Soybean

Introduction
Regarding the use of fat in the diet of ruminants (Jenkins, 1993), studies on ruminal lipid metabolism focused on the manipulation of physicochemical events in the rumen, with the two following aims: (1) to control the antimicrobial effects of fatty acids so that additional fat can be fed to ruminants without disrupting ruminal fermentation and digestion and (2) to regulate microbial biohydrogenation to change the absorption of selected fatty acids that might enhance performance or improve the nutritional qualities of animal food products.

In cattle, soybean and corn oil represent sources of unsaturated fatty acids and enhance the content of conjugated linoleic acid (CLA), cis-9 and trans-11 isomer (Duckett et al. 2009; Cooke et al. 2011; Choi et al. 2013), which has anticarcinogenic and antiatherogenic effects in humans consuming beef (Scollan et al. 2001; Shingfield et al. 2011). Previous studies have shown that soybean and corn oil have the potential to provide energy for muscle gain and to decrease the size of adipose cells in subcutaneous tissue (Choi et al. 2013; Duckett et al. 2009).

Using palm oil as the source of saturated fatty acid requires a longer supplementation period and may significantly increase carcass adiposity. This might increase marbling scores without increasing the palmitic acid or reducing the oleic acid content of beef because saturated fatty acid
(palmitic acid) strongly promotes adipogenic gene expression in intramuscular preadipocytes (Choi et al. 2013).

However, fat supplementation for animals in pastures may decrease forage dry matter intake and animal performance (Carvalho et al. 2017). Lipids inhibit the growth of fibrolytic bacteria and strongly react with ruminal microorganism membranes, with lethal outcomes (Patra and Yu 2012; Huws et al. 2010).

When including fat supplements in the diet, it is important to avoid negative effects on the growth of fibrolytic bacteria; moreover, rumen-protected fat should be used because it is less harmful to ruminal bacteria and only becomes available in the intestine (Cooke et al. 2011). In this context, we hypothesise that supplementing beef cattle in the pasture with rumen-protected fat has no negative effects on nutrient intake and digestibility. The aim of this study was to determine the impacts of source fat (free oil or rumen protected) in supplements for beef cattle at pasture on nutrient intake and digestion.

### Material and methods

#### Experimental design and treatments

The experiment was performed during the transitional rainy-dry season from March to June 2017 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, Santo Antônio do Leverger, Mato Grosso, Brazil. The climate is classified as tropical climate (Aw in the Köppen international system), with an average maximum temperature of 32.8 °C and an average minimum temperature of 19.7 °C.

Five rumen-cannulated Nelore bulls, with median body weight (BW) of 467.8 ± 32.8 kg and an age of 26 months, were used to evaluate the effects of the inclusion of fat supplementation (free oil and rumen-protected fat) on nutrient intake and digestibility, ruminal pH and NH3-N, as well as N use efficiency over five 19-days periods. The bulls were allocated in a Latin square design (5 × 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 days for adaptation to the supplement and 5 days for sampling.

Initially, the bulls were labelled, weighed and distributed into five paddocks of 0.24 ha each. The animals were moved from the paddocks at each period. The paddocks consisted of *U. brizantha cv.* Marandu pasture under continuous stocking, and the canopy was maintained at a height of 30 cm. The paddocks were fitted with smooth wire fencing, waterers and individual feed bunks. Before being allocated to the paddocks, the animals were administered Ivermectin (Ivomec, Merial, Paulínea, BR) to control ecto- and endoparasites.

Table 1

| Item¹ | Supplement (g/kg of DM) | WF | PA | PS | SO | CO |
|-------|-------------------------|----|----|----|----|----|
| Fine ground corn     | 640 | 450 | 450 | 480 | 480 |
| Soybean meal         | 210 | 240 | 240 | 240 | 240 |
| Urea                | 100 | 100 | 100 | 100 | 100 |
| Mineral mixture²     | 50  | 50  | 50  | 50  | 50  |
| Soybean oil free     | -   | -   | -   | -   | -   |
| Corn oil free        | -   | -   | -   | -   | -   |
| Rumen-protected fat palm oil | -   | 160 | -   | -   | -   |
| Rumen-protected fat soybean oil | -   | -   | -   | -   | -   |

¹Treatments: WF, no additional fat; PA, rumen-protected fat palm oil; PS, rumen-protected fat soybean oil; SO, soybean-free oil; CO, corn-free oil. ²Mineral Mixture: 198 g/kg calcium; 60 g/kg phosphorous; 117 g/kg sodium; 5.1 g/kg magnesium; 12.6 g/kg sulphur; 17.7 mg/kg iodine; 425 mg/kg iron; 10; 4 mg/kg selenium; 80 mg/kg cobalt; 527 mg/kg manganese; 600 mg/kg fluorine; 1000 mg/kg copper and 3000 mg/kg zinc. ³DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent insoluble fibre; NDFAp, neutral detergent insoluble fibre corrected for contaminant ash and protein; NFC, non fibre carbohydrate; EE, ether extract; TDN, total digestive nutrients.
centimetres (Barthram 1985). Three forage samples and the average sward height were collected by clipping all forage within a 0.25-m² frame in each paddock at each sampling to a stubble height of 5.0 cm with hand shears; these samples represented the average forage height. The clipped samples were dried to constant weight at 55 °C for 72 h, and the dry weights of these clippings were multiplied by the paddock area to estimate forage mass (Detmann et al. 2016).

The forage samples for chemical composition analyses were collected manually using the hand plucking method (Johnson 1978), mimicking forage selection by grazing bulls. The samples collected from each pasture during each period were dried to constant weight under forced air at 55 °C for 72 h, and the dry weights of these clippings were multiplied by the paddock area to estimate forage mass (Detmann et al. 2016).

Intake estimation

Intake and nutrient digestibility were estimated throughout the experimental period between days 15 and 18, using the marker method with titanium dioxide and indigestible NDF (iNDF). To estimate the excretion of faecal matter (as dry weight), supplement intake and forage intake were used. Faecal samples were collected directly from the rectum (twice daily) on day 16 (−06:00 h and 14:00 h), day 17 (−08:00 h and 16:00 h), day 18 (−10:00 h to 18:00 h) and day 19 (−12:00 h and 20:00 h). The samples were dried (55 °C for 72 h) and combined to obtain one composite sample per day and animal.

An external marker, titanium dioxide (15 g/animal/day), was used to estimate the DM (dry matter) faecal excretion, estimated based on the ratio between the amount of marker supplied and its concentration in the faeces, according to Holleman and White (1989), using the following equation: FE (faecal excretion, g/day) = (TiO₂ provided (g/day)/TiO₂ concentration faeces (g/kg)) × 100. Titanium dioxide was packaged and introduced once daily into the rumen for 11 days; the first 7 days were needed to stabilise the faecal excretion of the marker and the last 4 days to collect the samples (Titgemeyer et al. 2001).

An internal marker, iNDF, was used for estimating the dry matter voluntary intake (DMI) (Valente et al. 2011) via the following equation:

\[ DMI(\text{kg/day}) = \left( \left[ \frac{FE \times \text{faecal iNDF}}{(100) \times 100} - \text{supplement iNDF} \right] + \text{forage iNDF} \right) + SDMI \]

where faecal iNDF = iNDF in the faeces (%); supplement iNDF = iNDF in the supplement (kg/day); forage iNDF = iNDF in the forage (kg/kg); SDMI = supplement dry matter intake (kg/day).

Samples of faeces (0.5 g), forage and supplement were placed in 5×5-cm polypropylene bags (nonwoven fabric, weight 100 g/m²) to determine the iNDF. The samples were weighed to allow 20 mg DM/cm² of surface area (Nocek 1988) and incubated in the rumen of a cannulated Nellore bull at pasture for a period of 288 h (Valente et al. 2011).

Nitrogen retained

Urine samples were collected on days 16 to 18 of each experimental period at the same time as the faecal samples by spontaneous urination (10-min collection time per animal). 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 analysed using the colorimetric method according to Fujihara et al. (1987) as described by Chen and Gomes (1992).

Urine volume was estimated in relation to animal body weight (kg of BW), daily creatinine excretion (mg/kg BW) and creatinine concentration (mg/L) in the urine (Chizzotti et al. 2008). The animals were weighed on days 1 and 19 of each period. 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 urea concentration and urinary volume after 24 h, which was then multiplied by 0.466, which corresponds to the nitrogen content in urine (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 faeces and urine.

Blood urea serum

Blood samples were collected, two samples at two times, on day 15 at 06:00 h and 14:00 h in each experimental period (5-min collection time per animal). Samples were collected from the caudal vein by puncture, using test tubes, and kept cool in a polystyrene cooler with ice. Subsequently, serum samples were taken (centrifuged at 2000×g), sent to the laboratory and analysed to determine the urea content.

Ruminal fermentation

The concentration of ammonia nitrogen (NH₃-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 (10-min collection time per animal). Rumen fluid was obtained from several sites within the rumen and strained through two layers of cheesecloth. Additionally, the pH was measured, using a digital pH metre (Benchtop PH/ORP Meter, PHS-3E,
Accuracy: ± 0.01 pH), immediately after collection. The samples were poured into 50-mL plastic flasks, spiked with 1 mL of 9.3 M H2SO4, and the contents were frozen at −20 °C for the subsequent analysis of NH3-N. Ruminal fluid NH3-N was analysed by distilling in a micro-Kjeldahl system, according to Detmann et al. (2012).

### Chemical composition analysis

For the proximate analysis, supplement ingredient samples, forage samples and faeces 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 iNDF analysis (Valente et al. 2011). For further analyses, 20 g of each sample was ground to pass through a 1-mm screen. The samples were analysed 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 fibre content was corrected for ash and protein (apNDF), index INCT-CA F-002/1, INCT-CA M-002/1 and INCT-CA N-004/1 according to Detmann et al. (2012). Nonfibre carbohydrates (NFCs) were quantified according to Hall (2000). Based on the feedstuff chemical composition, the TDN (total digestive nutrients) was assessed according to the NRC (2000).

### Statistical analysis

The forage was analysed using a GLM model in the SAS software package (Statistical Analysis System, version 9.3), with the following equation: \( Y_{ij} = \mu + A_i + P_m + \tau_j + e_{ij} \), where \( Y_{ij} \) is an observation of unit \( j \) in treatment \( i \); \( \mu \) is the overall mean; \( A_i \) is a random effect of the animal; \( P_m \) is a random effect of the period; \( \tau_j \) is the fixed effect of treatment \( j \); \( e_{ij} \) is the random error, with mean 0 and variance \( \sigma^2 \). In the analysis of variance, a value of 0.05 was considered significant (Tukey’s test). The paddocks represented the experimental unit.

Nutrient intake and digestibility and nitrogen balance were analysed using a mixed model in the SAS software package (Statistical Analysis System, version 9.3): \( y_{ilm(i)} = \mu + A_i + P_m + \tau_j + e_{ilm(i)} \), where \( y_{ilm(i)} \) is the observation \( lmi \) (bi); \( \mu \) is the overall mean; \( A_i \) is a random effect of the animal; \( P_m \) is a random effect of the period; \( \tau_j \) is the fixed effect of treatment \( j \); \( e_{ilm(i)} \) is the random error, with mean 0 and variance \( \sigma^2 \) and \( lmi \) represents five animals, periods and treatments.

Repeated measure (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 analysed using the variance structure in unstructured mode, ruminal ammonia was analysed using the variance structure in antedependence mode and urea serum was analysed 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 \( y_{ijk} = \mu + \tau_k + \delta_{ij} + \tau_k + (\tau^* t_k) + e_{ijk} \), where \( i \) represents five supplements (treatments); \( j \) is five animals (subjects); \( k \) is four times; \( y_{ijk} \) is observation \( ijk \); \( \mu \) is the overall mean; \( \tau_k \) is the fixed effect of treatment \( i \); \( t_k \) is the random effect of times \( k \); \( (\tau^* t_k) \) is the effect of interaction between treatment \( i \) and period \( k \) and \( \delta_{ij} \) is the random error with average 0 and variance \( \sigma^2 \). The variance between animals (subjects) within treatment \( ijk \) was equal to the covariance between repeated measurements within animals. In addition, \( e_{ijk} \) is the interaction between treatment \( i \) and period \( k \).

### Results

The average forage mass values for DM, green leaves, green stems and senescent matter were 4054.4, 1141.2, 1124.5 and 1788.7 kg/ha, respectively (Table 2).

The 3rd and 4th periods corresponded to April and May, and higher 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 average content of crude protein of hand-plucked forage (Table 2) during the experimental period was 120.5 g/kg DM. The different lipid sources evaluated in the supplements did not alter the intake of DM, forage, OM, CP NDF, apNDF, NFC and TDN (\( P > 0.05 \)) compared to WF (Table 3). In this study, supplementation with free or rumen-protected oil had no 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), the supplemental additional fat of 1.2 percentage points of the dietary DM (Table 3).

In relation to nutrient digestibility, DM, OM, CP, NDF, apNDF and NFC were not affected (\( P > 0.05 \)) by fat supplementation with free oil or rumen-protected fat (Table 4). Fat supplementation increased (\( P < 0.05 \)) the ether extract digestibility compared with the animals that did not receive lipid supplementation in the diet (Table 4).

The results of this study (Table 5) indicate that supplementation without or with additional fat did not affect (\( P > 0.05 \)) ruminal parameters such as ruminal pH and NH3-N.

The profile of ruminal pH in relation to time (Fig. 1a) was quadratic (\( P < 0.05 \)), and the curve of ruminal NH3-N in relation to time (Fig. 1b) was cubic (\( P < 0.05 \)).
Table 2  Forage mass and morphological components in the experimental periods

| Item | Periods | SEM | P value |
|------|---------|-----|---------|
|       | 1° | 2° | 3° | 4° | 5° |
| Forage mass (kg/ha) | | | | | |
| DM | 3605.2 | 3628.3 | 4534.8 | 4693.4 | 3810.2 | 315.57 | 0.06 |
| Green leaf | 823.5<sub>b</sub> | 1,189.4<sub>ab</sub> | 1,515.6<sup>a</sup> | 1,268.2<sub>ab</sub> | 909.4<sub>b</sub> | 169.92 | <0.01 |
| Green stem | 984.2 | 832.5 | 1162.9 | 1515.3 | 1127.5 | 157.61 | 0.07 |
| Senescent | 1797.2 | 1606.4 | 1856.3 | 1909.9 | 1773.3 | 167.3 | 0.75 |
| Proportion (%) | | | | | |
| Green leaf (% DM) | 22.8<sub>b</sub> | 32.8<sub>ab</sub> | 33.4<sup>a</sup> | 27.0<sub>ab</sub> | 23.9<sub>b</sub> | 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<sub>b</sub> | 1.4<sup>a</sup> | 1.3<sub>ab</sub> | 0.9<sub>ab</sub> | 0.8<sub>b</sub> | 0.18 | <0.01 |
| Chemical composition (hand planked methodology; g/kg) | | | | | |
| DM | 360.25<sup>a</sup> | 268.56<sub>c</sub> | 294.11<sub>bc</sub> | 344.75<sup>a</sup> | 327.06<sub>ab</sub> | 16.59 | <0.01 |
| OM | 911.6<sub>b</sub> | 914.8<sub>c</sub> | 921.2<sub>a</sub> | 916.5<sub>ab</sub> | 926.9<sub>b</sub> | 1.80 | <0.01 |
| CP | 123.4<sub>b</sub> | 126.1<sub>ab</sub> | 116.1<sub>c</sub> | 105.8<sub>b</sub> | 130.9<sub>a</sub> | 2.94 | <0.01 |
| NDF | 639.0<sub>b</sub> | 673.5<sub>s</sub> | 609.5<sub>b</sub> | 623.3<sub>b</sub> | 643.3<sub>ab</sub> | 12.86 | <0.01 |
| apNDF | 599.2<sub>b</sub> | 631.6<sub>s</sub> | 582.9<sub>b</sub> | 594.6<sub>b</sub> | 614.3<sub>ab</sub> | 9.38 | <0.01 |
| NFC | 168.6<sub>bc</sub> | 140.0<sub>c</sub> | 202.5<sub>a</sub> | 191.1<sub>ab</sub> | 154.6<sub>c</sub> | 10.51 | <0.01 |
| EE | 20.3<sub>bc</sub> | 17.1<sub>c</sub> | 19.7<sub>bc</sub> | 24.8<sub>ab</sub> | 27.2<sub>a</sub> | 1.75 | <0.01 |

Means with the same letters in the row are not different according to Tukey’s test at P > 0.05. 1DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent insoluble fibre; apNDF, neutral detergent insoluble fibre corrected for ash and protein; NFC, nonfibre carbohydrate; EE, ether extract. 2DM dry matter of forage availability

Table 3  Dry matter and nutrient intake according to the source of fat supplemented (free oil 130 g/kg DM supplement or rumen-protected oil 160 g/kg DM supplement) to grazing bulls

| Item | Treatment<sup>1</sup> | SEM | P value |
|------|------------------|-----|---------|
|       | WF | PA | PS | 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 |
| apNDF | 5.12 | 5.11 | 4.68 | 5.19 | 5.09 | 0.85 | 0.98 |
| EE | 0.21<sub>b</sub> | 0.32<sub>a</sub> | 0.31<sub>a</sub> | 0.31<sub>a</sub> | 0.32<sub>a</sub> | 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 BW | | | | | |
| 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 the F test at the level of P > 0.05. 1Treatment: WF, no additional fat; PA, rumen-protected fat palm oil; PS, rumen-protected fat soybean oil; SO, soybean-free oil; CO, corn-free oil. 2DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent insoluble fibre; apNDF, neutral detergent fibre corrected for ash and protein; NFC, nonfibre carbohydrate; EE, ether extract.
Nitrogen (N) intake, N excretion in the faeces and urine, apparent N digested and N retained were not affected by fat supplementation (Table 6).

Discussion

Dry matter availability was close to the recommended value of 4262 kg/ha, and the green leaf mass was greater than the recommended value of 1108 kg/ha to avoid animal selectivity (Euclides et al. 1992). The average content of crude protein of hand-plucked forage was higher than the value of 70 g/kg DM recommended by Van Soest (1994) to meet the minimum nitrogenous compound requirements so that the activity of rumen microorganisms is not limited. The different lipid sources evaluated as supplements did not alter the nutrient intake compared to WF. For ruminants at pasture, Hess et al. (2008) determined that a limit of supplemental additional fat of 2.0 percentage points (p.p.) of the dietary DM will prevent negative effects on ruminants fed high-forage diets, which was observed in the current study.

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

In this study, the lipid level of the diet based on rumen-protected fat soybean oil or fee oil was 34 g/kg DM, with a supplemental additional fat of 1.2 percentage points of the dietary DM, no provide negative effects on intake. The greater amounts EE of DM in the diet (> 3 5 g/kg EE of DM) might have 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 the diet, with the supplemental additional fat of 2.4 percentage points of the dietary DM; the authors indicated that this value was sufficient to decrease DM, forage and NDF intake.

Jenkins (1993) and Patra and Yu (2012) emphasise that the elevated contents 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 can react with the membranes of ruminal microorganisms, which will lead to a decrease in the microbial population and can negatively impact microorganisms that ferment fibre, subsequently reducing DM intake.

| Item | Treatment | SEM | P value |
|------|-----------|-----|---------|
|     | WF | PA | PS | 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 | 0.500 | 0.488 | 0.511 | 0.497 | 0.502 | 0.03 | 0.96 |
| NDF | 0.574 | 0.536 | 0.548 | 0.536 | 0.562 | 0.02 | 0.38 |
| apNDF | 0.590 | 0.552 | 0.566 | 0.562 | 0.588 | 0.02 | 0.42 |
| EE | 0.136 | 0.444 | 0.456 | 0.402 | 0.426 | 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, no additional fat; PA, rumen-protected fat palm oil; PS, rumen-protected fat soybean oil; SO, soybean-free oil; CO, corn-free oil. **DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent insoluble fibre; apNDF, neutral detergent fibre corrected for ash and protein; NFC, non-fibre carbohydrate; EE, ether extract; TDN, total digestible nutrient**

| Item | Treatment | SEM | P value |
|------|-----------|-----|---------|
|     | WF | PA | PS | CO | SO |
| pH | | | | | | 6.35 | 6.42 | 6.40 | 6.32 | 6.23 | 0.084 | 0.22 | <0.01 | 0.94 |
| NH₃-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, no additional fat; PA, rumen-protected fat palm oil; PS, rumen-protected fat soybean oil; SO, soybean-free oil; CO, corn-free oil
In this study, supplementation with free oil or rumen-protected oil increased the fat intake in animals. According to Doreau 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.

Nutrient digestibility was not affected (P > 0.05) by fat supplementation with free oil or rumen-protected fat. Jenkins and Palmquist (1984) reported that less than 10% added fat can decrease the ruminal digestibility of structural carbohydrates by 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 summarised results from previous studies and indicated that an optimal inclusion rate for supplemental fat is less than 3% of DM if the goal is to maximise the intake of forage-based diets.

Ether extract digestibility increased with fat supplementation. A previous study has shown that a lipid intake greater than 500 g/day per animal could exceed the maximum intestinal capacity to digest lipids (Brandt, 1995); however, 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 ruminal pH and NH$_3$-N contents were not affected by fat supplementation. Fat supplementation may reduce the ruminal pH due to an increase in fat levels, which could be explained by the glycerol content in the rumen for microbial fermentation, promoting the release of volatile fatty acids by the potential reduction in ruminal pH (Nagaraja et al. 1997; Patra and Yu 2012). However, in this study, the lipid levels in the diet had no effect on ruminal pH.

The ruminal ammonia concentration was appropriate for optimising microbial growth and the further use of fibre substrate in 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 maximises 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 maximising the fermentation rate.

The curve of ruminal pH with a minor value is because of supplement intake. The curve of ruminal NH$_3$-N presented two peaks: the first one was related to supplementation and the second one to the greater intake of forage at the end of the day. According to Reis et al. (2009), peak forage intake occurs after supplement intake, which could contribute to higher ruminal NH$_3$-N contents.

The values of N excretion and N retained corroborate with the results obtained by Jose Neto et al. (2016) and Silva et al. (2021). The reported 51.44 g/day N retention for cattle fed fat supplementation (free oil and rumen-protected oil) means that bulls should have gained over 1.5 kg/day, assuming 16% protein in the tissue. In reality, the bulls gained an average of 0.6 kg/day throughout the experimental period (data not shown). Potential sampling error can also include loss of feed or drying of faecal samples. Although the N content in faeces was not determined in wet samples in the present study, Walter et al. (2012) reported an average N difference was only 0.6% between the dried and wet faecal samples. Other sources of unaccounted for N losses could be in the form of gaseous N$_2$ or nitrate and nitrite formation by rumen microbes (Spanghero and Kowalski 1997).

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 lipid sources are 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 rumen-protected fat) was not sufficient to promote deleterious effects. Thus, there is potential for fat use as a
supplement for beef cattle at pasture at a level below 40 g EE/kg DM diet using free oil (130 g/kg DM supplement) or rumen-protected oil (160 g/kg DM supplement). 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, whereas 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 (2 g/kg BW) with free oil (130 g/kg DM supplement) or rumen-protected oil (160 g/kg DM supplement) did not interfere with nutrient intake and digestibility.

**Acknowledgements** We appreciate CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and UFMT (Universidade Federal de Mato Grosso).

**Author contribution** L. F., J. Z., P. S. and Y. S. conceived and designed research. L. F., P. S. and Y. S. conducted experiments. J. Z. and N. P. contributed new reagents or analytical tools. L. F., J. Z., M. F. and N. P. analysed data. L. F., J. Z., N. P., M. F. and A. P. wrote the manuscript. All authors read and approved the manuscript.

**Data availability** The trial is the availability of data and material.

**Code availability** Not applicable.

**Declarations**

**Ethics approval** The trial was performed at the Faculdade de Agro-nomia 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.

**Conflict of interest** The authors declare no competing interests.

**References**

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.

AOAC. 1995. Official methods of analysis. 16th ed. AOAC Int., (Arlington, VA).

Barthram, G.T. 1985. Experimental techniques: The HFRO sward
stock. In: The Hill Farming Research Organization biennial report 1984/1985. Hill Farming Research Organization, Penicuik, Scot-
land, 29–30.

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.

Carvalho, I.P.C. de, Fiorentinia, G., Castagnino, P. de s., Jesusa, R.B. de, Messanaa, J.D., Granja-Salcedoa, Y.T., Detmann, E., Padmanabh, J., McSweeney, C.S., Berchielli, T.T. 2017. Sup-
plementation with lipid sources alters the ruminal fermentation and duodenal flow of fatty acids in grazing Nellore steers, Animal Feed Science and Technology, 227, 142-153.

Chen, X.B.; Gomes, M.J. 1992. Estimation of microbial protein supply
to sheep and cattle based on urinary excretion of purine deriva-
tives—an overview of the technical details. Bucksburn Aberdeen:
Rowett Research Institute, 21.

Chizzotti, M. L., S. C. Valadares Filho, R. F. D. Valadares, F. H. M. Chizzotti, and L. O. Tedeschi. 2008. Determination of creatinine
ecretion and evaluation of spot urine sampling in Holstein cattle. Livestock Science, 113, (2–3):218–225.

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.

Cooke, R.F., Bohnt, D.W., Morriel, P., Hess, B.W., and Mills, R.R. 2011. Effects of polyunsaturated fatty acid supplementation on ruminal in situ forage degradability, performance, and physi-
ological responses of feeder cattle, Journal of Animal Science, 89, 3677–3689.

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.

Detmann, E., Souza, M.A., Valadares Filho, S.C., Queiroz, A.C., Ber-
chielli, T.T., Saliba, E.O.S., Cabral, L.S., Pina, D.S., Ladeira, M.M., Azvedo, J.A.G., 2012. Métodos para análise de alimen-
tos – INCT – Ciência Animal, (Suprema Gráfica: Visconde do Rio Branco).

Detmann, E.; Paulino, M.F.; Valadares Filho, S.C. 2010. Otimi-
zação do uso de recursos forrageiros basais. In: SIMPÓSIO DE PRODUÇÃO DE GADO DE CORTE, 7, 2010, Viçosa, MG: DZO-UFV, 191–240.

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 Tech-
ology, 150, 187–196.

Duckett, S.K.; Pratt, S.L., Pavan, E. 2009. Corn oil or corn grain sup-
plementation 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.

Euclides, V.P.B.; Macedo, M.C.M.; Oliveira, M.P. 1992. Avaliação de diferentes métodos de amostragem [para se estimar o valor nutri-
tivo de forragens] sob pastejo, Revista da Sociedade Brasileira de Zootecnia, 21, 4, 691–702.

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 of Agricultural Science, 109, 1, 7-12.

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.

Hall, M.B., 2000. Calculation of non-structural carbohydrate content of feeds that contain non-protein nitrogen. Gainesville: University of Florida, 25-32.

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.

Hollemann, 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.

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.

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.

Jenkins, T.C., 1993. Lipid metabolism in the rumin. Journal of Dairy Science, 76, 381–3863

Johnson, A.D. 1978. Sample preparation and chemical analysis of vegetation. In: Maneje, L.T., editor, Measurement of grassland vegetation and animal production. Commonwealth Agricultural Bureaux, Aberystwyth, UK, 96–102.

Jose Neto, A., Zervoudakis, J.T., da Silva-Marques, R.P., Silva, L.C.R.P., Hatamoto-Zervoudakis, L.K., Klopfenstein, T.J. 2016. Suitable strategy to improve nitrogen utilization and reduce the environmental impact of Nellore bulls supplemented on tropical pasture, Journal of Animal Science, 94, 1110–1122.

Leng, R.A. 1990. Factors affecting the utilization of “poor-quality” forages by ruminants particularly under tropical conditions. Nutri-
Rational Research and Review, 3, 277-303.

Mehrez, A.Z.,Ørskov, E.R., Mcdonald, I. 1977. Rates of rumen fer-
mentation in relation to ammonia concentration. British Journal of Nutrition, 38, 3, 437-443.

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.

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.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2016. Nutrient requirements of beef cattle. 8th ed. Natl Acad. Press, Washington, DC.

Patra, A.K., and Yu, Z. 2012. Effects of essential oils on methane
production, fermentation, abundance and diversity of rumen microbrial populations, Applied Environmental Microbiology, 78, 4271–4280.

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.

Reis, R.A., Ruggieri, A.C., Casagrande, D.R., Páscoa, A.G. 2009. Suplementação da dieta de bovinos de corte como estratégia do manejo das pastagens, Revista Brasileira de Zootecnia, 38, 147-159.

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, 1233-1234.

Rennó, L.N. 2003. Consumo, digestibilidade total e parcial, produção microbiana, parâmetros ruminais e excreções de ureia e creatinina em novilhos alimentados com dietas contendo quatro níveis de
ureia ou dois de proteína. 252f. Tese (Doutorado em Zootecnia) - Universidade Federal de Viçosa, Viçosa, MG.

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.

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.

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.

Silva, Y.R.V.B., Zervoudakis, J.T., Hatamoto-Zervoudakis, L.K., Abreu, M.L.C., Cabral, L.S., Freiria, L.B., Silva, P.I.J.L.R., Possamai, A.J. 2021. Supplementation with different protein profiles for grazing beef cattle supplemented in tropical grass during the rainy-dry transition season, Tropical Animal Health and Production, 53, 29, 1-10.

Spanghero, M., Kowalski, Z.M.1997. Critical analysis of N balance experiments with lactating cows. Livestock Production Science, 52,113–122.

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.

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.

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.

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.

Van Soest, P.J. 1994. Nutrition ecology of the ruminant. 2.ed. Ithaca: Cornell University, 476.

Walter, L.J., McAllister, T.A., Yang, W.Z., Beauchemin, K.A., He, M., McKinnon, J.J., 2012. Comparison of wheat or corn dried distillers’ grains with soluble on rumen fermentation and nutrient digestibility by feedlot heifers. Journal of Animal Science, 90, 1291–1300.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.